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Diagnostic value of serum immunoglobulin G4, immunoglobulin E and anti-lactoferrin antibodies in type 1 autoimmune pancreatitis and immune-associated cholangitis in regular clinical circumstances**

Wartość diagnostyczna stężenia immunoglobuliny G4, immunoglobuliny E i przeciwciał przeciwlaktoferynowych w autoimmunologicznym zapaleniu trzustki typu 1 i immunologicznym zapaleniu dróg żółciowych w typowych uwarunkowaniach klinicznych

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Keywords

immunoglobulin G4, IgG4-related disease, autoimmune pancreatitis, IgG4-associated cholangitis

Słowa kluczowe

immunoglobulina G4, choroba IgG4 zależna, autoimmunologiczne zapalenie trzustki, immunologiczne zapalenie dróg żółciowych

Summary

Introduction. Most common presentations of IgG4-related disease are autoimmune pancreatitis (AIP) and IgG4-associated cholangitis (IAC) with clinical images frequently imitating a pancreatic or bile duct cancer.

Aim. To evaluate the diagnostic significance of serum levels of IgG4, antibodies against lactoferrin (ALA) and immunoglobulin E (IgE).

Material and methods. The study involved 16 patients with AIP type 1 and/or IAC, 20 control subjects and 80 patients with pancreatobiliary diseases of different etiology requiring meticulous differentiation with IgG4-related disease. Based on final clinical diagnosis 5 subgroups were identified: AIP type 1 and/or IAC (AIP/IAC, n = 16), primary sclerosing cholangitis (PSC, n = 20), chronic alcoholic pancreatitis (ChP, n = 20), pancreatic cancer (PanCa, n = 20) and cholangiocarcinoma (CCA, n = 20).

Results. In AIP/IAC group the serum level of IgG4 was elevated in 81% of patients, and exceeded doubled limit of upper reference value in 54% of patients. Percentage of abnormal IgG4 results was 35% in CCA, 30% in PanCa, 15% in PSC and 15% in ChP. Based on ROC analysis the optimal cut-off for IgG4 in differentiation of AIP/IAC with other diseases was 127 mg/dl (sensitivity and specificity of 81 and 90%, respectively). Increasing of IgG4 cut-off allowed detection of AIP/IAC with very high specificity. Serum ALA and IgE concentrations were not helpful either in detection or differential diagnosis of AIP/IAC.

Conclusions. Serum IgG4 is important criterion of AIP/IAC but false positive results occurring in malignant diseases, in particular cholangiocarcinoma, should be taken into account.

Streszczenie

Wstęp. Najczęstszymi manifestacjami klinicznymi choroby IgG4 zależnej są autoimmunologiczne zapalenie trzustki (AIP) i immunologiczne zapalenie dróg żółciowych (IAP), których objawy często naśladują raka trzustki lub dróg żółciowych.

Cel. Ocena znaczenia diagnostycznego stężenia surowiczego IgG4, przeciwciał przeciwlaktoferynowych (ALA) i immunoglobuliny E (IgE) w diagnostyce różnicowej AIP/IAC.

Materiał i metody. Do badania włączono 16 chorych z AIP typu 1 i/lub IAC, 20 ochotników grupy kontrolnej oraz 80 pacjentów z chorobami trzustki lub dróg żółciowych o różnej etiologii wymagającymi różnicowania z chorobą zależną od IgG4. Na podstawie ostatecznego rozpoznania klinicznego wyodrębniono 5 podgrup: AIP lub IAC (AIP/IAC, n = 16), pierwotne stwardniające zapalenie dróg żółciowych (PSC, n = 20), przewlekłe alkoholowe zapalenie trzustki (ChP, n = 20), rak trzustki (PanCa, n = 20) i rak zewnątrzwątrobowych przewodów żółciowych (CCA, n = 20).

Wyniki. W grupie AIP/IAC stężenie IgG4 było zwiększone u 81% chorych i przekraczało dwukrotnie górną granicę wartości referencyjnych u 54% chorych. Odsetek nieprawidłowych wyników IgG4 u chorych z CCA wynosił 35%, z PanCa 30%, a z PSC i ChP po 15%. Na podstawie analizy ROC optymalnym punktem odcięcia dla stężenia IgG4 w różnico-

Conflict of interest
Konflikt interesów

None
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waniu AIP/IAC z innymi chorobami było 127 mg/dl (czułość i swoistość odpowiednio 81% i 90%). Zwiększanie punktu odcięcia dla stężenia IgG4 pozwalało na wykrywanie AIP/IAC z bardzo wysoką swoistością. Stężenia surowicze ALA i IgE nie były pomocne w wykrywaniu i diagnostyce różnicowej AIP/IAC.

Wniosek. Stężenie surowicze IgG4 jest ważnym kryterium diagnostycznym AIP/IAC, lecz należy brać pod uwagę ich wyniki fałszywie pozytywne w chorobach nowotworowych, zwłaszcza raku przewodów żółciowych.

INTRODUCTION

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a multifaceted systemic disorder that includes various organ manifestations, among which the pancreas and biliary tree are most frequently affected. The predominant involvement of pancreas is known as autoimmune pancreatitis (AIP) and that of biliary tree as IgG4-associated cholangitis (IAC). IgG4-RD responds to corticosteroid treatment when diagnosed early enough, but if it remains untreated may lead to end-stage organ failure such as liver cirrhosis or fatal complications (1, 2). Liver cirrhosis was found in 8% of IAC patients (3).

Because the histology in AIP and IAC patients is often unavailable, the differentiation of these conditions with more common neoplastic and other inflammatory pancreatobiliary diseases can be extremely challenging. There is evidence that significant number of patients with IgG4-RD is falsely diagnosed as cancer and undergoes surgical instead of pharmacological treatment. Erdogan et al. reviewed post-surgery histology of 185 patients operated for biliary cancer, and in 32 patients the diagnosis of cancer was not confirmed, being in 50% of cases compatible with IAC (4). In another study, 25% of 122 patients transplanted for primary sclerosing cholangitis (PSC) showed on explanted hepatic histology moderate or marked IgG4-positive staining (5). These two studies stress the importance of pre-surgery diagnosis, but despite using panels of composite laboratory, radiological and histological parameters the gold standards for diagnosing AIP and IAC are still lacking (6-8).

IgG4-RD is often associated with elevated IgG4 serum level and is characterized by IgG4-positive plasma cells tissue infiltrates. IgG4 is one of the four subtypes of IgG, forming less than 5% of this protein in healthy population. High IgG4 serum level was first reported in patients with AIP and later was also associated with other organ manifestations of IgG4-RD, including IAC. Although elevated IgG4 level is an important diagnostic clue in the current diagnostic panels of AIP and IAC, this parameter presents several flaws such as poorly known reference values, unsatisfactory inter-laboratory reproducibility, and in particular limited specificity for IgG4-RD (9, 10).

Lactoferrin is a multifunctional glycoprotein of the transferrin family that has antimicrobial activity and is part of the innate mucosal defense. This protein is widely represented in various secretory fluids, such as milk, saliva, tears and bile. It is also present in secondary granules of neutrophils and is secreted by pancreatic acinar cells. Lactoferrin may serve as biomarker of inflammation and necrosis (11, 12). Anti-lactoferrin antibodies (ALA) may be found in 75% of patients with AIP (13). These antibodies may also emerge in autoimmune liver and bowel diseases, lupus erythematodes or type 1 diabetes. Moreover, Hardt et al. detected ALA in 21% of patients with non-alcoholic chronic pancreatitis suspected of AIP (14).

Recent studies have shown that patients with AIP demonstrate increased serum levels of IgE (15, 16). In addition, elevated IgE was not associated with allergic reactions and did not correlate with severity of AIP, therefore, it was conceived that this immunoglobulin might be a biomarker of subclinical IgG4-RD when IgG4 level is still normal.

AIM

The purpose of this study was to determine the ability of IgG4, ALA and IgE to identify clinically overt AIP and IAC amongst many other pancreatobiliary diseases difficult or impossible to distinguish from IgG4-RD without histology. For this reason we measured serum levels of these biomarkers not only in IgG4-RD but also in patients with wide spectrum of clinically similar diseases.

MATERIAL AND METHODS

Ninety six patients with pancreatobiliary diseases consecutively admitted to the tertiary gastro-hepatological center between January 2013 and December 2014 were included to the study. Final clinical diagnosis was based on abdominal imaging methods (ultrasound, computed tomography, magnetic resonance, endoscopic retrograde cholangiopancreatography), histopathological/cytological examination of biopsy samples and serum level of CA19-9, associated with median 17 months clinical follow-up.

According to final clinical diagnosis the patients were enrolled to one of 5 following subgroups: cholangiocarcinoma (CCA; n = 20), pancreatic cancer (PanCa; n = 20), alcoholic chronic pancreatitis (ChP; n = 20), primary sclerosing cholangitis (PSC; n = 20) and autoimmune pancreatitis and/or IgG4-associated cholangitis (AIP/IAC; n = 16). The control group consisted of 20 patients with functional gastroenterological diseases having neither pancreatobiliary problem nor autoimmune disease.

The initial criterion of recruitment to AIP/IAC group was one of the following: 1) diffusely or segmentally enlarged pancreas, 2) solid tumor of the pancreas

that was not diagnosed as cancer in at least two fine-needle aspiration biopsies or post-surgery histology, and 3) common biliary duct stricture at cholangiography (magnetic resonance or endoscopic).

The diagnosis of AIP was based on the criteria established by international consensus from Fukuoka, 2010 (6) and IAC was diagnosed according to latest Japanese criteria (8). Initially, 25 patients were suspected to have AIP/IAC, but ultimately 16 patients were included to this group: 8 patients were diagnosed with AIP-1 and coexisting IAC, 3 with AIP alone, and 5 with IAC alone. Reasons for exclusion of 9 patients were: lack of strong evidence against pancreatic or bile duct malignancy (n = 4), suspicion of genetic background of chronic pancreatitis (n = 2), loss of the patient from the follow-up before final diagnosis could be established (n = 2) and death from other causes (n = 1).

Exclusion criteria from all investigated groups were: type 2 autoimmune pancreatitis, recent acute pancreatitis, serum level of triglycerides > 600 mg/dl, increased serum calcium level, family history of pancreatitis, HIV infection, history of sepsis or hypovolemic shock and liver or kidney transplantation.

Serum samples

Peripheral blood morphology (Sysmex XT-1800i) and serum biochemistry (Olympus AU680) were determined on the day of admission. Serum level of CA19-9 was measured by immunoassay (CMIA Architect i2000SR). Serum levels of γ-globulin, immunoglobulin G and autoantibodies (antinuclear, anti-smooth muscle, antimitochondrial) were determined only in patients with AIP/IAC. Blood samples for non-routine examinations were centrifuged for 15 minutes at 1500 g and immediately stored in small aliquots at -80°C, until further elaboration. Serum level of IgG4 was measured by nephelometry (Minineph Human IgG4 KIT, Binding Site). Anti-lactoferrin antibodies and IgE levels were assayed using a commercially available enzyme-linked immunosorbent kits (Demeditec Diagnostics, Kiel, Germany).

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethics Committee of Silesian Medical University. The informed consent was obtained from all patients.

Statistical analysis

Continuous parameters were reported as a mean ± SD and as a median with interquartile range (IQR 25^{th}-75^{th} percentile). Normality test was performed for all quantitative variables. Non-parametric statistical methods were used for non-normally distributed data. For multiple comparisons Kruskal-Wallis test was used. Testing of significance was carried out using the T-test or Mann-Whitney U test.

Discrete data were summarized as frequencies and percentages. The Chi-Square test was used for comparison of categorical data. Correlations were assessed by Spearman's test, and the correlation coefficient r was used to measure the strength and the direction of relationship.

Receiver operating characteristic (ROC) curve analysis was carried out and area under the ROC curve (AUROC) was calculated to evaluate accuracy of IgG4, ALA and IgE for diagnosis of AIP/IAC. A two-tailed $p < 0.05$ was considered significant.

RESULTS

Of 16 patients with AIP/IAC eight patients met the criteria for "definite diagnosis", and eight patients for "probable diagnosis". Clinical characteristics of patients with AIP/IAC and other pancreatobiliary diseases are shown in table 1. The patients did not differ according to body mass index (BMI). The highest serum CA19-9 and bilirubin levels were found in patients with pancreatic cancer and cholangiocarcinoma.

In AIP/IAC patients the mean ± SD levels of γ-globulin and immunoglobulin G (IgG) were 1.80 ± 1.11 g/dl and 1.94 ± 1.03 g/dl, respectively. In eight (61.5%) patients the IgG level was higher than 1.60 g/dl. Antinuclear (ANA), anti-smooth muscle (ASMA) and antimitochondrial (AMA) antibodies were found in this group in 9 (56.3%), 6 (37.5%) and 2 (12.5%) patients, respectively.

Among different groups of patients the highest level of IgG4 was found in patients with AIP/IAC (tab. 2).

Tab. 1. Characteristics of 20 controls and 96 patients with different pancreatobiliary diseases

Parameter	Control	PSC	Chronic pancreatitis	Pancreatic cancer	Cholangio-carcinoma	AIP/IAC	*p*
Age (years)	63 ± 15.5	40.6 ± 13.5	56.5 ± 13.8	66.1 ± 11.6	63.2 ± 11.9	52.5 ± 16.1	0.00001
Males/females	6/14	12/8	16/4	9/11	8/12	8/8	0.00001
BMI (kg/m^2)	26.2 ± 5.9	22.9 ± 4.30	22.9 ± 2.7	23.6 ± 3.8	23.3 ± 3.0	24.7 ± 4.6	0.2014
Alanine aminotransferase (U/l)	25.5 ± 13.2	126 ± 97.7	67.3 ± 78.9	219 ± 167	149 ± 96.9	86.3 ± 85.4	0.00001
Alkaline phosphatase (UI/l)	57.8 ± 12.8	384 ± 289	338 ± 431	631 ± 399	561 ± 291	346 ± 332	0.00001
Bilirubin (mg/dl)	0.8 ± 0.3	3.2 ± 5.9	2.0 ± 3.3	14.7 ± 12.6	19.5 ± 11.6	2.4 ± 2.6	0.00001
C-reactive protein (mg/l)	8.10 ± 22.7	7.1 ± 9.0	19.1 ± 43.4	42.3 ± 50.6	18.3 ± 13.4	22.9 ± 35.3	0.00001
CA19-9 (U/ml)	9.6 ± 8.2	26.1 ± 41.0	33.7 ± 54.7	1280 ± 2300	6244 ± 13882	19.4 ± 23.9	0.00001

p – test probability value calculated using the Kruskal-Wallis test

Tab. 2. Serum levels of IgG4, ALA and IgE in patients with different biliary and pancreatic diseases

Group	IgG4 (mg/dl)		ALA (U/ml)		IgE (U/ml)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Control	37.3 ± 27.0	31.2	6.3 ± 1.4	6.4	63.7 ± 121	12.0
PSC	49.3 ± 42.2	38.6	8.8 ± 5.7	7.3	70 ± 131	17.8
ChP	57.4 ± 62.8	47.9	5.8 ± 2.5	6.4	158 ± 196	66.5
PanCa	58.9 ± 57.5	44.7	6.2 ± 3.5	5.0	79.4 ± 143	12.4
CCA	97.7 ± 86.1[a]	82.9	15.5 ± 20[c]	7.6	91 ± 120[b]	56.2
AIP/IAC	404 ± 710[b]	203	11.9 ± 9.8[d]	9.1	82.4 ± 116	44.4
p	0.0001		0.0003		0.4987	

[a]significantly higher than in control group ($p < 0.05$) and not different from AIP/IAC
[b]significantly higher than in control ($p < 0.001$), PSC ($p < 0.01$), ChP ($p < 0 < 0.01$) and PanCa ($p < 0.01$)
[c]significantly higher than in ChP ($p < 0.05$) and PanCa ($p < 0.01$)
[d]significantly higher than ChP ($p < 0.05$) and PanCa ($p < 0.01$)
PSC – primary sclerosing cholangitis; ChP – chronic pancreatitis; PanCa – pancreatic cancer; CCA – cholangiocarcinoma; AIP/IAC – autoimmune pancreatitis/immune-associated cholangiopathy; IgG4 – immunoglobulin G4; ALA – anti-lactoferrin antibody; IgE – immunoglobulin E; *p* – test probability value calculated using the Kruskal-Wallis test
IgG4: AIP/IAP different from control ($p = 0.0002$), PSC ($p = 0.0028$), ChP ($p = 0.0059$) and PanCa ($p = 0.0075$)
ALA: AIP/IAP different from ChP ($p = 0.042$) and PanCa ($p = 0.0071$)

In 13 (81%) AIP/IAC patients the serum level of IgG4 was higher than 113 mg/dl (upper limit of normal – ULN) and in 7 (44%) patients it was higher than 226 mg/dl (double ULN). In comparison the serum IgG4 was elevated in 35% of patients with CCA, and in 30% of patients with pancreatic cancer. IgG4 level in patients with CCA was significantly higher as compared to control subjects and was not different from AIP/IAC patients. Figure 1a shows the prevalence rates of increased level of IgG4 in all investigated groups. AUROC for identification of AIP/IAC with IgG4 was 0.83 (95% CI: 0.68-0.98), with the optimal cut-off defined as 127 mg/dl (fig. 2a). Efficacy of IgG4 for AIP/IAC diagnosis with different cut-off values is shown in table 3. If the cut-off was set at the double ULN then 92% of patients not having the AIP/IAC were identified, while those with AIP/IAC were correctly diagnosed in less than half of the cases (sensitivity was 44%).

The frequencies of normal and elevated ALA and IgE results in different groups of patients are shown in figures 1b, c. Patients with AIP/IAC had significantly higher level of ALA than patients with chronic pancreatitis (11.9 ± 9.8 U/ml vs 5.8 ± 2.5 U/ml; $p < 0.0003$) and pancreatic cancer (vs 6.2 ± 3.5 U/ml, $p < 0.0003$) but not different from CCA and PSC patients. There was no significant difference in serum concentration of IgE between the investigated groups (tab. 2). AUROC for AIP/IAC diagnosis was 0.70 (95% CI: 0.57-0.83) for ALA and 0.52 (95% CI: 0.37-0.67) for IgE (fig. 2b, c). In AIP/IAC patients IgG4 correlated with IgE ($r = 0.84$, $p < 0.001$) and IgG ($r = 0.64$; $p < 0.01$). In these patients IgE correlated also with IgG ($r = 0.63$; $p < 0.01$) (tab. 4).

Tab. 3. The performance of serum IgG4 (different cut-offs), anti-lactoferrin antibodies (ALA) and IgE for diagnosis of AIP/IAC

Biomarker	AIP/IAC (n = 16)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG4 ≥ 113 mg/dl (cut-off derived from diagnostic criteria)	13	81	87	50	97
IgG4 ≥ 127 mg/dl (cut-off derived from ROC)	13	81	90	57	97
IgG4 ≥ 226 mg/dl (2 x ULN)	7	44	98	78	92
IgG4 ≥ 452 mg/dl (4 x ULN)	2	12	100	88	100
ALA ≥ 8.44 IU/ml	10	62.5	76	29.4	92.7
IgE ≥ 36.6 IU/ml	10	62.5	59	19.6	90.8

ULN – upper limit of normal; AIP/IAC – autoimmune pancreatitis/immune-associated cholangiopathy; IgG4 – immunoglobulin G4; PPV – positive predictive value; NPV – negative predictive value

Tab. 4. Correlation of the concentration of IgG4, ALA, IgE, γ-globulin, IgG and CA19-9 in the group AIP/IAC

Independent factors	Related factors		
	IgG4	ALA	IgE
ϒ-globulin	0.64 (0.0079**)	-0.07 (0.7986)	0.52 (0.0385*)
Immunoglobulin G	0.64 (0.0074**)	-0.11 (0.6917)	0.63 (0.0090**)
CA19-9	-0.05 (0.8626)	0.07 (0.7904)	0.11 (0.6723)
IgG4	1	-0.10 (0.7078)	0.84 (0.0001***)
ALA	-0.10 (0.7078)	1	-0.16 (0.5597)
IgE	0.84 (0.0001***)	-0.16 (0.5597)	1

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

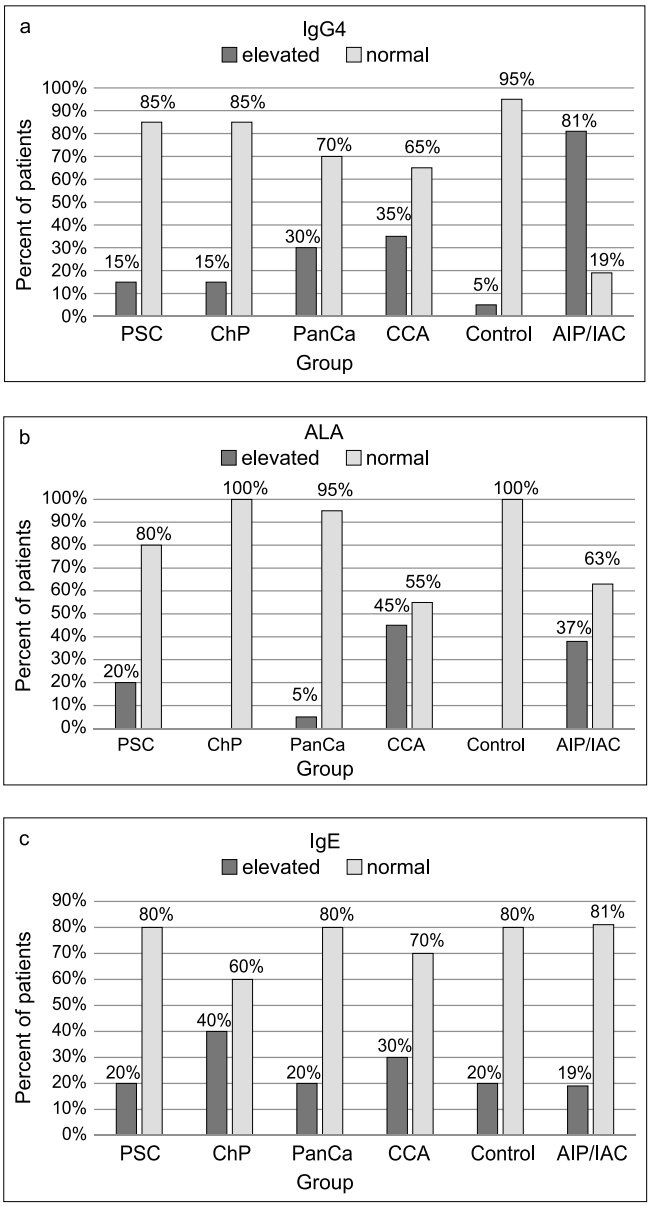

Fig. 1a-c. The prevalence rate of normal and elevated serum levels of IgG4, ALA and IgE in patients with AIP and IAC
PSC – primary sclerosing cholangitis; ChP – chronic pancreatitis; PanCa – pancreatic cancer; CCA – cholangiocarcinoma; AIP/IAC – autoimmune pancreatitis/immune-associated cholangiopathy; IgG4 – immunoglobulin G4; ALA – anti-lactoferrin antibody; IgE – immunoglobulin E

DISCUSSION

IgG4-RD is rare disease with AIP and IAC as most common manifestations. The diagnosis of these disorders is a result of meticulous analysis of laboratory tests, clinical and imaging features and histological characteristics. The aim of this study was to re-evaluate diagnostic significance of serum IgG4 level in AIP and IAC patients in the clinical setting when many other pancreatobiliary diseases must be taken into account. We extended the laboratory panel by ALA and IgE serum levels.

To examine the accuracy of IgG4 we enrolled to the study patients with diseases, which often imitate AIP/IAC such as pancreatic and biliary cancer, PSC

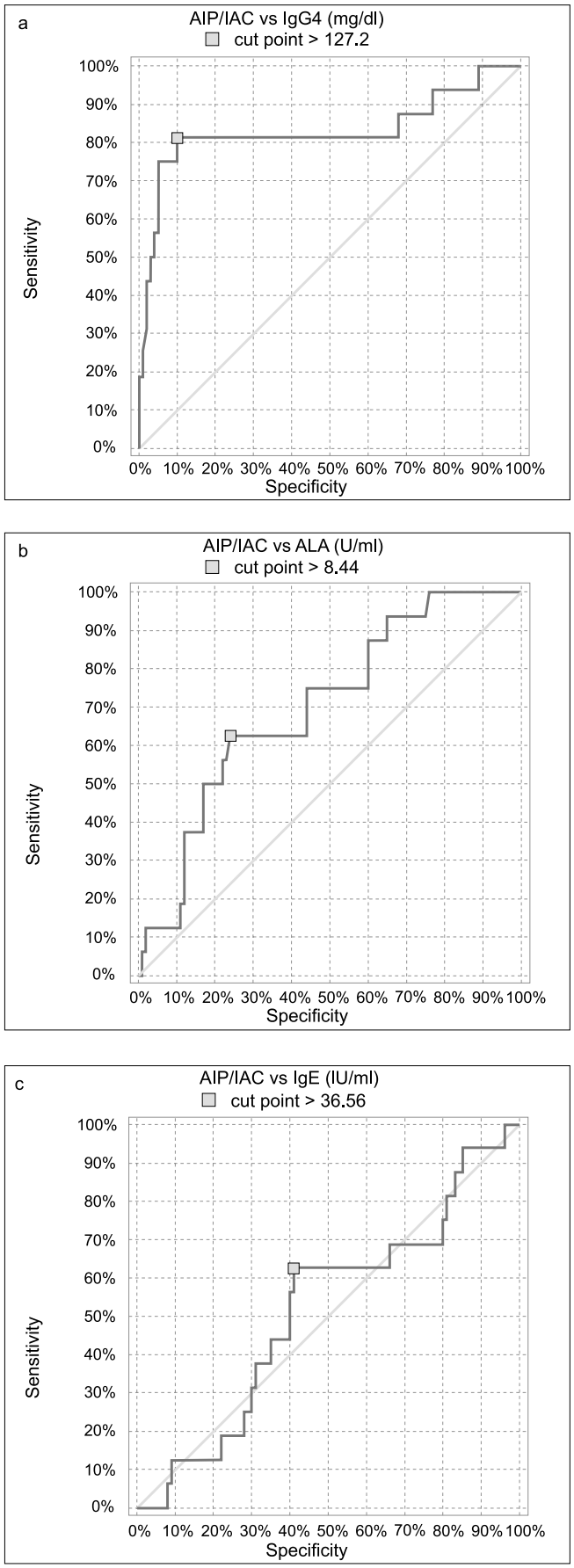

Fig. 2a-c. Receiver operating characteristic (ROC) curves for IgG4, ALA and IgE for diagnosis of AIP/IAC. Area under curves were 0.83 (95% CI: 0.68-0.98), 0.70 (95% CI: 0.57-0.83) and 0.52 (95% CI: 0.37-0.67) for IgG4, ALA and IgE, respectively

and chronic pancreatitis. Average age of our AIP/IAC patients was similar to reported in previous studies (17-19), however, half of our participants were women that challenges general view on dominance of male sex (in literature female/male ratio ranges from 1:3 to 1:6). Definite diagnosis of AIP with missing histology and unknown effect of corticosteroid therapy is achievable in about 70% of cases (1). According to international consensus criteria the diagnosis of AIP or IAC in our study was definite in 8 patients and in the remaining 8 patients was probable. IAC is usually associated with edema of the pancreas or other organs in the context of IgG4-RD. In our study isolated IAC was diagnosed in 5 patients (38.5%) that is considerably more often than in hitherto published series (20, 21).

Up to now, IgG4 is the only acceptable serological biomarker of AIP type 1 and IAC, but its role has been better established among Asian than Caucasian populations. First reports on diagnostic performance of IgG4 in AIP in terms of sensitivity and specificity yielded very good results, being 95% or higher (22). According to current knowledge the sensitivity of serum IgG4 in diagnosis of AIP or IAC ranges widely from 50 to 95%, while specificity retains excellent rates between 89 and 100% (18). Conflicting data on sensitivity of IgG4 may result from assuming variable diagnostic criteria of IgG4-RD, different IgG4 cut-off levels or enrollment of patients with AIP type 2 having normal IgG4 level.

For evaluation of diagnostic efficacy of IgG4 we used two thresholds of ULN, namely 113 mg/dl according to the instruction of the kit producer and 127 mg/dl calculated from ROC analysis. The latter best distinguished AIP/IAC from other benign or malignant pancreatobiliary diseases. Elevated level of IgG4 was found in 81% of AIP/IAC patients, and in 54% of them exceeded double ULN that according to current criteria is a strong predictor of IgG4-RD (level 1 of HISORt) (23). Categorizing patients to the AIP/IAC based on IgG4 equal or greater than 127 mg/dl was associated with the diagnostic sensitivity of 81% and specificity of 90%. Unfortunately, the positive prediction of this disease only slightly exceeded 50% chance of correct diagnosis, while IgG4-RD could be excluded with narrow margin of uncertainty (NPV was 97%). Similar data can be found in other studies. In English study the measurement of IgG4 distinguished AIP from pancreatic cancer, PSC and acute or chronic pancreatitis with 95% sensitivity and 90% specificity (3). The highest specificity was associated with IgG4 set at 220 mg/dl. In the study from Mayo Clinic diagnostic sensitivity and specificity for IgG4 higher than 140 mg/dl was 76 and 93%, respectively (24). According to expectations increasing the level of ULN considerably deteriorated the sensitivity of this test. If double ULN (226 mg/dl) was applied, the diagnostic sensitivity of IgG4 in our study fell to 44% and specificity was 98% (PPV 78%, NPV 92%). Nevertheless, one should keep in mind a possibility of IgG4-negative variants of this disease, occurring in 20-30% of cases (25). Besides, IgG4 level may differ according to the stage of the disease, being the lowest in later stages of IgG4-RD (26).

In our study serum level of IgG4 was increased in 30% patients with pancreatic cancer, and in 15% of patients with PSC or alcoholic chronic pancreatitis, although in these diseases mean concentrations was much lower than in AIP/IAC group. These results are in accordance with literature reporting elevations of IgG4 in 5-16% in PSC and about 10% in chronic pancreatitis (27-29). Mildly elevated IgG4 was also occasionally found in healthy subjects (in a single person in our study). Discrimination between IAC and cholangiocarcinoma is even more difficult than in the case of pancreatic cancer, and according to some investigators as high as four-fold increase of IgG4 may safely rule out CCA (24). In our study elevated IgG4 was found in 35% of patients with biliary cancer and in 2 patients its level was higher than double ULN.

Although in our study the serum level of ALA exceeded the ULN in 38% of AIP/IAC cases, such elevation was also found in 45% of CCA and 5% of pancreatic cancer. High prevalence of increased ALA in biliary cancer merits further research. Another investigated parameter was immunoglobulin E, whose increased level was reported in 30-90% of AIP patients with a suggestion that it may be useful in differentiation between AIP and pancreatic cancer (15, 16). In our study the measurement of IgE had no diagnostic significance in AIP/IAC. Increased level of this immunoglobulin was found in each group with the frequencies ranging from 19 to 40% with highest prevalence in alcoholic chronic pancreatitis.

CONCLUSIONS

To summarize, the serum level of IgG4 is useful in differentiating AIP/IAC from pancreatic cancer, chronic pancreatitis and PSC, but not CCA. Serum concentrations of ALA and IgE do not have any significance in diagnosis and differentiation of AIP/IAC with other pancreatobiliary diseases. Our results need confirmation in studies enrolling larger number of patients with this rare disease. Longitudinal assessment of IgG4 serum level in different stages of IgG4-RD would be warranted.

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