Original Research

Value of Serum IgG4 in the Diagnosis of Autoimmune Pancreatitis and in Distinguishing it from Acute and Chronic Pancreatitis of Other Etiology

Xiaoqin Zhu, MD, PhD;[1] M. Rabie Al-Turkmani, PhD;[1,2] Lokinendi.V. Rao, PhD[1,2]*

[1] *Department of Pathology, UMass Memorial Medical Center and University of Massachusetts Medical School, Worcester, MA*
[2] *Quest Diagnostics, North Region, Marlborough, MA*

Immunoglobulin G4 (IgG4)-related autoimmune pancreatitis (AIP) is one of the most common clinical presentations of the IgG4-related disease and distinct from acute pancreatitis and chronic pancreatitis derived from other etiology. Elevated serum IgG4 concentration has been established as one of the diagnostic criteria for AIP. However, a subset of patients of AIP have normal serum IgG4. It is essential to establish the value of serum IgG4 level in identifying AIP patients in clinical setting, and its association with the severity of the disease and the pathology diagnosis. We performed a retrospective study involving 67 patients who were identified to have elevated serum IgG4 measurements (>86 mg/dL) from a pool of 833 patients in our institute from 2012 to 2015. The concentration of serum IgG4 was significantly higher in AIP compared to acute pancreatitis and chronic pancreatitis of other etiology ($p<0.01$). The sensitivity of distinguishing AIP from acute and chronic pancreatitis of other etiology increased from 61% to 100% as the cutoff value for serum IgG4 was set from 135 mg/dL to 86 mg/dL. On the other hand, the specificity was decreased from 98% to 91%. A cutoff value of 120 mg/dL gave rise to the highest specificity without sacrificing the sensitivity. The level of serum IgG4 exhibited a trend to associate with the severity of IgG4-related AIP determined clinically based on the endoscopic ultrasound findings, although it did not reach statistical significance (p value = 0.05). All the pathology-proven cases of IgG4-related AIP exhibited elevated serum IgG4 concentration. Only one of eight cases of non-AIP cases confirmed by pathology showed elevated serum IgG4.

[N A J Med Sci. 2017;10(3):94-99. **DOI:** *10.7156/najms.2017.1003094]*

Key Words: *IgG4-related disease, serum IgG4, autoimmune pancreatitis, acute pancreatitis, chronic pancreatitis*

INTROUCTION

Chronic pancreatitis is usually characterized by a relentless and progressive loss of pancreatic parenchymal tissue, eventually leading to endocrine and exocrine insufficiency. The major causes of chronic pancreatitis include heavy consumption of alcohol, obstruction of pancreatic duct, pancreas divisum or idiopathic etiology.[1] Autoimmune pancreatitis (AIP) was introduced by Yoshida et al. in 1995 as one type of chronic pancreatitis that is associated with autoimmune manifestations revealed on laboratory, histologic and clinical testing.[2] In 2001, Hamano et al. reported high serum IgG4 concentration in the patients with sclerosing pancreatitis, which was a historical hallmark of AIP and IgG4-related disease (IgG4-RD).[3]

IgG4-related AIP is also named as type-1 AIP, distinct from type-2 AIP which is not associated with elevated IgG4 levels or extrapancreatic disease.[4] IgG4-RD is a systemic fibro-inflammatory disease that may affect almost any organ sites involving pancreatobiliary tract, liver, salivary gland, orbit, lymph node, retroperitoneum, aorta, mediastinum, soft tissue, kidney, breast, lung, and thyroid etc. IgG4-RD exhibited various clinical manifestations that are referable to the specific targeted organs. Patients with IgG4-related AIP often presented with common symptoms of chronic pancreatitis such as obstructive jaundice, weight loss, nonspecific abdominal pain, steatorrhea, and prior history of acute/chronic pancreatitis.[5] These symptoms are indistinguishable from chronic pancreatitis of other etiology. Because the steroid therapy is very effective in treating IgG4-related AIP, the diagnosis of IgG4-related AIP has significant impact on prognosis and treatment of the patient. A HISORt criteria was proposed by Mayo Clinic and were widely accepted.[6] These include five characteristics: (a) Diagnostic histology: Periductal lymphoplasmacytic infiltrate with storiform fibrosis and obliterative phlebitis; (b) Characteristic imaging: diffuse pancreatitic enlargement with irregular narrowing of main pancreatic duct; (c) Serology: elevated serum IgG4 with or

Received: 06/15/2017; Revised: 07/14/2017; Accepted: 07/20/2017
***Corresponding Author:** Department of Pathology, UMass Memorial Medical Center and University of Massachusetts Medical School, Worcester, MA. (Email: Lokinendi.Rao@umassmemorial.org)

without elevated serum total IgG; (d) Other organ involvement of bile duct retroperitoneum, salivary gland, or mediastinum; and (e) Response to steroid therapy. The diagnosis of IgG4-related AIP was made in those with more than one of the following: (a) diagnostic histology, (b) characteristic pancreatic imaging and abnormal serology, or (c) pancreatic disease with abnormal serology and/or other organ involvement that shows response to steroids.

Serum IgG4 concentrations are often considered useful in rendering the diagnosis, in assessing the response to the treatment and in predicting to the future therapeutic regimen.[7] In general, a serum IgG4 concentration > 135 mg/dL has been established as the cutoff value for the diagnosis of patients with IgG4-RD when normal serum IgG4 level ranges from 8 - 140 mg/dL measured by automated nephelometry.[5] However, the cutoff values of serum IgG4 for distinguishing AIP from other pancreatic disorders vary from study to study based on distinct test system.[8] Therefore, it is essential to provide an established cutoff value of serum IgG4 for the diagnosis of AIP in our system. In addition, it is undetermined whether the serum IgG4 level relates to the severity of the disease. Provided the verified application of endoscopic ultrasound (EUS) in diagnosing, ruling out and judging the severity of chronic pancreatitis,[9] we evaluated the association of serum IgG4 level with the EUS findings. Lastly we further examined the correlation of pathologic findings with serum IgG4 level in patients with or without AIP.

Table 1. Summary of the cases with elevated serum IgG4 (> 86 mg/dL).

Autoimmune pancreatitis	**23**
Suspected autoimmune pancreatitis/hepatitis	**9**
Suspected autoimmune pancreatitis	8
Suspected autoimmune hepatitis	1
Non-IgG4-related diseases	**35**
1. Acute pancreatitis	10
2. Chronic pancreatitis	4
3. interstitial lung disease	
Interstitial lung fibrosis	1
Sarcoidosis	2
4. Infectious disease	
Pneumonia and upper respiratory infection	6
C-diff infection	2
Soft tissue infection	2
Recurrent otitis	1
Hepatitis C	1
5. Autoimmune diseases	
Primary sclerosing cholangitis	2
Sjogren's syndrome	1
6. Miscellaneous	
Fibromyalgia syndrome	1
Recurrent neck lymph nodes	1
IgA immunodeficiency	1

METHODS

A medical record search identified 833 patients who underwent serum IgG4 testing at UMass Memorial Medical Center from January 2012 to July 2015. Out of 833 patients, 60 patients had acute pancreatitis and 131 patients had chronic pancreatitis. Among the patients with chronic pancreatitis, 23 patients were clinically diagnosed as AIP and 8 patients were suspected to have AIP. All of the patients who were diagnosed or suspected to have AIP exhibited elevated serum IgG4 (> 86 mg/dL). The diagnosis of definitive AIP was rendered in patients with elevated serum IgG4 when pathology diagnosis was achieved and/or a characteristic pancreatic imaging and marked improvement/resolution of pancreatic manifestation with steroid treatment were demonstrated. Patients were suspected to have AIP if they had never had undergone a tissue biopsy or been monitored with steroid treatment, but had chronic pancreatitis and a typical radiographic appearance. In these patients, all other etiologies of chronic pancreatitis, including alcohol, obstruction or hyperglyceridemia have been ruled out. There were 36 patients with elevated serum IgG4 and non-AIP diseases including one patient with suspected autoimmune hepatitis (**Table 1**).

The severity of chronic pancreatitis was determined by EUS findings according to EUS-based criteria for the diagnosis of chronic pancreatitis.[9-11] Pathologic diagnosis of IgG4-related AIP was made according to an international pathology consensus guideline including at least two of the three major histopathologic features of the disease: a lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis.[12] Additionally, immunostaining of patients' biopsy samples had to demonstrate >50 IgG4+ plasma cells per high power field (HPF) and an IgG4/IgG ratio > 40%.

Total IgG, and IgG subclasses (IgG1, IgG2, IgG3, and IgG4) were measured by Turbidimetry, using SPAPLUS® (The Binding Site Inc; San Diego, CA). The reference range of IgG4 was 4-86 mg/dL.

The statistical analyses were performed using ANOVA Welch's Test and Games-Howell pairwise comparison test, with Minitab software 17 (Minitab Inc, State College, PA).

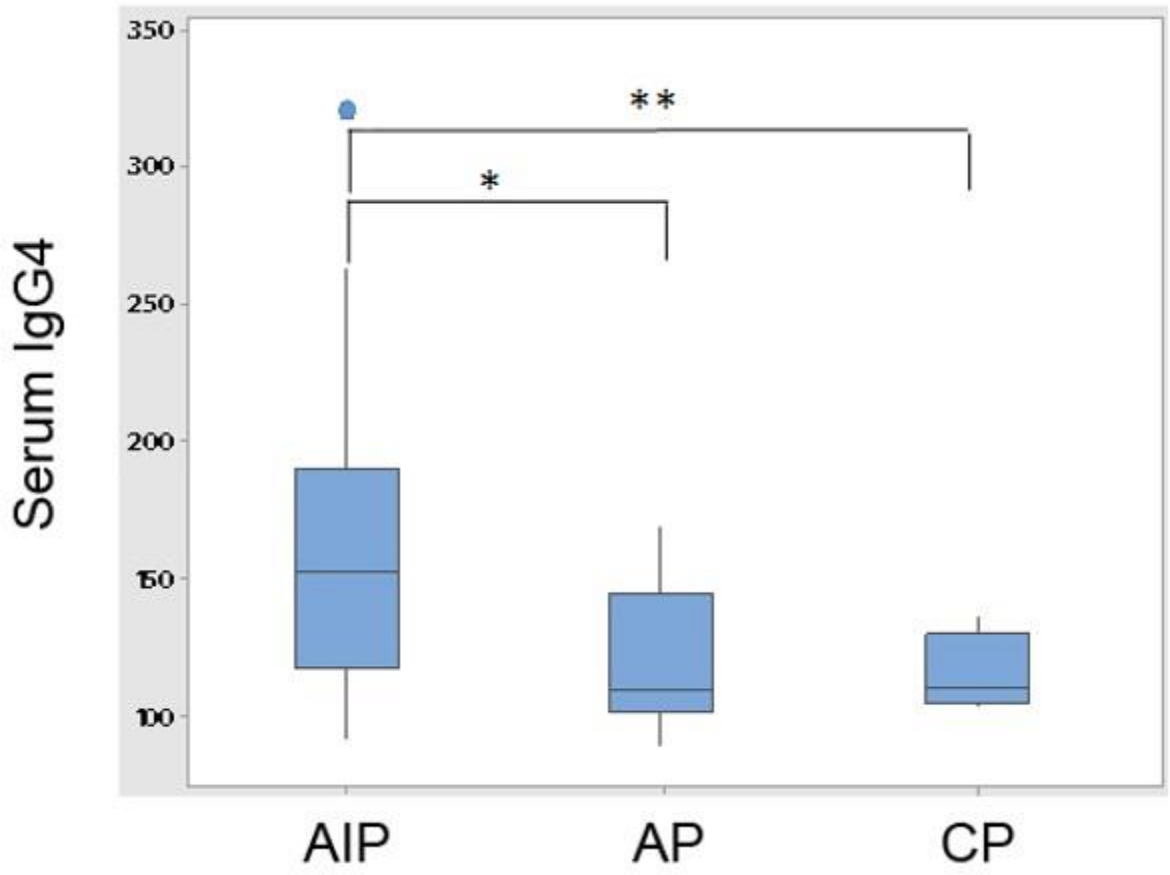

Figure 1. Boxplot of serum IgG4 in AIP, AP and CP.

Table 2. Sensitivity, specificity, positive predicative value (PPV) and negative predicative value (NPV) of different cutoff value of serum IgG4 for diagnosis of AIP.

IgG4 cutoff value (mg/dL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgG4 > 86	100	91	69	100
IgG4 > 110	81	96	78	96
IgG4 > 120	71	98	85	95
IgG4 > 135	61	98	83	95

RESULTS

Diagnosis Associated with Elevated Serum Igg4

Table 1 shows the distribution of diseases for cases with elevated IgG4 level (> 86 mg/dL). Among 67 patients, 34% were diagnosed as autoimmune pancreatitis, 12% were suspected to autoimmune pancreatitis, and 52% had other non-IgG4-related diseases including acute and chronic pancreatitis, interstitial lung disease, infectious disease and other autoimmune diseases, etc.

Clinical Features

The age of 23 patients with AIP (mean age = 45 years; range: 27 - 68 years) was similar to those of the 160 patients with

acute or chronic pancreatitis of other etiologies (mean age = 48 years; range: 20 - 82 years) (t-test; p value = 0.50). There was a male predominance in AIP (male to female ratio = 2.3) compared to acute pancreatitis and other forms of chronic pancreatitis (male to female ratio = 0.9) ($\chi 2$ test; p value = 0.047).

Comparison of Serum Igg4 Levels in Different Diseases

We compared the mean concentration of serum IgG4 in patients with autoimmune pancreatitis, acute and chronic pancreatitis (**Figure 1**). The average level of serum IgG4 in suspected AIP was 170±80 mg/dL, similar to that of diagnosed AIP (167±66 mg/dL). Ten of 60 patients with acute pancreatitis and 4 of 100 patients with non-autoimmune chronic pancreatitis also had elevated serum IgG4 levels. The mean serum IgG4 concentrations in these patients were 120±30 and 115±14, respectively, significantly lower than that in both diagnosed and suspected AIP ($p < 0.01$). The mean value of extra-pancreatic diseases with elevated IgG4 was 129±67, which was not statistically lower than IgG4 level of AIP.

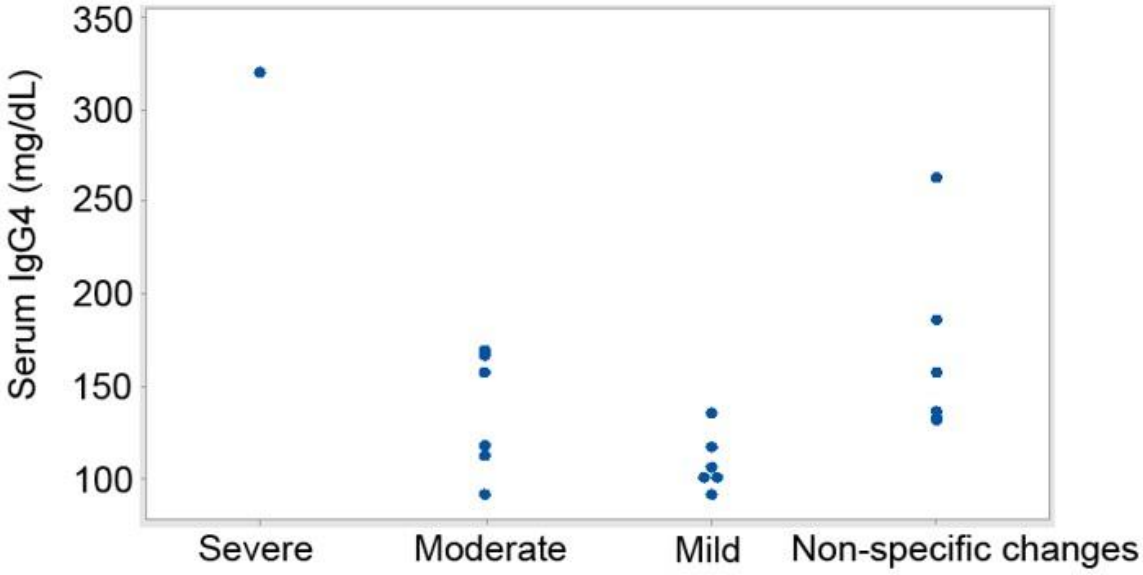

Figure 2. The level of serum IgG4 in autoimmune pancreatitis with various degree of severities is not significantly different (ANOVA Welch test, p-value = 0.05). The severity of the autoimmune pancreatitis is determined based on the endoscopic ultrasound reports.

- Severe: severe chronic pancreatitis
- Moderate: moderate chronic pancreatitis
- Mild: mild chronic pancreatitis
- Non-specific changes: hyperechoic strand/hypoechoic foci in pancreas

Table 3. Serum IgG4 level of pathology-proven non-IgG4-related chronic pancreatitis and IgG4-related autoimmune pancreatitis.

Pathology Diagnosis			
Non-IgG4-related CP		**IgG4-related AIP**	
Case #	Serum IgG4 (mg/dL)	Case #	Serum IgG4 (mg/dL)
1	17	9	133
2	34	10	105
3	74	11	155
4	53		
5	28		
6	27		
7	2		
8	136		

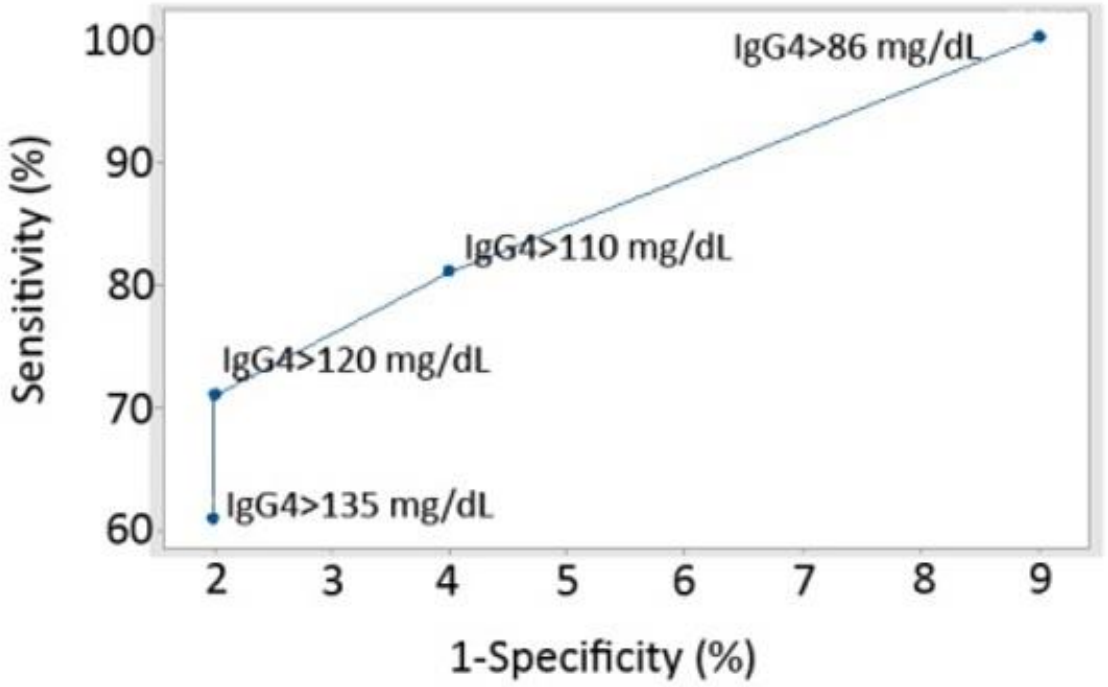

Figure 3. Receiver Operating Characteristic (ROC) curve of sensitivity vs 1-specificity at four cutoff levels of serum IgG4 concentration.

Evaluation of Cutoff Values of Serum Igg4 for Diagnosis of AIP

The cutoff values were evaluated at four different levels to detect AIP and separate it from acute or non-autoimmune chronic pancreatitis (**Table 2** and **Figure 3**). When the upper limit of the reference range (86 mg/dL) was applied as cutoff, the specificity and positive predictive value were 91% and 69%, respectively. At a cutoff of 110 mg/dL, the sensitivity was 81% and specificity was 96%. The positive and negative predicative values (PPV and NPV) were 78% and 96% at this cutoff. When the cutoff level increased to 120 mg/dL, the sensitivity dropped to 71% and specificity changed to 98%. PPV increased to 85% while NPV was similar at 95%. At 135 mg/dL cutoff value, sensitivity further decreased to 61% and specificity remained at 98%. The PPV and NPV were 83% and 95%, respectively. Therefore, a critical value of 120 mg/dL yielded the highest specificity and PPV without sacrificing the sensitivity and NPV.

Association of Serum Igg4 Level with Disease Severity

A trend was observed in the correlation between serum IgG4 and the severity of the disease. However, there is no statistically significant difference in serum IgG4 concentrations between patients with mild or moderate diseases ($p = 0.05$; **Figure 2**). The severity of chronic pancreatitis in 13 patients who underwent EUS was graded into mild, moderate or severe based on pancreatographic features. The mean value of serum IgG4 for 6 patients with each mild or moderate disease was 109±16 mg/dL and 137±33 mg/dL, respectively. However, only one patient was graded as severe and the serum IgG4 was 320 mg/dL, the highest level the system could detect.

Correlation of Pathologic Diagnosis with Serum Igg4 Level

In order to determine the proportion of patients with consensus IgG4-related AIP or lacking pathologic evidence of IgG4-related AIP had elevated serum IgG4, we found 11 patients who had chronic pancreatitis and also underwent tissue biopsy (**Table 3**). Among 8 cases which did not show pathologic evidence of IgG4-related pancreatitis, only 1 patient had elevated serum IgG4 at 136 mg/dL. All 3 patients that were proven to have IgG4-related AIP by biopsy had increased IgG4 levels in serum (105, 133, and 155 mg/dL).

DISCUSSION

IgG4-related AIP is a form of chronic pancreatitis which shows typical histopathologic features and clinical pictures of systemic involvement and serum IgG4 elevation, distinct from other non-autoimmune or type-2 autoimmune pancreatitis.[4] The analysis of clinical data from our institute revealed a male predominance in AIP with male to female ratio of 2.3, consistent with previous series of studies.[13]

Serum IgG4 concentrations have been demonstrated to be high in patients with AIP and can be utilized to distinguish this disorder from other diseases of the pancreas or biliary tract.[3] However, Ghazale et al revealed that mild (< 2-fold) elevations in serum IgG4 are also seen in up to 10% of subjects without AIP. With sensitivities and specificities of 76% and 93% at a cutoff of 140 mg/dL, IgG4 concentrations are inadequate for

the purpose of differentiating AIP from pancreatic cancer.[5] A recent study focused on analysis of multiorgan IgG4-RD did not prove the utility of serum IgG4 in diagnosing IgG4-RD due to elevated IgG4 in other non-IgG4-RD conditions and lack of elevation of IgG4 in some patients with IgG4-RD.[14] Similarly, we also found that around 50% of patients with increased IgG4 were associated with various non-IgG4-RD situations, including 17% or 4% of patients with acute or non-autoimmune chronic pancreatitis.

Our study demonstrated that the elevation of serum IgG4 concentrations in acute and non-autoimmune chronic pancreatitis was significantly lower than that in patients with AIP. At a cutoff of 135 mg/dL, a well-accepted cutoff value for diagnosis of AIP, the sensitivity and specificity were 61% and 98%, and PPV and NPV were 83% and 95%. As the cutoff decreased to 120 mg/dL, the sensitivity and PPV increased, and the specificity and NPV remained unchanged. Although a cutoff of 110 mg/dL gave a higher sensitivity, specificity and PPV dropped simultaneously. The widely adopted serum IgG4 > 135 mg/dL as a cutoff value for diagnosis of IgG4-RD was initially determined by comparing patients with IgG4-related AIP and those with pancreatic cancer.[3] The study from Ghazale et al., revealed that 9% of patients with pancreatic cancers had elevation of IgG4 between 140 and 280 mg/dL or higher.[5] However, our observation showed that more than 70% of patients with acute or non-autoimmune chronic pancreatitis and elevated IgG4 had IgG4 levels lower than 135 mg/dL. Meanwhile, only 61% of patients with AIP had IgG4 higher than 135 mg/dL. Therefore, we propose that the cutoff of IgG4 for differentiation of AIP from acute or non-autoimmune chronic pancreatitis to be at least 120 mg/dL. It is worthy to point out that the reference range of serum IgG4 was 8-140 mg/dL in many studies,5 while the reference range of serum IgG4 in our test system is 4-86 mg/dL. The difference in the reference range of serum IgG4 may account for the lower threshold for AIP using our system.

Our study is the first to analyze the correlation of serum IgG4 levels with the severity of chronic pancreatitis based on EUS findings. Our data revealed a trend of higher IgG4 levels with increasing severity on imaging, although it did not reach statistical significance. The limited number of cases prohibited further investigation of this observation. However, the analysis is meaningful as it shows the connection between two commonly applied methods for examining AIP. Increasing the statistical power is needed for future studies for this purpose.

Pathologic diagnosis has been recognized as the gold standard for the diagnosis of AIP. Association between the pathologic diagnosis and serum IgG4 remains to be determined. This include two frequently asked questions: 1) What proportion of patients with consensus IgG4-related RD pathology have elevated IgG4 levels; 2) Among the patients lacking pathologic evidence of IgG4-RD, what percentage have elevated IgG4.[15] Recent studies involving multi-organ IgG4-RD pointed out that a substantial subset of patients with biopsy-proven IgG4-RD do not have elevated serum IgG4.[14,16] Our study is specifically focused on IgG4-related AIP. All three cases of biopsy-proven IgG4-related AIP had elevated IgG4 ranging from 105 – 155 mg/dL. Only one of eight cases of non-IgG4-related chronic pancreatitis had elevated IgG4 at 136 mg/dL. A study to look into more cases and multicenter cohorts is warranted.

In conclusion, a serum IgG4 cutoff value of 120 mg/dL can be used to distinguish AIP from acute pancreatitis and chronic pancreatitis of other etiology. Serum IgG4 levels exhibit a trend to correspond with disease severity of chronic pancreatitis determined by EUS. They also show correlation with pathologic result from the cases in our institute.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

REFERENCES

1. Steer ML, Waxman I, Freedman S. Chronic pancreatitis. N Engl J Med. 1995;332:1482-1490.
2. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Digest Dis Sci. 1995;40:1561-1568.
3. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001;344:732-738.
4. Morse B, Centeno B, Vignesh S. Autoimmune pancreatitis: updated concepts of a challenging diagnosis. Am J Med. 2014;127:1010 e1-9.
5. Ghazale A, Chari ST, Smyrk TC, et al. Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer. Am J Gastroenterol. 2007;102:1646-1653.
6. Chari ST, Smyrk TC, Levy MJ, et al. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. Clin Gastroenterol Hepatol. 2006;4:1010-1016; quiz 934.
7. Tabata T, Kamisawa T, Takuma K, et al. Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease. Intern Med. 2011;50:69-75.
8. Lian M-J, Liu S, Wu G-Y, Liu S-Y. Serum IgG4 and IgG for the diagnosis of autoimmune pancreatitis: A systematic review with meta-analysis. Clin Res Hepatol Gastroenterol. 2016;40:99-109.
9. Sahai AV, Zimmerman M, Aabakken L, et al. Prospective assessment of the ability of endoscopic ultrasound to diagnose, exclude, or establish the severity of chronic pancreatitis found by endoscopic retrograde cholangiopancreatography. Gastrointest Endosc. 1998;48:18-25.
10. Axon AT, Classen M, Cotton PB, Cremer M, Freeny PC, Lees WR. Pancreatography in chronic pancreatitis: international definitions. Gut. 1984;25:1107-1112.
11. Catalano MF, Sahai A, Levy M, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointest Endosc. 2009;69:1251-1261.
12. Deshpande V, Zen Y, Chan JK, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol. 2012;25:1181-1192.
13. Deshpande V, Mino-Kenudson M, Brugge W, Lauwers GY. Autoimmune Pancreatitis: More Than Just a Pancreatic Disease? A Contemporary Review of Its Pathology. Arch Pathol Lab Med. 2005;129:1148-1154.
14. Carruthers MN, Khosroshahi A, Augustin T, Deshpande V, Stone JH. The diagnostic utility of serum IgG4 concentrations in IgG4-related disease. Ann Rheum Dis. 2015;74:14-18.
15. Fox RI, Fox CM. IgG4 levels and plasmablasts as a marker for IgG4-related disease (IgG4-RD). Ann Rheum Dis. 2015;74:1-3.
16. Hart PA, Smyrk TC, Chari ST. Lymphoplasmacytic sclerosing pancreatitis without IgG4 tissue infiltration or serum IgG4 elevation: IgG4-related disease without IgG4. Mod Pathol. 2015;28:238-247.