

Potential Value of Serum Total IgE for Differentiation between Autoimmune Pancreatitis and Pancreatic Cancer

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Abstract

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

Introduction

Autoimmune pancreatitis (AIP) is the pancreatic manifestation of a systemic inflammatory disorder, characterized by infiltration of IgG₄-positive plasma cells and marked interstitial fibrosis, which is responsive to steroid therapy [1, 2]. Extrapaneatic manifestations of the disease include cholangitis, retroperitoneal fibrosis, Mikulicz's disease, interstitial nephritis and pseudotumours of lungs and mediastinum [1, 3]. If located to the pancreatobiliary region, the disorder may mimic cancer of the pancreatic head or cholangiocarcinoma. In approximately 5% of pancreatoduodenectomies performed owing to suspicion of pancreatic carcinoma, the resected lesion turns out to be a benign, inflammatory lesion [4]. Hamano *et al.* [5], who were the first to report high serum IgG₄ concentrations in 20 Japanese patients with autoimmune pancreatitis, found that a serum IgG₄ level of >1.35 g/l had 95% sensitivity and 97% specificity for the differentiation of AIP from pancreatic cancer. They suggested that the measurement of serum IgG₄ in patients suspected of having pancreatic cancer may help reduce the incidence of unnecessary surgery. In a western population of 45

patients, Ghazale *et al.* [6] noted a 76% sensitivity and 93% specificity of a serum IgG₄ level of >1.40 g/l. This difference in sensitivity between Japanese and western populations may be caused by the occurrence of two subsets in AIP: type 1 AIP and type 2 AIP [7], of which only type 1 is characterized by elevated serum IgG₄ and an increased IgG₄-positive lymphoplasmacytic infiltrate.

The pathogenesis of AIP is unclear. The inflammatory reaction has both characteristics of autoimmune (Th1-mediated) and allergic (Th2-mediated) responses [8, 9]. Okazaki *et al.* [8] proposed a biphasic mechanism consisting of 'induction' by pro-inflammatory cytokines produced by Th1 cells and 'progression' by Th2 immune responses. When an allergy-like reaction is part of the progression of the disease, IgE levels might be elevated in AIP. Hamano *et al.* [5] found no significant difference in total IgE level between 20 patients with sclerosing pancreatitis and 20 normal individuals. Umemura *et al.* [10] found an elevated serum total IgE in 35% of 17 patients with autoimmune pancreatitis. After 4 weeks of glucocorticoid therapy of seven patients, both IgG₄ and IgE decreased to a level of about 30% of the median pre-treatment value [10]. Masaki *et al.* [11] reported a 20-fold

higher total IgE level in 64 patients with IgG₄-positive multi-organ lymphoproliferative syndrome compared with 33 patients with Sjögren's syndrome (Ss); the total IgG₄ level of these 64 patients was about 30-fold higher than that in the 33 patients with Ss. In this study, we further investigated the relationship between serum total IgE and total IgG₄ in patients with AIP, in patients with pancreatic carcinoma and in patients with atopic disease.

Patients and methods

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

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

For statistical analysis of the data, we used GRAPH PAD PRISM for Windows, version 5.01, Graph Pad Software, San Diego, CA, USA. For comparison between groups, the Mann–Whitney test was applied; the relationship between total IgG₄ and total IgE within groups was analysed by the Spearman rank correlation test. For analysis of the difference between receiver operating characteristic (ROC) curves, derived from the same samples,

we used MEDCALC Version 11.3, Medcalc Software, B-9030 Mariakerke, Belgium.

Results

Both total IgE and total IgG₄ levels of the 13 patients with AIP were significantly higher than those in 12 patients with pancreatic carcinoma ($P = 0.0004$ and $P = 0.015$, respectively) (Figs. 1 and 2). No significant difference was observed for total IgE and total IgG₄ between the 13 patients with AIP and 14 patients with atopy ($P = 0.14$ and $P = 0.07$, respectively). Total IgE level in the atopic group was significantly higher than that in the patients

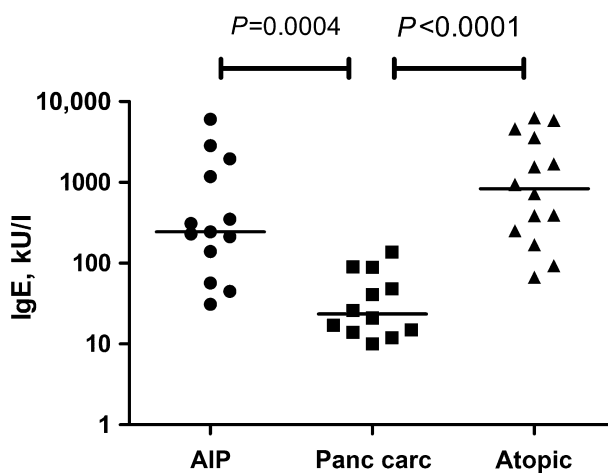


Figure 1 Serum total IgE in autoimmune pancreatitis, pancreatic carcinoma and atopics. Horizontal bars: median IgE level: 244, 24 and 833 kU/l.

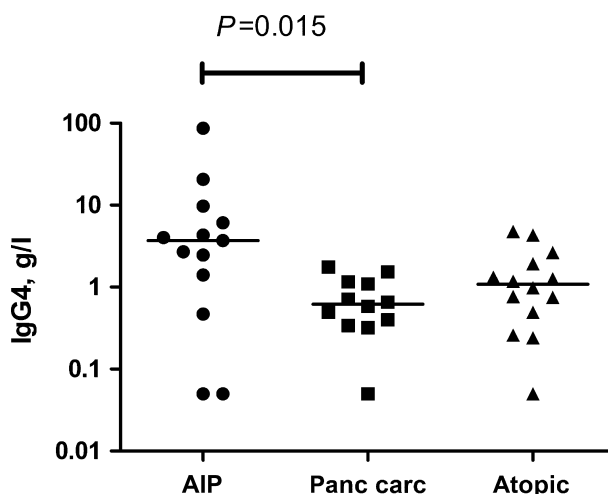


Figure 2 Serum total IgG₄ in autoimmune pancreatitis, pancreatic carcinoma and atopics. Horizontal bars: median IgG₄ level: 3.70, 0.62 and 1.08 g/l. Three sera with a total IgG₄ level of <0.05 g/L were assigned a value of 0.05 g/L.

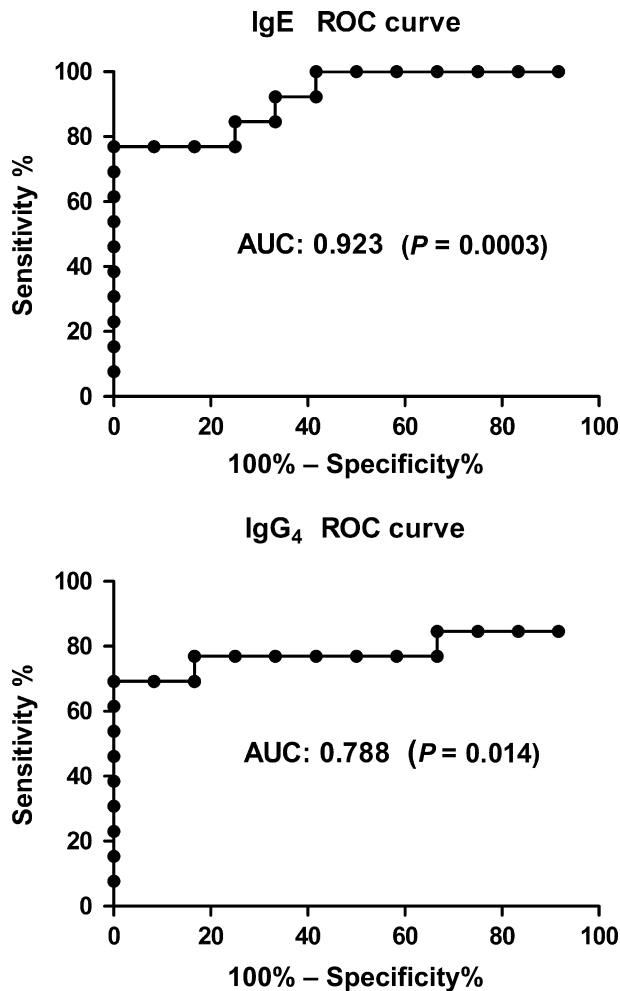


Figure 3 Receiver operating characteristic curves for the combined data from autoimmune pancreatitis patients and patients with pancreatic carcinoma.

with pancreatic carcinoma ($P < 0.0001$); this was not the case for total IgG₄ ($P = 0.21$) (Figs. 1 and 2). The diagnostic power of serum IgE and IgG₄ for discrimination between AIP and pancreatic carcinoma was analysed by the calculation of ROC curves (Fig. 3) for the combined data from the patients with AIP and the patients with pancreatic carcinoma. At a level of 1.6 g/l, total IgG₄ had 69% sensitivity and 92% specificity; at a level of 138 kU/l, total IgE had 77% sensitivity and 100% specificity. Although the area under the curve (AUC) for IgE (0.923) was larger than the AUC for IgG₄ (0.788), this difference did not reach statistical significance ($P = 0.15$), probably because of the small number of patients involved.

There was a significant correlation between total IgE and total IgG₄ levels in the patients with AIP, as well as in the patients with IgE-mediated allergy ($r_s = 0.82$, $P = 0.0006$, respectively, $r_s = 0.88$, $P < 0.0001$) (Fig. 4). In the samples from patients with pancreatic carcinoma, low levels of both IgE and IgG₄ were found, without a

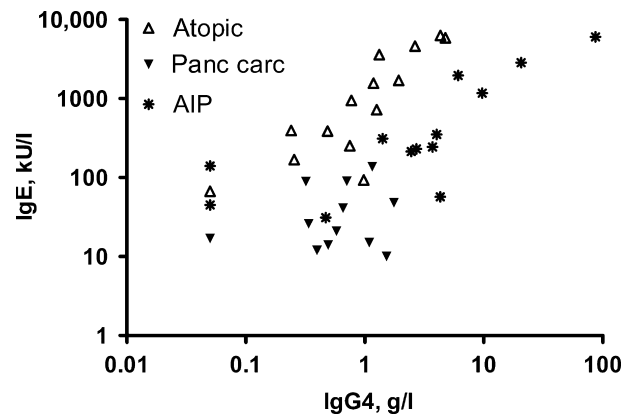


Figure 4 Relationship between total IgG₄ and total IgE in autoimmune pancreatitis (AIP), pancreatic carcinoma and atopy. AIP: $r_s = 0.82$, $P = 0.0006$ and atopy: $r_s = 0.88$, $P < 0.0001$. Three sera with a total IgG₄ level of <0.05 g/l were assigned a value of 0.05 g/l.

significant correlation between these two variables ($r_s = 0.14$, $P = 0.65$). The IgE and IgG₄ levels in Fig. 4 suggest that the patients with AIP and atopy have a different aetiology; however, the patients with pancreatic carcinoma patients show a trend similar to the AIP group. Indeed, when we compared the IgE/IgG₄ ratio's of the individual samples of the three different groups, we found a significant difference between patients with AIP and atopy. In Fig. 5, the IgE/IgG₄ ratio is depicted for the same sera shown in Fig. 4. The IgE/IgG₄ ratio for sera from patients with atopy was significantly different ($P = 0.0012$) from this ratio in sera from patients with AIP and also significantly different from patients with pancreatic carcinoma ($P < 0.0001$); interestingly,

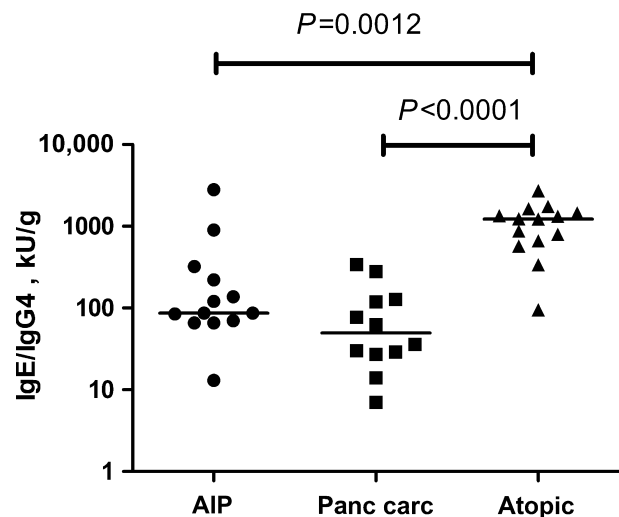


Figure 5 Serum IgE/IgG₄ ratio in autoimmune pancreatitis, pancreatic carcinoma and atopy. Horizontal bars: median IgE/IgG₄ ratio 87, 49 and 1234 kU/g. Three sera with a total IgG₄ level of <0.05 g/l were assigned a value of 0.05 g/l.

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

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

Discussion

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

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

In the study of Hamano *et al.* [5], total IgE was not significantly different between patients with AIP and normal individuals, whereas in the present study IgE was significantly higher in patients with AIP than in patients with pancreatic carcinoma. The ELISA used by Hamano *et al.* for total IgE analysis was not specified; we used the automated ImmunoCAP system with calibrators traceable to the WHO preparation 75/502 for human IgE. Our results are in line with the data published by Masaki *et al.* [11], who reported a 20-fold higher total IgE level in 64 patients with IgG₄-positive multi-organ lymphoproliferative syndrome compared with 33 patients with Sjögren's syndrome. The correlation we observed between serum total IgE and IgG₄ (Fig. 4) could theoretically be explained by a novel heterophilic antibody activity of

IgG₄, proposed by Ito *et al.* [18]. The significant difference between the IgE/IgG₄ ratios of patients with AIP and patients with atopy (Fig. 5), however, makes this explanation unlikely. Furthermore, we found no evidence for heterophilic antibody interference in the total IgE assay that was used in the present study, even in sera that showed high interference in the Unicap tryptase immunoassay (see Table S1).

To our knowledge, this is the first report that describes a correlation between serum total IgG₄ and total IgE in atopic individuals and in patients with autoimmune pancreatitis. Meiler *et al.* [19] showed that T-regulatory cells regulate antibody isotypes, which may contribute to the suppression of inflammatory diseases by the induction of specific IgG₄ and by the suppression of specific IgE. It is remarkable therefore to find a close association between total IgG₄ and total IgE both in atopic individuals and in patients with autoimmune pancreatitis. Zen *et al.* [9] concluded that the predominant Th2 and regulatory immune reactions in AIP might reflect an allergic nature in their pathogenesis. The simultaneous elevation of both IgG₄ and IgE in AIP and the correlation between these two markers support this view. Choi *et al.* [20] recently suggested that in AIP, there may be a defect in the function of regulatory T cells, which normally prevents autoimmune disease progression via a suppressor mechanism. Regulatory T cells also play a role in the suppression of IgE-mediated allergy [19, 21]. An abnormality in regulatory T cells therefore could be the common cause of elevated IgE and IgG₄ in AIP.

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

Disclosure of potential conflict of interest

All authors declare that they have no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Serum tryptase, human anti-mouse antibodies (Hama) and total IgE in sera before and after incubation of these sera in Heterophilic blocking tubes (HBT).

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Supporting information for the paper:

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

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

Tryptase ug/l without HBT	Tryptase ug/l with HBT	Hama ng/ml without HBT	Hama ng/ml with HBT	Tot.IgE kU/l without HBT	Tot.IgE kU/l with HBT
6.91	6.59	<1.5	<1.5	413	372
7.96	5.37	21.1	<1.5	5.34	4.93
4.77	4.45	<1.5	<1.5	5.92	4.73
5.92	4.02	3.4	<1.5	53	50.2
52.1	5.78	25.9	1.6	83.7	97.4
3.34	3.37	<1.5	<1.5	13.5	13.2
6.65	6.4	<1.5	<1.5	230	219
4.38	4.4	1.6	<1.5	31	27.2
21.3	14.4	40.8	<1.5	1109	1140
10.1	4.36	43.2	<1.5	20.6	16.4
7.08	5.18	5.6	<1.5	19.2	21
13.8	4.36	92.1	<1.5	49.2	47.1
5.11	3.33	46.7	<1.5	41.2	37.4
84.6	7.47	254	<1.5	3.95	3.86
11,8	9.03	21.7	<1.5	368	323

Estimation of serum tryptase and Hama and incubation of sera in HBT were performed as described in:

van Toorenenbergen AW, Hooijkaas H, Klein Heerenbrink G, Dufour-van den

Goorbergh DM. Heterophilic antibody interference in a tryptase immunoassay. Clin

Biochem 2008;41:331–334.

In four sera HBT treatment caused > 50% reduction of serum tryptase, indicating that heterophilic antibodies were present that caused a falsely elevated serum tryptase. HBT treatment decreased the levels of Hama to amounts below (or borderline above) the detection limit of the Hama assay; in contrast, there was no significant difference ($p = 0.094$) between total IgE in sera before and after HBT treatment.