AUTOIMMUNE PANCREATITIS

DIAGNOSTIC AND IMMUNOLOGICAL ASPECTS

Marianne van Heerde

Autoimmune pancreatitis
diagnostic and immunological aspects

Marianne van Heerde

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Chapter 1

Introduction, aims and outline

INTRODUCTION

DISEASE PRESENTATION AND EPIDEMIOLOGY

Autoimmune pancreatitis (AIP) is the pancreatic manifestation of a systemic fibro- inflammatory disease, characterized by infiltration with lymphoplasmacytic cells and extensive fibrosis, which leads to morphological changes (swelling, mass forming) and organ dysfunction.[1] Often, but not always, the disease is characterized by elevated levels of serum IgG4 and IgG4 positive plasma cells in the affected tissues.[2-3] The disease was initially described by Sarles in 1961.[4] A major breakthrough was the identification in 2001 of serum IgG4 levels as biomarker.[2] IgG4 levels are elevated in 68-95% of patients with AIP.[2, 5-9] The concept of IgG4-related systemic disease was adopted in 2003.[10] Currently two types of AIP are recognized: type 1 which is associated with IgG4-related disease, and type 2, which has substantial clinical overlap but distinctive pathological features.[11] There are little data on global incidence and prevalence of AIP. Virtually all data regarding epidemiology comes from Japan. Based on these data, the estimated prevalence is 2.2/100.000, with an annual incidence rate of 0.9/100.000.[12] It is believed that AIP accounts for 5-6% of all patients with chronic pancreatitis. In patients who underwent resection for presumed malignancy, AIP is found in 2.5%.[13] The male to female ratio is 3.7 and the mean age is 63 years.[12] The typical clinical presentation is that of an older man with jaundice, diffuse or focal pancreatic enlargement or mass, substantial weightloss and recent onset diabetes.
Other organ involvement is common in the course of AIP. Usually it coincides with or follows the pancreatic manifestation, but sometimes it may herald AIP. The biliary tree, salivary glands, retroperitoneum, kidneys and lymph nodes are involved most frequently, which may lead to sclerosing cholangitis, Sjögren like syndrome, retroperitoneal fibrosis, interstitial nephritis, pseudotumors in lungs or liver and generalized or localized lymphadenopathy.[1, 14]
Spontaneous remissions and relapses are common. The disease is highly responsive to steroids[15-16] and this can be used as a diagnostic tool.[17]

DIAGNOSIS

Because of its clinical resemblance with other pancreatobiliary diseases, including malignancy, diagnosis is often troublesome (Figure 1-3). Correct diagnosis at an early stage is crucial to prevent both unnecessary and potentially harmful surgery in AIP patients, as well as steroid trials with

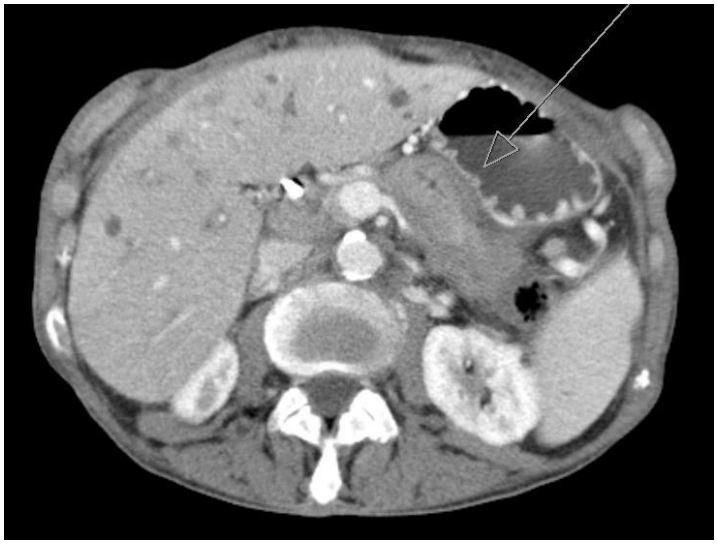

Figure 1 CT scan.
This patient was primarily diagnosed with cholangiocarcinoma with liver metastases. Two years later, a diagnosis of AIP with sclerosing cholangitis was made. The liver lesions proved to be cysts, diffuse pancreatic swelling with hypodense rim was already present initially, but not recognized.

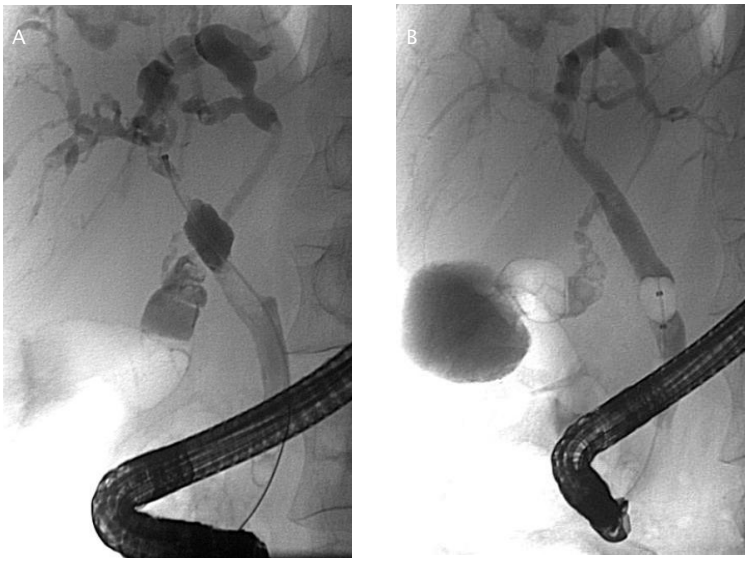

Figure 2 ERCP of same patient as **Figure 1**.
A. Two stenoses of hepatocholedochal duct, a biliary stent was placed.
B. Response to steroid therapy

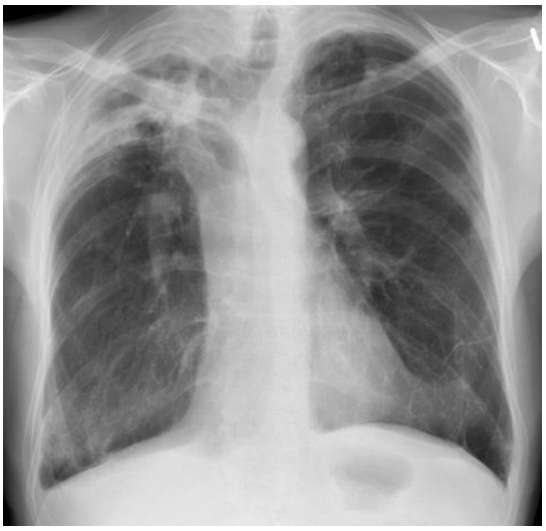

Figure 3 Nodular pulmonary lesion in the right upper lobe, suspicious of malignant tumor. Histology showed fibrosis and IgG4 positive cells.

subsequent delay of effective treatment in cancer patients.[18] Therefore exclusion of malignancy is the first step in the diagnostic process. To confirm a diagnosis of AIP, histology is considered the gold standard.[19] In resection specimens diagnosis can be made relatively easy, but is represents failure of timely diagnosis. Cytology is important to exclude malignancy, but lacks sufficient diagnostic performance to establish the diagnosis of AIP.[20] Fine needle biopsy is promising in expert hands[21-22] but is not routinely available in everyday gastroenterology practice. There is no single diagnostic test to diagnose AIP, but diagnosis can be confirmed by the use of diagnostic criteria, combining radiological (diffuse or focal enlargement, occasionally with rim enhancement, diffuse or segmental narrowing of the pancreatic duct), histological (lymphoplasmacytic sclerosing pancreatitis and IgG4 staining in type 1, granulocyitic epithelial lesions in type 2), serological (IgG4, IgG and the presence of autoantibodies like rheumatoid factor or antinuclear antibody) and other criteria such as other organ involvement and response to steroid therapy (Table 1). Numerous sets of diagnostic criteria have been proposed. The Asian criteria[23] and HISORt criteria[19] are used most frequently. In the Asian criteria, radiology is crucial, in particular diagnostic pancreatography. HISORt criteria enable diagnosis through different approaches: IgG4, steroid trial or histology. Recently the extensive International Consensus Diagnostic Criteria of AIP have been accepted and published.[24] For use in clinical practice, these

Table 1 Diagnostic criteria for autoimmune pancreatitis

Radiology	Diffuse or focal enlarged pancreas with hypodense rim Diffuse or focal narrowing of pancreatic duct Rarely pancreatic calcifications or cysts
Serology	Elevated IgG or IgG4 (>2x ULN), presence of autoantibodies (ANA, RF)
Other organ involvement	Sclerosing cholangitis, parotid/lacrimal gland enlargement, retroperitoneal fibrosis, interstitial nephritis, mediastinal or generalized lymphadenopathy, inflammatory tumors lungs and liver, inflammatory bowel disease
Response to therapy	prednisone 0.5 mg/kg/d, during 2 weeks
Histology	Type 1: lymphoplasmacytic infiltration with fibrosis or IgG4 positive plasmacells (>10 /hpf) Type 2: idiopathic duct centric pancreatitis or granulocyte epithelial lesion

Simplified summary of Asian and HISORt criteria.[19,23] Abbreviations ULN = upper limit of normal, hpf = high power field.

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.[25] Moreover, to avoid post-ERCP pancreatitis, gastroenterologists usually do not attempt to deliberately cannulate and fill the pancreatic duct.[26] A non-invasive modality such as magnetic resonance cholangio-pancreatography (MRCP) is still inferior to ERCP in detecting the subtle ductal changes in AIP.[27] 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 kind of difficult diagnostic situations which might improve sensitivity, but consequently complicate its application. This renders ICDC unsuitable for use in routine daily practice.

PATHOGENESIS

The pathogenesis of AIP remains to be elucidated. The current understanding is that in genetically susceptible persons,[28-29] an autoimmune reaction (Th1 mediated) or infectious agent (through molecular mimicry or innate immunity) triggers a Th2 mediated (allergic) immune response which leads to excessive or inappropriate B cell activation.[30-33] It is not known whether IgG4, classically known as an anti-inflammatory immunoglobulin,[34] is pathogenic or an epiphenomenon. Although numerous general and

pancreas-specific auto antigens have been proposed as initial trigger,[35] sufficient evidence is lacking. In 2009 *Helicobacter pylori* was launched as causal agent,[36] but results have not been confirmed yet.

TREATMENT AND PROGNOSIS

The treatment of AIP consists of three modalities: immunosuppression, supportive therapy and surgery. Although surgery is considered synonymous with failure of the diagnostic process, it is still inevitable in a substantial number of patients, mainly because malignancy could not be ruled out properly. In high volume centers, mortality is less than 5%[37] and is still decreasing.[38-39] Relapse is less common after surgery than after steroid therapy (20% versus 25-60%).[40-41] Supportive therapy includes biliary decompression by endoscopic stent placement (which also provides important diagnostic information), suppletion of pancreatic enzymes in case of exocrine insufficiency and treatment of diabetes. Steroid therapy is the corner stone in AIP treatment and is highly effective. Response rates are excellent (98%),[42] but relapses are common. Recommended induction doses are 30 to 40mg/day during 4 weeks, comparable with schemes used in other autoimmune diseases. Since AIP is highly responsive to steroids, the need of such high induction dose is debatable. We noticed excellent responses in several patients with low dose prednisone (10 to 20 mg), but this finding was neither confirmed in literature nor commonplace. After the initial phase, the dose can be tapered gradually (usually 5mg/week) and stopped after 3 months (recommended in the USA). In a nationwide study in Japan, long-term (2-3 years) maintenance therapy was recommended with low dose (2.5 to 10mg/day). The relapse rate with maintenance treatment was 23%, which was significantly lower than in patients who stopped (34%, p=0.048).[42] Despite the excellent initial response rates, relapse is common, especially in patients with proximal biliary involvement.[40-41] Persistently high values of IgG4 are not predictive of relapse.[41] In case of relapse, another course of steroids is equally effective.[42] Step up therapy with immunomodulators like azathioprine, 6-mercaptopurine or mycophenolate mofetil can be used.[43] Although the steroid-sparing concept is attractive and has been proven effective in other autoimmune disease, its efficacy in AIP has not been demonstrated conclusively. Recently selective B-cell depletion therapy by rituximab, a monoclonal antibody directed against the CD20 antigen on B-cells, was shown to be effective in steroid-refractory or relapsing disease.[43]

In most patients the long-term prognosis is excellent. A small subset of patients will be diagnosed in the chronic phase, with chronic pancreatitis (with or without exocrine insufficiency), or less common with pseudocyst formation, pancreatic duct stones or vascular complications like splenic vein thrombosis. Sequels of extrapancreatic manifestations like liver fibrosis / cirrhosis and chronic renal failure may cause substantial morbidity and mortality. AIP may be a risk factor of pancreatobiliary cancer, but the exact risk and its subsequent implications for long term follow-up and surveillance are unknown.[44]

AIMS

We aimed to characterize a group of 114 AIP patients and explore the performance of the three major diagnostic criteria systems in this group. Second we aimed to determine the extent and cause of misdiagnosis in patients that underwent resection for presumed malignancy of the pancreatic head. Furthermore we examined the serological profile, including serum total IgE and tumor marker Ca 19-9 in AIP and other pancreatobiliary disorders with a dual purpose: explore their value in differential diagnosis with malignancy and gain insight into pathogenesis. Finally we aimed to compare the efficacy of low dose versus high dose steroid induction therapy.

OUTLINE

In Chapter 2 a multicenter national cohort of 114 AIP patients is characterized. In this retrospective study the performance of the three major diagnostic criteria systems of AIP is investigated. Ultimately, recommendations are made which system should preferably be used in specific situations. In Chapter 3 we retrospectively analyze all pancreatoduodenectomies performed for presumed malignancy of the pancreatic head in the Erasmus University Medical Center, in a 9 year period. The number and type of benign diseases are established including the prevalence of AIP. The preoperative work up is evaluated and recommendations are made how to prevent unnecessary surgery.

The tumor marker Ca 19-9 is often elevated in pancreatic and biliary carcinoma. Marked elevation (>300 kU/L) is generally considered highly specific for malignancy.[45] In AIP however, very high levels are sometimes observed and

may prompt clinicians to embark on surgery. In search of an optimal cut-off level of Ca 19-9 to differentiate between AIP and pancreatic cancer, we study the Ca 19-9 levels in AIP and other benign and malignant pancreatobiliary diseases in Chapter 4. Moreover, IgG4 and Ca 19-9 levels are combined to improve diagnostic performance.

We are in need of new, preferably serological tests with sufficient sensitivity and specificity. Evidence shows that both autoimmune and allergic mechanisms are important in the inflammatory cascade observed in AIP. Most studies on serological markers focus on autoimmunity. This is also reflected in the fact that the presence of auto-antibodies is incorporated in several diagnostic criteria systems of AIP. In search of the triggering antigen, numerous general and pancreas-specific antigens are proposed. In general the results of these studies on auto-antibodies lack validation and reproducibility. Compared to the numerous studies on autoimmunity, the role of allergic mechanisms has been underexposed. In chapter 5 a pilot study describes the relation between serum total IgE and IgG4 in AIP, atopic allergy and pancreatic carcinoma. Furthermore the potential value of serum total IgE in the differentiation between AIP and pancreatic carcinoma is studied. In Chapter 6 an extensive serological profile, including several auto-antibodies, is determined in AIP and various pancreatobiliary diseases. The diagnostic performance of each test is assessed to differentiate between AIP and malignancy. Ultimately a logistic regression analysis is performed to detect combinations of tests that reliably predict AIP.

Chapter 7 focuses on steroid treatment in AIP. Guided by treatment regimens of other gastrointestinal autoimmune diseases like inflammatory bowel disease, current guidelines recommend high doses of steroid induction therapy. Steroids, especially high dose, are notorious because of side-effects like triggering or worsening of pre-existing diabetes mellitus, osteoporosis and psychological disturbances. These side effects are particularly inconvenient in the older diabetic AIP patient. AIP is responsive to steroids to such an extent that the rationale of high dose should be questioned. Chapter 7 shows the results of a retrospective study comparing the efficacy of low and high dose corticosteroid induction therapy.

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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 have been proposed including the Asian, HISORt and International Consensus Diagnostic Criteria. Few studies have compared the diagnostic performance of these scoring systems. We aimed to explore the diagnostic performance of these criteria in a group of 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 mainly type 1 AIP patients, the three major diagnostic scoring systems for AIP proved to be complementary rather than overlapping. Our data indicate that one-fifth of patients suffer from AIP while they do not meet any of these scoring systems. The Asian, HISORt and International Consensus Diagnostic Criteria should be considered as useful clinical tools but not as gold standard for the diagnosis.

INTRODUCTION

Autoimmune pancreatitis (AIP) 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 International Consensus Diagnostic Criteria (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 kind 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 to 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 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 response to steroids or 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. All authors had access to the study data, reviewed and approved the final manuscript.

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.

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 [†]	16/114	14%
	Biliodigestive anastomosis	2/114	2%
	Liver-kidney transplantation	1/114	1%
	Chemoradiation	1/114	1%
Diagnostic delay			
	median (IQR), months[‡]	4.3 (2.0-18.8)	

*IQR: inter quartile range, *Age at time of initial symptoms, [Δ] pANCA, ALF or ACA-II, [¥] with respect to onset of jaundice or overt pancreatic disease, [†] explorative laparotomy, diagnostic laparoscopy, [‡] time between date diagnosis and date symptoms*

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

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

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).

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	Patients no.
Criteria positive	82%	93/114
3x positive	18%	20/114
2x positive	35%	40/114
1x positive	29%	33/114
Criteria negative	18%	21/114
Histology	33%	7/21
Unexplained pancreatic disease +biliary disease/OOI† + response to steroids	38%	8/21
Unexplained pancreatic disease + biliary disease/OOI + IgG4 positive	38%	8/21

†*OOI: other organ involvement*

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

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 n.o.s.	6%	12%	32%	0%

† *Pancreatogram present ,‡normal appearance of pancreas on computed tomogram. Systems were scored with initial clinical findings, e.i. EUS FNA was included, histology obtained by resection or laparotomy biopsy was excluded*

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 are 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 international consensus diagnostic criteria 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

Prevalence of autoimmune pancreatitis and other benign disorders in pancreatoduodenectomy for presumed malignancy of the pancreatic head

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ABSTRACT

Background Occasionally patients undergoing resection for presumed malignancy of the pancreatic head are diagnosed with benign disease postoperatively. Autoimmune pancreatitis (AIP) is a rare disease that mimics pancreatic cancer. We aimed to determine the prevalence of benign disease and AIP in patients who underwent pancreatoduodenectomy (PD) over a 9 year period, and to explore if and how surgery could have been avoided.

Methods All patients undergoing PD between 2000 and 2009 in a tertiary referral centre were analyzed retrospectively. In cancer-negative cases, postoperative diagnosis was reassessed. Preoperative index of suspicion of malignancy was scored as non-specific, suggestive or high. In AIP patients diagnostic criteria systems were checked.

Results 274 PDs were performed for presumed malignancy. The prevalence of benign disease was 8.4%, overall prevalence of AIP was 2.6%. Based on preoperative index of suspicion of malignancy, surgery could have been avoided in 3 non- AIP patients. All AIP patients had sufficient index to justify surgery. If diagnostic criteria would have been checked however, surgery could have been avoided in one to five AIP patients.

Conclusions The prevalence of benign disease in patients who underwent PD for presumed malignancy was 8.4%, nearly one third attributable to AIP. Although misdiagnosis of AIP as carcinoma is a problem of limited quantitative importance, every effort to establish the correct diagnosis should be undertaken considering the major therapeutic consequences. IgG4 measurement and systematic use of diagnostic criteria systems are recommended for every candidate patient for PD when there is no histological proof of malignancy.

INTRODUCTION

Nowadays, routine work up consists of CT scan, frequently combined with endoscopic ultrasonography (EUS) and fine needle aspiration (FNA) cytology. Although the sensitivity of EUS with FNA is approximately 80% and the specificity of positive cytology approaches 100%,[1] false negative results are common and the negative predictive value of these tests is low.[2] Therefore, if a person presents with a mass in the pancreatic head without metastases, a PD will usually be considered as it is the only curative option. Five to 11% of patients however are found to have a benign disease on postoperative histological examination.[3-8] In large volume centers the mortality of this operation is less than 5%[9] and morbidity is substantial 46%.[4]

Autoimmune pancreatitis (AIP) is a rare disease that may present with a pancreatic head mass, jaundice and weight loss, and thus may mimic pancreatic carcinoma clinically. Biliary involvement (distal and proximal) is common, sometimes without overt pancreatic disease, mimicking cholangiocarcinoma. The disease is highly responsive to steroids,[10] and this feature can be used as a diagnostic tool.[11] The exact pathogenesis is unknown. In 68 to 95% of patients IgG4 serum levels are elevated.[12-16] AIP can be associated with extrapancreatobiliary manifestations like retroperitoneal fibrosis, Sjögren's disease, rheumatoid arthritis, inflammatory bowel disease, interstitial nephritis, thyroïditis or inflammatory tumors in lungs, mediastinum or liver. According to several large retrospective series, 23 to 38% of benign PDs are due to autoimmune pancreatitis.[3-4] Increasing knowledge and awareness of this intriguing disease is expected to avoid unnecessary surgery in a substantial amount of patients. Unfortunately there is no single diagnostic test. Several diagnostic criteria systems of AIP have been proposed including the HISORt and Asian criteria.[17-18] The aims of this study were first to determine the prevalence of benign disease and in particular of AIP in patients who underwent PD for presumed malignancy in the past decade, second to investigate if there was any decline in misdiagnosis over time, and third to assess if and how unnecessary surgery possibly could have been avoided.

METHODS

STUDY POPULATION

All patients undergoing PD between January 1 2000 and January 31 2009 in a tertiary referral center with multidisciplinary approach to pancreatic and biliary disease were retrospectively analyzed. Patients were included if the indication for surgery was suspicion of malignancy in the pancreatic head. If postoperative diagnosis did not harbor a benign or malignant neoplasm it was classified as a benign PD. Demographic characteristics (age, gender and mortality) were evaluated in all patients. In benign PDs postoperative diagnosis was reassessed by revision of histological and clinical data.

The following clinical data were extracted from patient case records: age, gender, diabetes mellitus, history of chronic pancreatitis, autoimmune disease, smoking, alcohol consumption, jaundice, weight loss and pain. Laboratory results of bilirubin, Ca19-9, total IgG, IgG4, and autoantibodies (RF, ANF) were recorded. Relevant radiological and endoscopic studies (ultrasound US, computed tomography CT, magnetic resonance imaging MRI, endoscopic retrograde cholangio pancreatography ERCP, endoscopic ultrasound EUS) were reviewed. Based on these data and -if available- preoperative cytological or histological examination, a preoperative index of suspicion of malignancy (non-specific, suggestive, highly suspicious)[6] was calculated (detailed information in Addendum Table 3). Van Gulik et al described this system in 1999, using US and ERCP features of malignant and inflammatory lesions in the pancreatic head. We added clinical symptoms (weight loss, jaundice and pain), level of Ca19-9,[19] EUS features[8] and pathology findings (preoperative histology or cytology). For each examination, suspicion of cancer was scored on a 0/+/++ scale. Retrospectively, surgery was considered unnecessary when preoperative findings were non-specific. In AIP patients, the HISORt and Asian diagnostic criteria systems (Addendum Table 4) were applied on preoperative data, to determine if and how surgery could have been avoided.

HISTOPATHOLOGIC EVALUATION

Resection specimens were revised by two expert pathologists familiar with pancreatic disease and with special interest in AIP. Immunostaining for IgG4 was performed using a monoclonal mouse anti-human IgG4 (Zymed Laboratories, San Francisco, USA), with a working dilution of 1:100. The presence of >10

IgG4-positive plasma cells in at least one HPF at a magnification of x400 was considered suggestive of AIP. Each specimen was evaluated for the presence of microscopic AIP features, as previously established in several series of resection specimens.[20-27] A classical histological triade is recognized in 80%: dense lymphoplasmacytic infiltration, cuff like periductal fibrosis and obliterative phlebitis (venulitis). Other common features are: perineural inflammation, acinar atrophy or fibrosis, storiform (spindle shaped) fibrosis, granulomas and the presence of neutrophils and eosinophils. More recently, two subtypes of autoimmune pancreatitis have been distinguished, each with a distinct clinical and histopathological picture: the predominant lobular type (AIP-PL or type 1) and predominant ductal type (AIP-PD, type 2).[21] AIP type 1 represents the "classic" lymphoplasmacytic sclerosing pancreatitis, more prevalent in older men, and is strongly associated with retroperitoneal fibrosis and biliary strictures, the latter often becoming prominent after pancreaticoduodenectomy. Especially this type of AIP is associated with an elevated serum IgG4 and the presence of IgG4 positive plasma cells in tissue. The less well-known AIP type 2 is characterized by the presence of so-called GELs: granulocytic epithelial lesions, which represent destruction of of pancreatic interlobular ductal epithelium.[26] This subtype is more prevalent in younger patients, more often associated with ulcerative colitis or Crohn's disease and generally shows no recurrence after resection. It is less associated with increase of IgG4. While AIP type 1 usually presents with typical histological pattern, AIP type 2 could be more difficult to diagnose, both preoperatively on biopsy material as well as on resection specimens. The typical fibrosis is missing and IgG4 staining is less useful.[20-27]

Suggestive of other forms of chronic pancreatitis are pseudocysts and calcifications, irregular ductal dilation, mucoprotein plugs and necrosis (suggestive of chronic alcoholic or obstructive pancreatitis), pancreas divisum or inflammation of the duodenal wall (groove pancreatitis).[3]

STATISTICAL ANALYSIS

Chi square and unpaired t-test were used to compare gender and age between malignant and benign postoperative diagnosis. Fischer's exact test and unpaired t-test were used to compare differences in characteristics and symptoms of patients with benign pancreatoduodenectomies. Two tailed p values of <0.05 were considered statistically significant.

RESULTS

Of 288 pancreatoduodenectomies performed during 2000-2009, 274 were performed for presumed malignancy. Twenty-three (8.4%) of 274 resections were negative for neoplastic disease (Figure 1). Patients with malignancy were significantly older (mean 63.7±10.1) than those with benign disease (mean 58.6±12.7) (p = 0.004). There was no difference in gender (p= 0.832). Overall operative mortality was 20/288 (6.9%) but mortality was not observed in the benign PD cases. Mortality did not differ between the first and second half of the study period (7.1% versus 6.8%, p = 1.0).

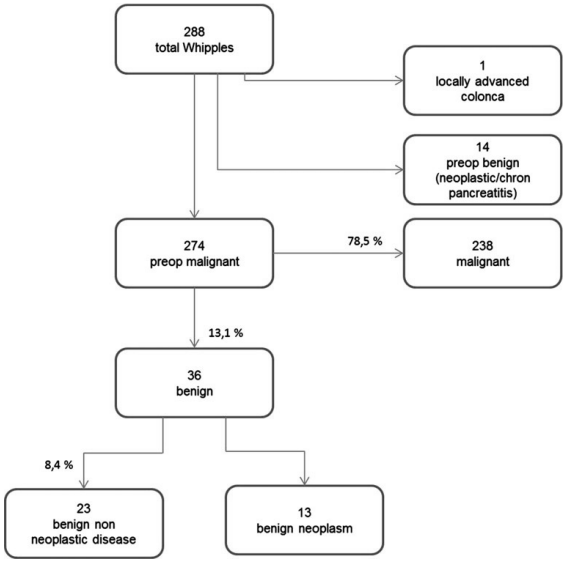

Figure 1 Flow chart of patient inclusion

In Table 1 postoperative diagnoses of 23 benign PDs are summarized.
AIP was diagnosed in 30.4%, that is 2.6% of total PDs performed for presumed malignancy. Clinical characteristics and symptoms of benign PDs (AIP and non AIP) are summarized in Table 2. No statistical differences were noted between AIP and non AIP except for pre-operative presence of diabetes mellitus, being more frequent in AIP patients (71% versus 19%, p = 0.026).

The prevalence of misdiagnosis in the first and second half of the study period showed a decline from 10.9% to 5.8% but it failed to gain statistical significance

Table 1 Clinicopathologic classification of disease in 23 benign pancreatoduodenectomies

	No of patients (%)
Chronic pancreatitis	
Alcoholic	3 (13.0%)
Obstructive	7 (30.4%)
Idiopathic	3 (13.0%)
Autoimmune	6 (26.1%)
Biliary tract disease	
Autoimmune	1 (4.3%)
Idiopathic	1 (4.3%)
Papillary fibrosis	1 (4.3%)
Crohn's disease (infiltrate)	1 (4.3%)

(p = 0.19). The proportion AIP among misdiagnosed patients remained constant (26.7% versus 37.5%, p = 0.66).

Based on the preoperative index of suspicion of malignancy (Table 3),

Table 2 Characteristics and symptoms of patients with benign pancreatoduodenectomy

	AIP	Non AIP	P value
No.	7	16	
M:F ratio	6.0	2.2	0.62
Mean age years (±SD)	53 (±19.7)	54 (±7.9)	0.65
Diabetes (de novo)	5 (2) (71%)	3 (2) (19%)	0.03
History of chronic pancreatitis	0	2 (13%)	1.00
Autoimmune disease	2 (29%)	1 (6%)	0.21
Smoking	5 (71%)	9 (56%)	0.66
Alcohol >2 U daily	1 (14%)	8 (50%)	0.18
Jaundice	6 (86%)	7 (44%)	1.00
Mean weight loss kg (±SD)	2.7 (±5.6)	7.0 (±7.7)	0.21
Pain - none / mild	5 (71%)	10 (63%)	1.00
- moderate/severe	2 (29%)	6 (37%)	1.00

Fisher's exact and unpaired t-test. SD = standard deviation.

postulating that for surgery findings should at least be suggestive, resection could have been avoided in three non AIP patients, one with alcoholic and two with obstructive chronic pancreatitis. The index of suspicion in these cases was non-specific. Radiology was indicative of chronic pancreatitis without clear signs of malignancy. The decision to operate was mainly based on symptoms (suggestive n=2 or non-specific n=1). The index of suspicion was

Table 3 Preoperative index of suspicion of malignancy and final diagnosis in benign pancreatoduodenectomy

No	M/F	Age	Symptoms	Ca19.9 kU/L	Pathology	Radiology	EUS	*Index* Suspicion	Final diagnosis
1	M	54	Non specific	<34	n.a.	Neoplasm	n.a.	Suggestive	CAP
11	M	52	Strong	<34	n.a.	Neoplasm	n.a.	Strong	CAP
12	M	59	Suggestive	<34	n.a.	CP	n.a.	Non specific	CAP
2	F	48	Non specific	<34	n.a.	Neoplasm & CP	n.a.	Suggestive	CIP
4	M	41	Strong	<34	n.a.	Non specific	n.a.	Suggestive	CIP
14	M	58	Non specific	<34	n.a.	Neoplasm	CP	Suggestive	CIP
3	F	50	Suggestive	<34	Benign	CP	n.a.	Non specific	COP
6	M	55	Strong	n.a.	n.a.	CP	n.a.	Suggestive	COP
8	F	48	Suggestive	<34	n.a.	Neoplasm & CP	n.a.	Suggestive	COP
17	M	57	Strong	50	n.a.	Neoplasm	n.a.	Strong	COP
18	M	71	Strong	<34	n.a.	Neoplasm & CP	n.a.	Strong	COP
20	M	68	Suggestive	1308	n.a.	Neoplasm & CP	CP	Strong	COP
21	F	52	Non specific	<34	n.a.	Non specific	n.a.	Non specific	COP
7	M	75	Suggestive	68	n.a.	Neoplasm	n.a.	Suggestive	AIP type 1
16	M	69	Strong	23284	n.a.	Neoplasm	n.a.	Strong	AIP type 1
15	M	33	Suggestive	<34	n.a.	Non specific	Neoplasm	Suggestive	AIP type 2
5	M	73	Suggestive	<34	Benign	Neoplasm	n.a.	Suggestive	AIP type 2
10	M	53	Suggestive	1689	Benign	CP	n.a.	Strong	AIP type 2
23	F	28	Suggestive	<34	Malignant	Neoplasm & CP	Neoplasm & CP	Strong	AIP type 2
19	M	40	Suggestive	<34	Benign	Neoplasm	Neoplasm	Suggestive	AIC
9	M	52	Non specific	n.a.	Benign	n.a.	n.a.	Non specific	Crohn's
13	M	66	Strong	<34	Atypical	CP	Non specific	Strong	IC
22	F	59	Suggestive	<34	Benign	Neoplasm	Non specific	Suggestive	Papillary fibrosis

n.a. = not available. Ca 19.9 normal <34 kU/L. CP = chronic pancreatitis. CAP = chronic alcoholic pancreatitis, CIP= chronic idiopathic pancreatitis, COP = chronic obstructive pancreatitis (stones, neoplasm, divisum), AIP = autoimmune pancreatitis. AIC = autoimmune cholangitis, IC = idiopathic cholangitis.

Table 4 Preoperative findings and work-up in patients diagnosed with AIP after pancreatoduodenectomy: did they meet the diagnostic criteria?

No	*Histology postop*	*Histology preop*	*Typical Imaging* (atypical)	*IgG*	*IgG4*	*AAB*	*Other organ involvement*	*Steroid trial*	*Asian positive* [5]	*HISORt positive*
5	AIP type 2	n.a.	No	n.a.	n.a.	n.a.	No	No	No	Possible[3]
7	AIP type 1	n.a.	No (focal mass)	n.a.	n.a.	n.a.	Hypothyroidism	No	No	Possible[3]
10	AIP type 2	n.a.	No	n.a.	n.a.	n.a.	No	No	No	Possible[3]
15	AIP type 2	n.a.	No (focal mass)	11.8[1]	1.39[1]	n.a.	No	No	No	No [4]
16	AIP type 1	n.a.	No (focal mass)	33.0[1]	13.6[1]	Negative	Retroperitoneal Fibrosis	No	No	Yes
19	AIC	n.a.	Biliary stricture	n.a.	n.a.	n.a.	No	No	No	Possible[3]
23	AIP type2	Malignant[2]	No (diffuse enlargement no rim, focal mass)	8.6	0.05	n.a.	No	No	No	No [4]

IgG normal <17.0 g/L, IgG4 nl <1.40 g/L. N.a. = not available. AAB = auto antibodies (RF, ANA). [1] Preoperative values, measured retrospectively, [2]cytology (EUS FNA), [3]if serology positive and/or suggestive pancreatogram, responsiveness to steroids would have confirmed diagnosis, [4]even if responsive to steroids, diagnostic criteria would not have been met, [5] none of the patients had adequate (mandatory) pancreatogram; patient 16 and 19 had double duct sign on ERCP with minimal contrast injection

also non-specific in another case finally diagnosed with Crohn's disease, but surgery could possibly not have been avoided since a tumor-like mass was found infiltrating both pancreas and colon ascendens and causing obstructive symptoms. The index of suspicion in all seven patients with AIP was sufficient to justify the operation (suggestive n=4; strong suspicion n=3). Important reasons to operate were marked elevation of Ca19-9 (levels as high as 23 284 kU/l), suggestive imaging (mass on EUS, double duct sign on CT/MRI or ERCP, regional adenopathy on CT or MRI) and (false) positive cytology (EUS-FNA). Based on diagnostic criteria systems for AIP however (Table 4), surgery could have been avoided in at least one case. This patient developed biliary strictures postoperatively, triggering clinicians to consider AIP. The preoperative IgG4 level (measured retrospectively) was very high (13.6 g/l). Based on the spectacular response to steroids postoperatively it is very likely that steroids would have prevented the operation. In four patients, findings at pancreatography and / or elevated IgG4 levels would have justified a steroid trial. However, none of the patients had an adequate pancreatogram and in only one case IgG4 had been measured preoperatively. In two AIP patients surgery seemed inevitable also in retrospect. Even if responsive to steroids, criteria would not be met (no other criterion present, IgG4 normal). In summary, surgery could have been avoided in at least 4 (which would reduce the percentage benign PDs to 6.9), but possibly 8 patients (three non AIP and five AIP) according to the index of suspicion for malignancy and the HISORt criteria. The pre-operative work-up in AIP patients was unsatisfactory.

DISCUSSION

The prevalence of benign disease in patients who underwent PD for presumed malignancy in our center was 8.4%. During a 9-year period seven patients were post-operatively diagnosed with AIP, corresponding with a total prevalence in this population of 2.6% and accounting for nearly one third of all benign cases. These findings show that AIP accounts for a significant proportion of incorrect preoperative diagnoses, but also indicate that, from a quantitative perspective, missing the diagnosis of AIP was a problem of limited magnitude. In our national AIP database, containing 130 patients, 20% underwent resection for presumed malignancy (unpublished data). Our data are compatible with other large series, reporting 5 to 11% [3-8] benign disease in patients after PD

for suspected malignancy, with AIP constituting 23 to 38% of benign cases.[3-4] The prevalence declined over time although this was not statistically significant.

A preoperative diagnosis of AIP was missed for several reasons. First we noted insufficient preoperative work up in patients finally diagnosed with AIP. IgG4 measurements were missing in 6/7 cases and adequate imaging of the pancreatic duct was not performed in any patient, both being crucial elements in either the American (HISORt) or Asian diagnostic strategy.[28] Second, the importance of Ca19-9 was overestimated. Levels of >300 U/ml are thought to be pathognomonic for malignancy[19] but markedly elevated levels were found in 2 of our AIP patients. The third reason is the mere fact that in some patients it may be virtually impossible to detect the disease without resecting the pancreas. In a recent study, in which a diagnostic strategy to distinguish AIP from pancreatic cancer based on HISORt criteria was tested, researchers from the US found that sensitivity of diagnostic criteria is 70%. In 30% of AIP cases however, the diagnosis could not be confirmed without a steroid trial, pancreatic core biopsy or surgical resection.[29]

Based on the index of suspicion of malignancy we used in this study, 3 non AIP patients underwent PD while the index was non-specific. Nowadays, we believe that the index should at least be suggestive before embarking on surgery. Although seemingly easy to use, this index is subject to personal interpretation, and discussion about findings to be interpreted as “suggestive” or “very suspicious” is inevitable. To better define the clinical usefulness of the index prospective validation studies are needed. Noteworthy, applying this index illustrated the fact that in patients with AIP findings may clearly suggest malignancy. Unnecessary surgery can be avoided only if this diagnosis is always considered and actively pursued.

Diagnosing AIP may be troublesome. The two main diagnostic systems (HISORt and Asian diagnostic criteria) are based on specific combinations of radiological (focal enlargement, sausage shaped pancreas with hypodense rim, diffuse or segmental narrowing of the pancreatic duct), serological (IgG4, IgG and the presence of autoantibodies like RF or ANA), and histological (pancreatic and or extrapancreatic tissue) findings, and the response to steroid therapy. An extensive discussion of the diagnostic criteria is beyond the scope of this article, but in preoperative work up the following clues are of key importance and should be looked for in every patient: elevated IgG4, narrowing of the

pancreatic duct (in contrast with ordinary carcinoma patient who usually presents with double duct sign) and evidence of extrapancreatobiliary involvement.

In our opinion a diagnostic strategy of measuring serum IgG4 levels in all patients suspected of pancreatic or cholangiocarcinoma could well be considered. Of all patients referred for presumed malignancy, 20% are candidates for surgery. With a prevalence of 2.5% among those undergoing PD, 200 patients would need to be screened to detect one case of AIP eligible for surgery. At approximately $50 per test, $10 000 would be spent for each patient preoperatively diagnosed with AIP, an amount considerably less than the costs of surgery and its associated morbidity (about $30 000).[2, 30] In resection for presumed hilar cholangiocarcinoma the percentage autoimmune cholangitis is probably higher (1.1-8.1%)[31] and less patients would need to be screened. In our center, with an annual volume of approximately 30 PDs, and taken into account that sensitivity of IgG4 is 68% to 95%,[12-16] it would take at least one and a half year of routine screening to detect one patient with AIP. Although this may seem a low yield of this screening strategy, this approach may still be defendable and worthwhile in the light of possible unnecessary major surgery, morbidity and mortality. This strategy would also allow to detect patients with AIP considered to have irresectable malignancies because of infiltration, lymphadenopathy or supposed metastases. This group is easily forgotten but not less important or tragic: be diagnosed with incurable cancer while steroids can heal. Although routine IgG4 measurement preoperatively has been gradually introduced in our center since 2006, we have not been able to prevent the one case that was diagnosed postoperatively after 2006. This young female with preoperative normal IgG4 and a cytology report of malignancy on EUS-FNA, was diagnosed with AIP type 2. It is only recently that AIP type 2 is acknowledged as a distinct phenotype. It is more difficult to detect because IgG4 is often not elevated and patient characteristics are very different from the classical jaundiced old man with weight loss and retroperitoneal fibrosis. This case reflects the lacuna in current diagnostic strategies, especially in IgG4 negative disease. Another important aspect and limitation of measuring IgG4 is that levels up to 2 times the upper limit of normal can also be found in patients with pancreatic cancer, primary sclerosing cholangitis and other pancreatic disease. The specificity of in particular slightly elevated levels is limited.[12-16, 32] If a cut off value is used of >2.8 g/L however, specificity rises to 98%.[29]

The second tool to detect AIP preoperatively is histology. In contrast to Asian criteria, the HISORt already diagnoses AIP if only histology is positive. This gives pancreatic core biopsy a special significance. Obviously, reliable histological assessment requires a dedicated pathologist, who is familiar with the histological features of pancreatic disease and IgG4 immunostaining. AIP can usually be diagnosed in resection specimens without great difficulty and be distinguished clearly from other types of pancreatitis and adenocarcinoma. IgG4 immunostaining however has limited sensitivity and specificity and shows overlaps between AIP, chronic pancreatitis and adenocarcinoma. Deshpande et al showed that IgG4 positive cells were identified in resection specimens in 42.9% cases of chronic pancreatitis and 52.6% cases of adenocarcinoma (using a working dilution of 1:50, scored in a 20x field). These findings suggest limited diagnostic value of pancreatic biopsy.[21] Data regarding the role of pancreatic biopsy, however, are sparse and disputed. Detlefsen et al[33] recognized AIP in pancreatic core biopsies using six microscopic features (granulocytic epithelial lesions -GELs-, >10 IgG4-positive per high power field -HPF- , >10 eosinophilic granulocytes/HPF, cellular fibrosis with inflammation, lymphoplasmacytic infiltration and venulitis). They were able to detect AIP in 76% when they used a cut off level of four features, rising to 86% when cases were added with three features including GELs. In this study there was no control group with adenocarcinoma. The Mayo Clinic group was able to detect AIP in EUS guided true cut biopsies in 100%.[34] Further studies are required to further establish the diagnostic significance of pancreatic biopsy in patients possibly suffering from AIP.

The third major diagnostic tool is pancreatography. Preoperative ultrasound and/or or CT showing a non-dilated pancreatic duct should always give rise to suspect AIP and not cancer. When MRI is performed, MRCP should be performed as well. Although a recently published randomized controlled trial showed that in carcinoma of the pancreatic head early surgery is superior to preoperative biliary drainage, most patients will still undergo an ERCP before surgery.[35] While gastroenterologists usually will not try to deliberately cannulate and fill the pancreatic duct, adequate pancreatography is helpful in establishing the correct diagnosis.

Finally, a two-week trial of corticosteroids[11] can confirm the diagnosis, but this should only be considered if other findings clearly suggest the possibility of AIP. We believe it is an important tool but should be left in experienced hands and

only after careful multidisciplinary review of all relevant data. Malignant tumors as well as benign non-autoimmune mediated inflammatory processes may respond to steroids to some degree and victims of the autoimmune hype have already been reported.[36]

CONCLUSIONS

Prevalence of benign disease in patients who underwent pancreatoduodenectomy for presumed malignancy is 8.4%. One third of these cases are diagnosed with AIP. In nine years the prevalence of benign PDs showed a non significant trend towards decline from 10.9% to 5.8%. The proportion AIP remained stable, at least partially due to insufficient preoperative work up. Routine work up for pancreatic cancer is not enough to detect these patients beforehand. IgG4 measurement and systematic use of diagnostic criteria systems should be considered in every patient eligible for PD but without preoperative histological confirmation of malignancy.

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ADDENDUM MATERIAL

Addendum Table 3 Index of suspicion of malignancy[6]

	Non specific	***Suggestive***	***Strong suspicion***
Clinical symptoms	Weight loss<5kg	Jaundice	>10kg weight loss
	Mild to moderate pain alone		+/- severe pain
Ca 19.9	<34 kU/l = normal	>34 and <300 kU/l	≥300 kU/l
Preoperative cytology or histology	Benign	Atypical	Malignant
Imaging	**Non specific**	**Sugg chronic pancreatitis**	**Suggestive neoplasm**
US, CT, MRI	Mass (solid / cystic)	Calcifications, pseudocyst	Double duct dilation
	Focal enlargement	Parenchymal atrophy	Blunt stenosis CBD or PD stenosis
	Single duct dilation	Tapering stenosis PD or CBD	Abnormal portal venous Doppler / encasement
	Thickening CBD wall	Diffuse enlargement / rim	Regional adenopathy
		PD irregularities	Infiltration
ERCP	Single duct dilation	CBD stenosis long, tapered, smooth	CBD stenosis (short, irregular, shouldering, excentric, complete)
		PD stenosis multiple, short, side branches	PD stenosis (single, >1cm, irregular, no side branche)
			Double duct sign
Endoscopic US	Single duct dilatation	Parenchymal / ductal criteria	Mass
	Thickening CBD wall		Lymphadenopathy
			Vascular involvement

PD: pancreatic duct, CBD: common bile duct

Addendum Table 4 *HISORT and Asian Diagnostic Criteria (summarized)*[17,18]

Criterion		
Histology		Lymphoplasmacytic sclerosing pancreatitis and >10 IgG4 positive plasmacells / high power field
Imaging	Typical	Diffusely enlarged pancreas with rim Diffusely irregular pancreatic duct (PD)
	Other	Focal mass or enlargement, focal PD stricture
		Atrophy, calcification, pancreatitis
Serology	IgG4 >1.40 g/L	Asian: IgG >17.0 g/L, presence of autoantibodies (ANA, RF)
Other Organ involvement		Biliary strictures, salivary glands, mediastinal lymphadenopathy, retroperitoneal fibrosis
Response to steroid therapy		

In HISORt criteria system diagnosis is made either if histology alone is positive (resection or pancreatic core biopsy), or if combination typical imaging and elevated IgG4, or if unexplained pancreatic disease with elevated IgG4 or other organ involvement and response to steroids. In Asian criteria typical imaging is mandatory either in combination with serology (IgG4, IgG and RF or ANA) or histology of pancreatic biopsy lesions or histology alone if ipositive in resected pancreas. Response to steroids is conducted only if typical imaging is present and after negative work up for pancreatic cancer. Other organ involvement is not included in Asian criteria.

Chapter 4

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 & aims Autoimmune pancreatitis (AIP) is often difficult to distinguish from pancreatic carcinoma or other pancreatobiliary diseases. Patients with malignancies are frequently identified based on high serum levels of carbohydrate antigen 19-9 (Ca 19-9), 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 patients with 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. Ca 19-9 was measured using an electrochemiluminescense immunoassay and IgG4 was measured using the Peliclass IgG subclass nephelometry kit. We associated protein levels with patient diagnoses and 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. Levels of IgG4 ranging from 1.4 to 2.6 g/L identified AIP patients with higher sensitivity (85%) but lower specificity (81%). 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 levels <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 carbonic 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.

PATIENTS AND METHODS

PATIENTS

Between March 2007 and May 2011 sera were prospectively obtained from consecutive patients presenting 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.

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. 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)

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]

*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.*

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, Supplementary Table). 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 bilirubine 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).

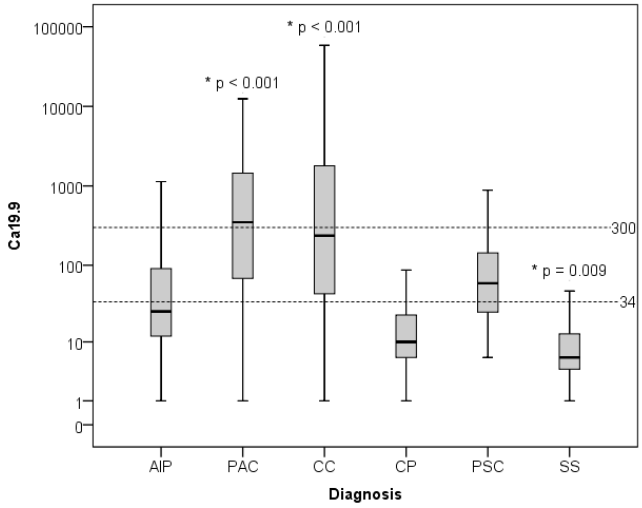

Figure 1 Ca 19-9 level in pancreatobiliary diseases and Sjögren's syndrome
*Levels of Ca 19-9 are expressed in U/ml, as grey boxes (interquartile range) with median (horizontal line within box) and whiskers (range that contains 95% of observations).. AIP: autoimmune pancreatitis, PAC: pancreatic adenocarcinoma,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

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 were studied. In AIP patients, Ca 19-9 levels were not significantly different between patients with or without mass presentation (p=0.24), steroid use (p=0.88) or proximal biliary involvement (p=0.17) (Table 2). 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.

Table 2 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.

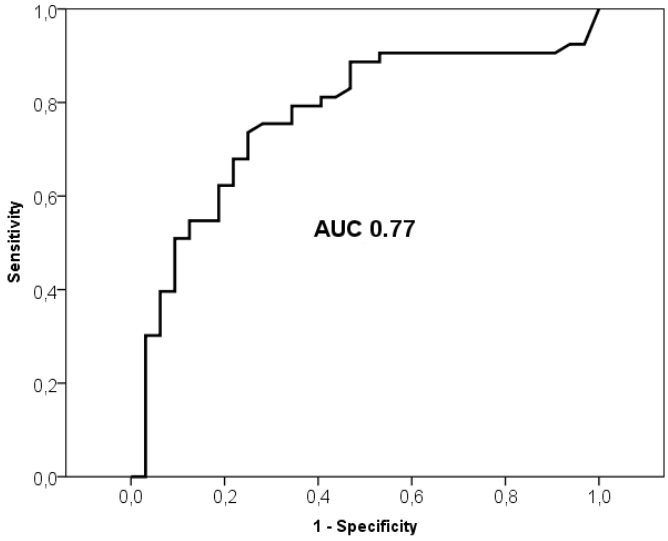

Figure 2 Receiver operating curve for Ca 19-9
The area under the curve (AUC) for the diagnosis of pancreatic carcinoma by means of Ca 19-9 levels was 0.77

The diagnostic performance of Ca 19-9 and IgG4, single and in combination, are shown in Table 3 and Figure 3. Ca 19-9 as a single marker had moderate sensitivity (73%), and specificity (74%). IgG4 levels higher than 1.4 g/L were

Table 3 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)	24.0	28.0	23.0	29.0	26.0
Sensitivity (%)	72.7	84.8	69.7	93.5	83.9
Specificity (%)	73.6	81.1	100.0	100.0	100.0
PPV (%)	63.2	73.7	100.0	100.0	100.0
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, c.i. = confidence interval. Value of Ca 19-9 in U/ml, IgG4 in g/L.

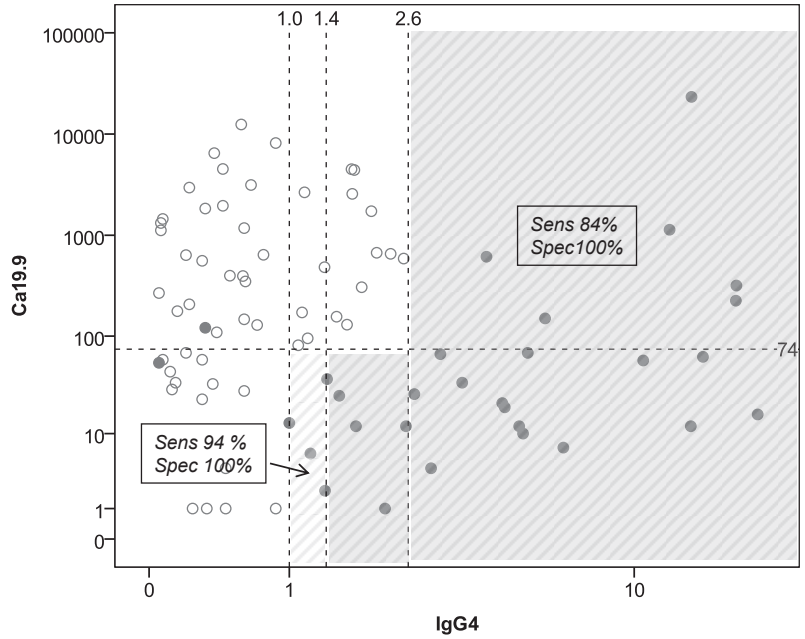

Figure 3 Combination of Ca 19-9 and IgG4 to differentiate AIP from pancreatic carcinoma

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).

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 distinct 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 patients 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, Ca 19-9 (<74 U/ml) and IgG4 (>2.6 g/L) would entail 27% and 30% false negative cases respectively. In other words, one quarter to one third of patients with cancer would be erroneously treated with prednisone and potentially deprived of a curative surgical resection. Furthermore, this corresponds with 26% false positive cases in case of Ca 19-9, one quarter of patients undergoing resection would be erroneously exposed to a surgical procedure with substantial morbidity (46%)[35] and mortality (though in large volume centers less than 5%[39]). 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 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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SUPPLEMENTARY MATERIAL

Supplementary Table 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.

Chapter 5

Potential value of serum total IgE for differentiation between autoimmune pancreatitis and pancreatic cancer

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ABSTRACT

Autoimmune pancreatitis (AIP) is associated with a marked elevation of serum total IgG4. Although there is evidence of autoimmunity in AIP, there are also signs of an allergic nature of its pathogenesis. Therefore we determined both IgE and IgG4 in 13 patients with AIP, in 12 patients with pancreatic carcinoma, and in 14 patients with atopic allergy, and investigated the relationship between IgE and IgG4. Total IgG4 was determined by automated nephelometry and total IgE by automated enzyme fluoroimmunoassay. Both total IgE and total IgG4 of AIP patients were significantly higher than these levels in patients with pancreatic carcinoma ($p= 0.0004$ and $p = 0.015$ respectively). There was a significant correlation between the total IgE and total IgG4 levels in patients with AIP and patients with atopic allergy ($r_s = 0.82$, $p=0.0006$ and $r_s = 0.88$, $p<0.0001$, respectively). The IgE/ IgG4 ratio in sera from patients with atopic allergy was significantly different ($p=0.0012$) from this ratio in sera from patients with AIP. These results suggest that analysis of total IgE in serum might be useful in the differentiation between autoimmune pancreatitis and pancreatic carcinoma.

INTRODUCTION

Autoimmune pancreatitis (AIP) is the pancreatic manifestation of a systemic inflammatory disorder, characterized by infiltration of IgG4 positive plasma cells and marked interstitial fibrosis, which is responsive to steroid therapy.[1,2] Extrapancreatic manifestations of the disease include cholangitis, retroperitoneal fibrosis, Mikulicz's disease, interstitial nephritis and pseudotumours of lungs en mediastinum.[1,3] If located to the pancreatobiliary region, the disorder may mimic cancer of the pancreatic head or cholangiocarcinoma. In approximately 5 % of pancreatoduodenectomies performed for suspicion of pancreatic carcinoma the resected lesion turns out to be a benign, inflammatory lesion.[4] Hamano et al.,[5] who were the first to report high serum IgG4 concentrations in 20 Japanese patients with autoimmune pancreatitis, found that a serum IgG4 level of >1.35 g/L had 95% sensitivity and 97% specificity for the differentiation of AIP from pancreatic cancer. They suggested that the measurement of serum IgG4 in patients suspected of having pancreatic cancer may help reduce the incidence of unnecessary surgery. In a western population of 45 patients, Ghazale et al.[6] noted a 76% sensitivity and 93% specificity of a serum IgG4 level of >1.40 g/L. This difference in sensitivity between Japanese and western populations may be caused by the occurrence of two subsets in AIP: type 1 AIP and type 2 AIP,[7] of which only type 1 is characterized by elevated serum IgG4 and an increased IgG4-positive lymphoplasmacytic infiltrate. The pathogenesis of AIP is unclear. The inflammatory reaction has both characteristics of autoimmune (Th1- mediated) and allergic (Th2- mediated) responses.[8,9] Okazaki et al.[8] proposed a biphasic mechanism consisting of "induction" by pro-inflammatory cytokines produced by Th1 cells and "progression" by Th2 immune responses. When an allergy-like reaction is part of the progression of the disease, IgE levels might be elevated in AIP. Hamano et al.[5] found no significant difference in total IgE level between 20 patients with sclerosing pancreatitis and 20 normal individuals. Umemura et al.[10] found an elevated serum total IgE in 35% of 17 patients with autoimmune pancreatitis. After 4 weeks of glucocortocoid therapy of 7 patients, both IgG4 and IgE decreased to a level of about 30% of the median pretreatment value.[10] Masaki et al.[11] reported a 20-fold higher total IgE level in 64 patients with IgG4-positive multi-organ lymphoproliferative syndrome compared with 33 patients with Sjögren's syndrome (Ss), the total IgG4 level of these 64 patients was about 30-fold higher than this level in the 33 Ss patients. In the present study we further investigated the relationship between serum total IgE and total IgG4 in patients with AIP, in patients with pancreatic carcinoma and in patients with atopic disease.

PATIENTS AND METHODS

Sera from patients with AIP and from patients with pancreatic carcinoma were collected simultaneously with samples drawn for routine clinical chemistry analysis, after informed consent was obtained. Of the patients diagnosed with AIP, using the HISORt diagnostic criteria proposed by the Mayo Clinic,[6] 11 patients had autoimmune pancreatocholangitis, 1 patient had autoimmune pancreatitis and 1 patient (with a total serum IgG4 of 87 g/L) had a provisional diagnosis of cholangitis and IgG4 associated autoimmune hepatitis, which has been recognised by Umemura et al as the hepatic manifestation of IgG4 related disease.[10] The medical history of these 13 patients did not mention inhalant or food allergies. Sera with total IgE levels evenly distributed over the range 0-7500 kU/L were selected from left-over samples, previously submitted for routine IgE antibody measurements. Sera from patients with cystic fibrosis, some of which have an elevated total IgE caused by allergic bronchopulmonary aspergillosis,[12] were excluded, because of frequent pancreatic pathology in these patients.[13] The median age of the patients with AIP, pancreatic carcinoma, or atopic allergy was 69, 66, and 37 years, and the male/female ratio 12/1, 5/7 and 9/5 respectively. Sera were stored at -20° C. Total IgE was determined on the ImmunoCAP 250 system, according to the manufacturer's instructions (Phadia, Nieuwegein, Netherlands). IgG subclasses were determined on the Immage 800 analyzer (Beckman Coulter, Mijdrecht, Netherlands) using the Peliclass IgG subclass nephelometry kit according to the manufacturer's instructions (Sanquin, Amsterdam, Netherlands). This manufacturer gives a value of 1.4 g/L as upper reference limit for serum total IgG4 in adults.

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

For statistical analysis of the data we used Graph Pad Prism for Windows, version 5.01, Graph Pad Software, San Diego, CA, USA. For comparison between groups the Mann-Whitney test was applied. The relation between total IgG4 and total IgE within groups was analysed by the Spearman rank correlation test. For analysis of the difference between ROC curves, derived from the same samples, we used Medcalc Version 11.3, Medcalc Software, B-9030 Mariakerke, Belgium.

RESULTS

Both total IgE and total IgG4 of the 13 AIP patients were significantly higher than these levels in 12 patients with pancreatic carcinoma (p= 0.0004 and p = 0.015 respectively) (Fig 1 and 2). No significant difference was observed for total IgE and total IgG4 between the 13 AIP patients and 14 atopic patients (p=0.14 and p= 0.07 respectively). Total IgE in the atopic group was significantly higher than this level in the patients with pancreatic carcinoma (p <0.0001). This was not the case for total IgG4 (p=0.21) (Fig 1 and 2). The diagnostic power of serum IgE and IgG4 for discrimination between AIP and pancreatic carcinoma was analyzed by calculation of receiver operating characteristic (ROC) curves (fig 3) for the combined data from the AIP patients and the patients with pancreatic carcinoma. At a level of 1.6 g/L, total IgG4 had 69% sensitivity and 92% specificity. At a level of 138 kU/L, total IgE had 77% sensitivity and 100% specificity. Although the area under the curve (AUC) for IgE (0.923) was larger than the AUC for IgG4 (0.788), this difference did not reach statistical significance (p=0.15), probably because of the small number of patients involved.

There was a significant correlation between total IgE and total IgG4 levels in the AIP patients, as well as in the patients with IgE-mediated allergy (r_s = 0.82, p=0.0006, respectively r_s = 0.88, p<0.0001) (Figure 4). In the samples from

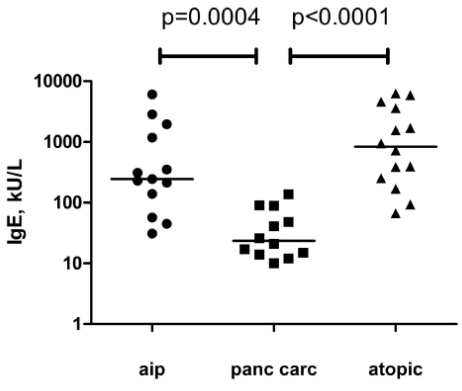

Figure 1 Serum total IgE in AIP, pancreas carcinoma and atopics
Horizontal bars: median IgE level: 244, 24, and 833 kU/l.

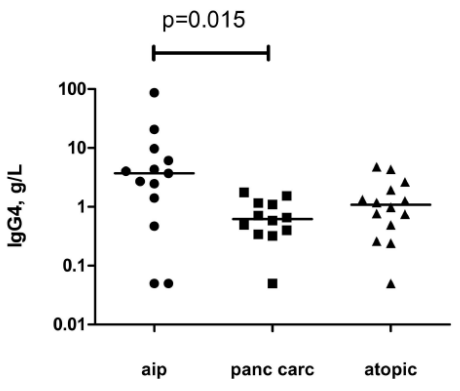

Figure 2 Serum total IgG4 in AIP, pancreas carcinoma and atopics
Horizontal bars: median IgG4 level: 3.70, 0.62, and 1.08 g/l. Three sera with a total IgG4 level of <0.05 g/L were assigned a value of 0.05 g/L.

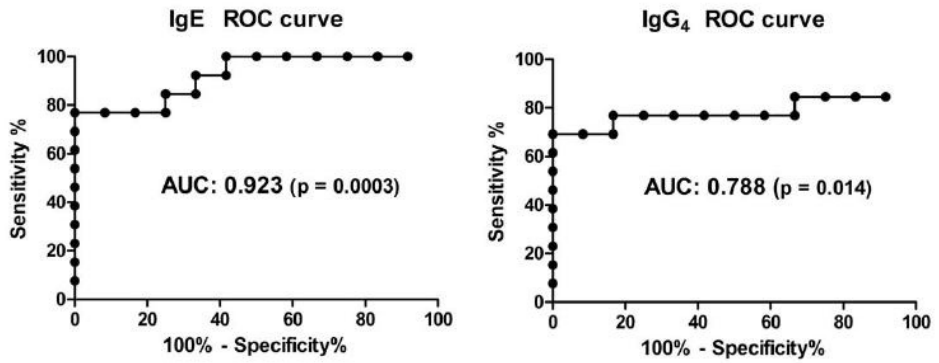

Figure 3 ROC curves for the combined data from AIP patients and patients with pancreatic carcinoma

patients with pancreatic carcinoma low levels of both IgE and IgG4 were found, without a significant correlation between these two variables ($r_s = 0.14$, p=0.65). The IgE and IgG4 levels in Figure 4 suggest that the AIP patients and the atopic patients have a different etiology. However, the pancreas-carcinoma patients show a trend similar to the AIP group. Indeed, when we compared the IgE/ IgG4 ratio's of the individual samples of the three different groups, we found a significant difference between AIP patients and atopic patients. In Figure 5 the IgE/ IgG4 ratio is depicted for the same sera shown in Figure 4. The IgE/ IgG4 ratio for sera from patients with atopy was significantly different (p=0.0012) from this ratio in sera from patients with AIP and also significantly different from patients with pancreatic carcinoma (p<0.0001). Interestingly,

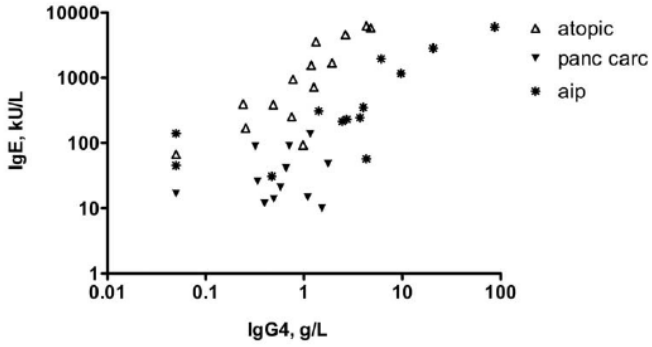

Figure 4 Relationship between total IgG4 and total IgE in AIP, pancreatic carcinoma and atopics *AIP : $r_s = 0.82$, p=0.0006 and atopics : $r_s = 0.88$, p<0.0001. Three sera with a total IgG4 level of <0.05 g/L were assigned a value of 0.05 g/L.*

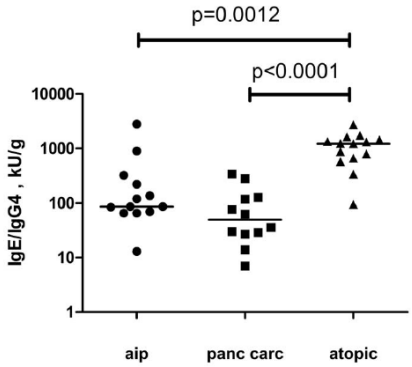

Figure 5 Serum IgE/IgG4 ratio in AIP, pancreatic carcinoma and atopics
Horizontal bars: median IgE/IgG4 ratio 87, 49, and 1234 kU/g. Three sera with a total IgG4 level of <0.05 g/L were assigned a value of 0.05 g/L.

the IgE/ IgG4 ratio in sera from patients with pancreatic carcinoma was not significantly different from this ratio in sera from AIP patients (p=0.09).

The serum with a total IgG4 level of 87 g/L was tested for the presence of a monoclonal immunoglobulin by serum electrophoresis/immunofixation with the Sebia Hydrasys system (Sebia, Vilvoorde, Belgium). However, a polyclonally elevated IgG was observed.

DISCUSSION

IgG4 is the least abundant of the four human IgG subclasses. Aalberse et al[14,15] have shown that prolonged allergen-exposure leads to the formation of specific IgG4 antibodies, whose main function was thought to suppress IgE-mediated inflammation. Likewise, Okazaki et al.[8] indicated that in autoimmune pancreatitis, IgG4 or IgG4-immunecomplexes are unlikely to be pathogenic factors, but may also act as anti-inflammatory factors. In autoimmune pancreatitis and associated pathology, total IgG4 can exceed the concentrations of the other subclasses. Kawa et al.[16] described total IgG4 levels up to 40 g/L. One of the patients in this study had a total IgG4 level of 87 g/l, a level that would suggest the presence of a monoclonal gamma-globulin. However, immunofixation/electrophoresis showed that serum from this patient contained highly elevated polyclonal IgG.

The median total IgE level of the patients with pancreatic cancer (24 kU/L) is close to a recently published value (21.7 kU/L) for non-atopic individuals.[17] The IgE/ IgG4 ratio in atopic patients was significantly higher than this ratio in AIP patients and in patients with pancreatic carcinoma (Figure 4). The difference in median age between the atopic patients (37 years) and the AIP patients (69 years) does not explain the difference in IgE/ IgG4 ratios: the median total IgE level of the US population (including atopic individuals) was 38.0 kU/l for 30-39 year-old persons and 36.6 kU/l for 60-69 year-old individuals in 2005.[17]

In the study of Hamano et al.[5] total IgE was not significantly different between AIP patients and normal individuals, whereas in the present study IgE was significantly higher in AIP patients that in patients with pancreatic carcinoma. The Elisa used by Hamano et al. for total IgE analysis was not specified. We used the automated ImmunoCAP system with calibrators traceable to the WHO preparation 75/502 for human IgE. Our results are in line with the data published by Masaki et al.,[11] who reported a 20-fold higher total IgE level in 64 patients with IgG4-positive multi-organ lymphoproliferative syndrome compared with 33 patients with Sjögren's syndrome. The correlation we observed between serum total IgE and IgG4 (Figure 4) could theoretically be explained by a novel heterophilic antibody activity of IgG4, proposed by Ito et al..[18] The significant difference between the IgE/IgG4 ratio's of AIP patients and atopic patients (Figure 5) however makes this explanation unlikely. Furthermore, we found no evidence for heterophilic antibody interference in the total IgE assay, that was used in the present study, even in sera that showed high interference in the Unicap tryptase immunoassay (Supplementary Table).

To our knowledge this is the first report that describes a correlation between serum total IgG4 and total IgE in atopic individuals and in patients with autoimmune pancreatitis. Meiler et al.[19] showed that T regulatory cells regulate antibody isotypes, which may contribute to the suppression of inflammatory diseases by the induction of specific IgG4 and by the suppression of specific IgE. It is remarkable therefore to find a close association between total IgG4 and total IgE both in atopic individuals and in patients with autoimmune pancreatitis. Zen et al.[9] concluded that the predominant Th2 and regulatory immune reactions in AIP might reflect an allergic nature in their pathogenesis. The simultaneous elevation of both IgG4 and IgE in AIP and the correlation between these two markers supports this view. Choi et al.[20] recently suggested that in AIP there may be a defect in the function of regulatory

T cells, which normally prevents autoimmune disease progression via a suppressor mechanism. Regulatory T cells also play a role in the suppression of IgE-mediated allergy.[19,21] An abnormality in regulatory T cells therefore could be the common cause of elevated IgE and IgG4 in AIP.

In summary, our results suggest that analysis of total IgE in serum might be useful in the differentiation between autoimmune pancreatitis and pancreatic carcinoma. A limitation of our study is the small number of patients involved. A more accurate estimation of the diagnostic value of total IgE would require a larger number of patients. Metz[22] indicated that about 100 samples are needed for meaningful conclusions to be drawn from ROC analysis.

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SUPPLEMENTARY MATERIAL

Supplementary Table *Serum tryptase, human anti-mouse antibodies (Hama) and total IgE in sera before and after incubation of these sera in Heterophilic blocking tubes (HBT).*

Tryptase	Tryptase	Hama	Hama	Total IgE	Total IgE
ug/l	ug/l	ng/ml	ng/ml	kU/l	kU/l
without HBT	with HBT	without HBT	with HBT	without HBT	with HBT
6.91	6.59	<1.5	<1.5	413	372
7.96	5.37	21.1	<1.5	5.34	4.93
4.77	4.45	<1.5	<1.5	5.92	4.73
5.92	4.02	3.4	<1.5	53	50.2
52.1	5.78	25.9	1.6	83.7	97.4
3.34	3.37	<1.5	<1.5	13.5	13.2
6.65	6.4	<1.5	<1.5	230	219
4.38	4.4	1,6	<1.5	31	27.2
21.3	14.4	40.8	<1.5	1109	1140
10.1	4.36	43.2	<1.5	20.6	16.4
7.08	5.18	5.6	<1.5	19.2	21
13.8	4.36	92.1	<1.5	49.2	47.1
5.11	3.33	46.7	<1.5	41.2	37.4
84.6	7.47	254	<1.5	3.95	3.86
11.8	9.03	21.7	<1.5	368	323

Estimation of serum tryptase and Hama and incubation of sera in HBT were performed as described in: van Toorenenbergen AW, Hooijkaas H, Klein Heerenbrink G, Dufour-van den Goorbergh DM. Heterophilic antibody interference in a tryptase immunoassay. Clin Biochem 2008;41:331-334. In four sera HBT treatment caused >50% reduction of serum tryptase, indicating that heterophilic antibodies were present that caused a falsely elevated serum tryptase. HBT treatment decreased the levels of Hama to amounts below (or borderline above) the detection limit of the Hama assay; in contrast, there was no significant difference (p = 0.094) between total IgE in sera before and after HBT treatment.

Chapter 6

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

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Submitted

ABSTRACT

Background There is no single reliable test for diagnosing autoimmune pancreatitis (AIP) and differentiating this disease from other disorders. Several serological markers have been proposed but most of these lack sufficient validation.

Objective To determine the diagnostic value of autoantibodies (antinuclear antibody/ANA, rheumatoid factor/RF, anti carbonic anhydrase II/ACA II, anti lactoferrin/ALF, perinuclear anti neutrophil cytoplasmic antibody/p-ANCA, extractable nuclear antigens/ENA) in groups of patients with AIP, various benign and malignant pancreatobiliary diseases and Sjögren's syndrome. In comparison we also determined the diagnostic test characteristics of serum IgG, IgG subclasses, IgE and Ca 19-9.

Design Prospective single-center study. Inclusion of consecutive patients presenting with AIP (n=33), pancreatic carcinoma (n=53), cholangiocarcinoma (n=32), chronic pancreatitis (n=30), PSC (n=30) and Sjögren's syndrome (n=31). Diagnostic characteristics and optimal cut-off levels were assessed to differentiate between AIP and malignancy. Logistic regression was performed to detect combinations of tests that predict AIP.

Results The seroprevalence of ANA, RF, ACA II and ALF was low and overall comparable for AIP, chronic pancreatitis, pancreatic cancer and cholangiocarcinoma. None of the tested autoantibodies was found to be discriminative or diagnostically helpful. IgG4 was the best single diagnostic test (AUC 0.89). The optimal cut-off level was 2.8 g/L (2 x upper limit of normal). Combination of IgG4, IgG3 and Ca 19-9 predicts probability of AIP with an AUC of 0.93.

Conclusions Testing for autoantibodies did not appear to be clinically useful in the diagnosis of AIP in this Western population. IgG4 was confirmed to be the best single test to differentiate between AIP and malignancy, with an optimal cut-off level of twice the upper limit of normal. Combining IgG4, IgG3 and Ca 19-9 can be used to reliably predict a diagnosis of AIP.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a distinct fibroinflammatory disease of the pancreas that is characteristically responsive to corticosteroids.[1-2] Currently two types of AIP are recognized. Type 1 AIP is a manifestation of IgG4 related disease, type 2 has substantial clinical overlap with type I, but is characterized by normal IgG4 levels and distinctive pathological features.[3] In its classical form, the disease presents like pancreatic carcinoma. Extrapancreatic biliary involvement may be prominent, resembling cholangiocarcinoma or primary sclerosing cholangitis. Other extrapancreatic manifestations include retroperitoneal fibrosis and sialoadenitis, mimicking Sjögren's syndrome.[4] The pathogenesis of AIP is not completely understood. The current understanding is that in genetic susceptible persons,[5-6] an autoimmune reaction (Th1 mediated) to auto antigens, or infectious agent (*H pylori*[7-10]) triggers a Th2 mediated (allergic) response which leads to excessive or inappropriate B cell activation.[11-14] It is not known whether IgG4 (or IgG4-immune complexes), classically known as an anti-inflammatory globulin, is pathogenic or an epiphenomenon.[15]

A large number of serological markers such as IgG4,[16-22] total IgG,[16-20, 23-27] and the presence of autoantibodies such as antinuclear antibody (ANA),[20, 28-34] rheumatoid factor (RF),[20, 29-30, 32-33, 35-37] anti carbonic anhydrase II (ACA II)[30, 32-33, 38-42] and anti lactoferrin (ALF)[30, 32, 43] have been proposed as useful diagnostic tests (supplementary Table 1). Although it has become clear that IgG4 is the best single marker of AIP, levels may be normal in otherwise characteristic AIP and may be elevated in other conditions including PSC and pancreatic cancer. The diagnostic significance of autoantibodies has not been well defined. Nevertheless the presence of autoantibodies forms one of the components of several (Asian, Japanese)[44] but not all (HISORt,[45-46] International Consensus[47]) diagnostic criteria systems developed for diagnosing the disease. The superiority of one of these systems over the other has not been established.

Given these uncertainties the primary aim of the present study was to assess the seroprevalence and diagnostic value of a set of previously proposed autoantibodies in a population of Western AIP patients and in groups of patients with disorders that may mimic or are associated with AIP. A secondary aim was to compare the diagnostic significance of these antibodies with that of measuring IgG, IgG subclasses, IgE and tumor marker Ca 19-9.

METHODS

PATIENT SELECTION

Between March 2007 and May 2011 sera were obtained from consecutive individuals presenting with AIP, pancreatic carcinoma, cholangiocarcinoma, chronic (alcoholic, obstructive or idiopathic) pancreatitis and primary sclerosing cholangitis. Serum aliquots of patients with Sjögren's syndrome were obtained from an existing serum bank. After written informed consent was obtained, sera were collected together with samples drawn for routine clinical analysis and processed immediately or stored at -80˚C. The diagnosis of AIP was established according to the International Consensus Diagnostic Criteria,[47] Asian,[44] or HISORT[45-46] criteria, a combination of unexplained pancreatic disease, biliary disease/extrapancreatic manifestations and either response to steroids or IgG4-positive serology.[48] These diagnostic criteria were also systematically applied to all chronic pancreatitis cases to exclude misclassification. All cases with pancreatic carcinoma and cholangiocarcinoma were histologically confirmed. PSC and Sjögrens syndrome were diagnosed according to accepted criteria.[49-50]

LABORATORY INVESTIGATION

The following laboratory investigations were performed: measurement of immunoglobulins total IgG, IgG subclasses 1 to 4 and IgE and the presence of the autoantibodies ANA, RF, pANCA, Extractable Nuclear Antigen (ENA) aSSa and aSSb, ACA II and ALF. We also included the tumor marker Ca 19-9 as high levels are considered indicative of malignancy.[51-53] Finally we developed a test to detect anti (pancreatic) elastase antibodies (AE). Commercially available tests were used for IgG and IgG subclasses, IgE, ANA, RF, pANCA, ENA (aSSa and aSSb) and Ca 19-9. For ACA II, ALF and AE, ELISA's were developed in a uniform matter. For a detailed description of each test, see Supplementary File 1.

STATISTICAL ANALYSIS

Frequencies of positive tests were determined in each group. One way analysis and pair wise comparisons with Bonferroni correction for multiple testing were performed to detect significant differences between groups. Diagnostic characteristics of each test were calculated using 2x2 tables: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV),

likelihood ratio of positive test (LR+) and likelihood ratio of negative test (LR-). Receiver operating curves (ROC) were constructed to assess optimal cut-off levels and calculate the area under the ROC curve (AUC). Logistic regression was performed to detect combinations of tests that predict AIP. Statistical analysis was carried out using SPSS Statistics 17.0 Software (IBM, New York, USA). Two-sided p-values ≤0.05 were considered statistically significant.

ETHICAL CONCERNS

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

RESULTS

The patient groups differed significantly in terms of age, sex and bilirubin level (Table 1), but these differences were not attributable to differences between AIP and control groups. AIP patients were mainly type 1. Proximal biliary involvement was noted in 50%, 28% were on low dose maintenance prednisone (2.5-10 mg/d).

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]
bilirubin (µmol/L)	10(8-34)	15(9-57)	23(8-57)	7(5-11)	38(15-109)	0(0-1)	<0.001[1]

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

The seroprevalence of antibodies previously proposed as indicative for AIP, in particular ANA, RF, ACA II and LF, was low and overall comparable for patients with AIP, chronic pancreatitis, pancreatic cancer and cholangiocarcinoma. No significant differences between the study groups were detected. Only few cases with anti elastase antibodies were found, and this test was not found

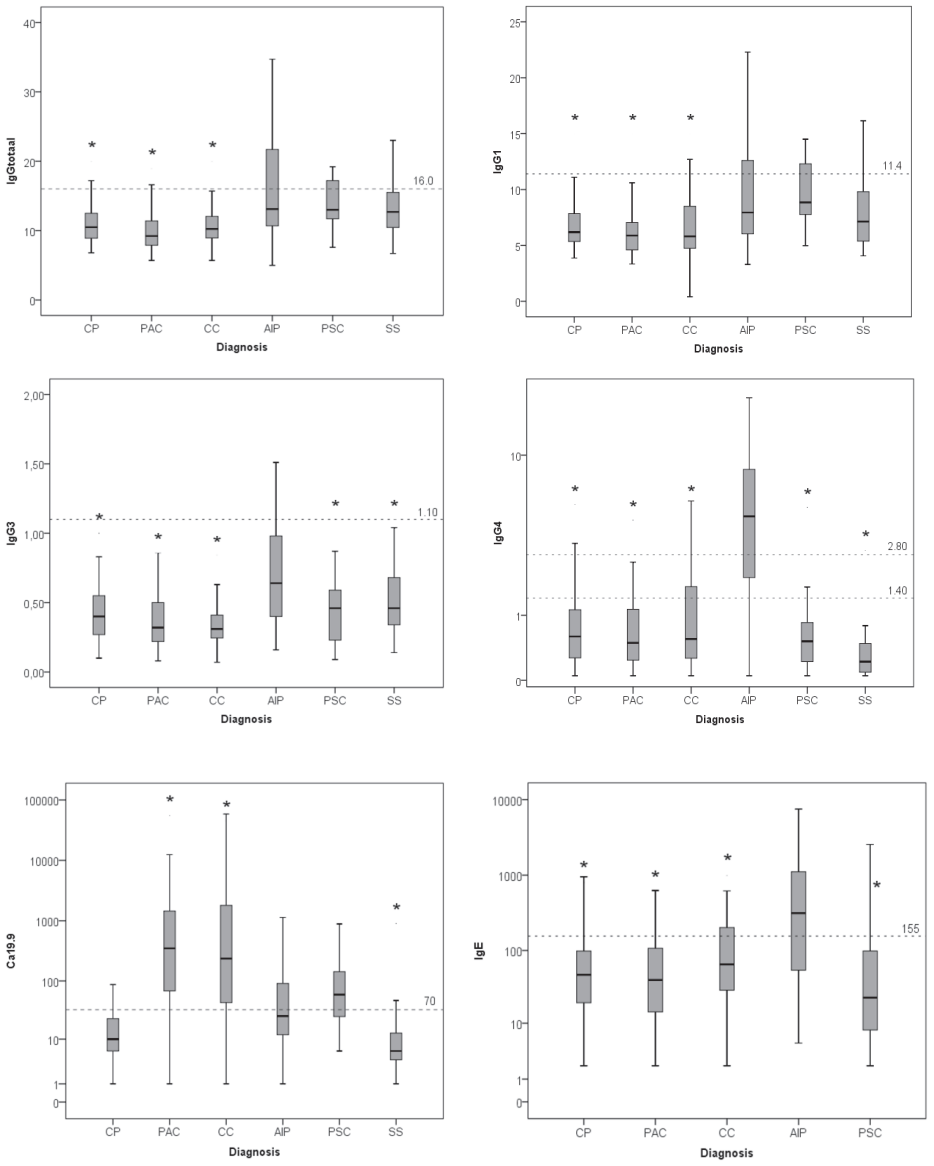

Figure 1 Immunoglobulin G, IgG subclasses, IgE and Ca 19-9 in AIP, other pancreatobiliary diseases and Sjögren's syndrome

*Box plot. The box shows the median value and the interquartile (25 to 75 percentile) range, the whiskers the range of the upper and lower quartile with the minimal and maximal values. *p<0.05 between AIP and group. CP=chronic pancreatitis, PAC=pancreatic carcinoma, CC=cholangiocarcinoma, AIP=autoimmune pancreatitis, PSC=primary sclerosing cholangitis, SS=Sjögren's syndrome. IgG2 is not shown, no significant differences were detected.*

Table 2 Seroprevalence of autoantibodies in AIP, other pancreatobiliary diseases and Sjögren's syndrome

	NON AUTOIMMUNE			***AUTOIMMUNE***			
	Benign	***Malignant***					
	Chronic pancreatitis	***Pancreatic carcinoma***	***Cholangio carcinoma***	***Autoimmune pancreatitis***	***Primary sclerosing cholangitis***	***Sjögren's syndrome***	***p-overall***
Number	***52***	***53***	***32***	***33***	***30***	***31***	
ACA II	11.5	9.4	3.1	12.1	23.3	16.1	0.18
ALF	21.2	17.0	12.5	9.1	53.3†	12,9	<0.01
AE	5.8	1.9	0.0	6.1	10.0	0.0	0.15
ANA	23.0	15.1	25.0	27.3	16.7	77.4†	<0.001
pANCA	0.0	0.0	0.0	6.3	20.0†	3.2	<0.001
RF IgM	3.8	13.2	18.8	21.9	10.0	54.8†	<0.001
aSSa	1.9	1.9	0.3	0	0.0	67.7†	<0.001
aSSb	0.0	0.0	0.0	3.1	0.0	48.4†	<0.001

Autoantibodies in % positive. P-overall: one way analysis of variance (to detect differences between groups). †: p<0.05 between AIP and group (post hoc paired test with correction for multiple testing).

Table 3 Diagnostic performance of IgG, IgG subclasses, IgE, autoantibodies and Ca19.9 in differentiating between AIP and malignancy (pancreatic cancer and cholangiocarcinoma)

	True positive (n)	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*LR +*	*LR-*	*AUC Total (95% CI)*
IgG >16.0 g/l	14	42.4	95.3	77.8	81.0	9.0	0.60	0.74 (0.63-0.85)
IgG1 >11.4 g/l	9	27.3	92.9	60.0	76.7	3.8	0.78	0.70 (0.59-0.81)
IgG2 >6.4 g/l	2	60.6	91.8	22.2	71.6	7.4	0.43	0.51 (0.38-0.63)
IgG3 >1.1 g/l	6	18.2	98.8	85.7	75.5	91	0.83	0.76 (0.66-0.86)
IgG4 >1.4 g/l	26	83.9	77.6	57.8	93.0	3.7	0.21	0.89 (0.81-0.97)†
IgG4 >2.8 g/l*	20	64.5	97.6	90.9	88.3	26.9	0.36	0.89 (0.81-0.97)†
IgE >155 kU/l‡	16	66.7	81.4	55.2	88.0	3.6	0.41	0.75 (0.63-0.88)
ACA II positive	4	12.1	92.9	40.0	73.1	1.7	0.95	0.40 (0.28-0.52)
ALF positive	3	9.1	84.7	18.8	70.6	0.6	1.07	0.56 (0.44-0.67)
AE positive	2	6.1	98.8	66.7	73.0	30.5	0.95	0.40 (0.29-0.51)
ANA positive	9	27.3	81.2	36.0	74.2	1.5	0.90	0.45 (0.33-0.57)
pANCA positive	2	6.7	100	100	73.9	∞	0.93	0.47 (0.35-0.59)
RF IgM positive	7	21.9	84.7	35.0	74.2	1.4	0.92	0.47 (0.35-0.59)
aSSa positive	0	0.0	96.5	0.0	71.9	0.0	1.04	0.52 (0.40-0.63)
aSSb positive	1	3.1	100	100	73.3	∞	0	0.48 (0.37-0.60)
Ca 19.9 <34 kU/L	18	56.3	80.0	51.4	82.9	2.8	0.55	0.76 (0.67-0.86)

*†AUC IgG4 total. * IgG4 level of 2.8 g/l was the best cut off value for detecting AIP by using ROC analysis. ‡IgE level of 155 kU/l was the best cut- off level for detecting AIP by using ROC analysis. PPV: positive predictive value, NPV: negative predictive value, LR+: likelihood ratio of positive test, LR-: likelihood ratio of negative test, AUC: area under the curve (95% confidence interval).*

to be discriminative. Lactoferrin antibodies and pANCA were more frequent in primary sclerosing cholangitis. ANA, RF IgM and aSSa and aSSb were indicative of Sjögren's syndrome.

IgG, IgG1, IgG3, IgG4, IgE and Ca 19-9 levels significantly differed between AIP and malignancy (Figure 1, Supplementary Table 2). The most striking difference between AIP and the other groups was the IgG4 level. IgE levels were also significantly higher in AIP but there was noticeable overlap with other patient groups. In patients with pancreatic cancer and cholangiocarcinoma the highest Ca 19-9 levels were found but substantially elevated levels were also frequently observed in AIP and PSC. The diagnostic performance of each single test in differentiating between AIP and malignancy is shown in Table 3.

IgG4 was the best single test to discriminate between AIP and malignancy, with an AUC of 0.89, followed by IgG3 (0.76), Ca 19-9 (0.76), IgE (0.75), IgG (0.74) and IgG1 (0.70). The highest likelihood ratios for positive test (LR+, tests that rule in AIP with respect to malignancy) were found for IgG3 >1.1 g/L (LR+ 91), IgG4 >2.8 g/L (LR + 26.9), and the presence of elastase antibodies (LR+ 30.5). The sensitivity of IgG3 levels >1.1 g/L and the presence of elastase antibodies however, was extremely low (18.2 and 6.1% respectively). The lowest likelihood ratios for negative tests (LR-, tests that rule out AIP with respect to malignancy) were IgG4 <1.4 g/L (LR- 0.21), IgG4 <2.8 g/L (LR- 0.36), IgE <155 kU/L (LR-0.41) and IgG2 <6.4 g/L (LR- 0.43).

The optimal cut-off level of IgG4 is 2.8 g/L, that is twice the upper limit of normal (Table 4). Although the sensitivity of a cut-off level of 1.4 g/L, which is

Table 4 Optimal cut-off levels of IgG (subclasses), IgE and Ca 19-9 for differentiating between AIP and malignancy

	Normal range	***Optimal cut- off level***	***Sensitivity (%)***	***Specificity (%)***
IgG (g/l)	7.0-16.0	10.6	75.8	64.7
IgG1 (g/l)	4.9-11.4	6.8	66.7	69.4
IgG3 (g/l)	0.20-1.10	0.37	81.8	59.5
IgG4 (g/l)	0.08-1.40	2.80 g/l	64.5	97.6
IgE (kU/l)	*n.a.*	155 kU/l	66.7	81.4
Ca 19.9 (kU/L)	0-34	70*	70.6	75.0

**Levels lower than 70 U/ml are indicative of AIP, n.a.: not available.*

the generally accepted upper limit of normal, is higher (83.9%), the specificity of IgG4 levels >1.4 g/L is quite low (77.6%).

The result of the logistic regression analysis is shown in Figure 2 and Supplementary File 2. Combining IgG4, IgG3 and Ca 19-9 levels predicts probability of AIP with an AUC of 0.93, which is better than for IgG4 alone.

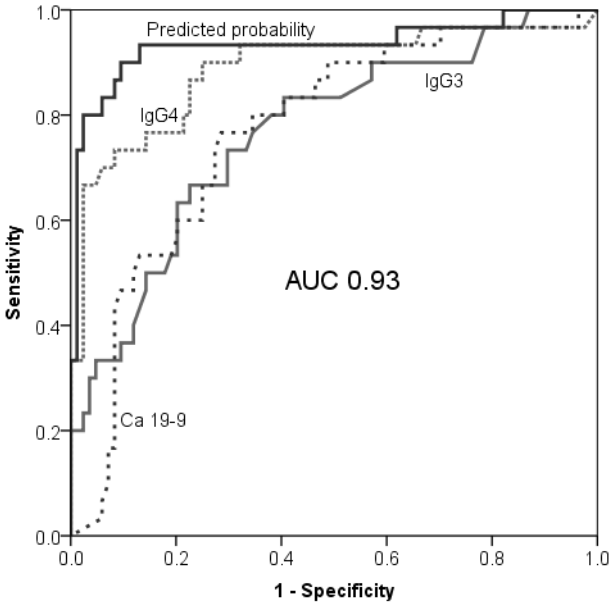

Figure 2 Combination of IgG4, IgG3 and Ca 19-9 in discriminating between AIP and malignancy ROC curves of Ca 19-9, IgG3 and IgG4 are shown as single test and as predicted probability of AIP (logistic regression analysis).

DISCUSSION

The results of the present study indicate that testing for autoantibodies such as ANA, RF, ACA II and ALF is not useful in diagnosing AIP, at least not in a Western patient population. This study confirms that IgG4 remains the single most important diagnostic laboratory test, with an optimal cut-off level of twice the upper limit of normal.

In previous studies, mainly performed in Asia, substantial higher frequencies of positive tests for ANA, RF, ACA II and ALF in AIP were reported.[20, 28-33, 35-43] As in both these series and in our study the large majority of patients were classified

as type I, differences in AIP subtype do not readily explain the diverging results. Possibly these could be related to different genetic background of the study populations. Further it may be possible that laboratory technical issues could at least partially be involved in explaining different outcomes. This could be subject for further research.

Obviously, based on our results testing for autoantibodies cannot be advocated in the workup of patients possibly suffering from AIP, or for differentiating AIP from other benign and malignant pancreatobiliary disorders. The results further suggest that systems developed for assisting in the diagnosis, in particular the Japanese and the Asian consensus criteria should be modified with respect to the presence or absence of detectable autoantibodies, at least when used in non-Asian populations. Other proposed diagnostic systems, such as the HISORt and the International consensus criteria, do not include the presence of autoantibodies. Our findings strongly support the major diagnostic weight given to elevated serum IgG4 levels in these systems. In line with previous reports[17] we confirm that IgG4 levels higher than twice the upper limit of normal clearly suggest a diagnosis of AIP. In clinical practice elevated but lower (intermediate) levels should be interpreted with great caution as the specificity of this finding is suboptimal. This study found that, in addition to IgG4, total IgG, IgG1, IgG3, IgE and Ca 19-9 levels also differed significantly between AIP and malignancy. However, their test characteristics are insufficient to be regarded as useful single diagnostic tests. The combination of IgG4, IgG3 and Ca 19-9 predicted probability of AIP with an AUC of 0.93, which was better than IgG4 alone. The concept of combining these tests to improve diagnosis is attractive, since they are readily available. However, it was a result of logistic regression analysis (Formula available at Supplementary file 2) and should not be interpreted as combining three single tests with their respective cut-off values. The value of this combination test needs to be addressed in a validation study.

As far as we are aware this is one of the few studies addressing the diagnostic value of antibody testing in AIP. This was a prospective study not only including patients with a definitive diagnosis of AIP but also a number of relevant and well defined reference groups. In all cases with malignancies diagnosis was histologically confirmed. Follow up of AIP patients was sufficient enough to exclude malignancy (at least 2 years). A limitation is that the results apply to a Western patient population and may not be applicable to other populations.

The use of diagnostic criteria as a selection criterion for AIP (which include IgG, IgG4 and autoantibodies) may have introduced selection bias. Also the results were derived from a population of largely AIP type 1 patients and may not necessarily apply to AIP type 2. Further, although we sincerely attempted to include all consecutive AIP patients, the number of cases is moderate, reflecting the relative rarity of the disease. Finally, 28% of AIP patients were on low dose maintenance prednisone (2.5-10 mg/d), which may have lowered levels of immunoglobulins or autoantibodies. However, although steroid therapy has shown to lower IgG4 levels, they usually still remain elevated despite remission. In a previous study, the sensitivity of IgG4 did not change significantly when AIP patients with steroid treatment were excluded.[17] In our study, results on autoantibodies did not differ between patients with or without prednisone.

For diagnostic purpose testing for autoantibodies including ANA, RF, ACA II and ALF is not useful. What this means for their putative role in pathogenesis remains unclear. Generally, autoimmunity is still considered the major trigger of inflammatory cascade in AIP. However, accumulating evidence suggests that allergic (Th2 mediated) reactions play an important role in the pathogenesis of AIP.[15,55] Generally, IgG4 can be induced by repeated antigen exposures and is controlled, like IgE production, by Th2 cells. Whether the causal antigen in AIP is endogenous or exogenous, still remains to be elucidated. In any case, with its male predominance and striking sensitivity to steroids, AIP clearly does not meet the classical phenotype for autoimmune disease.

In conclusion, this study shows that testing for autoantibodies, previously proposed to be of diagnostic value, is not useful in the workup of patients with a possible diagnosis of AIP. IgG4 is the best single test to differentiate between AIP and other pancreaticobiliary disorders, with an optimal cut-off level of 2.8 g/L (twice the upper limit of normal).

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FILE 1: SEROLOGIC TESTS

Immunoglobulines

IgG and IgG subclasses were determined on the Immage 800 Analyzer (Beckman Coulter, Mijdrecht, the Netherlands) using Peliclass IgG subclass nephelometry kit according to manufacturer's instructions (Sanquin, Amsterdam, the Netherlands). The upper limits provided by the manufacturer were 16.0 g/L (IgG), 11.40 (IgG1), 6.40 (IgG2), 1.10 (IgG3) and 1.40 (IgG4) g/L. Total IgE was determined on the ImmunoCAP 250 system, according to the manufacturer's instructions (Phadia, Nieuwegein, the Netherlands). No upper limit was given, results in kU/L.

Ca19-9

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.

Autoantibodies: immunofluorescence

ANA were determined by routine laboratory indirect immunofluorescence technique using Hep-2000 slides (Immuno Concepts, N.A. Ltd, Sacramento, California). As cut off value titer 80 was used. ANCA were also determined by indirect immunofluorescence according daily routine of the laboratory. Ethanol Fixed Human Neutrophil Slides (IgG) were used for screening and positive results were confirmed on Formalin Fixed Human Neutrophil slides (IgG) (both provided by INOVA Diagnostics, Inc, San Diego, California). A cut-off titer of 10 on Ethanol slides, and 20 on formalin slides was used, respectively.

Autoantibodies: ELISA

RF IgM was determined by an in-house ELISA which is used in the daily routine laboratory diagnostics.

Alle sera were screened for the presence of ENA by QUANTA Lite™ ENA 6 ELISA kit IgG (INOVA Diagnostics, Inc, San Diego, California). If this screening test was

positive, several tests were performed to confirm the presence of anti-SS-A and/or anti-SS-B antibodies: QUANTA Lite™ SS-A/SS-B (ENA) ELISA Kit IgG (INOVA Diagnostics, Inc, San Diego, California), ANA Profile 3 Line blot IgG (EuroImmun, Lubeck, Germany), EliA Ro/ La IgG (Phadia, Uppsala, Sweden) and INNO-LIA ANA Update (Innogenetics NV, Gent, Belgium. Two positive results were concerned as a positive test for one of these antibodies.

ACA II, ALF and AE: home made ELISA

Because no commercial tests were available, ACAII, ALF and AE were tested in a homemade ELISA. High binding plastic micro titer plates (Greiner Bio-one, Netherlands) were coated with CAII (Sigma-Aldrich, Germany), E (Calbiochem,Netherlands) or LF (Sigma-Aldrich, Germany , all 1 mg/ml) in NH4HCO3 (0.1M) at 37˚C overnight. After washing three times with PBS/0.05% Tween 20 wells were blocked with 0.3% casein overnight at room temperature. After washing three times serum samples and controls (diluted 1:100 in Trisbactopepton/0.05% Tween 20) were incubated for 1 hour at 37˚C. The microtiter plates were washed thoroughly with PBS 0.05%Tween and incubated another hour at 37˚C with anti-human-IgG peroxidase (home made) diluted 1:100 in Trisbactopepton/Tween 20. After washing the substrate buffer was applied for 30 minutes, after which stop solution (H2SO4) was added. The optical density (OD) was read at 450 nm. OD values exceeding 2SDs of the reference material from healthy blood donors(n=100) were considered positive.

SUPPLEMENTARY FILE 2: PREDICTED PROBABILITY

The probability of AIP can be predicted using the following formula:

Probability of AIP = -0.39 + 2.45 · IgG3 + 3.55·(log IgG4) - 1.24 (log Ca19-9)

Supplementary Table 1 *Reported seroprevalence of abnormal IgG, IgG4 and IgE levels and of autoantibodies in AIP and other pancreatobiliary diseases, Sjögren's syndrome and healthy controls*

	AIP	***PAC***	***CC***	***CP***	***PSC***	***SS***	***Control***	***Reference***
Elevated IgG4	68 - 95%	0 - 10%	13.5%	0 - 15%	0 - 36%	0%	2 - 5%	16-22
Elevated IgG	37 - 80%	3 - 5%	-	1 - 10%	30%	-	10%	16-20, 23-27
Elevated IgE	35%-77%	0%	-	-	-	-	-	54-55
ANA	43 - 80%	-	-	6%	-	30 - 90%	4%	20, 28-33
RF	13 - 30%	-	-	0%	-	75 - 95%	3 - 25%	20, 29-33, 35-37
aSSa	0 - 2%	-	-	-	-	70 - 100%	0%	20, 28, 31
aSSb	0 - 2%	-	-	-	-	60 - 100%	0%	20, 28, 31
pANCA	-	-	-	-	30 - 80%	-	0%	28, 56-57
ACA II	10 - 89%	0%	-	0 - 46%	-	62 - 68%	0 - 10%	30, 32-33, 38-42
ACA IV	27%	14%	-	13%	-	45%	-	41
ALF	23 - 76%	-	-	0%	-	-	-	30, 32, 43
aPSTI	31%	0%	-	0%	-	-	0%	30, 58
AMY-2A	100%	0%	-	0%	-	-	-	59
aHSP10	92%	8%	-	8%	-	-	-	60
aPBP	94%	5%	-	0%	-	-	0%	7

AIP=autoimmune pancreatitis, PAC= pancreatic adenocarcinoma, C=cholangiocarcinoma, CP=chronic pancreatitis, PSC=primary sclerosing cholangitis, SS=Sjögren's syndrome. ANA=antinuclear antibody, RF=rheumatoid factor IgM, aSSa/b=anti-R0 and anti-La antibody associated with Sjögren's syndrome, pANCA=perinuclear anti neutrophil cytoplasmic antibody, ACA= carbonic anhydrase antibody, ALF= lactoferrin antibody, aPSTI=pancreatic secretory trypsin inhibitor antibody, AMY-2A=amylase alpha-2A antibody, aHSP10=heat shock protein antibody, aPBP=plasminogen binding protein antibody

Supplementary Table 2 *Immunoglobulin G, IgE and Ca 19-9 in pancreatobiliary diseases and Sjögren's syndrome*

	NON AUTOIMMUNE			***AUTOIMMUNE***			***p-overall***
	Benign	***Malignant***					
	Chronic pancreatitis	***Pancreatic carcinoma***	***Cholangio carcinoma***	***Autoimmune pancreatitis***	***Primary sclerosing cholangitis***	***Sjögren's syndrome***	
IgG (g/l)	10.5 (8.9-12.6)†	9.2 (7.9-11.4)†	10.3 (8.9-12.1)†	13.1 (10.6-24.1)	13.0 (11.6-17.3)	12.7 (10.4-15.8)	<0.001
IgG1 (g/l)	6.2 (5.3-7.9)†	5.9 (4.5-7.3)†	5.8 (4.7-8.5)†	7.9 (5.9-12.8)	8.9 (7.5-12.4)	7.1 (5.4-10.1)	<0.001
IgG2 (g/l)	3.5 (2.6-5.1)	3.2 (2.4-3.9)	3.7 (2.6-5.1)	3.3 (2.1-5.1)	4.0 (2.5-5.4)	2.8 (1.8-4.4)	0.12
IgG3 (g/l)	0.4 (0.3-0.6)†	0.3 (0.2-0.5)†	0.3 (0.2-0.4)†	0.6 (0.4-1.0)	0.5 (0.2-0.6)†	0.5 (0.3-0.7)†	<0.001
IgG4 (g/l)	0.6 (0.3-1.1)†	0.5 (0.2-1.1)†	0.6 (0.3-1.7)†	4.7 (1.8-10.5)	0.5 (0.2-0.9)†	0.2 (0.1-0.5)†	<0.001
IgE (kU/l)	47 (19-102)†	40 (14-110)†	65 (28-204)†	314 (52-1190)	23 (7-156)†	n.a.	<0.001
Ca 19-9 (kU/L)	10 (6-24)	349 (63-1588)†	247 (41-2175)†	26 (12-108)	59 (23-154)	6 (4-15)†	<0.001

Values of antibodies and Ca 19.9 in median (interquartile range). N.a.: not available. P-overall: one way analysis of variance (to detect differences between groups). †: p<0.05 between AIP and group (post hoc paired test with correction for multiple testing).

Chapter 7

Comparable efficacy of low and high dose induction corticosteroid treatment in autoimmune pancreatitis Low dose steroids effective alternative in AIP

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ABSTRACT

Objective To compare efficacy of high versus low doses of prednisone for induction of remission in 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 dose of prednisone of 10 mg/d (n=5), 15 mg/d (n=2), 20 mg/d (n=7), 30 mg/d (n=15), 40 mg/d (n=34), and 60 mg/d (n=2). 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 all patients responded clinically well. After 6 months, treatment response with respect to symptomatic, radiological and laboratory improvement was comparable for the different dosage groups.

Conclusions In patients presenting with AIP no evidence was found for a superior treatment effect of high-dose prednisone compared with low-dose (≤20 mg/d) treatment. Based on these retrospective data the general recommendation to start with high-dose therapy may be reconsidered.

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 IgG4-related disease, a systemic disorder that may not only involve the pancreas but 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/d, resulting in daily starting doses of 30-40 mg.[8-9] 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.[10-13] These negative treatment effects may even be more important in patients presenting with AIP since this is a population characterized by relatively advanced age, (de novo) diabetes mellitus and obstructive jaundice. Further, 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 remission induction therapy.

MATERIALS AND METHODS

PATIENTS AND TREATMENT

A retrospective, multicenter study was conducted among patients diagnosed with AIP between May 1992 and March 2012. Data were retrieved from electronic medical record systems and by reviewing paper hospital charts. Patients were included if they fulfilled the ICDC, Asian or HISORt 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 as remission induction therapy.[7,14-15] Patients were excluded if: 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; 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.

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. Additionally, 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 immunoglobulin-G4 (IgG4), immunoglobulin-G (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.

STATISTICAL ANALYSIS

Patients were categorized into three prednisone dosage groups (low dose: 10-20 mg/d, medium dose: 30 mg/d and high dose: 40-60 mg/d).

Statistical analysis was performed using Fisher's 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's 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 two described periods. The random intercept allows for adjustment of the individual baseline biochemical values.

To correct for multiple testing, a p-value <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 males and 8 females with a median age of 63 years) were included (Figure 1). A recent onset of diabetes mellitus (<1 year) was seen in 24/65 (38%) patients. Extrapancreatic manifestations were observed in 48/65 (74%) patients, including extrapancreatic sclerosing cholangitis in 38/65 (59%) 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.

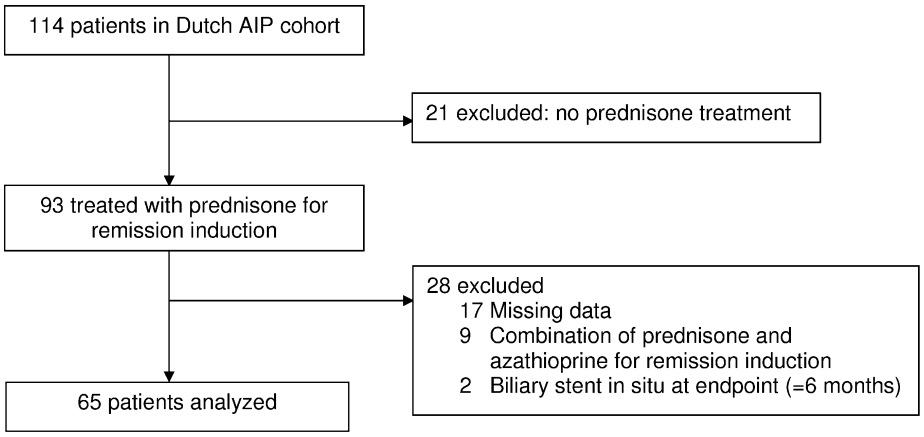

Figure 1 Study enrollment and exclusion.
AIP indicates autoimmune pancreatitis.

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 (Table 2). The mean prednisone induction dosage in the low dose group (10-20 mg) was 0.22 mg/kg/d, in the medium dose group (30 mg) was 0.41 mg/kg/d and in the high dose group (40-60 mg) was 0.55 mg/kg/d.

There were no significant differences in baseline characteristics including gender, age, presenting symptoms, laboratory and imaging results between the treatment groups (Table 1). In general, the initial dose was administered for 2-4 weeks and gradually tapered by 5 mg every 2 weeks. In patients treated with 10-20 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 28/65 (43%) patients were treated for distal biliary obstruction by endoscopic insertion of plastic endoprotheses: 6/14 patients (43%) in the low-dose group, 5/15 (33%) in the medium-dose group and 17/36 (47%) in the high-dose group. These stents were removed after a median of 10 weeks (IQR 5-15) weeks.

During the follow-up period of 6 months, azathioprine was added to steroid therapy in 12/65 (18%) patients: 6/14, 1/15 and 5/29 in the low-, medium- and high-dose group, respectively. Seven of the 12 (58%) patients who were treated with azathioprine took this drug during at least two months. The number of

Table 1 Baseline patient characteristics

	AIP patients	Low dose	Medium dose	High dose	Dose categorized: L-M-H
	(n=65)	(n=14)	(n=15)	(n=36)	(p-value)
Male, n (%)	57 (88%)	13 (93%)	12 (80%)	32 (89%)	0.596[1]
Age at onset, median (IQR), y	63 (53-71)	67 (62-74)	59 (49-66)	62 (44-72)	0.015[2]
Weight, median (IQR), kg	74 (70-83)	72 (58-84)	74 (67-79)	74 (70-87)	0.423[2]
Initial symptoms, n (%)					
Jaundice	47 (73%)	11 (79%)	11 (73%)	25 (71%)	0.930[1]
Weight loss	55 (87%)	13 (93%)	12 (80%)	30 (88%)	0.682[1]
Recent onset diabetes mellitus	24 (38%)	6 (43%)	5 (33%)	13 (37%)	0.885[1]
Steatorrhea	40 (69%)	12 (86%)	12 (80%)	16 (55%)	0.099[1]
Abdominal discomfort	34 (53%)	6 (43%)	7 (47%)	21 (60%)	0.500[1]
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)	0.018[2]
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)	0.044[2]
Total bilirubin, µmol/L (N=≤16)	49 (14-151)	42 (17-150)	79 (18-182)	49 (13-138)	0.780[2]
ALP, U/L (N=≤114)	425 (235-618)	564 (309-768)	468 (333-794)	379 (203-590)	0.204[2]
ASAT, U/L (N=≤34)	126 (55-186)	139 (77-168)	146 (72-176)	93 (38-223)	0.630[2]
ALAT, U/L (N=≤44)	129 (67-306)	130 (65-213)	137 (112-461)	127 (49-337)	0.530[2]
Elevated IgG4, n (%)	41 (77%)	11 (100%)	7 (70%)	23 (72%)	0.113[1]
Radiology, n (%)					
Pancreatic enlargement[3]					0.680[1]
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%)	0.546[1]
Extrapancreatic lesions, n (%)	48 (74%)	12 (86%)	11 (73%)	25 (69%)	0.554[1]

** p <0.01; [1] Fisher's exact test; [2] Kruskal-Wallis test, IQR= interquartile range. AIP indicates autoimmune pancreatitis; IgG4, immunoglobulin-G4; IgG, immunoglobulin-G; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.*

Table 2 Steroid remission induction therapy

Dose prednisone (mg/d)	No. of patients	Treatment group
10	5	Low
15	2	Low
20	7	Low
30	15	Medium
40	34	High
60	2	High

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, while 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 12/15 and 20/36 in the medium- and high-dose group, respectively (p=0.003). No relapses were observed in those patients in whom prednisone was discontinued.

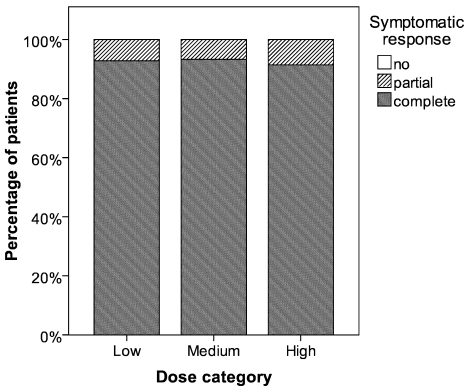

Figure 2 Symptomatic response after 6 months of prednisone remission induction therapy. Results were categorized in 3 treatment groups, low dose (10-20 mg/d), medium dose (30 mg/d) and high dose (40-60 mg/d) prednisone.

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, IgG normalized in all patients. IgG4 showed a rapid decline in the majority of patients, but levels remained elevated in 76% (Figure 3A).

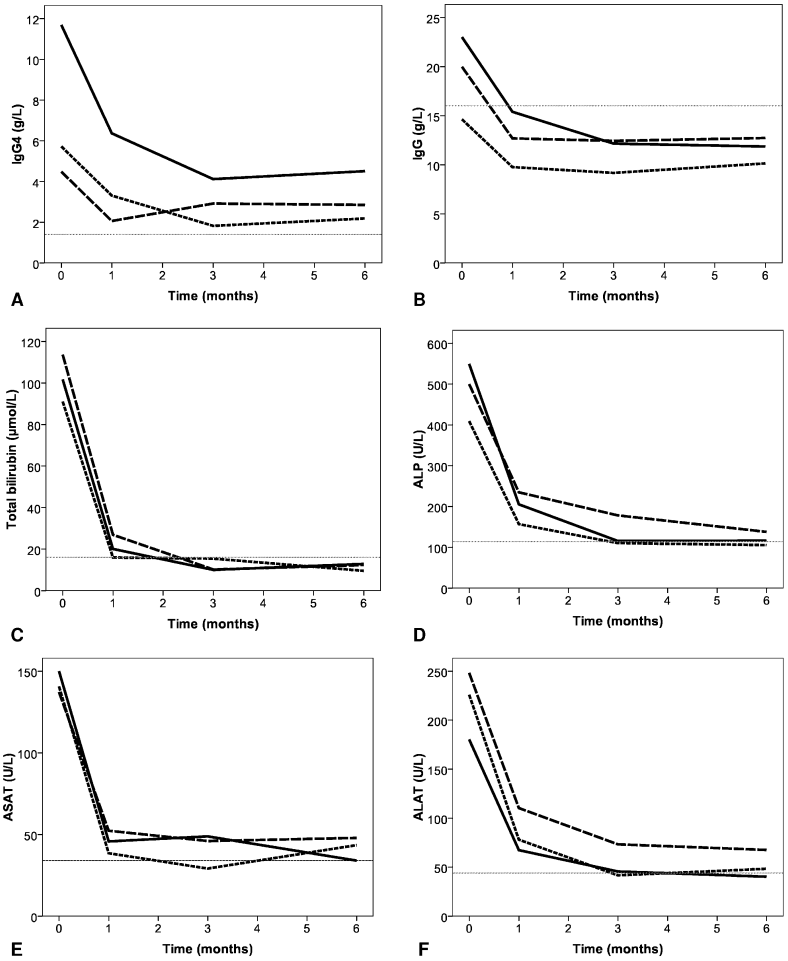

Figure 3 Biochemical mean response during 6 months of prednisone remission induction therapy: IgG4 (**A**), IgG total (**B**), Total Bilirubin (**C**), Alkaline Phosphatase (**D**), ASAT (**E**), ALAT (**F**). —— low dose (10-20 mg/d), −− medium dose (30 mg/d) and ···· high dose (40-60 mg/d) prednisone.
IgG4 indicates immunoglobulin-G4; IgG, immunoglobulin-G; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

At baseline the majority of patients had abnormal serum liver tests.
In conjunction with clinical improvement, in all patients rapid declines in total bilirubin, alkaline phosphatase, ASAT and ALAT were observed, which persisted after stent removal (Figure 3C-F).

Treatment response as assessed by biochemical parameters was not associated with doses of prednisone (Table 3). 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).

Table 3 Response to treatment during 6 months of treatment. Estimated mean decline the first month and from month 1 to month 6 by dosage.

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

** 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), t=months y=lab value). ** p <0.01. IgG4 indicates immunoglobulin-G4; IgG, immunoglobulin-G; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.*

RADIOLOGICAL RESPONSE

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

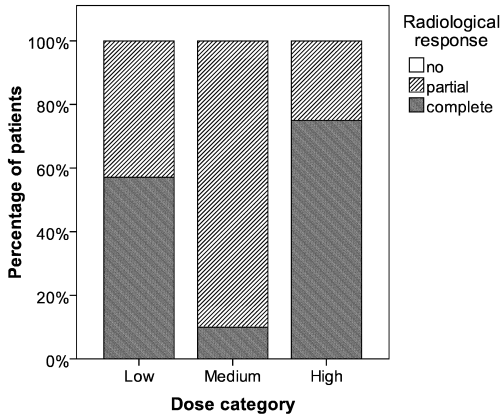

Figure 4 Radiological response after 6 months of prednisone remission induction therapy. Results were categorized in 3 treatment groups, low dose (10-20 mg/d), medium dose (30 mg/d) and high dose (40-60 mg/d) prednisone.

DISCUSSION

This study shows comparable therapeutic efficacy over a range of prednisone induction doses in AIP. Importantly, no evidence was found for an inferior treatment effect of low (10-20 mg/d) prednisone as compared to high (40-60 mg/d) prednisone.

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 regimes. 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.[9,16-18] Reports on corticosteroid induction doses lower than 15 mg/d are scarce. One case report described successful treatment with prednisolone 5 mg/d.[19] 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.[6,17,20-21] High-dose steroid therapy, especially during an extended period (>1 week), poses a substantial risk for significant side effects.[10-13] Frequently observed important side-effects in elderly populations are inducing, or worsening of

pre-existing, diabetes mellitus, osteoporosis, opportunistic infections, cataract and psychological disturbances. Diabetes mellitus or worsening of glycaemic control is frequent in individuals presenting with AIP.[4,18,20,22] 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 9-40% developed diabetes mellitus upon treatment with steroids. Older age and obesity were identified as independent risk factors.[11,12] Additionally, Gurwitz et al[13] 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 1-39 mg/d, to 3.02 for 40-70 mg/d and to 5.82 for 80-119 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.[23]

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. Due to the retrospective design of this study we were not able to retrieve sufficient and/or reliable data for assessing potential adverse treatment effects e.g. on glucose tolerance, body weight and blood pressure.

Differences in biochemical response adjusted for baseline biochemical values 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. The broken stick model was used to take into account the structural changes of gradient between the first month of follow-up and the next 5 months (Figure 2).

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.[24] Since 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 three 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 3-4 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-months 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.

The results of the present study await confirmation, ideally by controlled trials comparing the efficacy and tolerance of low- and high-dose induction corticosteroid therapy. However, the relative rarity of the condition makes it difficult to perform such studies.

In conclusion, in this retrospective series response to therapy was comparable for AIP patients treated with doses of prednisone in the range of 10 mg/d-60 mg/d. These results suggest that low-dose (<20 mg/d) prednisone as initial treatment for AIP is an effective alternative to currently recommended higher doses.

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Chapter 8

Summary and general discussion
Samenvatting en conclusies

SUMMARY AND GENERAL DISCUSSION

Autoimmune pancreatitis is the pancreatic manifestation of a benign systemic disease of unknown cause, that shows striking similarity with other pancreatobiliary diseases and is highly responsive to steroids. Two types of AIP are recognized: type 1-IgG4 related disease and type 2, which has substantial clinical overlap but distinct pathological features. The main clinical challenge is its differential diagnosis with malignancy. There is no single diagnostic test. Numerous serological tests have been proposed, but most of them lack sufficient validation. Diagnosis is made by different sets of diagnostic criteria, each with their own focus on particular aspects of the disease, not all equally useful or easy to use. We aimed to characterize a group of 114 AIP patients and explore the performance of the three major diagnostic criteria systems in this group. Second we aimed to establish the extent and cause of misdiagnosis in patients that underwent resection for presumed malignancy of the pancreatic head. Furthermore we examined the serological profile, including serum total IgE and tumor marker Ca 19-9 in AIP and other pancreatobiliary disorders with a dual purpose: establish their value in differential diagnosis with malignancy and gain insight into pathogenesis. Finally we aimed to compare the efficacy of low dose versus high dose steroid induction therapy.

The introduction presents AIP in a broader perspective. Chapter 2 describes the characteristics and clinical presentation of 114 patients with AIP. The performance of the three major diagnostic criteria systems for AIP is studied. Recommendations are made which system should preferably be used, both generally and in specific diagnostic dilemmas. Chapter 3 is a retrospective study on the prevalence of AIP and other benign disorders in patients that underwent pancreatoduodenectomy for presumed malignancy of the pancreatic head. The preoperative work up is studied and causes of misdiagnosis are suggested. Ultimately recommendations are made how to avoid unnecessary surgery in AIP patients. In Chapter 4 the tumor marker Ca 19-9 is studied in AIP and other benign and malignant pancreatobiliary disorders. High levels of Ca 19-9 are considered distinctive for cancer. Its value in differential diagnosis between AIP and pancreatic cancer is examined, both as a single marker and in combination with IgG4. Optimal cut-off levels of both markers are assessed. In Chapter 5 a pilot study is conducted on possible allergic mechanisms in AIP, by studying serum total IgE and its relation to IgG4 in AIP, atopic allergy and pancreatic cancer. We also investigate the potential value of IgE in differential

diagnosis with pancreatic cancer. In Chapter 6, an extensive immunological profile is assessed in AIP and other benign and malignant pancreatobiliary disorders. Both general (eg antinuclear antibody, rheumatoid factor) and pancreas specific autoantibodies (anti carbonic anhydrase II, anti lactoferrin), considered important in pathogenesis and diagnosis, are tested. Ultimately, a logistic regression analysis is performed to detect combinations of tests that reliably predict a diagnosis of AIP. Finally, in Chapter 7 a retrospective study compares the efficacy of high versus low doses of prednisone for induction of remission in AIP.

CHARACTERIZATION OF A GROUP OF 114 AIP PATIENTS AND THE PERFORMANCE OF THE THREE MAJOR DIAGNOSTIC CRITERIA SYSTEMS IN THIS GROUP

Main findings

In general, our series resembles other large cohorts of AIP patients. Only three cases of histologically confirmed type 2 AIP were reported. Main differences with other cohorts were: a high percentage of elevated Ca 19-9 levels (58% compared to an average of 25%), 5 cases of AIP associated prostatitis (which is not very common) and no case of associated inflammatory bowel disease, corresponding with a virtual absence of type 2 AIP. Response (steroid treatment) and recurrence rates (98% and 37%) matched those reported in literature.
At initial presentation, 82% of patients with AIP were 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. In 18% all systems failed to confirm the diagnosis. The highest percentage of patients met the International Consensus Diagnostic Criteria (ICDC, 68%), followed by HISORt criteria (52%) and Asian (33%). 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 (51%). If a pancreatogram was available, Asian, ICDC and HISORt criteria performed equally well (62% vs 67%, p=0.698 and 53%, p=0.452, respectively). If abdominal CT showed no pancreatic abnormalities (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 (18%), AIP was never diagnosed according to the HISORt criteria, while the Asian criteria and ICDC established the diagnosis in 42% and 58% respectively (p=0.517) (Chapter 2)

Conclusions and further research

The three major diagnostic criteria systems are helpful tools but should not be regarded as absolute, gold standard. Based on the results and the usability of the systems, we recommend the use of HISORt criteria, except for IgG4 negative disease, which is not covered by HISORt. If a pancreatogram is available, the relatively straightforward Asian criteria are equally effective (unless CT shows normal appearing pancreas, which is not covered by Asian criteria). ICDC can be used if diagnosis is still not confirmed, but these criteria are very extensive and difficult to use. Noteworthy is the fact that the vast majority (82%) were correctly diagnosed without histology, that is before operation (which is regarded failure of timely diagnosis) or histological biopsy (which is not widely available in the Netherlands).

A large, preferably multicenter, international prospective study is needed to validate the diagnostic criteria of AIP, preferably in a diagnostic algorithm. Little is known concerning the specificity of the criteria, that is false positivity in cancer patients, chronic pancreatitis or sclerosing cholangitis. Furthermore, efforts should be made to simplify the International Consensus Diagnostic Criteria.

THE EXTENT AND CAUSE OF MISDIAGNOSIS IN PATIENTS THAT UNDERWENT PANCREATODUODENECTOMY FOR PRESUMED MALIGNANCY OF THE PANCREATIC HEAD

Main findings

In our cohort of 114 AIP patients, one third (32%) had a history of pancreatic resection (16%), explorative surgery (14%) or palliative surgery (2%) for presumed malignancy, while in retrospect 82% of patients were correctly identified preoperatively according to any of the diagnostic criteria systems (Chapter 2). The prevalence of benign disease in patients who underwent pancreatoduodenectomy in our center was 8.4%. During a 9-year period, seven patients were postoperatively diagnosed with AIP, corresponding with a total prevalence of 2.6% and accounting for nearly one-third of all benign cases. These numbers matched those reported in literature. Based on a preoperative index of suspicion of malignancy, surgery could have been avoided in 3 benign non-AIP patients. In all AIP patients preoperative findings clearly suggested

malignancy. A preoperative diagnosis of AIP was missed for several reasons: IgG4 measurements were missing in 6/7 cases, adequate imaging of the pancreatic duct was not performed in all and the importance of Ca 19-9 levels was overestimated (2/7 cases). However if diagnostic criteria for AIP would have been checked, surgery could have been avoided in one to five AIP patients. In two AIP patients, surgery seemed inevitable also in retrospect (Chapter 3).

Conclusions and further research

AIP accounts for a significant proportion of incorrect preoperative diagnosis. From a quantitative perspective, missing the diagnosis of AIP was a problem of limited magnitude. However considering the major therapeutic consequences, every effort should be undertaken to establish the correct diagnosis. Routine work-up for pancreatic cancer is not enough to detect these patients beforehand. IgG4 measurements and systematic use of diagnostic criteria systems are recommended for every candidate patient for pancreatoduodenectomy when there is no histological proof of malignancy. Important clues for diagnosis of AIP are: elevated IgG4, narrowing of the pancreatic duct (in contrast with pancreatic carcinoma which usually presents dwith double duct dilation) and evidence of extrapancreatic involvement.

To define the clinical usefulness of the preoperative index of suspicion of malignancy we used in this study, prospective validation studies are needed. In line with this and as already mentioned above, a multicenter, international prospective validation study of diagnostic algorithms for AIP is highly desirable.

THE VALUE OF (AUTO)ANTIBODIES AND THE TUMOR MARKER CA 19-9 IN DIFFERENTIAL DIAGNOSIS BETWEEN AIP AND OTHER PANCREATOBILIARY DISEASES

Main findings

First, Ca 19-9 and IgG4 levels were studied in several pancreatobiliary diseases, ultimately to establish their value in differentiation between AIP and pancreatic carcinoma (Chapter 4). Low levels of Ca 19-9 were an independent predictor of AIP (OR=0.28; 95%CI(0.13-0.59), p=0.0001). The optimal cut-off level of Ca 19-9 for detection of AIP was 74 U/ml (upper limit), yielding a sensitivity of

73% and specificity of 74%. The optimal cut-off level of IgG4 for differentiation with pancreatic cancer was 2.6 g/L (lower limit) yielding a sensitivity of 70% and specificity of 100%. Intermediate levels of IgG4 (between 1.4 and 2.6) show higher sensitivity (85%), albeit a lower specificity of 81%. If low levels of Ca 19-9 (<74 U/ml) were combined with IgG4 >1.0 g/L, specificity rose to 100% with a sensitivity of 93%. Thus, low levels of Ca 19-9 levels improved both the moderate specificity of intermediate IgG4 levels, as the poor sensitivity of high IgG4 levels.

Second, the diagnostic value of autoantibodies was studied in groups of patients with AIP, various benign and malignant pancreatobiliary diseases and Sjögren's syndrome (Chapter 6). In comparison we also determined the diagnostic test characteristics of serum IgG, IgG subclasses, IgE and Ca 19-9. The seroprevalence of ANA, RF, anticarbonic anhydrase II and anti lactoferrin was low and overall comparable for AIP, chronic pancreatitis, pancreatic cancer and cholangiocarcinoma. None of the tested autoantibodies was found to be discriminative or diagnostically helpful. IgG4 was the best single diagnostic test (AUC 0.89). The optimal cut-off level was 2.8 g/L (2x upper limit of normal), with a sensitivity of 65% and specificity of 98%. IgG, IgG1, IgG3, IgE and Ca 19-9 levels significantly differed between AIP and malignancy. However, their test characteristics are insufficient to be regarded as as useful single diagnostics tests. Logistic regression analysis showed that a combination of IgG4, IgG3 and Ca 19-9 predicted probability of AIP with an AUC of 0.93. This combination test awaits confirmation in a validation cohort.

Conclusions and further research

Testing of autoantibodies is not useful in diagnosing AIP. IgG4 is the best single test to differentiate between AIP and malignancy, with an optimal cut-off level of 2.6 g/L (with respect to pancreatic cancer) or 2.8 g/L (pancreatic carcinoma and cholangiocarcinoma combined), that is twice the upper limit of normal. This corresponds with high specificity (100 and 98% respectively), but poor sensitivity (70 and 65% respectively). The diagnostic performance of Ca 19-9 as a single test to differentiate between AIP and malignancy is poor. However, low levels of Ca 19-9 (<74 U/ml) improve both the moderate specificity of intermediate IgG4 levels, as the poor sensitivity of high IgG4 levels. Combining IgG4, IgG3 and Ca 19-9 may be used to reliably predict a diagnosis of AIP, but a validation study is needed to determine its use in clinical practice.

(AUTO)ANTIBODIES AND GAINING INSIGHT IN PATHOGENESIS OF AIP

Autoimmunity is generally considered the major trigger of the inflammatory cascade in AIP, but the exact cause remains to be elucidated. Several critical comments can be made regarding the presumed predominant autoimmune nature of AIP. First, the male predominance and striking steroid responsiveness do not match the classical phenotype of autoimmune disease. Second, the presence of autoantibodies did not appear to be useful for diagnostic purpose (Chapter 6). However, what this means for their putative role in pathogenesis remains unclear. In general, studies on autoantibodies in AIP lack validation and reproducibility. The latest candidate antigens have been discovered by screening AIP sera with a pancreas cDNA library, PCR to produce the peptide and subsequent confirmation of the presence of corresponding antibodies with ELISA, Western blot or other technique. Most of these highly specific antibodies (to pancreatic secretory trypsin inhibitor PTSI, amylase-alpha-2A, heat shock protein antibody) show excellent specificity with regard to pancreatic cancer, but studies are characterized by very small patient numbers (usally less than 10) and await confirmation. For example, to date even the promising a-PBP (Helicobacter associated) antibody that was discovered by Frulloni et al in 2009, has not been validated. In our opinion it is unlikely that a complex and systemic disease can be explained by one autoantigen. The detection of certain antibodies does not prove their role in pathogenesis and may reflect a secondary response to inflammation. Furthermore, from an etiological point of view it makes no sense that a pancreas specific antigen causes a systemic disease. Besides autoimmune (Th1) reactions, allergic (Th2) responses are also recognized in the pathogenesis of AIP. IgG4 can be induced by prolonged or repeated antigen exposures and is controlled, like IgE production, by type T helper (Th2) cells. The presence of allergen-specific IgG4 generally means that tolerance inducing mechanisms are activated. IgG4 is inefficient in activating complement and forms unstable molecules that are unable to cross-link antigens. This may contribute to its anti-inflammatory function. In pemphigus, IgG4 antibodies have been shown to be pathogenic in mice. The role of IgG4 in AIP, pathogenic or anti-inflammatory, is not known. Th2 cytokines interleukin 4 and 13 enhance the production of both IgG4 and IgE (both located on the same chromosome). Interleukin 10, produced by regulatory T cells shifts the balance between IgG4 and IgE, favoring IgG4 (the so called 'modified Th2-response'). Compared to numerous studies on autoimmunity in AIP, the role of allergy has been underexposed.

Main findings

In a pilot study on IgE and IgG4 in 13 patients with AIP (Chapter 5), 12 patients with pancreatic carcinoma and 14 patients with atopic allergy, IgG4 and IgE levels were studied. Both total IgE and IgG4 levels in patients with AIP were significantly higher than those in patients with pancreatic carcinoma (p=0.0004 and p=0.015 respectively). There was a significant correlation between the total IgE and total IgG4 levels in patients with AIP and atopic allergy (r_s = 0.82, p=0.0006 and r_s = 0.88, p<0.0001, respectively), supporting the view that allergic reactions are important in pathogenesis of AIP. The IgE/IgG4 ratio in sera from patients with atopic allergy was significantly different (p=0.0012) from this ratio in sera from patients with AIP, but this ratio did not differ between AIP and pancreatic cancer (p=0.09). We were not able to validate the promising results of this pilot study on serum total IgE in the differential diagnosis between AIP and pancreatic cancer (AUC of 0.92) in our larger prospective study of 33 AIP patients (AUC 0.75) (Chapter 6), most likely due to missing values in the AIP group. The optimal cut-off of IgE was 155 kU/L, yielding a sensitivity of 67% and a specificity of 81%.

Conclusions and further research

The presence of autoantibodies in AIP is not helpful in diagnosis, but no conclusions can be made regarding their role in pathogenesis. Elevated total IgE and the correlation between IgE and IgG4 in AIP support the view that allergic mechanisms are important in the pathogenesis of AIP. Shifting the focus from the initiating auto antibody to the effector IgG4 (and IgE) producing B-cell will likely provide more insight in pathogenesis.

THE EFFICACY OF LOW-DOSE VERSUS HIGH-DOSE CORTICOSTEROID INDUCTION THERAPY IN AIP

Main findings

A retrospective study compared high-dose (>20mg/day) versus low-dose (≤20mg/day) steroid therapy for induction of remission (Chapter 7). A total of 65 patients were studied (14 on low dose, 51 on high dose). 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 six months all patients responded clinically well. Treatment response with respect to symptomatic, radiological and laboratory improvement was comparable for the different dosage groups.

Conclusions and further research

In a retrospective unblinded study, no evidence was found for a superior treatment effect of high-dose steroid therapy compared to low-dose. Based on these data the general recommendation to start with high-dose steroid therapy may be reconsidered. These results should be confirmed in a prospective randomized controlled trial. Looking beyond the horizon, patients included in this trial on steroid induction therapy could be enrolled in a prospective study on the maintenance treatment of AIP. Once remission has been achieved, patients are randomized to two different treatment arms: low dose maintenance steroid therapy and wait-and-see. In case of recurrence off steroids, low dose maintenance steroid therapy is compared to wait-and-see after remission induction (pulsed steroid therapy). If recurrence occurs on steroids, or if a patient is steroid dependent, immunosuppressive therapy is started, comparing high dose azathioprine (2.5 mg/kg) with a combination of low dose azathioprine (50mg) and low dose prednisone (like in autoimmune hepatitis). The relative rarity of the condition makes it difficult to perform such studies and once again affirms the importance of international consensus and collaboration.

SAMENVATTING EN CONCLUSIES

Autoimmuun pancreatitis (AIP) is de pancreatische manifestatie van een goedaardige systeemaandoening, waarvan de pathogenese niet volledig gekend is. Klinisch vertoont AIP sterke gelijkenis met andere benigne en maligne aandoeningen van pancreas en galwegen. Veel patiënten ondergaan hierdoor onnodige zware chirurgische ingrepen zoals pancreatoduodenectomie of partiële hepatectomie, of worden ten onrechte geclassificeerd als irresectabel of gemetastaseerd. De ziekte respondeert meestal uitstekend op corticosteroïden. Ondanks deze opvallend goede respons worden van oudsher hoge doseringen prednison aanbevolen om remissie te induceren. Er bestaan twee types AIP, met substantiële klinische overlap maar karakteristieke histologische eigenschappen: type 1 (geassocieerd met IgG4 gerelateerde ziekte) en type 2. Het stellen van de diagnose AIP is vaak erg lastig. Er bestaat geen enkelvoudige diagnostische test. IgG4 is vaak, maar niet altijd verhoogd. Licht verhoogde waarden (tussen 1.4 en 2.8 g/l) worden ook aangetroffen bij patiënten met chronische pancreatitis of maligniteit. Talloze serologische testen zijn onderzocht, met name de aanwezigheid van verschillende autoantilichamen, waarbij de daarbij behorende autoantigenen in het algemeen ook worden beschouwd als de oorzaak van de inflammatoire cascade in AIP. De meeste van deze testen zijn echter onvoldoende gevalideerd. De diagnose wordt gesteld door het gebruik van verschillende diagnostische criteria systemen, die niet allemaal even bruikbaar of toepasbaar zijn in de dagelijkse praktijk.

Het eerste doel van dit proefschrift was het karakteriseren van een groep van 114 patiënten met AIP, waarbij de drie belangrijkste diagnostische criteria systemen werden getoetst. Ten tweede werd de omvang en de oorzaak van misdiagnose onderzocht bij patiënten die een pancreatoduodenectomie (Whipple operatie) ondergingen onder verdenking van maligniteit van de pancreaskop. Verder werd een uitgebreid serologisch profiel vastgesteld in AIP en diverse pancreatobiliaire aandoeningen, (inclusief serum totaal IgE en de tumor marker Ca 19.9) met een tweeledig doel: de waarde vaststellen van deze markers als diagnostische test en om inzicht te krijgen in de pathogenese van AIP. Tot slot werd de effectiviteit onderzocht van lage versus hoge dosis prednison remissie inductie therapie.

De introductie (hoofdstuk 1) beschrijft de ziektepresentatie, epidemiologie, diagnose, pathogenese en behandeling van AIP. Hoofdstuk 2 beschrijft

de karakteristieken van een cohort van 114 patiënten met AIP. De drie belangrijkste diagnostische criteria worden getoetst, waarbij er uiteindelijk aanbevelingen worden gedaan welk systeem het beste kan worden gebruikt, zowel in het algemeen als in specifieke diagnostische dilemma's. Hoofdstuk 3 is een retrospectieve studie naar de prevalentie van AIP en andere benigne aandoeningen bij patiënten die een pancreatoduodenectomie (Whipple operatie) ondergingen onder verdenking van pancreascarcinoom. De preoperatieve work up wordt bestudeerd, waarbij mogelijke oorzaken van foutieve diagnose worden onderzocht. Uiteindelijk worden aanbevelingen gedaan om onnodige chirurgie te voorkomen bij AIP patiënten. In hoofdstuk 4 wordt de tumor marker Ca 19.9 bestudeerd bij AIP en andere benigne en maligne aandoeningen van pancreas en galwegen. Hoge waarden van Ca 19.9 worden beschouwd als suggestief voor maligniteit. De waarde in de differentiaal diagnose met pancreascarcinoom wordt onderzocht, zowel als enkelvoudige test, als in combinatie met IgG4, waarbij optimale afkapwaarden worden vastgesteld. Hoofdstuk 5 is een pilot studie naar mogelijke allergische mechanismen bij AIP. Serum IgE en de relatie tot IgG4 worden onderzocht in AIP, atopische allergie en pancreascarcinoom. Ook wordt de potentiële waarde van IgE bij het stellen van de diagnose AIP vastgesteld. In hoofdstuk 6 wordt een uitgebreid serologisch profiel bepaald bij AIP en andere benigne en maligne aandoeningen van pancreas en galwegen. Het doel is om de waarde vast te stellen van het testen van autoantistoffen, die belangrijk worden geacht in de diagnose en pathogenese van AIP. De diagnostische karakteristieken van multipele testen (IgG, IgG subklassen, IgE en Ca 19.9) worden bepaald in het onderscheid tussen AIP en maligniteit. Uiteindelijk wordt een logistische regressie analyse verricht om combinaties van testen op te sporen die voorspellend zijn voor AIP. Tot slot wordt in hoofdstuk 7 een retrospectieve studie beschreven die de effectiviteit vergelijkt van lage versus hoge dosis prednison inductie behandeling.

HET KARAKTERISEREN VAN EEN GROEP VAN 114 PATIËNTEN MET AIP EN TOETSING VAN DE DRIE BELANGRIJKSTE DIAGNOSTISCHE CRITERIA SYSTEMEN

Belangrijkste resultaten

In het algemeen zijn de karakteristieken van onze cohort vergelijkbaar met andere beschreven cohorten. Er waren slechts 3 gevallen van type 2 AIP. De belangrijkste verschillen met andere cohorten waren een hoog percentage

verhoogde Ca 19.9 waarden (58% versus gemiddelde van 25%) en kleine verschillen in extrapancreatische manifestaties: 5 gevallen van autoimmuun prostatitis (weinig gerapporteerde orgaanmanifestatie) en het ontbreken van inflammatoire darmaandoeningen. Op basis van de gegevens bij initiële presentatie (dus voordat eventuele operatie plaatsvond) kon 82% van de patiënten correct worden geïdentificeerd met behulp van de drie belangrijkste diagnostische criteria systemen, zonder histologisch bewijs. In 18% van de patiënten kon de diagnose niet worden gesteld. De Internationale Consensus Diagnostische Criteria (ICDC) voldeden bij 68%, de HISORt bij 52% en de Aziatische criteria bij 33% van de patiënten. De Aziatische criteria deden het slechter omdat er slechts in 51% een diagnostisch pancreatogram voorhanden was, wat een obligaat criterium is in dit systeem. Indien wel een pancreatogram was verricht, bleken de drie systemen gelijkwaardig te zijn. Indien CT een ogenschijnlijk normaal pancreas toonde (21%), bleek HISORt het enige systeem dat de diagnose kon bevestigen (door middel van prednison proefbehandeling). Bij normale IgG4 waarden (18%) kon de diagnose niet worden gesteld met HISORt criteria (hoofdstuk 2).

Conclusies en toekomstig onderzoek

De drie belangrijkste diagnostische criteria systemen zijn belangrijke hulpmiddelen maar mogen niet als absolute gouden standaard worden beschouwd. De HISORt criteria zijn het meest bruikbaar. Als een pancreatogram voorhanden is (optioneel), of bij IgG4 negatieve ziekte, worden de relatief eenvoudige Aziatische criteria aanbevolen. De zeer uitgebreide ICDC kunnen worden gebruikt als de HISORt of Aziatische criteria niet voldoen en de verdenking op AIP blijft bestaan.

Een prospectieve studie, bij voorkeur multicentrisch en internationaal, is nodig om de diagnostische criteria van AIP te valideren, bij voorkeur in de vorm van een diagnostisch algoritme. Er is weinig bekend over de specificiteit van de criteria systemen, dat wil zeggen de vals positiviteit met betrekking tot maligniteit, chronische pancreatitis of PSC. Daarenboven zouden de internationale consensus criteria moeten worden vereenvoudigd, zodat ze ook toepasbaar zijn in de dagelijkse praktijk.

DE OMVANG EN OORZAAK VAN MISDIAGNOSE BIJ PATIËNTEN DIE EEN WHIPPLE OPERATIE ONDERGAAN IN VERBAND MET VERDENKING MALIGNITEIT

Belangrijkste resultaten

In onze cohort bleek 32% van de patiënten een voorgeschiedenis te hebben van Whipple operatie (16%), exploratieve laparotomie (14%) of palliatieve chirurgie (2%) wegens vermeende maligniteit, terwijl 82% in retrospectie preoperatief al voldeden aan de diagnostische criteria voor AIP (hoofdstuk 2). De prevalentie van benigne aandoeningen bij patiënten die een Whipple operatie ondergingen onder verdenking van pancreascarcinoom was 8.4%, waarvan 2.6% te wijten was aan AIP (hoofdstuk 3). Op basis van een preoperatieve klinische index van verdenking op maligniteit, bleken 3 patiënten (niet AIP) ten onrechte geopereerd te zijn. Alle patiënten met AIP hadden een hoge verdenking op maligniteit. Een preoperatieve diagnose van AIP werd gemist doordat sleutelkenmerken tot het stellen van de diagnose AIP ontbraken (IgG4, pancreatogram) of doordat het belang van een sterk verhoogd Ca 19.9 werd overschat. In 2 van de 7 AIP patiënten was chirurgie onvermijdelijk, ook in retrospectie.

Conclusies en toekomstig onderzoek

Bij 2.6% van de patiënten die worden geopereerd voor pancreaskopcarcinoom werd achteraf de diagnose AIP gesteld, dat wil zeggen een derde van alle benigne diagnoses (8.4%). Alle patiënten met AIP hadden sterke klinische verdenking op maligniteit. Om de diagnose preoperatief te kunnen stellen moet actief naar sleutelkenmerken van AIP worden gezocht: verhoogd IgG4, pancreatogram, extrapancreatische manifestaties en in geselecteerde gevallen een proefbehandeling met prednison. Prospectief onderzoek is nodig om de klinische bruikbaarheid van de index van verdenking op maligniteit vast te stellen. Zoals eerder genoemd is een multicentrische internationale studie nodig naar diagnostische algoritmes voor AIP.

DE WAARDE VAN (AUTO)ANTILICHAMEN EN DE TUMOR MARKER CA 19.9 BIJ HET STELLEN VAN DE DIAGNOSE AIP

Belangrijkste resultaten

Ca 19.9 en IgG4 waarden werden bestudeerd in verschillende pancreatobiliaire aandoeningen en AIP, met als uiteindelijk doel hun waarde te bepalen in de differentiaal diagnose AIP en pancreascarcinoom (hoofdstuk 4). Lage Ca 19-9 waarden bleken een onafhankelijke voorspeller te zijn voor AIP (OR=0.28; 95%CI(0.13-0.59), p=0.0001). De optimale afkapwaarde van Ca 19-9 voor de detectie van AIP was 74 U/ml, met een sensitiviteit van 73% en specificiteit van 74%. De optimale afkapwaarde van IgG4 voor de detectie van AIP bleek 2.6 g/l (ondergrens), met een sensitiviteit van 70% en specificiteit van 100%. Licht verhoogde (intermediaire) waarden van IgG4 (tussen 1.4 en 2.6 g/l) zijn sensitiever (85%) maar dit gaat ten koste van een lagere specificiteit van 81%. Door laag Ca 19.9 (<74 kU/l) te combineren met een IgG4 van >1.0, stijgt de specificiteit naar 100% en de sensitiviteit naar 94%. In hoofdstuk 6 werd de diagnostische waarde van autoantilichamen onderzocht bij groepen patiënten met AIP, maligne en benigne pancreatobiliaire aandoeningen en syndroom van Sjögren. Ook werden de testkarakteristieken vergeleken tussen IgG, IgG subklassen, IgE en Ca 19.9, voor het onderscheid tussen AIP en maligniteit (pancreas- en cholangiocarcinoom gecombineerd). De prevalentie van de autoantistoffen ANA, rheuma factor IgM, anticarboanhydrase II en antilactoferrine was laag en vergelijkbaar tussen AIP, chronische pancreatitis, pancreas- en cholangiocarcinoom. Geen van deze testen bleek bruikbaar voor het stellen van de diagnose AIP. IgG4 werd bevestigd als beste enkelvoudige test (oppervlakte onder de receiver operating curve AUC 0.89), met een optimale afkapwaarde van >2.8 g/l, dat wil zeggen twee maal de bovengrens van normaal, met een sensitiviteit van 65% en specificiteit van 98%. IgG, IgG1, IgG3, IgE en Ca 19.9 waren significant verschillend tussen AIP en maligniteit, maar hun testkarakteristieken waren onvoldoende om als betrouwbare enkelvoudige test te kunnen worden beschouwd. Logistische regressie analyse toonde dat een combinatie van IgG4, IgG3 en Ca 19.9 de meest betrouwbare voorspeller voor AIP was, met een AUC van 0.93.

Conclusies en toekomstig onderzoek

Het bepalen van autoantistoffen is niet nuttig bij het stellen van de diagnose AIP. IgG4 is de beste enkelvoudige test om AIP te onderscheiden van maligniteit, met een afkapwaarde van twee maal de bovenlimiet van normaal (>2.6 g/L ten opzichte van pancreascarcinoom en >2.8 g/l ten opzichte van pancreas- en cholangiocarcinoom gecombineerd). Dit correspondeert met een hoge specificiteit (respectievelijk 100 en 98%), maar een matige sensitiviteit (respectievelijk 70 en 65%). Verhoogde waarden van de tumor marker Ca 19.9 zijn niet bruikbaar in de differentiaal diagnose tussen AIP en pancreascarcinoom. Echter, lage waarden van Ca 19-9 (<74 kU/l), gecombineerd met een IgG4 >1.0 g/L heeft een sensitiviteit van 94% en specificiteit van 100%. Ca 19-9 verbetert dus het discriminerend vermogen van IgG4. Een combinatie van IgG4, IgG3 en Ca 19.9 bleek de beste serologische voorspeller van de diagnose AIP, maar deze combinatie moet nog worden gevalideerd alvorens de waarde ervan kan worden bepaald in de dagelijkse praktijk.

INZICHT VERKRIJGEN IN DE PATHOGENESE VAN AIP

Autoimmuniteit wordt in het algemeen beschouwd als de belangrijkste trigger van de inflammatoire cascade bij AIP, maar de exacte oorzaak is niet bekend. Er zijn diverse argumenten aan te voeren die tegen het veronderstelde predominant autoimmune karakter van deze ziekte pleiten. De ziekte komt vooral voor bij mannen en reageert in het algemeen bijzonder goed en snel op prednison. Dit komt niet overeen met het klassieke fenotype van autoimmuunziekte. Het bepalen van bepaalde autoantistoffen die als belangrijk werden beschouwd voor de pathogenese, bleek geen nut te hebben bij het stellen van de diagnose. Dit sluit hun vermeende rol in de pathogenese van de ziekte niet uit, maar relativeert op zijn minst het belang ervan. In het algemeen worden studies naar autoantistoffen gekenmerkt door het ontbreken van goede validatie studies, wat mede samenhangt met hun algemene gebrek aan reproduceerbaarheid. De recentste kandidaat autoantigenen werden opgespoord door min of meer gelijkaardige en complexe technieken: het screenen van patiënt sera met een zogenaamde pancreas-cDNA bibliotheek, waarop het peptide geproduceerd wordt door middel van PCR, waarna de aanwezigheid van de corresponderende autoantistof wordt bevestigd met ELISA, Western blot of andere techniek. In onze opinie is het onwaarschijnlijk

dat een complexe systeemaandoening kan worden verklaard door één autoantigen, waarbij het niet perse logisch dat dit antigen in het pancreas moet worden gezocht. Het is zeer wel mogelijk dat autoantistoffen ontstaan als reactie op inflammatie (ten gevolge van het vrijstellen van lichaamseigen stoffen) en dus een secundair fenomeen vertegenwoordigen. Behalve autoimmuun (Th1 cel) reacties, zijn er ook aanwijzingen dat allergische (Th1) reacties een rol spelen in de pathogenese van AIP. De productie van allergeen specifiek IgG4 betekent normaal gesproken dat tolerantie inducerende mechanismen worden geactiveerd. IgG4 is niet goed in staat om complement te activeren en vormt instabiele moleculen die niet goed in staat zijn om antigenen te binden. Dit draagt bij aan de anti-inflammatoire eigenschappen van IgG4. In muizen studies bij pemphigus bleken IgG4-complexen echter wel degelijk pathogeen te kunnen zijn. Het is niet bekend of IgG4 bij AIP een anti-inflammatoir karakter heeft, of toch pathogene eigenschappen bezit. Th2 cytokines, o.a. interleukine 10, verschuiven de balans van IgE productie naar IgG4, dit is de zogenaamde "modified Th2-response". Dit vindt plaats in de uiteindelijke effector cel: de immuunglobuline producerende B lymfocyt (plasma cel). Allergische fenomenen bij AIP zijn veel minder goed onderzocht dan autoimmuunreacties.

Belangrijkste resultaten

In een pilot studie werden IgE en IgG4 waarden bepaald bij AIP (n=13), pancreascarcinoom (n=12) en patiënten met atopische allergie (n=14) (hoofdstuk 5). Zowel IgG4 als IgE waarden waren significant hoger bij patiënten met AIP dan bij pancreascarcinoom. Er was een significante correlatie tussen IgE en IgG4 bij patiënten met AIP en allergie, niet bij pancreascarcinoom. De IgE/IgG4 ratio van patiënten met allergie was signifcant verschillend van deze ratio bij AIP, maar deze ratio was niet verschillend tussen AIP en pancreascarcinoom. IgE had in deze pilot studie een uitstekende AUC van 0.92 bij de differentiatie tussen AIP en pancreascarcinoom. Dit kon niet worden gevalideerd in onze prospectieve studie in een cohort van 33 AIP patiënten, waar de AUC 0.75 bleek te zijn, meest waarschijnlijk ten gevolge van ontbrekende waarden in de AIP groep. De optimale afkapwaarde van IgE was 155 kU/l, met een sensitiviteit van 67% en specificiteit van 81% (hoofdstuk 6).

Conclusies en toekomstig onderzoek

De prevalentie van autoantistoffen in AIP is laag en bleek niet nuttig bij het stellen van de diagnose AIP, maar op basis van deze resultaten er kunnen geen conclusies worden getrokken ten aanzien van hun rol in de pathogenese. Verhoogde IgE waarden en de correlatie tussen IgE en IgG4 in AIP ondersteunen de hypothese dat allergische mechanismen een rol spelen in de pathogenese van AIP. Het is aannemelijk dat verder inzicht kan worden verkregen in de pathogenese van AIP als de huidige focus op de rol van autoantigenen, wat toch min of meer als zoeken naar indirecte bewijslast kan worden beschouwd, zal verschuiven naar de rol van allergie en de IgG4 producerende B-lymphocyt.

DE EFFECITVITEIT VAN LAGE VERSUS HOGE DOSIS PREDNISON INDUCTIE THERAPIE BIJ AIP

Belangrijkste resultaten

In een retrospectieve studie werd de effectiviteit vergeleken van hoge dosis (>20mg/dag) versus lage dosis (≤20mg/dag) prednison inductie therapie (hoofdstuk 7). In totaal werden 65 patiënten geïncludeerd, waarvan 14 lage en 51 hoge dosis hadden gekregen. Er waren geen significante verschillen in basis karakteristieken tussen de beide behandelgroepen, inclusief leeftijd, symptomen en laboratorium afwijkingen. Tijdens een follow up periode van zes maanden respondeerden alle patiënten goed. De respons met betrekking tot symptomen, radiologische afwijkingen en laboratoriumafwijkingen was vergelijkbaar tussen beide behandelgroepen.

Conclusies en toekomstig onderzoek

In een retrospectieve, niet geblindeerde studie bleek een hoge dosis prednison (>20mg/dag) niet effectiever te zijn dan een lage dosis in het induceren van remissie in AIP. Prospectief gerandomiseerd gecontroleerd onderzoek is nodig naar de inductie behandeling van AIP (lage versus hoge dosis prednison), vooraleer deze resultaten kunnen worden geëxtrapoleerd naar de dagelijkse praktijk. Idealiter zou deze studie gekoppeld moeten worden aan een prospectieve studie met betrekking tot de onderhoudsbehandeling van AIP. Zodra door middel van inductie therapie remissie is bereikt, zouden patiënten kunnen worden gerandomiseerd naar twee verschillende behandel

armen: lage dosis prednison (5-10mg) onderhoudsbehandeling en wait-and see. Bij recidief na staken prednison zou moeten worden vergeleken tussen wait-and-see na hernieuwde inductie kuur (prednison puls therapie) en alsnog onderhoudsbehandeling met lage dosis prednison. Bij het eerste recidief tijdens prednison of bewezen prednison afhankelijkheid zou immuunsuppressieve behandeling kunnen worden gestart, waarbij hoge dosis azathioprine kan worden vergeleken met combinatie azathioprine 50mg met lage dosis prednison (conform autoimmuun hepatitis). De zeldzaamheid van de ziekte maakt het moeilijk om zulke studies te verrichten, tenzij dit in internationaal verband zou worden verricht. Dit onderstreept nogmaals het belang van nationale en internationale consensus en samenwerking.

Chapter 9

Dankwoord
Curriculum vitae
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DANKWOORD

Achteraf blijven er bepaalde dingen hangen.

Ritjes tussen Rotterdam en Delft, in een bloedhete auto, met een piepschuimen doos gevuld met diepgevroren buisjes. Gescharrel in de talloze diepvriezers van het MDL lab, die plots bleken te zijn verhuisd naar niet nader omschreven lades van vrij ontoegankelijke diepvriezers in een duistere uithoek van het EMC. De blaren die dat opleverde omdat ik het aantrekken van handschoenen maar tijdsverlies vond. Dagen lang stickertjes op buizen plakken, buisjes tellen en doosjes vullen op het MDL lab. Sessies met Bettina Hansen, die als een soort tovenares in hoog tempo onbegrijpelijke formules in SPSS invoerde, waar ik zo door werd overdonderd dat ik vreesde het nimmer te kunnen vatten. De immense opluchting toen het statistiek lampje ook voor mij bleek aan te gaan. Het schrijven van het eerste artikel, hoofdstuk 3, te midden van gejuich en getoeter van Nederlands eerste groepswedstrijd in het WK. Vakanties doorgebracht bij mijn ouders, zodat Ben en Marie buiten konden spelen terwijl ik boven, net als vroeger, mijn 'huiswerk' kon maken. De koppen koffie en plakken cake die me dan werden toegestopt en de kusjes en knuffels die de kinderen tussendoor even kwamen stelen. De weken die ik in aan bed gekluisterd lag: in 2007 door dreigende vroeggeboorte van Ben en Marie (inclusief door gynaecoloog opgelegd leesverbod op medische literatuur) terwijl intussen het huis werd afgebroken, en in het voorjaar van 2012 door een hernia, waar mijn enige activiteit erin bestond onder invloed van hoge dosis pijnstillers en frustraties in hoog tempo artikelen te produceren, liggend onder het meest fantastische gadget wat ik in tijden heb aangeschaft: de 'Laptop Laidback'. *Ach, het was een leuke tijd* (citeer Herman Finkers).

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CURRICULUM VITAE

Marianne van Heerde werd geboren op 9 januari 1973 te Vollenhove (Overijssel). Na het behalen van het VWO eindexamen aan de Christelijke Scholen Gemeenschap te Emmeloord in 1991, ging zij geneeskunde studeren in Antwerpen (B). Hier haalde zij in 1994 haar kandidaats examen, gevolgd door het arts examen in 1998, beide met grote onderscheiding. Aansluitend werkte ze als arts assistent niet in opleiding (ANIO) in het Ignatius Ziekenhuis te Breda (dr B Veldhuijzen) en het Sophia Ziekenhuis te Zwolle (Dr van Marwijk Kooy, opleider interne geneeskunde, en dr F. Nelis, D. Westerveld en J. Vecht, MDL artsen). Vanaf 1999 werkte ze als arts onderzoeker op de afdeling gastroenterologie & hepatologie in het Academisch Ziekenhuis van Utrecht (dr J. van Hattum), waar ze participeerde in de opzet van de CIRA trial (Hepatitis C). Na een half jaar droeg zij dit over aan Hanneke van Soest (nu MDL arts te Den Haag), die in 2011 promoveerde op dit onderwerp. Hierna werkte Marianne twee en half jaar als huisarts in opleiding in Berchem (Groepspraktijk Fruithof, Carl Stubbe) en Schoten (Martine Joossens en Sonja De Locht), in de provincie Antwerpen. Zij behaalde het diploma huisartsgeneeskunde in 2002. Intussen deed zij ervaring op als consultatiebureau-arts (Kind en Gezin, Antwerpen) en in de spoedeisende geneeskunde (Henry Serruys, Oostende). Na een drie maanden durende reis door Zuid Amerika, kwam ze via het Sophia Ziekenhuis (inmiddels Isala Klinieken) te Zwolle, terecht in Rotterdam, waar zij werd aangenomen voor de opleiding Maag-, Darm en Leverziekten (Prof dr E.J. Kuipers, Dr R.A. de Man). In 2004 startte zij de vooropleiding interne geneeskunde in het IJselland Ziekenhuis te Capelle a/d IJssel (opleider dr A. Fischer). De intensive care stage volgde zij in het St Franciscus Gasthuis te Rotterdam (drs A. Rietveld). Hierna werkte zij 4 jaar als MDL arts in opleiding in het Erasmus Medisch Centrum te Rotterdam (dr R.A. de Man, opleider). Op 6 juni 2010 werd zij geregistreerd als MDL arts. Sinds 1 september 2010 werkt zij als MDL arts in het Amphia Ziekenhuis te Breda.

Marianne woont sinds 2006 samen met Darek Sikorski. In 2007 kregen zij een tweeling: Marie en Ben.

LIST OF PUBLICATIONS

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2. Rituximab for the treatment of glomerulonephritis in hepatitis C associated cryoglobulinaemia. Korte M, van Heerde M, Betjes M, de Man R. Neth J Medicine. 2008; 66: 27-30

3. Potential value of serum total IgE for differentiation between autoimmune pancreatitis and pancreatic cancer. van Toorenenbergen AW, van Heerde MJ, van Buuren HR. Scand J Immunol 2010; 72: 444-8

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6. Comparable efficacy of low and high dose induction corticosteroid treatment in autoimmune pancreatitis. Buijs J, van Heerde MJ, Rauws EA, Maillette de Buy Wenniger LJ, Hansen BE, Biermann K, Verheij J, Vleggaar FP, Brink MA, Beuers UH, Kuipers EJ, Bruno MJ, van Buuren HR. *Pancreas, accepted May 2013.*

7. Serum level of Ca 19-9 increases ability of IgG4 test to distinguish patients with autoimmune pancreatitis from those with pancreatic carcinoma. van Heerde MJ, Buijs J, Hansen BE, de Waart M, van Eijck CHJ, Kazemier G, Pek C, Poley JW, Bruno MJ, Kuipers EJ, van Buuren HR. *Submitted*

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10. IgG4-related prostatitis. Buijs J, Maillette de Buy Wenniger L, van Leenders GJLH, Verheij J, van Onna IEW, Hansen BE, van Heerde MJ, Krak NC, Beuers U, Bruno MJ, van Buuren HR. *Submitted*