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Value of anti-plasminogen binding peptide, anti-carbonic anhydrase II, immunoglobulin G4, and other serological markers for the differentiation of autoimmune pancreatitis and pancreatic cancer

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Abstract

The diagnosis of autoimmune pancreatitis (AIP) and its differential diagnosis from pancreatic cancer (PC) can be challenging. In this retrospective study, we aimed to evaluate the value of anti-plasminogen binding peptide (a-PBP), immunoglobulin G4 (IgG4), and anti-carbonic anhydrase-II (a-CA-II), together with other serological markers whose value is not fully elucidated.

The serum levels of a-PBP, IgG4, IgG, anti-nuclear antibodies (ANA), anti-lactoferrin (a-LF), a-CA-II, and rheumatoid factor (RF) were evaluated in patients with AIP (n=29), PC (n=17), pancreatic neuroendocrine neoplasm (P-NEN, n=12), and alcoholic chronic pancreatitis (ACP, n=41). ANCA were measured in the AIP patients.

There was no statistically significant difference in mean a-PBP values in AIP compared with PC. A ROC curve showed that, when using a cut-off of 38.3 U, low values of a-PBP had a sensitivity and specificity of 45% and 71% for differentiating AIP from PC. The sensitivity and specificity of IgG4 (cut-off 1.4 g/L) for differentiating AIP from PC was 45% and 88%, but rose to 52% and 88% when using a cut-off of 1.09 g/L. When using this cut-off, the sensitivity and specificity for differentiating type 1 AIP from PC was 68% and 88%. None of the other markers were significantly changed in AIP versus PC. For differentiation of type 1 and type 2 AIP, the only significant differences were IgG4 in type 1 AIP ($P < .01$), with a sensitivity of 68% and a specificity of 80%, and c-ANCA elevations found in some type 2 AIP patients ($P < .05$).

The only serological marker for which we found a statistically significant difference in mean values between AIP and PC was IgG4. However, the value of IgG4 for the distinction of AIP from PC was limited, probably in part due to the relatively high number of type 2 AIP patients in our study. In accord with recent publications, our data do not support a role of increased serum a-PBP for the diagnosis of AIP.

Abbreviations: a-CA-II = anti-carbonic anhydrase II, ACP = alcoholic chronic pancreatitis, a-LF = anti-lactoferrin, ANA = anti-nuclear antibodies, ANCA = anti-neutrophil cytoplasmic antibodies, a-PBP = anti-plasminogen binding peptide, AIP = autoimmune pancreatitis, IgG = immunoglobulin G, IgG4 = immunoglobulin G4, IgG4-RD = IgG4-related disease, IgG4-SC = IgG4-related sclerosing cholangitis, PC = pancreatic cancer, P-NEN = pancreatic neuroendocrine neoplasm, RF = rheumatoid factor.

Keywords: anti-neutrophil cytoplasmic antibodies, anti-carbonic anhydrase II, anti-lactoferrin, anti-plasminogen binding peptide, autoimmune pancreatitis, immunoglobulin G4

1. Introduction

In 1995, Yoshida et al[1] were among the first to describe autoimmune pancreatitis (AIP) as a new entity of pancreatitis. They characterized AIP as a chronic pancreatic disease showing autoantibodies, diffuse enlargement of the pancreas, irregular strictures of the main pancreatic duct, lymphoplasmacytic infiltration at histology, mild pancreas-related symptoms without any acute attacks, and good response to corticosteroids. AIP is now divided into two subtypes named type 1 and type 2 AIP, based on histological, clinical, and serological features.[2–5] Regarding clinical symptoms and imaging, both subtypes of AIP have a significant overlap with pancreatic cancer (PC), which makes AIP a relevant differential diagnosis of PC, despite its low incidence.[6] Also alcoholic chronic pancreatitis (ACP) and pancreatic neuroendocrine neoplasms (P-NENs) can represent a differential diagnosis in some instances. A pancreatic endoscopic ultrasound-guided fine-needle biopsy or a percutaneous core needle biopsy (CNB), an invasive procedure not without risk of complications, is often required for the diagnosis of AIP, especially when type 2 is

considered.[7,8] van Heerde et al[9] found benign conditions in approximately 8% of patients who had a pancreaticoduodenectomy performed because of suspicion of PC, and around one-third of these had AIP. Therefore, it is relevant to explore new methods for diagnosing AIP and differentiating it from PC preoperatively, particularly in geographical areas such as France or Denmark, with a relatively high proportion of the IgG4-negative subtype of AIP (type 2) and/or a relatively low sensitivity of serum IgG4 in type 1 AIP.[10,11] Different serological markers for the diagnosis of AIP have been tested, but for now, the results are incoherent and disappointing.[12–16] In the meta-analysis by Lian et al,[16] sensitivity and specificity for IgG4 in differentiating AIP from PC ranged from 52–95% to 81–99%. The present study intended to revisit the usefulness of the serum markers anti-plasminogen binding peptide antibodies (a-PBP), IgG4, IgG, anti-carbonic anhydrase II antibodies (a-CA-II), anti-lactoferrin antibodies (a-LF), anti-nuclear antibodies (ANA), and rheumatoid factor (RF) for the differentiation of AIP from PC.[13,17] Besides, anti-neutrophil cytoplasmic antibodies (ANCA) were measured in the AIP patients.

2. Methods

The Regional Committees of Health Research Ethics of Southern Denmark approved the study (project-ID: S-20150216). All participants were enrolled after written and informed consent.

2.1. Participating patients

The study was conducted at Odense University Hospital (OUH). Patients with AIP were included from the diagnostic registry at OUH and the Danish Pathology Registry (Patobank) for the period from 2005 to 2015. Twenty-two of the AIP patients were included in a previous study.[11] Eligible patients had their diagnosis established mainly by pancreatic core needle biopsy (CNB) and/or imaging and their clinical course, based on a slight modification of the revised Histology, Imaging, Serology, Other organ involvement and Response to steroid therapy (HISORt) criteria of the Mayo Clinic for the diagnosis of AIP, as described previously.[18] Even though endoscopic retrograde cholangio-pancreatography (ERCP) is not part of the HISORt criteria, we used diffuse or segmental narrowing of the main pancreatic duct with irregular wall (more than 1/3 length of the entire pancreas) without upstream dilatation of the main pancreatic duct as an additional criterion to the HISORt criteria.[18] Importantly, retrospectively, the included patients did fulfill the International Consensus Diagnostic Criteria (ICDC) published in 2011.[19] If pancreatic CNB was performed, the diagnosis was further verified by using a previously published scoring system for the diagnosis of AIP, consisting of 6 microscopic features.[20] Patients with ACP (n=41), PC (n=17), and P-NEN (n=12) were recruited from the outpatient clinic at the Department of Gastroenterology, Odense University Hospital (OUH). The diagnosis of PC (ductal adenocarcinoma) and P-NEN was based on microscopy (fine needle aspiration [FNA] cytology, CNB, or resection specimen), imaging and follow-up (at least 6 months). The diagnosis of ACP was based on clinical features and morphological features (calcifications and ductal changes) and/or pancreatic functional insufficiency, according to the Multiple risk factors, Alcohol, Nicotine, Nutrition, Hereditary, Efferent duct, Immunological, Miscellaneous (M-ANNHEIM) criteria.[21] None of the ACP patients fulfilled the above-mentioned criteria for AIP. Sera were analyzed at the Statens Serum Institute (SSI) for

autoantibodies, serum IgG, and serum IgG4. Further, patient records were scrutinized for follow-up data, recurrence of disease, and other diseases connected to the primary condition, AIP. If recorded data were found to be incomplete, the patient was contacted for completion of the medical history.

2.2. Serological analysis

In this study, the cut-offs of the assays used were adjusted as the mean values for healthy controls plus 2 to 3 times standard deviation. All sera were stored at −80°C until usage.

Anti-CA II antibodies (a-CA-II): ELISA procedures for serum a-CA-II were carried out as described by Ono et al[22] with some modifications. The 96-well microplate (Nunc Maxisorp, Thermo Scientific, Waltham, MA) was coated with 100 μL of human CA-II (Sigma, C6654, St. Louis, MO), 5 μg/mL in carbonate–bicarbonate buffer [pH 9.6] and incubated at 4°C overnight. The plate was blocked with 2% skimmed milk in phosphate buffered solution (PBS) for 1 hour at 37°C. An anti-CA-II sample was used to generate a standard curve consisting of seven calibrators (100, 50, 25, 12.5, 6.25, 3.125, and 1.56 units). All samples were diluted in 0.2% skimmed milk in PBS. After washing off unbound materials, 100 μL of diluted standard and samples were added in duplicates into the appropriate wells, followed by incubation for 1 hour at 37°C. After washing the plate 3 times, 100 μL of goat anti-human IgG conjugated with alkaline phosphatase (AP) (Sigma, A3188, 1:2000 dilution) was added to each well, followed by incubation for 1 hour at room temperature. The plate was washed again three times and 100 μL of freshly prepared 4-nitrophenyl phosphate disodium salt hexahydrate (Sigma, S0942) were added into each well, followed by incubation for 20 minutes at room temperature. Enzyme activity was read at 405 nm and 650 nm as a reference in an ELISA reader (VersaMax, Sunnyvale, CA). The absorbance value of each well was compared and the concentrations for all samples were calculated, using the 7-point standard curve and multiplied with dilution factor to obtain the result in Units/mL.

Anti-PBP antibodies (a-PBP): An enzyme-linked immunosorbent assay (ELISA) for anti-PBP-peptide IgG antibodies was performed. A microplate (Maxisorp) was coated with 100 μL of H-AKEERRY-OH (PBP, MW.951.01, Schafer-n, Copenhagen, Denmark, 2 mg/mL) in carbonate–bicarbonate buffer (dilution 1:100), pH 9.6, followed by incubation at 4°C overnight. The plate was washed three times with TTN buffer (0.05 M Tris, 0.3 M NaCl, and 1% Tween 20, pH 7.5). A positive anti-PBP sample (identified during screening at our laboratory) was used to generate a standard curve consisting of 7 calibrators (100, 50, 25, 12.5, 6.25, 3.125, and 1.56 Units). All samples were diluted in incubation buffer with 0.2% BSA and 100 μL of each diluted samples were added in duplicates into the appropriate wells, followed by incubation for 1 hour at room temperature. After washing the plate 3 times with TTN buffer, 100 μL of AP-conjugated goat anti-human IgG (Sigma, A3188, 1:2000 dilutions) was added to each well and incubated for 1 hour at room temperature. The plate was washed again 3 times with TTN buffer, and 100 μL of freshly prepared 4-nitrophenyl phosphate disodium salt hexahydrate (Sigma) was added into each well, followed by incubation for 20 minutes at room temperature. Enzyme activity was read at 405 nm and 650 nm as a reference, in an ELISA reader (VersaMax). The absorbance value of each well was compared and the concentrations for all samples were calculated, using the 7-point standard curve and multiplied with dilution factor to obtain the result in Units/mL.

Anti-neutrophil cytoplasmic antibody (ANCA) screening: The samples were diluted 1:10 in PBS and tested by indirect immunofluorescence technique, on ethanol and formalin-fixed human granulocytes, regarding the presence or absence of a cytoplasmic (c-ANCA) or perinuclear (p-ANCA) immunofluorescence pattern.

Anti-lactoferrin antibodies were measured using an ANCA Panel Kit (Wieslab, Lund, Sweden): The samples were diluted 1:80 in dilution buffer and incubated for 30 minutes at room temperature. The wells were then washed to remove unbound antibodies and other components. A conjugate of AP-labeled antibodies to human IgG (ready to use) was added to the wells and incubated for 60 minutes at room temperature. After a further washing step, detection of specific anti-lactoferrin antibodies was obtained by incubation with substrate solution. The amount of bound antibodies correlated to the color intensity and was measured at 405 nm and 650 nm as a reference in ELISA reader (VersaMax). If the samples optical density (OD) value was ≥0.4, an OD-ratio was calculated using the formula

$$OD\,Ratio = \frac{OD\,value\,sample}{OD\,value\,blank}$$

An OD ratio <3 indicated that the sample was negative and an OD ratio >3 that the sample was positive.

IgG and IgG4: All samples were measured using the BN ProSpec system ([BNPS], Siemens, Erlangen, Germany), according to the manufacturer's protocol. The concentrations for all samples were calculated using the standard curve in BNPS to obtain the results in g/L.

Anti-nuclear antibodies (ANA): ANA were determined using a Hep-2 based indirect immunofluorescence assay. The samples, a positive control (+), a blank control (PBS), and a negative control (–) were diluted at 1:160 in PBS. The diluted samples were incubated on Hep-2-slides (ANA 13 wells slides HEp-2 substrate, Immuno Concepts, Sacramento, CA, cat no. SA2013) for 30 minutes at room temperature. After washing the slides 3 times for 10 minutes in PBS, the slides were incubated with fluorescein isothiocyanate (FITC) Antibody Reagent (Immuno Concepts, cat no. 2075CS, ready to use) for 30 minutes. After washing the slides 3 times for 10 minutes, they were evaluated using a fluorescence microscope. The identification of ANA-positivity and its pattern was based on a combination of the strength of the fluorescent signal and its location inside the Hep-2 cells.

Rheumatoid factor (RF): IgM RF were determined using an in-house assay. Purified human IgG at 10 μg/mL in carbonate buffer (pH 9.6) was used (50 μL/well) to coat the wells of a microtiter plate under cover overnight at 4°C. Wells were then blocked in dilution buffer (200 μL/well) overnight at 4°C. Plates were centrifuged dry and kept up to 6 months in the dark at –20°C before use. For the analysis, plates were washed 3 times for 1 minute in washing buffer and 50 μL sera were applied to the wells in duplicate at a dilution of 1:100 in dilution buffer. The incubation included a 5-step dilution of an RF standard serum, a negative control, a positive control, and a blank (buffer alone). After incubation at room temperature for 1 hour on a shaker, the plate was washed for 3 times for 1 minute with washing buffer. Afterwards, horse radish peroxidase (HRP)-conjugated rabbit anti-human IgM (DAKO cat. no. P0215 [Agilent Technologies, Santa Clara, CA) was added to the wells at a 1:9000 dilution. After incubation for 1 hour and another washing step, wells were developed for HRP activity using o-phenylenediamine dihydrochloride/H_2O_2. Color development was stopped after

Table 1	

Type of tissue specimens contributing to the histological diagnosis of 29 patients with autoimmune pancreatitis (AIP).

Procedure	No. of AIP patients
Laparoscopic ultrasound-guided pancreatic core needle biopsy (CNB)	13
Endoscopic ultrasound (EUS)-guided pancreatic fine needle biopsy (FNB)	2
Percutaneous pancreatic CNB	5
Whipple's procedure	2
Left sided pancreatic resection	2
Liver CNB	1
No histology	4

approximately 15 minutes using 150 μL/well of 1 M H_2SO_4. Wells were read in an ELISA reader (Tecan Sunrise, Tecan, Männedorf, Switzerland) at 492 nm using 650 nm as a reference. U/mL values for the samples were finally calculated based on absorbance values and extrapolation to the standard curve.

2.3. Statistical analysis

For comparison of continuous data between 2 groups the Mann–Whitney U test was used. For comparison of 3 groups, analysis of variance (ANOVA) was used. Categorical data were compared using the chi-squared-test between >2 groups and Fisher exact test for 2 groups. A P value <.05 was considered statistically significant. ROC curve analysis was performed to evaluate the markers as diagnostic tools. For the diagnostic performance, 95% confidence intervals (CI) were calculated. STATA v. 15 was used (StataCorp LLC, College Station, TX).

3. Results

3.1. Clinical aspects

Sera were obtained from patients diagnosed with AIP (n=29), pancreatic cancer (PC) (n=17), pancreatic neuroendocrine neoplasm (P-NEN, n=12), and ACP (n=41). The PC cohort consisted of 8 men and 9 women, mean age 67 years (range: 54–87 years). Five of the P-NEN patients were women and 7 were men, with a mean age of 63 years (range: 40–79 years). The ACP cohort included 30 men and 11 women, mean age 58 years (range: 40–77 years). The AIP cohort consisted of 21 men and 8 women, mean age 56 years (range: 28–73 years). The male/female ratio was 3.75 in type 1 and 1.5 in type 2 AIP. Nineteen AIP patients (65.5%) were diagnosed with type 1 AIP and 10 (34.5%) with type 2. The majority of the diagnoses (n=25) were based on histological HISORt criteria that included pancreatic core needle biopsy (CNB) and/or pancreatic resection specimens (Table 1). Four patients had the AIP diagnosis established without biopsy or resection (Table 1). Four other patients (20% of type 1 AIP and 13.8% of all AIP) had a surgical procedure performed due to suspicion of PC (Table 1). Histological examination showed the microscopic features of type 1 AIP in these 4 patients. Type 1 AIP patients were significantly older than type 2 AIP (64.4 vs 40.9 years, P < .001, Table 2). The sex ratio showed a majority of men in both groups, which was higher in type 1 AIP (not statistically significant). Type 1 AIP patients presented more often with monosymptomatic jaundice when compared with type 2 (47% vs 20%, P=.23). Type 2 AIP patients significantly more often presented with acute pancreatitis (30% vs 0%, P=.03). We

Sex, age, and debut symptoms in type 1 and type 2 autoimmune pancreatitis (AIP) patients.

	Type 1 AIP (n = 19)	Type 2 AIP (n = 10)	*P*-value
Sex, male/female (ratio)	15/4 (3.75)	6/4 (1.5)	ns
Age, mean, years (SD)	64.4 (10.0)	40.9 (19.9)	<.001
Debut symptoms (%)			
Monosymptomatic jaundice	9 (47)	2 (20)	ns
Jaundice together with other symptoms	2 (10.5)	2 (20)	ns
Pancreatic insufficiency	3 (16)	1 (10)	ns
Random imaging finding	2 (10.5)	0	ns
Abdominal pain	4 (21)	4 (40)	ns
Weight loss	1 (5)	3 (30)	ns
Acute pancreatitis	0	3 (30)	0.03

ns = not significant.

detected no other significant differences regarding the clinical debut symptoms.

As shown in Table 3, extrapancreatic manifestations of IgG4-related disease (IgG4-RD) were found in 12 type 1 AIP patients (63%) but none of type 2 AIP (*P* < .005). Of those with other organ involvement (OOI) of IgG4-RD in type 1 AIP, IgG4-related sclerosing cholangitis (IgG4-SC) was most frequent, although not significantly (*P* = .07). Two type 1 AIP patients (10.5%) had involvement of 2 extrapancreatic organs. One type 1 AIP patient who also was reported in our earlier study had involvement of 4 extrapancreatic organs, including IgG4-SC, lymphadenopathy, perisplenitis, and lung disease.[11] Eight type 1 AIP patients (42%) and 3 type 2 AIP patients (30%, *P* = .69) had other autoimmune diseases. Inflammatory bowel disease (IBD, all ulcerative colitis) was exclusively observed in type 2 AIP (30% vs 0%, *P* = .03). Extrapancreatic malignancy was found in 8 AIP patients (all previous to the diagnosis of AIP, no currently active

disease). Six of these had type 1 AIP (31.5%) and 2 had type 2 (20%, *P* = .68). None of the AIP patients had hepatobiliary or pancreatic malignancies.

3.2. Serological findings

Table 4 shows the average concentrations of the examined serological markers. Serum IgG4 was significantly higher in AIP patients (mean 4.2 g/L) and especially type 1 AIP patients (mean 6.0 g/L) when compared with PC (mean 0.7 g/L), P-NEN (mean 0.7 g/L) and ACP (0.9 g/L) (*P* < .01, ANOVA) (Table 4). The sensitivity and specificity of IgG4 for differentiation of AIP from PC/ACP was 45% and 84% (Table 5). The sensitivity and specificity of IgG4 for differentiation of type 1 AIP from PC (or PC/ACP) was 58% and 84% (Table 5). The sensitivity of IgG4 for differentiation of type 1 and type 2 AIP was 68% and 80%.

There was a lower mean a-PBP value in the AIP group compared with the PC group, but this difference was not statistically significant (*P* = .1) (Table 4). However, we found a significant difference in mean a-PBP between the 3 groups, mainly due to higher values in ACP (*P* = .01). Hence, our data do not support that a-PBP antibodies are raised in AIP. RF were detected slightly more frequently in PC patients compared with AIP, but due to the low number of PC patients in whom this marker was measured, no statistical analyses were performed. There was no significant difference detected between the groups with regard to a-CA-II and ANA. The method of analysis for a-LF changed during the study. As a result, serum values were determined for 11 ACP, 4 PC, 7 P-NEN, and 12 AIP (8 type 1) patients, whereas categorical data (positive/negative) were determined for the remaining patients. Anti-LF serum values showed no significant difference between the groups. Categorical a-LF values were negative in the remaining patients.

Only markers with significant different mean values between the 3 groups (AIP, PC, and ACP) were used in the ROC curve

Extrapancreatic organ involvement and occurrence of malignancy in type 1 and 2 autoimmune pancreatitis (AIP).

	Type 1 AIP (n = 19)	Type 2 AIP (n = 10)	*P*-value
Organ involvement, IgG4-RD (%)	12 (63)	0	.001
IgG4-related sclerosing cholangitis	6 (31.5)	0	ns
Perisplenitis	1 (5)	0	ns
Lymphadenopathy	3 (16)	0	ns
Sialoadenitis	1 (5)	0	ns
Tubulointerstitial nephritis	1 (5)	0	ns
Lung disease	2 (10.5)	0	ns
Xerophthalmia	3 (16)	0	ns
Other autoimmune disease (%)	8 (42)	3 (30)	ns
Sarcoidosis	0	1 (10)	ns
Raynaud's phenomenon	2 (10.5)	0	ns
Polymyalgia rheumatica	4 (21)	0	ns
Scleroderma	1 (5)	0	ns
Arteritis temporalis	1 (5)	0	ns
Muscular dystrophy, unknown genesis	1 (5)	0	ns
Sjögren syndrome	1 (5)	0	ns
Hypothyroidism (unspecified)	2 (10.5)	0	ns
Ulcerative colitis	0	3 (30)	.03
Malignancy (%)	6 (31.5)	2 (20)	ns
Type of malignancy	Appendiceal mucinous neoplasm, testicular cancer, malignant melanoma, colorectal cancer, chronic myelomonocytic leukemia, urinary bladder cancer	Breast cancer, renal cell carcinoma	

ns = not significant.

Table 4

Serological findings in patients with type 1 and type 2 autoimmune pancreatitis (AIP), pancreatic cancer (PC), pancreatic neuroendocrine neoplasm (P-NEN), and alcoholic chronic pancreatitis (ACP).

		AIP n=29	PC n=17	AIP vs PC P*	P-NEN n=12	ACP n=41	AIP vs ACP P*	AIP, PC, ACP P#	AIP-1 n=19	AIP-2 n=10	AIP-1 vs AIP-2 P*
IgG, g/L	Mean	11.3	10.3	.67	11.6	10.3	.93	.55	12.1	9.8	.35
	SD	6	3.5		2.9	2.8			6.9	3.4	
IgG4, g/L	Mean	4.2	0.7	.04	0.72	0.9	.04	.01	6	1	<.01
	SD	8.5	0.8		0.65	0.8			10.1	1.4	
a-CA-II, U/mL	Mean	40	128	.18	61.8	83	.79	.28	41	36	.65
	SD	45	215		66.5	222			49	39	
a-PBP, U/mL	Mean	57	73	.10	80.5	97	<.01	.01	64	45	.17
	SD	48	39		67.7	60			49	46	
ANA	Positive (%)	45	55	.43£	50	56	.35£	.56£	42	50	.69
a-LF, U/mL	N	12	4		7	11					
	Mean	0.89			0.9	0.76	.04	.22			
	SD	0.09			0.2	0.24					
RF, U/mL	N	14	5		7	19					
	Cut-off ≥15 positive (%)	0	60		14	21	.07§				

a-CA-II = anti-carbonic anhydrase II, a-LF = anti-lactoferrin, ANA = anti-nuclear antibodies, a-PBP = anti-plasminogen binding peptide, IgG = immunoglobulin G, IgG4 = immunoglobulin G4, RF = rheumatoid factor.

* Mann–Whitney U test.

\# ANOVA test.

§ Fisher exact test.

£ Chi²-test.

analyses. For comparison, a ROC curve was also made for IgG (Fig. 1A). The results for IgG4 and anti-PBP are shown in Fig. 1B–D and Table 5. For IgG4, the recommended cut-off of 1.4 g/L for differentiating AIP from PC/ACP was confirmed as appropriate and used (Fig. 1B). For anti-PBP, a cutoff value of 38.3 U was selected (Fig. 1C). For differentiation between AIP type 1 and 2, a lower cut-off point for IgG4 of 1.09 performed better (Fig. 1D). The ROC curve for the sensitivity and specificity of IgG4 for differentiation of type 1 AIP and PC is shown in Fig. 1E. The frequencies of IgG4-positivity in AIP, type 1 AIP, type 2 AIP, ACP, and PC dependent on different cut-off values are presented in Table 6. The sensitivity and specificity of IgG4 dependent on different cut-off values is shown in Table 7.

We performed analysis of ANCA in 17 out of 19 type 1 AIP patients and in all type 2 AIP patients (data not shown). Samples from the type 1 AIP patients were c-ANCA-negative in all cases, whereas 30% of type 2 AIP samples were c-ANCA-positive ($P < .05$). Besides c-ANCA and IgG4, no other serological marker showed significant difference between type 1 and type 2 AIP. When regarding type 1 AIP alone and comparing these patients to the control groups, the sensitivity of IgG4 improved (Tables 5 and 7). A prevalence of AIP of 1.5% will result in a positive and negative predictive value of 3.8% and 99%, respectively. When combining the markers, the diagnostic performance did not improve.

4. Discussion

The aim of this study was to estimate the usefulness of a-PBP, serum IgG, serum IgG4, a-LF, a-CA-II, ANA, and RF for the differential diagnosis of AIP versus PC. Our data confirm that an

Table 5

Sensitivity and specificity of IgG4 and anti-PBP in differentiating autoimmune pancreatitis (AIP) from pancreatic cancer (PC), alcoholic chronic pancreatitis (ACP) and PC/ACP, and AIP Type 1 from PC/ACP and AIP Type 2.

AIP and PC/ACP

	Cut-off	Sensitivity	CI	Specificity	CI
IgG4	≥1.4	45%	26–64%	84%	73–93%
a-PBP	<38.3	45%	26–64%	81%	69–90%
AIP Type 1 and PC/ACP					
IgG4	≥1.4	58%	33–79%	84%	73–93%
a-PBP	<38.3	32%	26–64%	81%	69–90%
AIP and PC					
IgG4	≥1.4	45%	26–64%	88%	64–99%
a-PBP	<38.3	45%	26–64%	71%	44–90%
AIP and ACP					
IgG4	≥1.4	45%	26–64%	83%	64–94%
a-PBP	<38.3	45%	26–64%	85%	71–94%
AIP Type 1 and Type 2					
IgG4	≥1.09	68%	43–87%	80%	44–97%

IgG4 = Immunoglobulin G4, a-PBP = anti-plasminogen binding peptide. Because there were made no comparisons, no statistical tests were used. Instead, 95% confidence intervals (CI) are given.
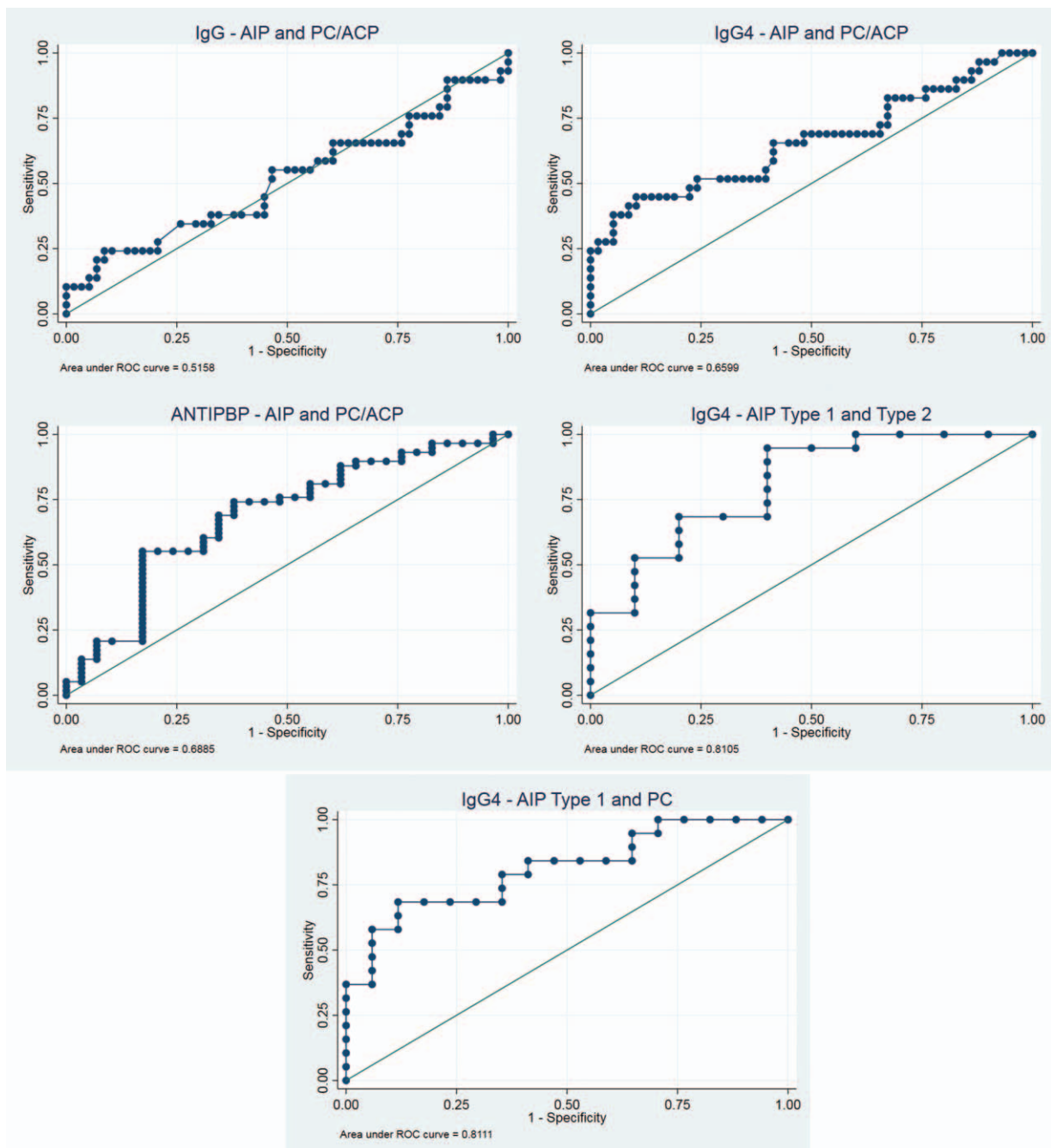
Figure 1. Sensitivity and specificity for differentiating autoimmune pancreatitis (AIP) from pancreatic cancer (PC)/alcoholic chronic pancreatitis (ACP) of (A) IgG, (B) IgG4, and (C) anti-plasminogen-binding peptide (anti-PBP). Sensitivity and specificity of IgG4 for differentiating (D) type 1 AIP from type 2 AIP, and (E) type 1 AIP from PC. Receiver operating characteristic (ROC) curves. AUC = area under the curve, CI = confidence interval, IgG = immunoglobulin G, IgG4 = immunoglobulin G4.

increased level of serum IgG4 is an independent predictor of type 1 AIP, although the sensitivity was lower than in East-Asian patients.[23–28] We also found that higher concentrations of serum IgG4 correlated with a higher probability of type 1 AIP. However, also a few PC and ACP patients showed elevated serum IgG4 levels, particularly in the range between once and twice the upper limit of normal, in accordance with published studies.[23,24] We were unable to confirm the reported utility of a high a-PBP value as a marker of AIP, in accord with recent studies from The Netherlands and the UK.[29,30]

In the present study, we found a higher number of AIP patients with a low a-PBP than PC patients, when using a cut-off of 38.3 U, but the sensitivity and specificity was very low. Moreover, as also at least some healthy patients will show low values, it is unlikely that this finding can be of diagnostic value. Hence, our data cannot confirm the report from Frulloni et al[13] who found significantly raised a-PBP in AIP patients, with both sensitivity and specificity >90% using 110 PC patients as a control group. Our results differ to such an extent, that it must be considered whether the same molecule has been measured. It would be

Table 6

Frequency of IgG4-positivity in autoimmune pancreatitis (AIP), type 1 AIP, type 2 AIP, pancreatic cancer (PC), and alcoholic chronic pancreatitis (ACP), dependent on the selected cut-off value for serum IgG4.

IgG4 cut-off	AIP	AIP Type 1	AIP Type 2	PC	ACP
n	29	19	10	17	41
1.09 g/L	52%	68%	20%	12%	29%
1.25 g/L	45%	58%	20%	12%	22%
1.40 g/L	45%	58%	20%	12%	17%
2.80 g/L	28%	37%	10%	6%	5%

IgG4 = Immunoglobulin G4.

interesting to identify the proportion of our enrolled patients who are *Helicobacter pylori* (*HP*) positive, since this condition is suspected of triggering the development of AIP and elevated PBP-values, but unfortunately, we did not evaluate this parameter in our patients.[31,32] It is also possible that the ratio of HP-positive patients was lower in our AIP cohort than in our PC and ACP cohorts, which may have contributed to the slightly higher number of AIP patients with low a-PBP values, as compared with the PC and ACP patients. The mean a-PBP values were higher in ACP than in PC. Again, it is possible that differences in HP-positivity may in part have contributed to these findings. The prevalence of *HP*-positive patients is likely to be higher in the original study on a-PBP, but this should also apply to their control groups.[13] A possible limitation of our study is that most patients had already received steroid treatment at the time of measurement of a-PBP, and also differences in the methods used for analysis may influence the results. Our measurement of a-PBP IgG antibodies was based on the method described in the original report, using the bacterial PB peptide of the AKEERRY sequence, similar to Buijs et al,[29] but without a biotinylated anchor sequence.[13] We used a traditional solid-phase immunoassay format (ELISA) with direct antigen coating and enzyme-generated colorimetric-based detection instead of the time-resolved fluorescence methodology used in the original publication.[13]

As expected and in accordance with earlier studies, we found a significant difference regarding mean serum IgG4 concentrations between AIP, PC, and ACP patients.[12,14,17,23–28] 45% of AIP, 58% of type 1 AIP, and 12% of PC patients had serum IgG4 values above the cut-off of 1.4 g/L. In comparison, Chang et al[12] reported IgG4 levels above this cut-off in 64.8% of AIP and 20% of PC patients. This low proportion of serum IgG4 elevation in the present series is probably due to that Chang reported of South-East Asian patients, where there is a higher rate of type 1 AIP and a lower rate of type 2 AIP, in contrast to Europe including Denmark.[10,12,25,26] In a recent study from the United

States, elevated serum IgG4 was found in 66% of AIP and 10% of PC.[14] When using a cut-off of 1.4 g/L, the sensitivity and specificity of serum IgG4 in differentiating AIP from PC were 45% and 88% in our study. In a recent meta-analysis, the respective values were 73% and 93%.[16] However, again, the proportion of type 1 AIP patients in the meta-analysis was probably higher than in our study.[16] However, even when taking this into account, only 58% of our type 1 AIP patients had IgG4 levels above the common cut-off of 1.4 g/L. Interestingly, this value rose to 68% when using a cut-off of 1.09 g/L, without increasing the number of IgG4-positive PC patients (Table 6). It is important to note the overlap in concentrations between the groups, resulting in 6% of PC patients and 5% ACP patients presenting with serum IgG4 values above twice upper limit of normal (2.8 g/L), in accordance with previous findings.[12,15,24,33] We found elevated serum IgG4 levels (>1.25 g/L) in two type 2 AIP patients (20%), which is consistent with previous findings.[34] In our study, a cut-off for IgG at 13.5 g/L had a sensitivity of 24% and a specificity of 82.7% for differentiating AIP from PC. In a recent meta-analysis, the respective values were 51% and 94%.[16]

Since there is no established normal range for the concentration of anti-CA-II, we only considered the differences in concentrations between the groups. Anti-CA-II was higher in the PC and ACP groups when compared with AIP, but the difference was not statistically significant. Okazaki et al[17] found elevation of anti-CA-II in 59% of AIP (n = 17) but in none of the ACP patients (n = 17). In the same year, another Japanese study reported elevation in 33% of AIP (n = 6) and none of ACP (n = 16).[35] Aparisi et al[36], on the other hand, reported anti-CA-II elevation in Spanish patients with ACP (10.5%) as well as in AIP (27.8%), more in line with our data. However, another Japanese group reported elevated anti-CA-II in 88.9% of AIP (n = 9) and 45.8% of ACP (n = 24), and in a recent Spanish study, similarly in 83% of AIP (n = 12), 50% of idiopathic chronic pancreatitis (n = 26), 29% of PC, and 9% of chronic pancreatitis (n = 23).[15,37] In an

Table 7

Sensitivity and specificity at various cut-off values of IgG4 for the differentiation of autoimmune pancreatitis (AIP) and type 1 AIP from pancreatic cancer (PC).

Cut-off value	AIP versus PC				AIP type 1 versus PC			
	Sensitivity	CI	Specificity	CI	Sensitivity	CI	Specificity	CI
1.09 g/L	52%	33–71%	88%	64–94%	68%	43–87%	88%	64–99%
1.25 g/L	45%	26–64%	88%	64–99%	58%	33–80%	88%	64–99%
1.4 g/L	45%	26–64%	88%	64–99%	58%	33–80%	88%	64–99%
2.8 g/L	28%	13–47%	94%	71–100%	37%	16–61%	94%	71–100%
3.6 g/L	28%	13–47%	100%	80–100%	37%	16–61%	100%	80–100%

Because there were made no comparisons, no statistical tests were used. Instead, 95% confidence intervals (CI) are given.

unselected cohort of CP patients back in 2000, Frulloni et al[38] reported a-CA-II elevation in 26%. Hence, the exact role of this marker for the differentiation of AIP from PC remains to be elucidated and should probably be performed in the frame of a large, international multicenter study to eliminate possible methodological differences. Because initial studies on a few AIP patients indicated usefulness of rheumatoid factor (RF) and a-lactoferrin (a-LF) antibodies in AIP, we also included these markers in our survey.[17,35] RF were detected more frequently in PC patients compared with AIP, but this may be due to the higher age of the PC patients. 45% of our AIP patients were ANA-positive. By comparison, others have found ANA-positivity in AIP ranging from 20.3% to 76.4%.[17,35,39] In the present study, c-ANCA was elevated in 30% of type 2 AIP and none of type 1 AIP. Hence, ANCA may be a potential marker that can contribute to the differentiation of type 1 from type 2 AIP, but larger studies are needed to evaluate this aspect.

The inconsistency of the markers tested in our study and in the various studies cited is remarkable and may be due to: different populations studied, as the IgG4-positive type 1 AIP is more common in Asia than in the West, differences in biochemical methods and cut-off values, lack of distinction between AIP subtypes and lack of an appropriate control group, publication bias, where negative results are less likely to be published, differences in treatment status of the included patients (i.e., before or after immunosuppressive treatment), and differences in diagnostic criteria and identification of patients. Our study has several limitations and strengths. The large majority of AIP cases were supported by pancreatic histology (24 of 29 cases), which should be considered a strength as many other serological studies are based on serum IgG4 as one of the diagnostic criteria, which may introduce selection bias and may contribute to some of the differences between our study and other studies. A weakness of our study is that serology might be affected by medical treatment, and most of our patients had started steroid treatment at time of blood sampling, due to the retrospective nature of this study. However, Ghazale et al[24] demonstrated that the exclusion of treated patients did not significantly alter the sensitivity and specificity of the serological markers. Unfortunately, only 17 PC patients were included in this study, which is a low number of patients, particularly compared with the number of included patients with AIP (n=29), which is a rare disease. The low number of PC patients included in this study may very well have influenced the results, and in particular the calculations regarding the statistical significance. Finally, a promising marker for the diagnosis of AIP is anti-amylase-α2A, with reported sensitivities and specificities ranging from 76%–88% and 78%–99%, respectively.[40,41] Unfortunately, we were not able to include this marker in the present study.

In conclusion, the only marker for which we found a statistically significant difference in mean values between AIP and PC was IgG4. However, the value of serum IgG4 for the distinction of AIP from PC and ACP was limited, probably in part due to the relatively high number of type 2 AIP patients in our study. For differentiation of type 1 AIP and PC, IgG4 had a sensitivity of 68% and a specificity of 88% when using a cut-off of 1.09 g/L. In accord with recent publications, our data do not support a role of increased a-PBP for the diagnosis of AIP.

Acknowledgments

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