Role of apoptosis inhibitor of macrophages in patients with IgG4-related disease/autoimmune pancreatitis and the clinical characteristics associated with this condition

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Abstract. Type 1 autoimmune pancreatitis (AIP) is a pancreatic manifestation of IgG4-related disease (IgG4-RD) and is a unique chronic inflammatory disease characterized by fibrosis. IgG4-RD is caused by an autoimmune mechanism that mimics malignant tumors and inflammatory disorders. Apoptosis inhibitor of macrophages (AIM) can function as a biomarker of autoimmune or inflammatory diseases with tissue fibrosis, including in inflammatory bowel disease and chronic liver disease. Therefore, the aim of the present study was to clarify the role of serum AIM levels and the clinical characteristics of patients with IgG4-RD and AIP. For this purpose, serum AIM concentrations were assessed using ELISA and the association between AIM and the laboratory and clinical data from patients with IgG4-RD/AIP, patients with pancreatic cancer (PC) and healthy controls (HCs), was determined. The results demonstrated that the serum AIM concentrations were not associated with the laboratory data. However, the serum AIM levels were significantly elevated in patients with AIP compared with the HCs and patients with PC. Furthermore, the serum AIM levels significantly decreased following steroid therapy in patients with AIP who were in remission. Overall, the present study demonstrates that serum AIM levels may be a potentially useful biomarker for the differential diagnosis of AIP and for evaluating the therapeutic reactivity of affected patients.

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Introduction

IgG4-related disease (IgG4-RD) is a multiorgan lymphoproliferative disease characterized in the majority of patients by increased serum IgG4 concentrations. It is considered to be a systemic, chronic, inflammatory disorder characterized by enlarged organs, the abundant infiltration of $IgG4^+$ plasmacytes and fibrosis in the involved organs. Yoshida *et al* (1) reported that chronic pancreatitis caused by an autoimmune mechanism responded well to steroid therapy. Moreover, Hamano *et al* (2) described this disorder as autoimmune pancreatitis (AIP) with high serum IgG4 concentrations. Therefore, AIP is described as a pancreatic manifestation of IgG4-RD (3,4). IgG4-RD is an immune-mediated condition that mimics malignant and inflammatory disorders.

IgG4-RD was initially identified among Japanese patients with sclerosing chronic pancreatitis. The extensive infiltration of IgG4-positive plasma cells and lymphocytes with fibrosis was found in the involved organs, which mimicked malignant tumors or inflammatory diseases. Although the established standard therapy for IgG4-RD and AIP involves the use of steroids, the high relapse rate among patients is concerning. Moreover, half of all patients with AIP exhibit focal (<33% of the whole pancreas) or segmental (>33 and <66%) pancreatic enlargement, which can be difficult to distinguish from pancreatic malignant tumors (5,6). Therefore, it is necessary to investigate novel therapeutics and diagnostic markers.

Differentiated and mature macrophages produce apoptosis inhibitor of macrophages (AIM), which is named based on its function of suppressing macrophage apoptosis (7,8). Arai and Miyazaki (9) suggested that blood AIM concentrations may function as a biomarker for obesity-associated autoimmune disease in humans. As a secretory protein, AIM is found in the blood and its concentration changes in various diseases (8,10,11). In a previous study, it was demonstrated that there was an association between AIM and hepatic fibrosis in chronic hepatitis C (12). It has also been determined that AIM may function as a biomarker for distinguishing between different inflammatory bowel diseases (13). It can thus be hypothesized that AIM may function as a biomarker for autoimmune and inflammatory diseases with tissue fibrosis, such

as IgG4-RD and AIP. However, to the best of our knowledge the clinical significance of AIM in IgG4-RD and AIP has not previously been investigated. Therefore, the aim of the present study was to investigate the role of serum AIM concentrations in patients with IgG4-RD and AIP.

Patients and methods

Patients. The present study was retrospective in nature. In total, 38 patients who fulfilled the comprehensive diagnostic criteria [APS 2011, ICDC, IgG4-RD 2011 (14-16)] of having IgG4-RD and AIP and who were admitted to Kagoshima University, Medical and Dental Hospital (Kagoshima, Japan) between January, 2006 and December, 2016 were enrolled in the study. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was performed in 27 patients with AIP to obtain pancreatic tissue and all tissue was subjected to immunohistochemical analysis. Baseline laboratory findings including serum amylase and IgG4 and CRP levels were retrieved from the patients' medical records. Organ involvement was determined from reviews of each patient's history and the results of a physical examination, computed tomography (CT) scan, magnetic resonance imaging, positron emission tomography/CT, laboratory analyses and tissue biopsies. Moreover, a history of malignant disease was determined from the medical records. Relapse was defined by a symptomatic status or having elevated serum IgG4 concentrations during maintenance therapy. A pathological diagnosis was not necessary (17). Written informed consent was obtained from all patients and healthy controls (HCs). The present study was approved by the Ethics Committee of Kagoshima University Medical and Dental Hospital (approval no. 28-241).

ELISA of serum AIM levels. Baseline serum samples were collected from 32 patients who had not been treated at the time of diagnosis and among these, patient serum samples from only 16 patients who were in remission on steroid therapy were collected before and after treatment. Samples were portioned and stored at -80˚C. The serum AIM concentrations in patients with untreated IgG4-RD with AIP (n=32), patients with AIP before and after treatment in remission (n=17), patients with pancreatic cancer (PC; n=30) who were admitted to the same hospital and healthy controls (HCs; n=32) were determined using ELISA kits (cat. no. KK901, Trans Genic, Inc.) according to the manufacturer's protocol. Briefly, serum samples (1:1,000) were incubated for 1 h at room temperature. The wells were washed three times with PBS and subsequently HRP-conjugated anti-human IgG antibody (1:30, included in the ELISA kit) was added followed by incubation for 10 min at room temperature. The HCs were subjects without AIP or PC and included students and staff recruited from the facility (Kagoshima University).

Immunohistological analysis. Pancreatic tissues from patients with AIP were obtained following EUS-FNA for immunohistological staining as previously described (13). Briefly, tissue samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 2-μm-thick sections, blocked with Protein Block Serum-Free (cat. no. X0909, Dako; Agilent Technologies, Inc.) for 3 h at room temperature and then incubated at room temperature overnight with the following antibodies against: Human AIM (1:400, cat. no. 54264, Anaspec, Inc.), human CD14 (1:200, cat. no. ab45870, Abcam) and human CD16 (1:500, cat. no. MCA2537, Bio-Rad Laboratories, Inc.). Secondary antibodies were donkey anti-rabbit IgG(H+L) Highly Cross Adsorbed Secondary Antibody Alexa Fluor 488 (cat. no. A21206, Thermo Fisher Scientific, Inc.), donkey anti-goat IgG(H+L) Cross Adsorbed Secondary Antibody Alexa Fluor 647 (cat. no. A21447, Thermo Fisher Scientific, Inc.), donkey anti-mouse IgG(H+L) Highly Cross Adsorbed Secondary Antibody Alexa Fluor 555 (cat. no. A231570, Thermo Fisher Scientific, Inc.), respectively (1:200). All were incubated at room temperature for 35 min. Fluorescence emission was analyzed using a fluorescence microscope (BZ9000; Keyence Corporation).

Statistical analysis. All data were analyzed using SPSS software version 24 (IBM Corp.) at least twice. Data were statistically analyzed using a non-parametric Mann-Whitney U test, Kruskal-Wallis test and Dunn's test as post hoc test or the Wilcoxon signed-rank test and Chi-squared test. Correlation coefficients were determined using the Spearman's rank correlation coefficient test. Data are presented as the mean±SD. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of all patients with IgG4-RD/AIP. The characteristics for all the 38 enrolled patients with IgG4-RD and AIP are summarized in Table I. The number and age of the patients was significantly higher for males [n=25 (65.8%); mean age, 69.2±9.1 years] than for females [n=13 (34.2%); mean age, 59.8±7.0 years]. The mean number of organs involved per patient was 2.4. At least two organs were involved in 25 (65.8%) patients. The serum IgG4 and IgG concentrations were 640.0±423.1 and 2,523.2±1,011.9 mg/dl, respectively. The serum concentrations of the soluble IL-2 receptor (1,186.8±524.2 U/ml; normal concentration, 121-613 U/ml) and IgE (966.3±1,185.8 U/ml; normal concentration, <232 U/ml) were elevated. The median serum AIM concentration was 3,891.9±8,374.5 ng/ml. No significant difference was observed in these biomarkers between the sexes.

Serum AIM concentrations are higher in patients with AIP compared with the HCs and patients with PC. Subsequently, the serum AIM concentrations in patients with AIP (n=32), PC (n=30) and the HCs (n=32) were compared. The mean serum AIM concentrations were 2,387.9±1,232.1, 1,740.2±1,470.2 and 1,313.1±631.0 ng/ml in patients with AIP, PC and the HCs, respectively. The serum AIM concentrations were significantly higher in patients with AIP compared with those with PC ($P<0.005$) and the HCs ($P<0.001$) No significant differences were observed in the serum AIM levels between the patients with PC and the HCs ($P=0.37$) (Fig. 1).

Serum AIM concentrations before and after treatment with steroids in patients with AIP. The serum AIM concentrations were compared in patients with AIP before and after treatment with steroid (prednisolones) who were in remission

Table I. Comparison of baseline characteristics and associations between serum AIM levels and clinical parameters in patients with IgG4-RD.

Characteristic	Total	Male	Female	P-value
Sex, n (%)	38	25 (65.8%)	13 (34.2%)	0.001
Age, years	64.8±10.3	69.2±9.1	59.8±7.0	0.002
Number of organs, n	2.4±1.3	2.6±1.4	1.9±0.9	0.159
IgG4, mg/dl	640.0±423.1	640.5±428.8	639.1±430.3	0.993
IgG, mg/dl	2,523.2±1,011.9	2,603.7±1,014.7	2,362.3±1,032.1	0.508
IgG4/IgG ratio	0.3±0.2	0.2± 0.1	0.27±0.13	0.543
IgE, IU/l	966.3±1,185.8	1,186.3±1,395.8	636.3±776.1	0.399
IgM, mg/dl	122.0±80.2	111.2±69.2	134.0±93.6	0.551
IgA, mg/dl	212.2±76.8	228.0±74.4	194.5±79.8	0.357
Amy, IU/l	119.2±184.1	142.9±227.3	77.9±41.9	0.338
P-Amy, IU/l	84.7±209.3	112.9±263.2	37.8±27.9	0.407
FBS, mg/dl	125.9±47.3	130.9±51.9	116.4±37.2	0.419
HbA1c, %	6.5±1.5	6.5±1.6	6.4±1.1	0.844
CEA, ng/ml	2.6±1.3	2.9±1.3	2.2±1.3	0.143
CA19-9, U/ml	48.5±126.1	33.3±52.1	77.5±206.0	0.499
sIL-2 receptor, U/ml	1,186.8±524.2	1,305.3±564.4	932.9±328.7	0.123
White blood cells, n	5,894±1,638	5,765±1,719	6,165±1,502	0.534
Neutrophils	3,419±1212	3,388±1357	3,508±724	0.826
Eosinophils	230±1423	224±134	244±178	0.752
AIM, ng/ml	3,891.9±8,374.5	4,329.5±9,908.0	1,505.4±559.0	0.287

Amy, amylase; P-Amy, pancreatic amylase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; sIL-2, soluble interleukin-2; AIM, apoptosis inhibitor of macrophages.

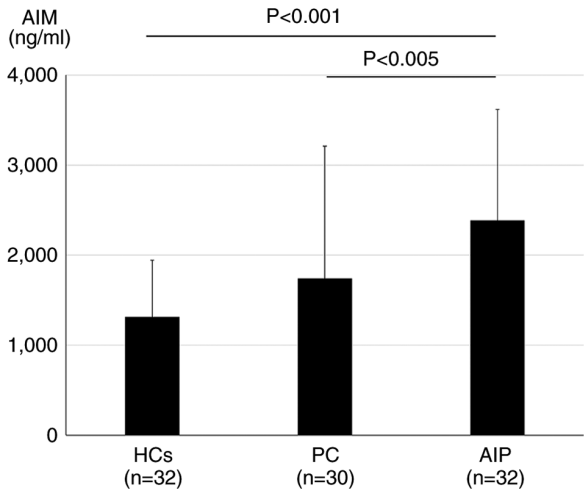

Figure 1. Serum AIM concentrations in patients with AIP, PC and HCs. Serum AIM concentrations in patients with AIP (n=32) were significantly higher compared with the HCs (n=32) and patients with PC (n=30). The serum AIM concentrations were as follows: AIP, 2,387.9±1,232.1 ng/ml; PC, 1,740.2±1,470.2 ng/ml, P<0.005 vs. AIP; and HC, 1,313.1±631.0 ng/ml, P<0.001 vs. AIP. Data were statistically analyzed using the Kruskal-Wallis test. AIM, apoptosis inhibitor of macrophages; AIP, autoimmune pancreatitis; PC, pancreatic cancer; HCs, healthy controls.

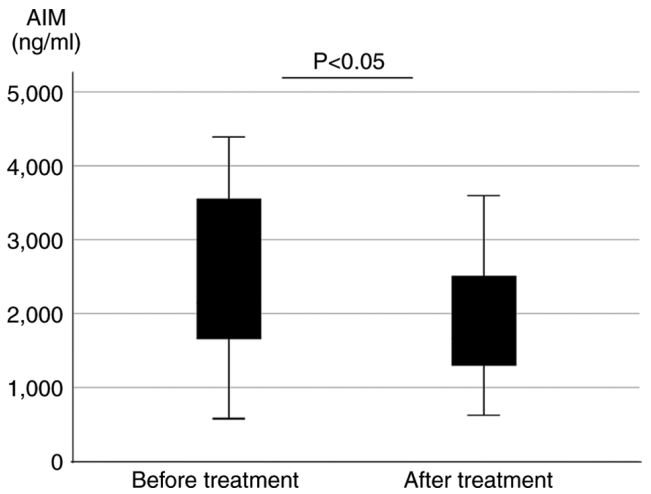

Figure 2. Serum AIM concentrations of patients with IgG4-RD/AIP before and after treatment with steroids. AIM levels significantly decreased following treatment (n=16). The serum AIM concentrations were as follows: Before treatment, 2,499.9±1,210.5 ng/ml; and after treatment, 1,913.6±924.2 ng/ml. P=0.016. Data were statistically analyzed using the Wilcoxon rank sums test. AIM, apoptosis inhibitor of macrophages; IgG4-RD, IgG4-related disease; AIP, autoimmune pancreatitis.

during treatment (n=16). Patients in the treated AIP group exhibited significantly decreased serum AIM concentrations after treatment (before treatment, 2,499.9±1,210.5 ng/ml; after treatment, 1,913.6±924.2 ng/ml; P=0.036; Fig. 2).

Associations between AIM and various parameters. Among the laboratory parameters analyzed, serum amylase levels (ϱ=0.378; P=0.043) significantly positively correlated with the serum AIM concentrations. In addition, IgG levels (ϱ=0.345; P=0.057) and age (ϱ=0.335; P=0.057) also positively

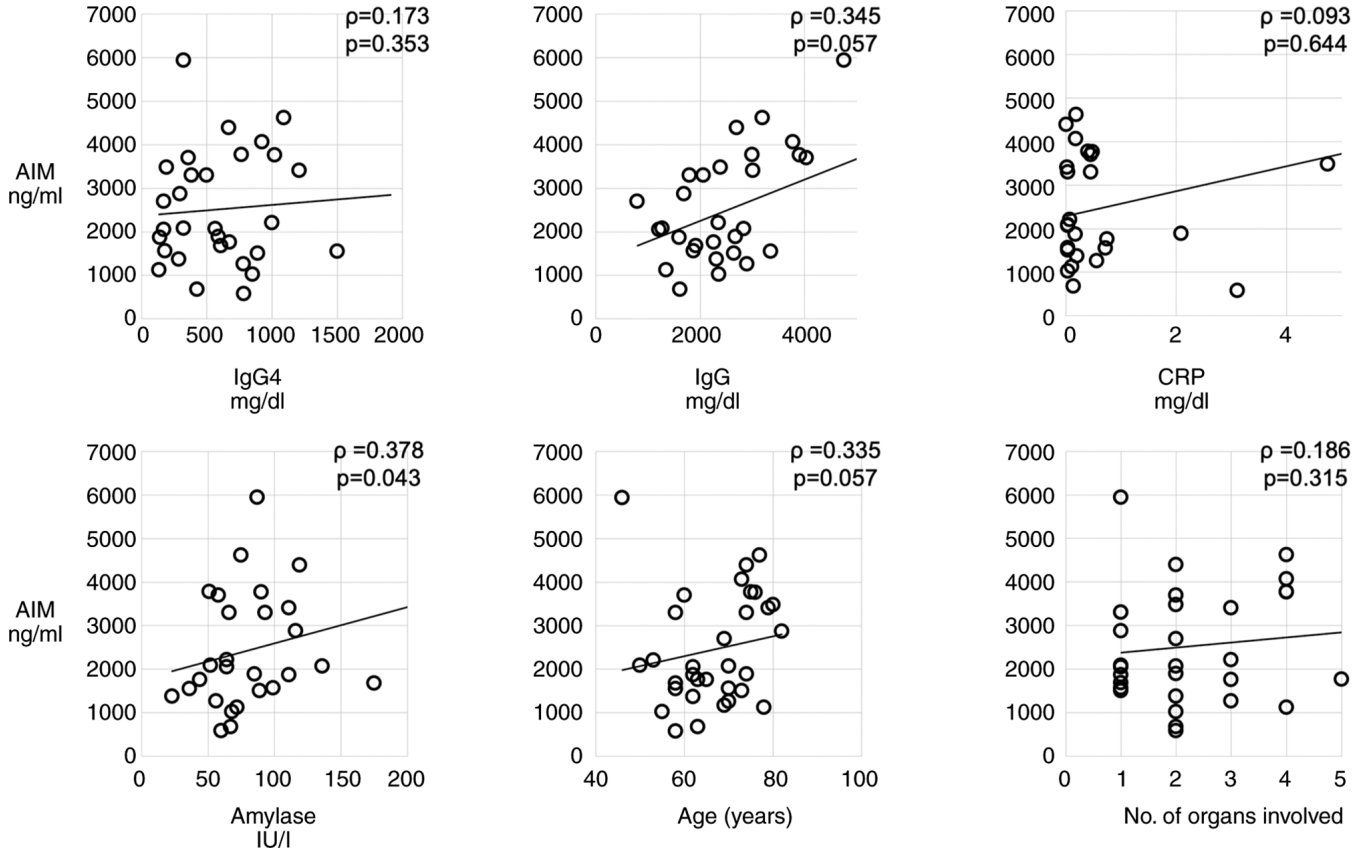

Figure 3. Association between the serum levels of AIM and clinical parameters in patients with IgG4-RD/AIP. Correlation coefficients were determined using Spearman's rank correlation coefficient analysis; n=33. AIM, apoptosis inhibitor of macrophages; IgG4-RD, IgG4-related disease; AIP, autoimmune pancreatitis.

correlated with the serum AIM concentrations. No significant correlations were observed between AIM and IgG4 (ϱ=0.173; P=0.353), CRP (ϱ=0.093; P=0.644) and the number of organs involved (ϱ=0.186; P=0.315) (Fig. 3).

AIM expression in macrophages of pancreatic tissue from patients with AIP. Tissue sections were assessed using immunohistochemistry to determine AIM protein expression levels in patients with AIP who had undergone EUS-FNA. Inflammatory cell infiltration was evident in pancreatic tissues from patients with AIP and immunostaining demonstrated AIM protein expression in mononuclear cells. Moreover, double immunostaining for CD14 and CD16 demonstrated that AIM-positive mononuclear cells predominantly expressed CD16. These results indicated that active macrophages in the pancreas expressed AIM (Fig. 4).

Characteristics and clinical parameters of patients with IgG4-RD under various parameters (age, number of organs involved, history of malignant diseases, relapse). The values for age (<70 and ≥70 years), number of organs involved (<2 and ≥2), a history of malignant diseases and relapse (positive or negative) and the results of their comparisons are presented in Tables II-V. The serum concentrations of IgG, amylase and AIM were higher in patients aged ≥70 years (Table II). Patients who had more than two organs involved had elevated levels of IgG4, fasting blood sugar and eosinophils (Table III). Patients with a history of malignancies had higher AIM levels and elevated levels of eosinophils (Table IV). The IgG4 levels did not differ significantly in terms of age (Table II) or relapse (Table V).

Discussion

To the best if our knowledge, this is the first investigation into serological AIM concentrations in patients with IgG4-RD/AIP. The present study found higher serum AIM concentrations in patients with AIP compared with patients with PC and the HCs. Furthermore, the serum AIM concentrations significantly decreased in patients with AIP who were in remission following treatment with steroids.

Differentiated and mature macrophages produce AIM, a secretory protein of ~50 kDa that consists of three scavenger receptor cysteine-rich domains and is named based on its function of suppressing macrophage apoptosis. AIM is an apoptosis inhibitor that supports the survival of macrophages against various apoptosis-inducing stimuli (7,8). The association between AIM and autoimmune diseases has previously been reported. Arai and Miyazaki (9) suggested that blood AIM concentrations may function as a biomarker for obesity-associated autoimmune diseases in humans, along with diabetes-associated antibodies against pancreatic β-cell antigens (including protein tyrosine phosphatase-like protein), chronic thyroiditis-associated anti-thyroid peroxidase and anti-thyroglobulin antibody. When macrophages take up oxidized low-density lipoprotein, AIM expression is induced via the activated nuclear receptors, liver X receptor/retinoid

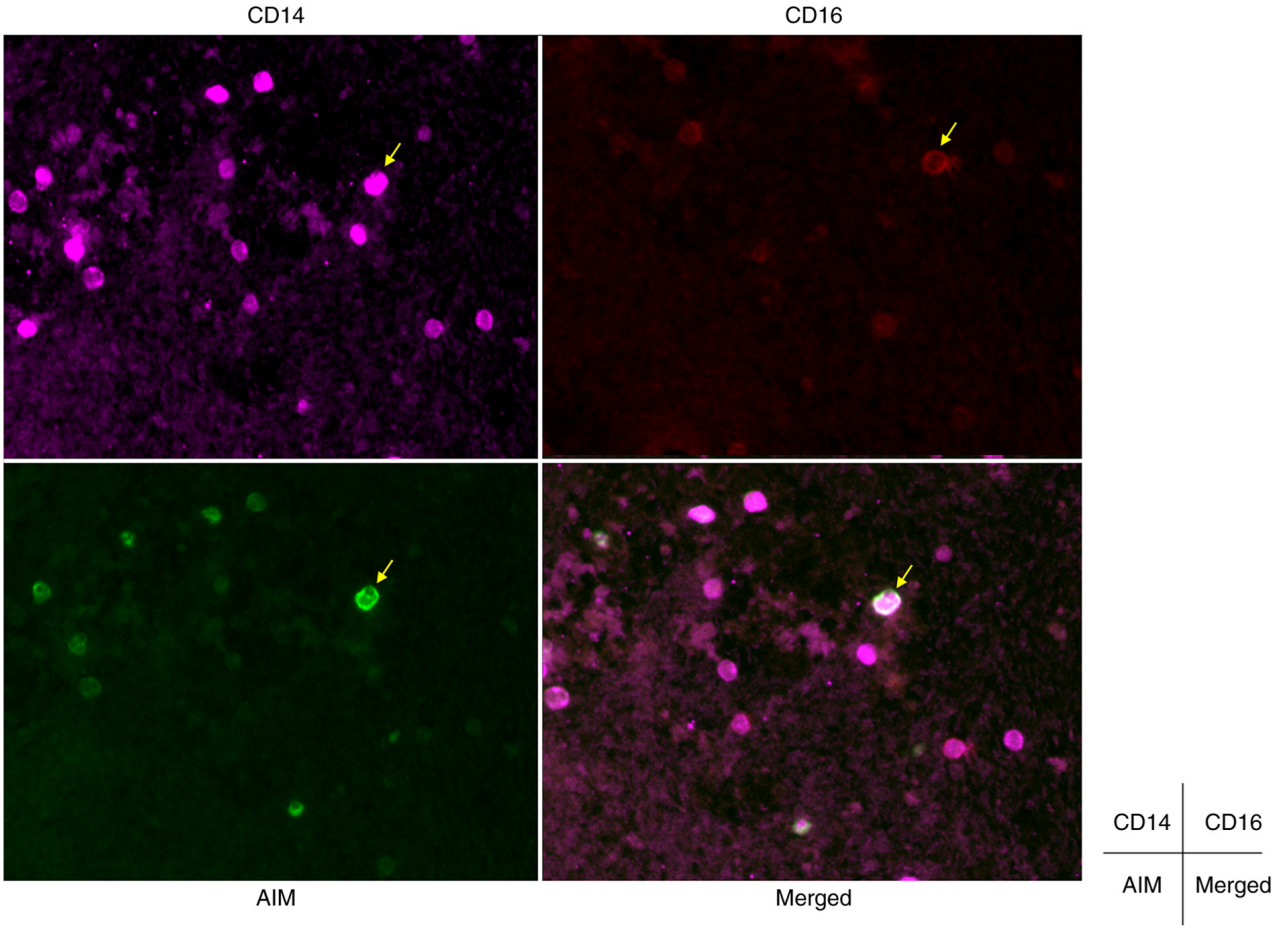

Figure 4. AIM expression in the pancreas tissues of patient with AIP (magnification, x100). AIM-positive mononuclear cells were stained with antibodies against CD14 and CD16. AIM was dominantly expressed in CD16-positive cells, which are indicated by the yellow arrows. AIM, apoptosis inhibitor of macrophages; AIP, autoimmune pancreatitis.

X receptor heterodimers (18,19). As a secretory protein, AIM is found in the blood and its concentration changes in response to various diseases (8,10,11). In a previous study, it was reported that there was an association between AIM and hepatic fibrosis in chronic hepatitis C. Therefore, it was hypothesized that the serum concentration of AIM may be a potential marker of hepatic fibrosis in chronic liver disease (12). Moreover, it has been demonstrated that AIM may function as a biomarker for distinguishing between different inflammatory bowel diseases. Furthermore, AIM is a novel biomarker of Crohn's disease, which can be used to distinguish Crohn's disease from ulcerative colitis and Behcet's disease (13). According to these aforementioned studies, as IgG4-RD and AIP are diseases that are characterized by tissue inflammation and fibrosis, it can be hypothesized that AIM may function as a diagnostic or active biomarker of autoimmune and inflammatory diseases with tissue fibrosis, such as IgG4-RD and AIP. In the present study, AIM was demonstrated to play a role as a potential biomarker for the differentiation of AIP and PC, as well as for disease activity. Furthermore, there was a significant positive correlation between AIM and amylase in the present study. Macrophages are the most abundant immune cells and play a crucial role in pancreatic tissue. Activated macrophages, which generate AIM in the pancreatic tissue, may be associated with pancreatitis and stimulate pancreatic exocrine function (20). The present study did not have height and weight data and was not able to analyze the association between AIM and BMI. This is an important point that needs to be analyzed in the future.

AIP is classified into the following three categories according to its swelling pattern: i) Diffuse; ii) focal; and iii) segmental. As the images of focal and segmental type AIP are similar to pancreatic tumors, AIP can be particularly difficult to distinguish from malignant tumors, even when using various different imaging modalities (21). Furthermore, a differential diagnosis between AIP and PC cannot be based only on serum IgG4 concentrations (22,23). Therefore, as a result of its complexity, numerous AIP cases using surgical resection as the initial treatment have been reported (24,25). Consequently, it is important to set diagnostic criteria to distinguish AIP from PC in order to avoid unnecessary invasive surgery. Therefore, a useful biomarker that can distinguish AIP from PC is required. In the present study, serum AIM concentrations in patients with AIP, PC and HCs were compared. The serum AIM concentrations were significantly higher in patients with AIP compared with HCs and patients with PC. The serum AIM concentrations may thus play a role as a potential biomarker that can distinguish between AIP and PC. The present study also obtained data on serum AIM levels in chronic pancreatitis (data not shown). The AIM value of AIP tended to be higher than that of chronic pancreatitis,

Table II. Comparison of baseline characteristics of patients with IgG4-RD under the parameter of age.

	Age, years		
Characteristic	<70 (n=21)	≥70 (n=17)	P-value
No. of organs involved	2.0±1.1	2.8±1.3	0.100
IgG4, mg/dl	550.3±384.6	740.3±452.8	0.196
IgG, mg/dl	2,321.3±1,192.0	2,748.9±734.3	0.042
IgG4/IgG ratio	0.2±0.1	0.3±0.1	0.950
IgE, IU/l	664.8±627.4	1,795.5±2,000.0	0.226
Amy, IU/l	72.8±36.1	168.6±256.6	**0.006**
P-Amy, IU/l	34.5±24.7	144.1±304.8	0.608
FBS, mg/dl	126.6±46.5	125.1±49.8	0.766
HbA1c, %	6.7±1.7	6.3±1.2	0.654
CEA, ng/ml	2.6±1.3	2.7±1.4	0.551
CA19-9, U/ml	72.3±179.5	27.4±40.3	1.000
sIL-2 receptor, U/ml	991.2±372.1	1381.5±596.1	0.133
White blood cells	6,147± 1,463	5,656±1,800	0.470
Neutrophils	3,494±1,056	3,358±1,358	0.755
Eosinophils	219±157	238±135	0.614
AIM, ng/ml	2,080.8±1,293.7	5,816.2±11,839.1	**0.034**

Values in bold font indicate statistically significant differences (P<0.05). Amy, amylase; P-Amy, pancreatic amylase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; sIL-2, soluble interleukin-2; AIM, apoptosis inhibitor of macrophages.

Table III. Comparison of baseline characteristics of patients with IgG4-RD under the parameter of number of organs involved.

	No. of organs involved		
Characteristic	<2 (n=11)	≥2 (n=25)	P-value
Age, years	62.2±11.5	67.9±8.4	0.104
IgG4, mg/d	383.5±219.9	721.8±425.2	**0.004**
IgG, mg/d	2,064.6±974.5	2,698.7±991.2	0.087
IgG4/IgG ratio	0.2±0.1	0.3±0.2	0.098
IgE, IU/l	674.2±618.3	1,228.0±1,540.0	0.554
Amy, IU/l	94.3±33.1	136.3±230.0	0.554
P-Amy, IU/l	42.1±27.7	106.0±255.7	0.493
FBS, mg/d	100.6±23.0	138.4±52.3	**0.009**
HbA1c, %	6.2±1.3	6.6±1.6	0.487
CEA, ng/m	2.4±0.7	2.7±1.5	0.402
CA19-9, U/m	8.3±5.7	66.7±149.5	0.231
sIL-2 receptor, U/ml	881.4±372.1	1,305.1±519.3	0.122
White blood cells, n	5,272±1230	6,194±17,20	0.177
Neutrophils	3,387±1,244	3,484±1,242	0.861
Eosinophils	148±50	253±156	**0.014**
AIM, ng/ml	2,614.3±1,352.3	4,741.9±10,448.3	0.530

Values in bold font indicate statistically significant differences (P<0.05). Amy, amylase; P-Amy, pancreatic amylase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; sIL-2, soluble interleukin-2; AIM, apoptosis inhibitor of macrophages.

although there was no significant difference. Thus, the authors aim to identify more cases in the future for further analysis.

Furthermore, the present study demonstrated that treatment with steroids significantly decreased the serum AIM

Table IV. Comparison of baseline characteristics of patients with IgG4-RD under the parameter of history of malignant diseases.

	History and number of malignancies		
Characteristic	(-) (n=28)	(+) (n=10)	P-value
No. of organs involved	2.4±1.3	2.3±1.3	0.903
IgG4, mg/dl	701.0± 442.5	481.5±337.3	0.189
IgG, mg/dl	2545.0±1013.2	2466.5±1061.0	0.958
IgG4/IgG ratio	0.3±0.1	0.2±0.1	0.189
IgE, IU/l	693.0±620.6	1718.0±2061.8	0.661
Amy, IU/l	172.9±210.9	92.3±37.1	0.757
P-Amy, IU/l	97.8±241.2	45.5±36.6	0.626
FBS, mg/dl	116.5±38.1	146.6±60.2	0.119
HbA1c, %	6.1±0.9	7.3±2.1	0.124
CEA, ng/ml	2.5±1.1	3.0±1.7	0.651
CA19-9, U/ml	55.3±175.8	30.9±51.9	0.902
sIL-2 receptor, U/ml	1204.1±500.7	1140.8±631.3	0.747
White blood cells	5980±1553	5684±1913	0.781
Neutrophils	3481±1153	3272±1416	0.815
Eosinophils	192±143	320±97	**0.004**
AIM, ng/ml	2306.5±1385.1	8119.7±15727.9	**0.049**

Values in bold font indicate statistically significant differences (P<0.05). Amy, amylase; P-Amy, pancreatic amylase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; sIL-2, soluble interleukin-2; AIM, apoptosis inhibitor of macrophages.

Table V. Comparison of baseline characteristics of patients with IgG4-RD under the parameter of disease relapse.

	No. of patients with disease relapse		
Characteristic	(-) (n=22)	(+) (n=8)	P-value
No. of organs involved	2.3±1.2	2.6±1.4	0.601
IgG4, mg/dl	640.9± 398.8	807.3±573.6	0.636
IgG, mg/dl	2521.2±1026.7	2598.8± 1019.6	0.784
IgG4/IgG ratio	0.2±0.1	0.3±0.1	0.469
IgE, IU/l	941.3±1532.6	1159.0±391.7	0.178
Amy, IU/l	93.1±35.8	215.6±369.8	0.449
P-Amy, IU/l	45.1±30.5	173.9±358.5	0.414
FBS, mg/dl	122.7±47.6	135.0±45.6	0.297
HbA1c, %	6.4±1.5	6.4±1.5	0.804
CEA, ng/ml	3.1±1.4	1.9±0.9	**0.041**
CA19-9, U/ml	53.9±161.2	21.4±21.0	0.935
sIL-2 receptor, U/ml	1004.9±384.9	1413.8±772.1	0.505
White blood cells	5846±1469	5941±1943	0.790
Neutrophils	3401±931	3797±1497	0.407
Eosinophils	260±142	201±142	0.332
AIM, ng/ml	4693.0±10729.3	2178.7±1149.8	0.725

Values in bold font indicate statistically significant differences (P<0.05). Amy, amylase; P-Amy, pancreatic amylase; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; sIL-2, soluble interleukin-2; AIM, apoptosis inhibitor of macrophages.

concentrations in patients with AIP who were in remission. To the best of our knowledge, an association between serum AIM concentrations and treatment outcomes for other autoimmune diseases has not yet been reported. An international survey

reported in 2013 demonstrated that 74% of patients with AIP were initially treated with steroids and the remission rate was 99.6% (681/684 patients) (26). However, the high relapse rate of AIP is an issue and of the 978 patients with AIP in that previous study, a total of 302 (31%) patients experienced relapse (26). It remains unknown how disease activity indexes or predictors of relapse influence parameters, such as the Crohn's disease Activity Index (CDAI) in Crohn's disease. Furthermore, there is no association between serum AIM concentrations and the CDAI in patients with Crohn's disease (13). However, the results of the present study suggested that serum AIM concentrations may potentially be an indicator of AIP.

It has previously been reported that IgG4-RD and AIP may exhibit aspects of paraneoplastic syndrome, which is an altered immune system response to a neoplasm (27-29). In the present study, 10/38 (26%) patients with IgG4-RD had a history of malignancy. Standardized incidence ratios (SIR) estimate an increased risk of overall cancer in patients with IgG4-RD patients (SIR, 2.57; 95% CI, 1.72-3.84) compared with the general population (30). Moreover, even though it is important to diagnose patients with IgG4-RD and AIP who have malignant diseases as it affects prognosis, the clinical characteristics remain unclear (31). AIM is also associated with certain malignancies (32). In the present study, the serum AIM concentrations were significantly higher in patients with IgG4-RD and a history of malignancies. These results suggest that the serum AIM concentrations may potentially serve as a biomarker to predict malignant disease in patients with IgG4-RD and AIP.

In the present study AIM expression in active macrophages in pancreatic tissues was demonstrated. Moreover, as macrophages play a crucial role in IgG4-RD (33), it can be hypothesized that AIM, which is produced by resident macrophages in pancreatic tissues, contributes to pancreatic inflammation and fibrosis, which may result in elevated serum AIM concentrations. However, there are limitations to the present study as it used a single-center design and only a small number of patients were enrolled. Therefore, a larger sample size should be used in future studies.

In conclusion, in the present study, serological AIM concentrations were significantly higher in patients with IgG4-RD/AIP compared with patients with PC. Furthermore, serum AIM concentrations decreased in patients treated with steroids who were in remission. The results of the present study suggest that serum AIM concentrations may potentially serve as a precise diagnostic and therapeutic biomarker in patients with IgG4-RD/AIP.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ST drafted the manuscript. ST, SK and AI were substantial contributions to the conception or design of the study, the acquisition, analysis or interpretation of data for the work, the drafting the manuscript and revising it critically for important intellectual content. MH, SA, FS and SH contributed to the acquisition, analysis and interpretation of the data, as well as the in writing and revision of the manuscript. SK and ST confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Written, informed consent was obtained from all patients. The present study was approved by the Ethics Committee of Kagoshima University Medical and Dental Hospital (Kagoshima, Japan; approval no. 28-241). All experimental procedures were performed according to the Ethical Principles for Medical Research Involving Human Subjects outlined in the World Medical Association Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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