

Differentiation of autoimmune pancreatitis from pancreas cancer: utility of anti-amylase and anti-carbonic anhydrase II autoantibodies

M. Sánchez-Castañón · G. de las Heras-Castañón ·
C. Gómez · M. López-Hoyos

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Abstract

Purpose To investigate the utility of different combinations of serum anti-carbonic anhydrase II antibodies (CA II Abs), anti- α amylase antibodies (AMY- α Abs) and IgG4 levels for the diagnosis of autoimmune pancreatitis (AIP).
Methods We recruited 93 patients with clinical suspicion for AIP and 94 patients as control groups between June 2003 and October 2009. Serum antibodies were measured using homemade enzyme linked immunosorbent assay and IgG4 levels were determined by nephelometry.
Results Both CA-II Abs and AMY- α Abs had the highest sensitivity (83%) although AMY- α Abs (89%) were more specific than CA-II Abs (75%). The presence of increased IgG4 levels was the most specific serological marker (94%), but it had the lowest sensitivity (58%). The combination of the three serological markers altogether had the highest specificity (99%) and positive predictive value (PPV) (86%), but they had a rather low sensitivity (50%). When we combined CA-II Abs and AMY- α Abs without IgG4 levels, we got the highest sensitivity (75%) and negative predictive value (98%) but the specificity and the PPV decreased to 93 and 50%, respectively. Importantly, AMY- α Abs were not detected in pancreas cancer.

Conclusions The presence of serum CA-II and AMY- α Abs with increased IgG4 is useful in the differential diagnosis of AIP from pancreatic cancer.

Keywords Autoimmune pancreatitis · Anti- α amylase antibodies · Anti-carbonic anhydrase II antibodies · Pancreatic cancer · Diagnosis · IgG4

Introduction

Autoimmune pancreatitis (AIP) is a chronic disease characterized by lymphoplasmacytic infiltration and fibrosis of the pancreas. Although the precise pathogenesis of AIP remains unknown, several evidences support the hypothesis of an autoimmune origin, that compromise exocrine and endocrine pancreatic function [1–3].

Despite the description of a number of organ- and not organ-specific autoantibodies (autoAbs) together with hypergammaglobulinemia and hyperIgG4 associated with AIP, there is a lack of specific serological markers for the diagnosis of AIP and their utility is not clear [3]. To date, most of the reports look at different autoAbs separately, but they do not have evaluated a panel of serum autoAbs. Autoantibodies against exocrine pancreatic antigens such as anti-lactoferrin antibodies (LF Abs), anti-carbonic anhydrase II antibodies (CA-II Abs) and anti-amylase α antibodies (AMY- α Abs) have been detected in patients with AIP [4–6]. CA-II and LF are present in the normal pancreas, although they are also found in the cells of several others organs including the lactating breast, biliary ducts, distal renal tubules, and salivary, bronchial and gastric glands. On the contrary, amylase α is a pancreas-specific antigen.

Most patients with AIP have alterations of the endocrine pancreas and develop diabetes mellitus [8, 9]. Both

M. Sánchez-Castañón · C. Gómez · M. López-Hoyos (✉)
Servicio Inmunología, Hospital Universitario Marqués de
Valdecilla-IFIMAV, Avda. Valdecilla s/n,
39008 Santander, Spain
e-mail: inmlhm@humv.es

G. de las Heras-Castañón
Gastroenterology Service, Hospital Universitario Marqués de
Valdecilla-IFIMAV, Santander, Spain

diseases are simultaneously diagnosed in many cases, but some cases show exacerbation of pre-existing diabetes mellitus with the onset of AIP. AIP is associated with other autoimmune diseases, such as Sjögren syndrome, primary biliary cirrhosis, primary sclerosing cholangitis, Crohn disease and systemic lupus erythematosus in approximately 20–40% of patients [2, 10–14]. On the other hand, it is difficult to distinguish AIP from other types of chronic pancreatitis or cancer of the pancreatic head [15–17].

In this work, we evaluated an assembled collection of frozen serum samples from patients with clinical suspicion for AIP, to investigate the utility of different combinations of serum CA-II Abs, AMY- α Abs and IgG4 levels for the diagnosis of AIP.

Materials and methods

Patients

This retrospective study involved 93 patients with clinical suspicion for AIP and 94 patients as control groups. Serum samples were collected between June 2003 and October 2009 and stored at -80°C . The local ethics committee approved the study, and all the subjects provided informed consent. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. We subdivided patients according to the type of pancreatic disease into the following groups: AIP ($n = 12$), chronic pancreatitis (CP; $n = 23$), idiopathic chronic pancreatitis ($n = 26$), acute pancreatitis ($n = 11$), pancreatic cancer ($n = 21$). Additionally, we included two other disease control groups: Sjögren's syndrome (SS, $n = 9$) and type-1 diabetes mellitus (T1DM, $n = 40$). We also included 45 healthy subjects. Table 1 shows the general demographic characteristics of the subjects included in the study.

Table 1 General characteristics of patients included in the study

	<i>n</i>	Age*	Sex (male/female)
AIP	12	67 (16–78)	11/1
CP	23	44 (24–72)	22/1
ICP	26	58 (11–87)	16/10
Acute pancreatitis	11	59 (33–86)	5/6
Pancreatic cancer	21	65 (38–84)	12/11
SS	9	63 (45–71)	0/9
T1DM	40	31 (5–67)	19/21
Healthy subjects	45	44 (23–86)	14/31

AIP autoimmune pancreatitis, CP chronic pancreatitis, ICP idiopathic chronic pancreatitis, SS Sjögren syndrome, T1DM type 1 diabetes mellitus

* Data are expressed as median, years (range)

Diagnosis of AIP was made by combination of the HISORt criteria [18], excluding the histological and serological study, and the diagnostic algorithm for AIP proposed by our group [5]. Although, the presence of high IgG4 serum levels is one of the HISORt criteria, we did not use it as inclusion criterion, but as a result, to avoid a bias in the inclusion of patients. Diagnosis of CP was made according to the existence of exocrine pancreatic failure, calcium deposits, ductal changes or cysts demonstrated in functional and morphological studies. The chronic pancreatitis group included 15 cases of chronic alcoholic pancreatitis, 5 cases of hereditary chronic pancreatitis and 3 cases of pancreas divisum.

Patients were diagnosed as having idiopathic CP when no apparent causes such as alcoholism, gallstones, or autoimmunity could be identified according to the Cambridge and Marsella criteria [19, 20]. Acute pancreatitis was defined by an elevation of serum amylase levels in association with characteristic clinical and radiological findings. The existence of a previous or concomitant history of AIP in pancreas cancer patients was discarded since they did not fulfil either the HISORt criteria [18] or ours [5]. Diagnosis of SS was performed according to the criteria established by the specific Study Group from the European Community [21] and diagnosis of T1DM was made according to the WHO criteria [22].

Detection of serological markers

Total IgG and IgG4 serum levels were measured by nephelometry (BNII Nephelometer, Siemens, Munich, Germany). Cut off values were set up at 1,200 mg/dl for IgG and 130 mg/dl for IgG4 at our laboratory with samples from 50 healthy subjects at our serum bank.

Serum levels of CA-II Abs were determined by enzyme linked immunosorbent assay (ELISA), as previously described [5]. Serum levels of LF Abs were analysed by ELISA (Orgentec Diagnostika GmbH, Mainz, Germany). Serum levels of AMY- α Abs were determined using an ELISA as previously described with minor modifications [6]. Briefly, microtiter plates were coated with 100 μL of 1 $\mu\text{g}/\text{mL}$ of AMY- α purified from human pancreas (Sigma Chemical Co., St. Louis, MO) overnight at 4°C . After three washes with phosphate-buffered saline containing 0.05% Tween 20 (PBST), the plates were coated with 1% bovine serum albumin (Sigma Chemical Co.) in PBS for 2 h at 4°C and then incubated with 100 μL of diluted (1:50) patient serum for 1 h at room temperature overnight at 4°C . After three washes with PBST, the plates were incubated with 100 μL of diluted (1:1,000) alkaline phosphatase-conjugated goat anti-human IgG antibody (Sigma Chemical Co.) at room temperature for 2 h. After three washes with PBST,

alkaline phosphatase activity retained in the wells of the microtiter plate was assayed by addition of 100 μ l of substrate solution containing 1.8 mmol/L *p*-nitrophenyl-phosphate in 100 mmol/L diethanolamine, pH 10.5, and 0.25 mmol/L $MgCl_2$. Sera were run in triplicate in two different tests and results for CA-II and AMY- α Abs were considered as positive when absorbance values were over the cut off. In case of discrepancy between tests, the serum sample was run in a third ELISA test. Cut off values were established as 3 standard deviations over the mean absorbance value obtained of 16 healthy control sera.

Statistical analysis

Statistical analysis of differences in the frequency of positive markers between AIP and the different patient groups and healthy subjects were calculated by the Chi-Square test. Differences were considered significant when $p < 0.05$.

The diagnostic value of each serological marker by separate and in combination was calculated as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). True positives cases were AIP patients with positive serological markers. False-positives cases were those patients with other pancreatic diseases and healthy subjects with positive serological markers. True negatives cases were those patients with other pancreatic diseases and healthy subjects with negative serological markers. Finally, false-negatives cases were AIP patients with negative serological markers.

Results

Prevalence of serological markers

Autoimmune pancreatitis

Table 2 shows the frequency of increased serum total IgG and IgG4 levels and the positivity for CA-II and AMY- α Abs in the different disease groups considered. A final diagnosis of AIP was established in 12 patients. Nine serum samples were available from 12 of these patients before steroid-therapy was started and in 3 patients serum samples were collected after treatment. Serum levels of both total IgG and IgG4 were elevated in 6/12 patients (50%). Besides, there was one patient with only total IgG increased and another one with only IgG4 increased. The median IgG and IgG4 level were 1,550 mg/dl (range 785–3,110) and 136 mg/dl (range 26–4,990), respectively (Table 3). Both CA-II and AMY- α Abs were detected in the sera of 10/12 patients (83%). In 8 of those 10 patients both CA-II and AMY- α Abs were positive concomitantly.

Importantly, 4 out of the 12 AIP (33.3%) patients developed pancreatic cancer during the course of the disease. Serum levels of both total IgG and IgG4 were elevated in 3/4 patients (75%). The median IgG and IgG4 level were 1,905 mg/dl (range 785–3,110) and 273 mg/dl (range 26–4,990), respectively. Besides, both CA-II and AMY- α Abs were simultaneously detected in the sera of all these patients. Differences in the frequencies of positive results between isolated AIP and AIP with pancreatic cancer were not significant. We also studied serum levels

Table 2 Serological markers of AIP in the study subjects

	IgG increased	IgG4 increased	CA-II Abs positivity	AMY- α Ab positivity
AIP	58 (7/12) ^a	58 (7/12) ^b	83 (10/12) ^c	83 (10/12) ^d
CP	17 (4/23)	0 (0/23)	9 (2/23)	13 (3/23)
ICP	38 (10/26)	8 (2/26)	50 (13/26)	31 (8/26)
Acute pancreatitis	27 (3/11)	(1/11)	54 (6/11)	18 (2/11)
Pancreatic cancer	10 (2/21)	14 (3/21)	29 (6/21)	0 (0/21)
SS	67 (6/9)	0 (0/9)	67 (6/9)	23 (2/9)
T1DM	ND	7 (3/40)	32 (13/40)	22 (9/40)
Healthy subjects	0 (0/45)	2 (1/45)	8 (4/45)	2 (1/45)

Results are expressed as percentage (number of positive cases/total number of cases)

Ab antibodies, AIP autoimmune pancreatitis, CP chronic pancreatitis, ICP idiopathic chronic pancreatitis, ND not determined, SS Sjögren's syndrome, T1DM type 1 diabetes mellitus

^a AIP versus CP: 0.03; AIP versus pancreatic cancer: 0.002; AIP versus healthy subjects: <0.0001

^b AIP versus CP: <0.0001; AIP versus ICP: 0.001; AIP versus acute pancreatitis: 0.013; AIP versus pancreatic cancer: 0.008; AIP versus SS: 0.005; AIP versus T1DM: <0.0001; AIP versus healthy subjects: <0.0001

^c AIP versus CP: <0.0001; AIP versus ICP: 0.05; AIP versus pancreatic cancer: 0.002; AIP versus T1DM: 0.002; AIP versus healthy subjects: <0.0001

^d AIP versus CP: <0.0001; AIP versus ICP: 0.003; AIP versus acute pancreatitis: 0.002; AIP versus pancreatic cancer: <0.0001; AIP versus SS: 0.005; AIP versus T1DM: <0.0001; AIP versus healthy subjects: <0.0001

Table 3 Serum IgG and IgG4 levels in the study subjects

	IgG (mg/dl)	IgG4 (mg/dl)
AIP	1,550 (785–3,110)	136 (26–4,490)
CP	1,010 (564–1,490)	28 (10–113)
Idiopathic CP	1,085 (495–1,620)	50 (3–205)
Acute pancreatitis	883 (789–1,580)	17 (1–190)
Pancreatic cancer	901 (523–2,500)	32 (1–278)
Sjögren's syndrome	2,300 (436–2,630)	20 (0.32–32)
Type 1 diabetes mellitus	ND	27 (1–218)
Healthy subjects	871 (528–1,230)	40 (2.27–164)

Results are expressed as median (range)

AIP autoimmune pancreatitis, CP chronic pancreatitis, ICP idiopathic chronic pancreatitis, SS Sjögren syndrome, T1DM type 1 diabetes mellitus

of LF Abs in AIP patients, although none of them were positive.

Non-AIP

Total IgG and IgG4 serum levels were elevated in 4/23 (17%) and 0/23 (0%) of CP patients, respectively. The median IgG and IgG4 level were 1010 mg/dl (range 564–1,490) and 28 mg/dl (range 10–113), respectively (Table 3). CA-II Abs were detected in 2/23 (9%) and AMY- α Abs in 3/23 (13%).

Total IgG and IgG4 serum levels were elevated in 10/26 (38%) and 2/26 (8%) of ICP patients, respectively. The median levels were 1,085 mg/dl (range 495–1,620) for IgG and 28 mg/dl (range 10–113) for IgG4 (Table 3). CA-II Abs were detected in 13/26 (50%) and AMY- α Abs in 8/26 (31%). Despite the presence of serum markers, these ICP patients were not considered as AIP since they did not show characteristic radiological finding.

Total IgG and IgG4 serum levels were elevated in 3/11 (27%) and 1/11 (9%) of acute pancreatitis patients, respectively. The median levels were 883 mg/dl (range 789–1,580) for IgG and 17 mg/dl (range 1–190) for IgG4. CA-II Abs were detected in 6/11 (54%) and AMY- α Ab in 2/11 (18%).

Total IgG and IgG4 serum levels were elevated in 2/21 (10%) and 3/21 (14%) of pancreatic cancer patients, respectively. The median levels were 901 mg/dl (range 523–2,500) for IgG and 32 mg/dl (range 1–278) for IgG4 (Table 3). CA-II Abs were detected in 6/21 (29%). However, no AMY- α Ab was found in the sera of any of these 21 patients.

Disease control groups

Among the 9 SS patients, 6/9 (67%), presented increased levels of total serum IgG. However, none of these patients

presented increased levels of serum IgG4. The median levels were 2,300 mg/dl (range 436–2,630) for IgG and 20 mg/dl (range 0.32–32) for IgG4 (Table 3). CA-II Abs were detected in 6/9 (67%) and AMY- α Abs in 2/9 (23%).

Among the 40 T1DM patients, 3/40 (7%) presented increased levels of serum IgG4. The median levels were 27 mg/dl (range 1–218) (Table 3). Total IgG was not measured in this group. CA-II Abs were detected in 13/40 (32%) and AMY- α Abs in 9/40 (22%). In all the patients with CA-II and/or AMY- α Abs but three, the presence of serum T1DM autoantibodies, anti-GAD 65 and/or IA-2, was detected. There was absence of any PAI- or T1DM-associated autoantibodies in 4/49 patients (10%).

Healthy subjects

None of the 45 healthy subjects presented increased levels of total serum IgG, whereas only, 1/45 (2%), presented increased levels of serum IgG4. The median levels were 871 mg/dl (range 528–1,230) for IgG and 40 mg/dl (range 2.27–164) for IgG4 (Table 3). CA-II Abs were detected in 4/45 (8%) and AMY- α Abs in 1/45 (2%).

Diagnostic value of CA-II Abs, AMY- α Abs and elevated IgG4 levels

Table 4 shows the sensitivity, specificity, PPV and NPV for AIP diagnosis based on elevated IgG4 levels, CA-II Abs and AMY- α Abs, considering the remaining disease groups of the study as control disease groups (ICP, AP, pancreas cancer, SS and type 1 diabetes mellitus).

In the present study, both CA-II Abs and AMY- α Abs had the highest sensitivity (83%) although the specificity was higher for AMY- α Abs (89%) than for CA-II Abs

Table 4 Diagnostic value of serological markers for AIP diagnosis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Elevated IgG4 levels	58	94	50	96
CA-II Abs	83	75	24	98
AMY- α Abs	83	89	42	98
Elevated IgG4 levels and CA-II Abs	50	98	67	95
Elevated IgG4 levels and AMY- α Abs	50	99	86	95
CA-II Abs and AMY- α Abs	75	93	50	98
All three: elevated IgG4 levels, CA-II Abs and AMY- α Abs	50	99	86	95

AIP autoimmune pancreatitis, Abs autoantibodies, CAII anti-carbonic anhydrase II, AMY- α anti-amylase α , PPV positive predictive value, NPV negative predictive value

Table 5 Diagnostic value of serological markers for AIP diagnosis excluding idiopathic CP as control group

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Elevated IgG4 levels	58	95	58	95
CA-II Abs	83	81	34	98
AMY- α Abs	83	94	63	98
Elevated IgG4 levels and CA-II Abs	50	98	75	94
Elevated IgG4 levels and AMY- α Abs	50	100	100	94
CA-II Ab and AMY- α Abs	75	97	75	97
All three: elevated IgG4 levels, CA-II Ab and AMY- α Abs	50	100	100	94

AIP autoimmune pancreatitis, Abs autoantibodies, CAII anti-carbonic anhydrase II, AMY- α anti-amylase α , PPV positive predictive value, NPV negative predictive value

(75%). The presence of increased IgG4 levels was the most specific serological marker (94%), but it had the lower sensitivity (58%). The NPV was higher for both CA-II Abs and AMY- α Abs (98%) than for elevated IgG4 levels (96%), whereas the PPV was higher for elevated IgG4 levels (50%) than for both AMY- α Ab (42%) and CA-II Ab (24%).

Table 5 shows the diagnostic value of serological markers excluding idiopathic CP as disease control group since some AIP patients might be misdiagnosed from or be in evolution to AIP. Our results showed that the specificity of both CA-II and AMY- α Abs increased to 81 and 94%, respectively. Whereas the PPV of elevated IgG4 levels, AMY- α Abs and CA-II Abs increased to 58, 63 and 34%, respectively.

Diagnostic value of combination CA-II Ab, AMY- α Ab and elevated IgG4 levels

In our study, the combination of elevated IgG4 levels and AMY- α Abs or the three serological markers altogether had the highest specificity (99%) and PPV (86%), but they had a rather low sensitivity (50%). When we combined CA-II Abs and AMY- α Abs, we got the highest sensitivity (75%) and NPV (98%). However, both the specificity and the PPV decreased to 93 and 50%, respectively (Table 4).

Since some of patients diagnosed of idiopathic CP may have an atypical presentation of AIP, we performed an additional analysis by excluding this group of patients. Our results showed that specificity and PPV of the combination of elevated IgG4 levels with AMY- α Abs and/or CA-II Abs increased to 100% (Table 5). When we combined CA-II and AMY- α Abs, the same sensitivity (75%) was obtained,

whereas both the specificity and PPV increased to 97 and 75%, respectively, and NPV decreased to 97% (Table 5).

Discussion

Autoimmune pancreatitis has a number of clinical, serological, morphological, and histopathological features. As there is currently no specific diagnostic serum marker for AIP, it should be diagnosed on the basis of a combination of abnormalities unique to AIP. In 2002, the Japan Pancreas Society established the “Diagnostic Criteria for Autoimmune Pancreatitis” [24, 25] that included the presence of autoantibodies, such as CA-II Ab or ANA. AIP is associated with hypergammaglobulinemia and elevated serum IgG4 levels. Hamano and colleagues [26] reported that an increase in serum IgG4 might distinguish AIP from other diseases of the pancreas. Autoantibodies such as ANA, anti-smooth muscle antibodies, rheumatoid factor, LF-Abs, anti-pancreatic secretory trypsin inhibitor antibodies (PSTI-Abs), CA-II and AMY- α Abs have been detected in patients with AIP [4–6, 27]. However, the distribution of these molecules is non-organ specific [28–30], and the prevalence of CA-II, LF, and PSTI-Abs in AIP is rather low, ranging from 42 to 73% [26, 27, 31]. Among all the autoantibodies, CA-II Abs have been demonstrated to be the most useful, together with the presence of increased serum IgG4 levels, in the diagnosis of AIP in our experience [5].

In the present study, we demonstrated a higher prevalence of elevated IgG4 levels (58%), AMY- α Abs (83%) and CA-II Abs (83%) in patients with AIP as compared to patients with other pancreatic diseases and controls group. We also searched for the presence of LF-Abs in AIP patients, although they were not found in the sera of any of these patients. Such a discrepancy with previous reports [4] could lie on the sensitivity of the ELISA method employed in the present study or even on a different genetic influence. Nonetheless, LF-Abs have not been postulated as AIP biomarkers up-to-date.

In many instances, AIP is difficult to distinguish from pancreatic cancer. Indeed, both diseases tend to occur in elderly men, and both can present with similar clinical features. Interestingly, when we compared patients with only AIP, AIP that developed pancreatic cancer, and pancreatic cancer, we found that CA-II Abs AMY- α Abs and increased IgG4 levels were detected mostly in the group of patients with AIP and, specially, in those AIP patients that developed cancer. No difference in the histological type of pancreatic cancer was observed and it was adenocarcinoma in both isolated cancer and cancer in the context of AIP. Because AMY- α Abs were not detected in sera from patients with pancreatic cancer, this serological marker

may be a useful tool for the differential diagnosis of AIP and pancreatic cancer. However, patients with AIP may finally develop pancreatic cancer having AMY- α Abs. In our cohort, the 75% of the four patients with AIP that suffered from pancreatic cancer showed increased serum levels of IgG4. Thus, in AIP patients with both CA-II and AMY- α Abs, the coexistence of persistently increased serum levels of IgG4 is a risk factor for developing pancreas cancer. Patients with AIP have been followed-up until present and clinical diagnosis has not changed and no new cases of pancreatic cancer have been detected.

On the other hand, we found higher levels of serum IgG in SS patients than in AIP patients. However, in accordance with the studies of Hamano et al. [26] and Aparisi et al. [5], the increase in IgG4 did not occur in our patients with SS. These results support the hypothesis that IgG4 may serve to identify two immunological types of autoimmune exocrinopathies, one with normal IgG4 levels, such as SS, and another with elevated IgG4 levels, such as AIP.

We demonstrated a high prevalence of CAII Abs in SS patients (67%). It is well documented that AIP is occasionally observed as a complication of SS [15, 32]. It has been hypothesised that the diseases may be manifestations of an autoimmune reaction against a common target antigen (in this case, CAII) that is expressed in the epithelial cells of distinct organs.

In T1DM patients, we have seen a rather high prevalence of CAII Abs (32%). CA II is localized in pancreatic ductal cells. Moreover, the islet precursor cells are also localized in the pancreatic ducts [33]. Therefore, an immune-mediated response against the islet precursor cells located in the pancreatic ducts might be related to the pathophysiology of type-1 diabetes [33]. In addition, we have found a rather high prevalence of AMY- α Abs in both T1DM and SS patients. Unfortunately, morphological and clinical studies were not performed at our institution to demonstrate progression to AIP.

In the present study, we compared the diagnostic value of each serological marker by separate and in combination. We found that both CA-II Abs and AMY- α Abs had the highest sensitivity and NPV, although the specificity was higher for AMY- α Abs than for CA-II Abs and the PPV was rather low for both serological markers. Elevated IgG4 levels had the highest specificity and PPV, but it had the lower sensitivity.

In a landmark study, Hamano et al. [26] reported that serum IgG4 levels were highly sensitive (95%) and highly specific (97%) for AIP. In accordance with Ghazale et al. [16], we show that elevated serum IgG4 levels are characteristic but not diagnostic of AIP. In our study, we found the sensitivity of serum IgG4 for AIP to be lower than that reported in the study by Hamano et al. [26] and Ghazale

et al. [16] (58 vs. 95% and 77% respectively). A possible explanation for this lower frequency could be that our patients had the recently described histologic variant of AIP named idiopathic duct centric pancreatitis (IDCP) [34, 35], rather than lymphoplasmacytic sclerosing pancreatitis (LPSP), which is the typical histology in AIP [34, 35]. IDCP is characterized by a neutrophil predominant lobular inflammation and a duct destructive infiltrate without obliterative phlebitis, a histologic pattern that is distinguishable from lymphoplasmacytic sclerosing pancreatitis LPSP [34]. It is unclear if these two entities represent different manifestations of the same disease or are in fact different diseases. In a recent study, Ghazale et al. [34] found that while LPSP consistently showed moderate to severe infiltration with IgG4-positive cells, IDCP rarely shows excess IgG4-positive cells. Probably, this latter entity may be associated with normal serum IgG4 levels. Unfortunately, pancreatic histology was not available in our AIP patients. The specificity of serum IgG4 for AIP was similar in our study compared with that reported by Hamano et al. [26] and Ghazale et al. [16].

According to the study of Hosoda et al. [36], we found that CA-II Abs were highly sensitive for AIP diagnosis. However, CA-II Abs were also detected in the sera of patients with other pancreatic diseases, such as pancreatic cancer (0 vs. 29%). Thus, in our hands CA-II Abs is not a useful tool for the differential diagnosis of AIP and pancreatic cancer.

Endo et al. [6] studied the presence of autoantibodies against CAII, LF and the recombinant AMY-2A in 13 serum samples from AIP patients. They found AMY-2A Abs to be the most specific marker for AIP patients. In our study, we found the same sensitivity both CA II and AMY- α Abs (83%). However, the specificity was higher for AMY- α Abs (89%) than for CA-II Abs (75%). Therefore, according with the study of Endo et al. [6], we concluded that AMY- α Abs might be a more useful marker for AIP than CAII and LF Abs.

Finally, after combining the three serological markers used in the present study, sensitivity dropped to 50% as would be expected, but specificity increased to 99%, yielding a high PPV for this combination (86%).

In conclusion, we describe the clinical utility of three serological markers in the differential diagnosis of AIP from other pancreatic diseases, especially pancreatic cancer. Despite a recent study identifies new target autoantigens involved in the humoral response in AIP [37], we have analyzed the three best-defined autoantigens to date. Combination of CA-II Abs and AMY-2A Abs with increased levels of IgG4 reaches a good specificity although at a cost of moderate sensitivity. Nonetheless, blinded prospective studies are necessary to define the true value of these serum markers.

Conflict of interest None of the authors has a conflict of interest to declare.

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