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ORIGINAL ARTICLE

Immunoglobulin subclass 4 for the diagnosis of immunoglobulin subclass 4-associated diseases in an unselected liver and pancreas clinic population

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Abstract

Aims: The diagnosis of autoimmune pancreatitis (AIP) and immunoglobulin subclass 4 (IgG_4)-associated cholangitis (IAC) is based on imaging studies, serology, histology and a response to steroid therapy. The major serological finding is an elevation of the serum IgG_4 concentration. Previous studies have shown that its sensitivity is about 70% and its specificity exceeds 90% at a cut-off of 140 mg/dl in selected patient populations. The aim of the present study was to assess the performance of serum IgG_4 as a diagnostic parameter in an unselected liver and pancreas clinic population.

Methods and results: IgG_4 was prospectively determined in 1412 patients and clinical diagnoses were recorded from a review of patient charts. The prevalence of AIP or IAC in the entire cohort was 1.1% (n = 15). The sensitivity of IgG_4 for the diagnosis of AIP and IAC was 80% and the specificity was 86% at a cut-off value of ≥135 mg/dl. The positive predictive value and the negative predictive value were 6% and 99.7%, respectively. The most common differential diagnosis in patients with elevated IgG_4 was liver cirrhosis.

Conclusion: IgG_4 has a reasonable sensitivity and specificity in a liver and pancreas clinic population, where liver cirrhosis appears to be the most frequent differential diagnosis for elevated IgG_4 concentrations.

Keywords

pancreatitis, cholangitis, malignancies, diagnostic performance, cirrhosis

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Introduction

Immunoglobulin subclass 4 (IgG_4)-associated diseases are an increasingly recognized group of autoimmune diseases,[1,2] which are characterized by sclerosis and lymphoplasmocytic infiltration of affected organs with IgG_4-positive cells.[2–5] The disease can affect the pancreas, biliary tract, salivary glands, retroperitoneum, lymph nodes, kidney, lungs or the prostate.[6,7] In the gastrointestinal tract, autoimmune pancreatitis (AIP) and IgG_4-associated cholangitis (IAC) are considered the most common manifestations.[8]

The prevalence of AIP ranges from 4% to 8% among patients with pancreatitis in different study cohorts.[9] Common differential diagnoses of AIP and IAC include chronic pancreatitis, pancreatic cancer and primary sclerosing cholangitis.[1,10,11] Male predominance and a mean age of 60 ± 10 years were consistently reported.[5,6,12,13] Clinicopathological and radiological features include jaundice, abdominal pain, and diabetes mellitus, extra and

The preliminary results of this study were presented as a poster at the AASLD (American Association for the Study of Liver Diseases) liver meeting 2010 in Boston.

intrahepatic biliary strictures, pancreatic duct encasement and a sausage shape of the pancreas on abdominal magnetic resonance imaging (MRI) or computed tomography (CT).[1,5,6,13]

Studies on IgG_4-related diseases were predominantly carried out in Asian countries,[14–16] the United States of America[5] and Europe,[9,17] where different diagnostic criteria for AIP have been proposed by several societies. The Japanese and Korean criteria were established in 2006 and 2007, respectively.[15,16] The HISORt criteria (**H**istology, **I**maging, **S**erology, **O**ther organ involvement, **R**esponse to **t**herapy) were proposed by Chari *et al*. in 2006.[5]

The typical histological finding of IgG_4-related diseases is infiltration of affected organs with IgG_4-positive plasma cells.[5,15,16] Imaging studies in patients with IAC or AIP show irregular narrowing of the bile ducts or the pancreatic duct by endoscopic retrograde cholangiopancreaticography (ERCP), and enlargement of affected organs or pseudotumour formation. Elevated serum IgG_4 and autoantibodies (ANA and RF) are commonly found in serological studies.[1,5,9,15,16] More specific antibodies directed against carbonic anhydrase II, SPINK1 or trypsinogen have been identified.[18] The performance of total IgG_4 serum concentrations for the diagnosis of IgG_4-related diseases has been evaluated in different studies. A cut-off for IgG_4 elevation was suggested at ≥135 mg/dl by Hamano *et al*. in 2001 with a sensitivity of 95% and a specificity of 97%.[4] The Mayo Clinic group reported a specificity and a sensitivity of 93% and 76%, respectively, at a cut-off level of >140 mg/dl. In patients with IgG_4 concentrations >280 mg/dl, a sensitivity of 53% and a specificity of 99% was found.[5] Based on these findings, the IgG_4 serum concentration has been proposed as a non-invasive parameter for the diagnosis of IgG_4-related disease and is included in the HISORt criteria.

The aim of the present study was to evaluate the sensitivity and specificity of a serum IgG_4 concentration in an unselected liver and pancreas clinic population.

Patients and methods

From January 2009 to May 2010, serum immunoglobulin subclasses were prospectively determined in all patients referred to the unit of gastroenterology and hepatology at the Medical University Innsbruck, Austria. To record clinical diagnoses, patients' charts were carefully reviewed and a diagnosis of AIP and AIC was made according to the HISORt and ASIAN criteria. IgG_4 concentrations were determined in patients' sera using the immunoglobulin subclass assay from Siemens N IGG4 (formerly Dade Behring, Marburg, Germany) on a Behring Nephelometryplatform (BN II). The interassay coefficients of variation were 3.7% at 0.195 g/l, 2.4% at 0.507 g/l and 3.5% at 0.729 g/l, respectively.

Statistical analysis

Results are shown as absolute numbers and medians (with range). Analysis of variables with a non-normal distribution was carried out using Mann–Whitney *U*-tests and means of normally distributed variables were compared with *t*-tests. Exact *P*-values for frequency tables were calculated using the chi-square test and association of variables was assessed by Pearson's correlation. A two-sided *P*-value of less than 0.05 was considered statistically significant. The diagnostic performance of IgG_4 was calculated by receiver-operating characteristic (ROC) analyses with the area-under-the-curve (AUC) statistic and a 95% confidence interval (CI). The statistical analysis of the data was performed in SPSS version 18.0 (SPSS, Chicago, IL, USA).

Table 1 Gender distribution and age in the entire patient cohort and in the subgroups of patients with pancreatitis and cholangitis

	n (females)	Median age (range) years
All patients	1412 (595)	54 (17–99)
Pancreatitis	142 (46)	54 (17–99)
Cholangitis	50 (13)	59 (25–81)

Results

The diagnostic performance of IgG_4 was determined in 1412 consecutive patients referred to our unit from January 2009 to May 2010. Baseline demographic characteristics are shown in Table 1. A diagnosis of pancreatitis or cholangitis was made in 192 patients (13%). Male gender was predominant in both diseases and median age did not significantly differ between these two groups.

The median IgG_4 concentration in patients with pancreatitis was 45.7 mg/dl (range: 1.8–1330 mg/dl), in patients with cholangitis 70.0 mg/dl (range: 6.6–669.0 mg/dl) and 47.5 mg/dl (range: 0.2–1230.0 mg/dl) in the remaining patient cohort. These differences did not reach statistical significance. An IgG_4 concentration of ≥135 mg/dl was found in 210 of 1412 patients (14.9%).

Review of patient charts in the entire cohort identified 22 patients with the clinical diagnosis AIP or IAC. Of these, 15 fulfilled HISORt and Asian criteria for the diagnosis of IgG_4-related diseases. A median IgG_4 concentration in patients with IAC according to HISORt was 240 mg/dl (range: 101–669) and 94 mg/dl (3.5–1330) in patients with AIP compared with 47.1 mg/dl in the remaining cohort ($P < 0.001$).

The prevalence of AIP and/or IAC was 1.1% in the entire cohort (15 of 1412) and 7.8% in the subgroup of 192 patients with pancreatitis or cholangitis. In all, 80% of patients with AIP or IAC (12 of 15) had an IgG_4 concentration above 135 mg/dl as compared with 14% of patients without AIP or IAC (198 of 1397). This results in a positive predictive value of 5.7%.

At a cut-off of ≥135 mg/dl, the sensitivity of IgG_4 for the diagnosis of AIP or IAC was 80% (95% CI: 55% to 93%) and the specificity was 86% [95% confidence interval (CI): 84% to 88%; Table 2]. The area under the ROC curve was 0.837 (95% CI: 0.713 to 0.960; Fig. 1) and the optimal cut-off in our patient cohort was ≥145 mg/dl with a sensitivity of 80% and a specificity of 88%.

To identify potential causes of elevated serum IgG_4 concentrations, the main diagnoses were recorded in all 210 patients with a

Table 2 Diagnostic performance of serum IgG4 concentration for the diagnosis of AIP or IAC at a cut off of ≥135 mg/dl

		95% confidence interval
Sensitivity	80%	55%–93%
Specificity	86%	84%–88%
Positive likelihood ratio	5.6	4.3–7.5
Negative likelihood ratio	0.2	0.1–0.6
Diagnostic odds ratio	24.2	6.8–86.6

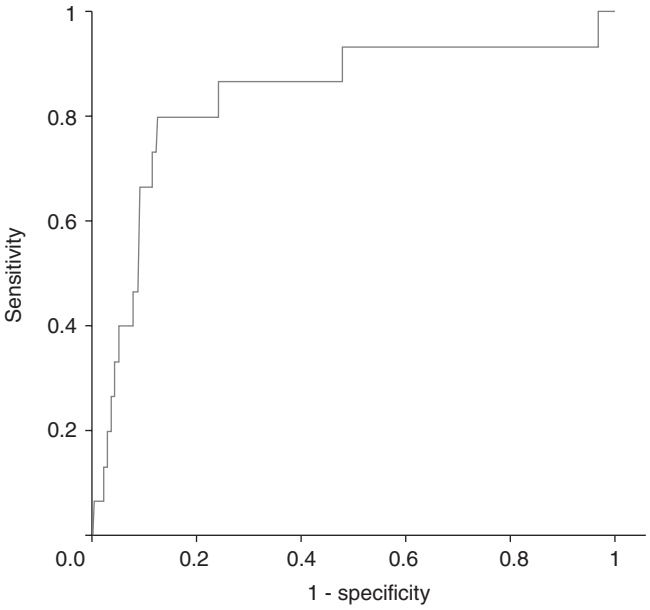

Figure 1 Receiver-operating curve (ROC) curve analysis of serum immunoglobulin subclass 4 (IgG_4) at a cut-off of ≥135 mg/dl for the diagnosis of autoimmune pancreatitis (AIP) and IgG_4-associated cholangitis or IAC

serum $IgG_4 \geq 135$ mg/dl (Table 3). Cirrhosis was the most frequent diagnosis and present in 35% of patients with an IgG_4 serum concentration ≥ 135 mg/dl. Further diagnoses commonly associated with $IgG_4 \geq 135$ mg/dl were fatty liver disease (17%), viral/toxic hepatitis (11%), haemochromatosis (4%), other autoimmune diseases (3%) and other gastrointestinal disorders including malignancies (14%). Median IgG_4 concentrations did not differ significantly between these groups.

An increased serum IgG_4 concentration in liver cirrhosis patients could be attributed to hypergammaglobulinaemia which is a common finding in patients with portal hypertension. Correlation analysis showed that IgG_4 was weakly correlated with total immunoglobulin in the entire cohort ($r = 0.4$, $P < 0.001$). However, the area under the ROC curve of the IgG_4/total IgG ratio (AUC: 0.838; 95% CI: 0.713 to 0.962) was not different from that of IgG_4, indicating that the diagnostic performance of IgG_4 cannot be improved by including total IgG.

Table 3 Diagnoses of patients with serum immunoglobulin subclass 4 (IgG_4) concentrations ≥ 135 mg/dl ($n = 210$)

Diagnosis	*n* (%)
Liver cirrhosis with or without HCC	72 (35%)
Steatosis	35 (17%)
Viral/toxic hepatitis	24 (11%)
Cholangitis including IAC	21 (10%)
Pancreatitis including AIP	8 (4%)
Hemochromatosis	8 (4%)
Other autoimmune diseases	6 (3%)
Cholecystitis/cholecystolithiasis	3 (1%)
Budd Chiari syndrome	2 (1%)
Other gastrointestinal diseases/malignancies	30 (14%)

HCC, hepatocellular carcinoma; IAC, IgG_4-associated cholangitis; AIP, autoimmune pancreatitis.

Discussion

Autoimmune pancreatitis and IAC are typically associated with elevated serum IgG_4 concentrations, where its positive and negative predictive values in a selected patient population are 36% and 99% at a threshold of 140 mg/dl.[10] A general gastroenterology and hepatology clinic population was investigated in the present study where a reasonable sensitivity (80%) and specificity (86%) of IgG_4 was found (Table 2).

A surprising result of the present study is the frequent increase in serum IgG_4 in patients with other underlying diseases, especially in patients with end-stage liver disease. The high number of false-positive results translates to a positive predictive value of only 6% for a cut-off of ≥135 mg/dl. Whether liver cirrhosis is a consequence of IAC in these patients or increased serum IgG_4 concentrations result from impaired metabolic function has to be established. Nevertheless, liver cirrhosis should be considered as a differential diagnosis in patients with unexplained IgG_4 elevation.

The optimal cut-off was 145 mg/dl in the present study, which is close to the cut-off of 140 mg/dl suggested in the HISORt criteria.[5] The varying cut-offs suggested by different groups may be a result of different assays used for measurement of serum IgG_4: Nephelometric assays were used in the present study and by the Mayo group,[5] whereas an enzyme-linked immunosorbent assay and a single radial immunodiffusion assay was used by Hamano *et al.*[4] As previously shown, even at a threshold of >200 mg/dl, pancreatic cancer or cholangiocarcinoma cannot be excluded[10] which illustrates the necessity for a biopsy to confirm the diagnosis.

The negative predictive value of IgG_4 was 99% in a previous study[10] which was confirmed in our study. An important differential diagnosis in patients with suspected AIP and negative IgG_4 is type II AIP, whose diagnosis relies on the histological finding of granulocytic epithelial lesions and narrowing of the pancreatic duct.[18] Findings from non-invasive tests that are indicative of type II AIP are the positivity for ANA, SMA or more specific autoan-

tibodies directed against carbonic anhydrase II, SPINK1 or trypsinogen.[18] Another condition where a false-negative IgG_4 test result could be expected is previous treatment with steroids.[19]

In conclusion, AIP and IAC are emerging entities, whose diagnosis relies on clinical, radiological, biochemical and histological criteria. In clinical practice, malignant diseases of the biliary tree and pancreas are important differential diagnoses. Clear diagnostic criteria, ideally based on non-invasive tests, are required to avoid unnecessary surgery and to discriminate inflammatory from neoplastic diseases. Practical limitations of HISORt criteria are that invasive procedures are required to obtain histology and that the response to steroids can only be assessed after the presumed diagnosis has been made.

The present study confirms that the proposed threshold for IgG_4 is in the range of $\geq$135 mg/dl and shows that in spite of its excellent negative predictive value, its positive predictive value renders it insufficient to be used as an isolated parameter. Furthermore, serum IgG_4 is not suited as a first-line screening test, owing to the low prevalence of IgG_4-related diseases and the high rate of false-positive results. Particularly liver cirrhosis is an important differential diagnosis and commonly associated with elevated IgG_4.

Conflicts of interest

None declared.

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