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Characteristic findings in images of extra-pancreatic lesions associated with autoimmune pancreatitis☆

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ABSTRACT

Purpose: Autoimmune pancreatitis is a unique form of chronic pancreatitis characterized by a variety of extra-pancreatic involvements which are frequently misdiagnosed as lesions of corresponding organs. The purpose of this study was to clarify the diagnostic imaging features of extra-pancreatic lesions associated with autoimmune pancreatitis.
Materials and methods: We retrospectively analyzed diagnostic images of 90 patients with autoimmune pancreatitis who underwent computer-assisted tomography, magnetic resonance imaging, and/or gallium-67 scintigraphy before steroid therapy was initiated.
Results: AIP was frequently (92.2%) accompanied by a variety of extra-pancreatic lesions, including swelling of lachrymal and salivary gland lesions (47.5%), lung hilar lymphadenopathy (78.3%), a variety of lung lesions (51.2%), wall thickening of bile ducts (77.8%), peri-pancreatic or para-aortic lymphadenopathy (56.0%), retroperitoneal fibrosis (19.8%), a variety of renal lesions (14.4%), and mass lesions of the *ligamentum teres* (2.2%). Characteristic findings in CT and MRI included lymphadenopathies of the hilar, peri-pancreatic, and para-aortic regions; wall thickening of the bile duct; and soft tissue masses in the kidney, ureters, aorta, paravertebral region, *ligamentum teres*, and orbit.
Conclusions: Recognition of the diagnostic features in the images of various involved organs will assist in the diagnosis of autoimmune pancreatitis and in differential diagnoses between autoimmune pancreatitis-associated extra-pancreatic lesions and lesions due to other pathologies.

1. Introduction

Autoimmune pancreatitis (AIP) is a unique form of chronic pancreatitis characterized by a preponderance of elderly male sufferers, minimal abdominal pain, irregular narrowing of the main pancreatic duct, and swelling of the pancreatic parenchyma [1–12]. The pathogenesis is thought to involve an autoimmune mechanism based on the presence of various serum autoantibodies, hypergammaglobulinemia, histological evidence of lymphoplasmacytic inflammation and fibrosis, and a favorable response to glucocorticoid treatment [13–21]. This disease has been occasionally misdiagnosed as pancreatic cancer, leading to unnecessary surgery [22,23]. It is therefore imperative to improve the diagnostic accuracy for AIP.

The characteristic features of AIP include a high serum IgG4 concentration and complications involving various extra-pancreatic lesions. Over 90% of patients exhibit high serum IgG4 concentrations, reflecting infiltration of abundant IgG4-bearing plasma cells and disease activity [11] in the pancreatic lesion [12,18]. Thus, a serum assay for IgG4 provides a useful tool for the diagnosis and monitoring of this disease. In addition, abundant IgG4-bearing plasma cells are a histological hallmark that can be used in differentiating between AIP and malignant conditions.

Other prominent features of AIP involve a variety of extra-pancreatic complications, including sclerosing cholangitis [2,7,17,19,20], lachrymal and salivary gland abnormalities [18,24], hypothyroidism [25], hilar lymphadenopathy [26], retroperitoneal fibrosis [12,17,27–29], interstitial pneumonia [30,31], and tubulointerstitial nephritis [32,33]. Some of these extra-pancreatic lesions exhibit pathologies similar to those found in pancreatic

Abbreviations: AIP, autoimmune pancreatitis; CT, computer-assisted tomography; Ga-67, gallium-67; LIP, lymphocytic interstitial pneumonia; MRI, magnetic resonance imaging; MRCP, magnetic resonance cholangiopancreatography; NSIP, nonspecific interstitial pneumonia; PSC, primary sclerosing cholangitis.

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lesions, including infiltration of abundant IgG4-bearing plasma cells [12,18,26,29]. In addition, the presence of multiple extra-pancreatic lesions suggests a systemic disease associated with IgG4 [12]. Most reports regarding AIP-associated extra-pancreatic lesions have been published as single cases, small series of cases, or cases restricted to specific lesions. There have been only a few detailed reports addressing the broad spectrum of manifestations [34,35,36], particularly with regard to imaging that might be useful in the diagnosis of extra-pancreatic lesions associated with AIP.

It is uncertain whether there are imaging characteristics that might be useful for differentiation between IgG4-related extra-pancreatic lesions and other diseases affecting the same organs. In this study, we aimed to characterize and identify useful imaging features of a large cohort in the diagnosis of extra-pancreatic manifestations of AIP to differentiate them from other diseases affecting the same organs.

2. Materials and methods

We systematically reviewed diagnostic images for 90 patients with AIP, 75 men and 15 women aged 38–79 (median age, 63.1 years old), treated in our hospital and affiliated hospitals between September, 1994 and March, 2008. Diagnosis of AIP was based on the criteria proposed by the Japanese Pancreatic Society in 2002 [37] and the revised version in 2006 [38].

We analyzed images from computer-assisted tomography (CT), magnetic resonance imaging (MRI), and/or gallium-67 (Ga-67) scintigraphy that were taken at admission or during an active stage of the disease before the initiation of steroid therapy. CT examination was performed with single-detector row helical CT (HiSpeed Advantage; GE Medical Systems, Milwaukee, WI, USA) from 1994 to 2003 and with multi-detector row helical CT from 2003 to 2008 (LightSpeed Ultra or LightSpeed VCT; GE Medical Systems, Milwaukee, WI, USA). Because of the length of the period under review, the model of the CT scanner, slice thickness (1.25–10 mm) and the protocol of the contrast-enhanced CT varied. MRI was performed with a 1.5-T superconductor unit (Magnetom Symphony, Siemens Medical Solution, Erlangen, Germany) from 1994 to 2006 and with a 3-T superconductor unit (Magnetom Trio, Siemens Medical Solution, Erlangen, Germany) from 2007 to 2008. The slice thickness (2–10 mm), image sequence and the protocol of the contrast-enhanced MRI varied for the same reason. Ga-67 scintigraphy was performed with a single-head rectangular gamma camera (SNC-510R; Shimazu, Japan) from 1994 to 2002 and a triple-head rotating gamma camera (PRISM IRIX; Philips Medical Systems, Best, The Netherlands) or a large field-of-view dual-detector gamma camera with a mounted CT scanner (Millennium VG, GE Medical Systems, Milwaukee, WI, USA) from 2003 to 2008.

Abdominal and thoracic CT images were available in all 90 and 69 patients, respectively. Abdominal MRI and neck MRI were available in 78 and 40 patients, respectively. Ga-67 scintigram was available in 80 patients. Systemic image analysis using CT, MRI and Ga-67 was performed, even if they showed no specific symptoms of the extra-pancreatic organs, because lesions in extra-pancreatic organs found on images were not always associated with symptoms. Image findings were reviewed by two radiologists (Y.F. and M.K.) in consensus.

Table 1
Summary of the prevalence and distribution of extra-pancreatic lesions.

Organ	No. of cases	Percentage
Total extra-pancreatic lesions	83/90	92.2%
Lachrymal or salivary grand	38/80	47.5%
Hilar lymph node (CT)	54/69	78.3%
Hilar lymph node (Ga-67 scintigraphy)	60/80	75%
Lung	25/46	54.3%
Bile duct	63/81	77.8%
Peri-pancreatic or para-aortic lymph node	51/90	57%
Kidneys	13/90	14.4%
Retroperitoneum	17/86	19.8%
Ligamentum teres	2/90	2.2%
Prostate	8/80	10.0%

The total number of examined cases in each row is different among respective lesions because CT, MRI or Ga-67 scintigraphy were not performed in all the organs, and some images were not available.

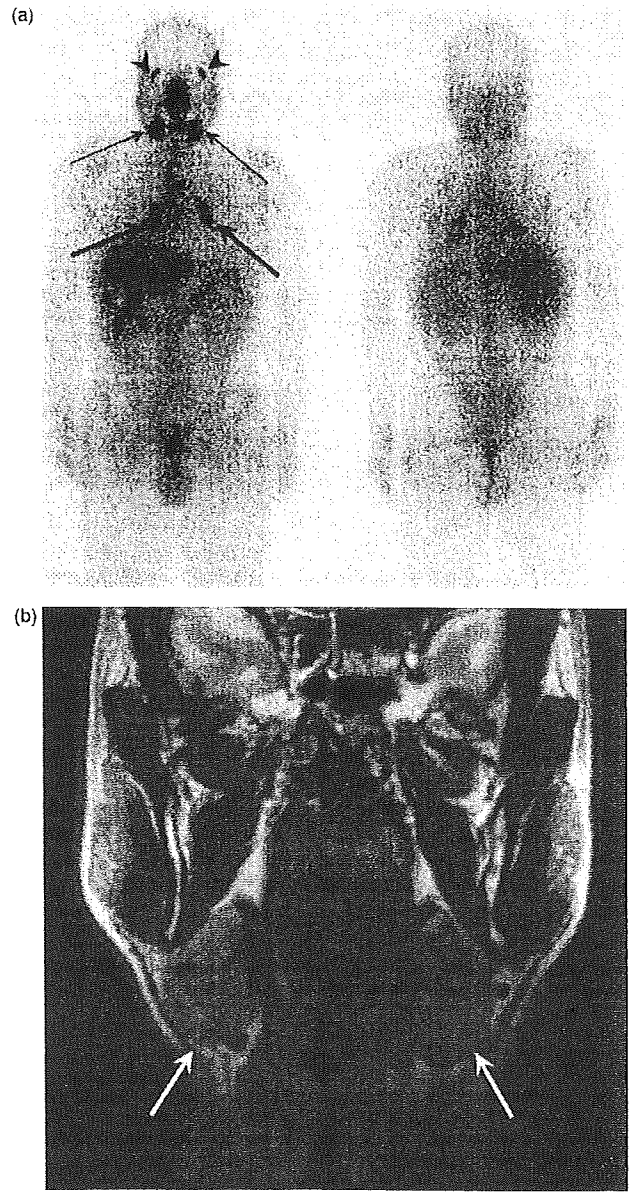

Fig. 1. Lachrymal and salivary lesions in a 67-year-old man visualized with Ga-67 scintigraphy and MRI. (a) Ga-67 scintigraphy shows increased uptake in the hilar (arrows), submandibular (thin arrows), and lachrymal glands (arrow heads). (b) Coronal T2-weighted images show bilateral submandibular gland swellings that are homogeneous without dilatation of the ducts (white arrows).

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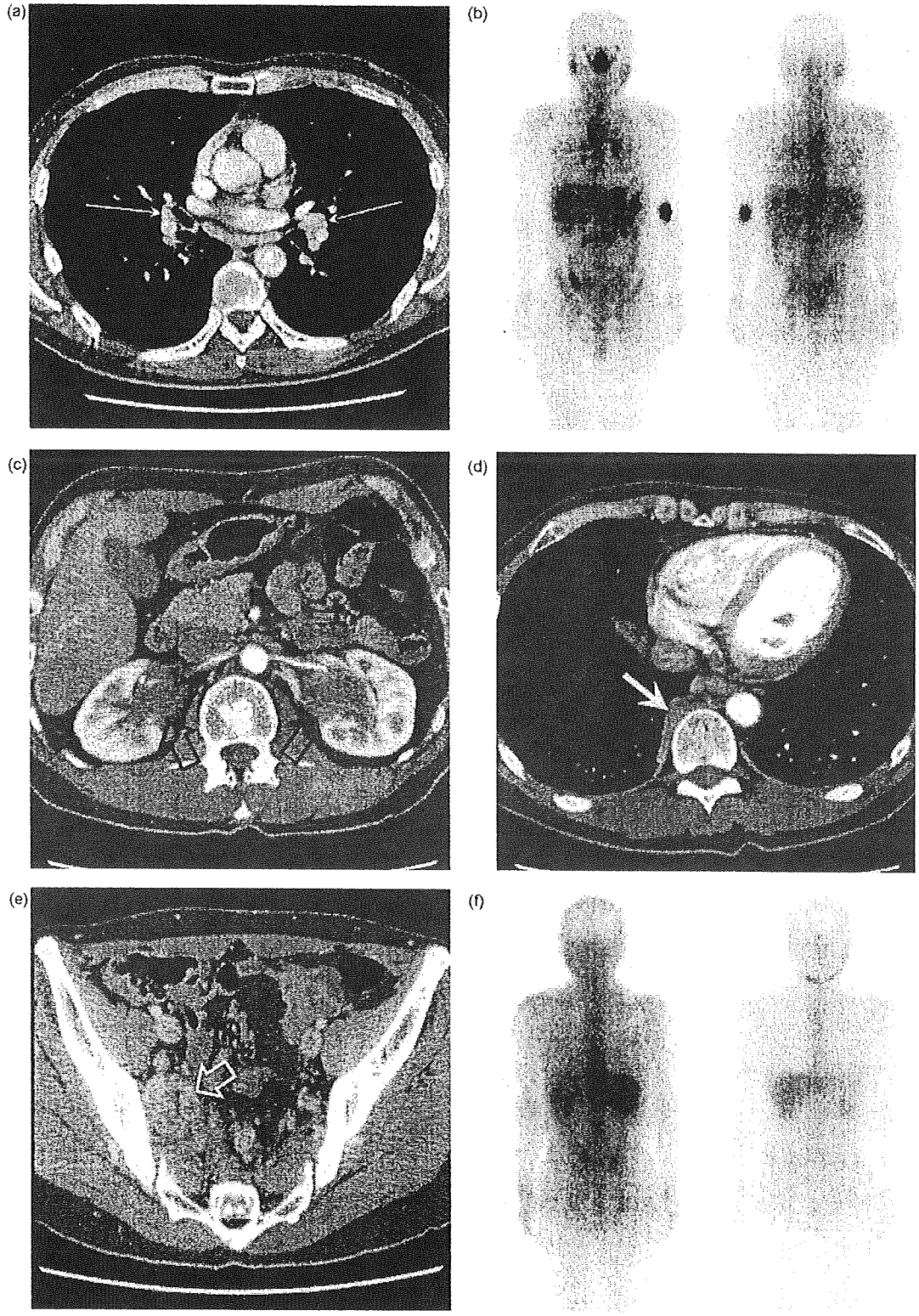

Fig. 2. Hillar lymphadenopathy in a 50-year-old man. (a) Dynamic contrast-enhanced CT shows hilar lymphadenopathy (long arrows). (b) Ga-67 scintigraphy demonstrates an increased uptake in right parotid gland and right retroperitoneum, as well as hilum. (c–e) Another slice of the dynamic contrast-enhanced CT identifies bilateral renal lesions (open arrows in c), paravertebral mass (white arrow in d) and retroperitoneal lesion (white open arrow in e) (to be describe). (f) After corticosteroid therapy, the accumulation of Ga-67 disappeared in each lesion.

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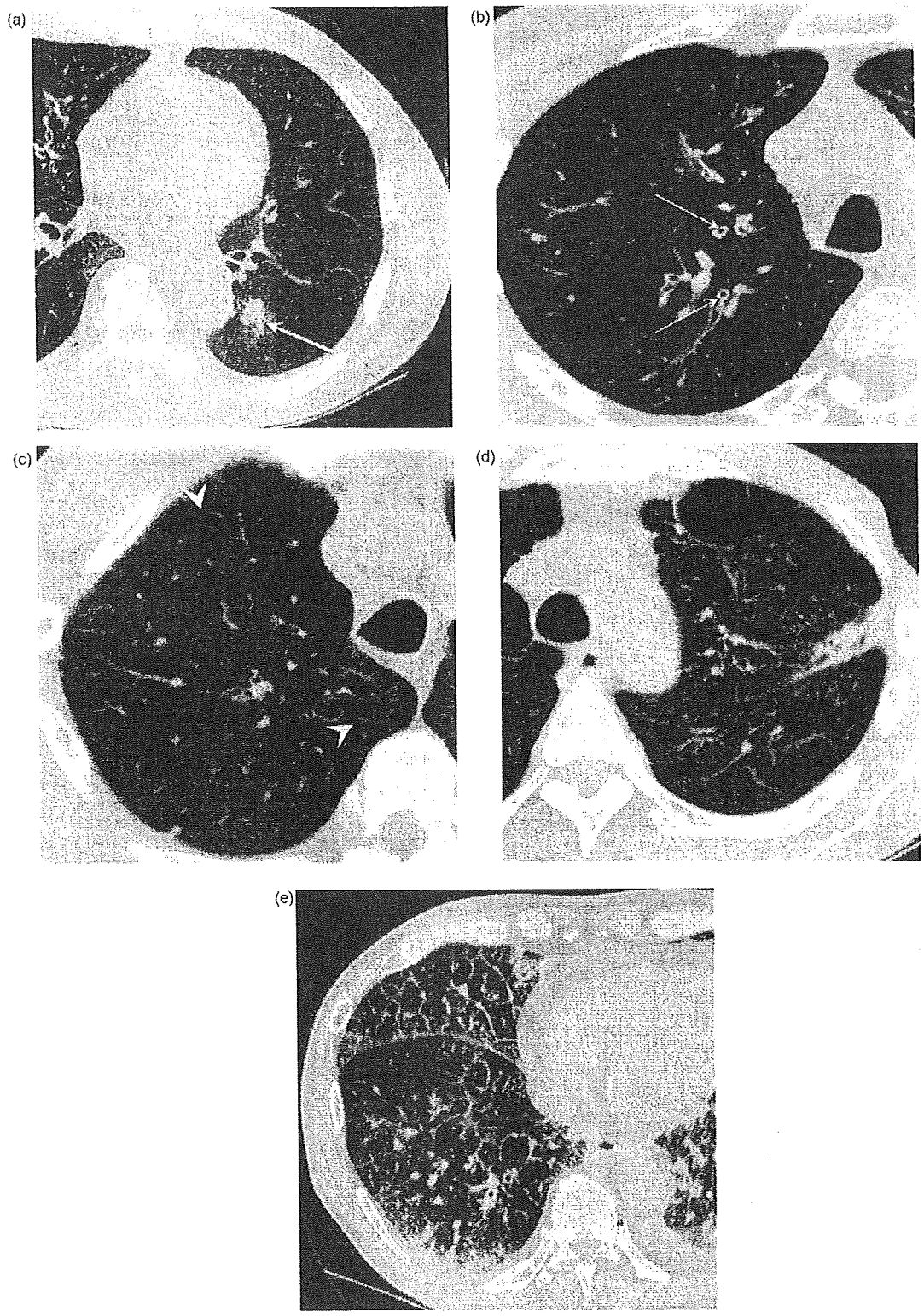

Fig. 3. Lung lesions. (a–d) Thin-sliced CT before corticosteroid therapy shows an irregular nodular lesion in the left lower lobe (white arrow in a), bronchial thickening in the right upper lobe (thin white arrows in b), diffuse interlobular thickening in the right upper lobe (arrow heads in c), and subpleural consolidation in the left lower lobe (open arrow in d). (e) In another case, a coarse reticulation consistent with thickening of interlobular septa is mixed with multiple subpleural consolidations in the right lower lobe.

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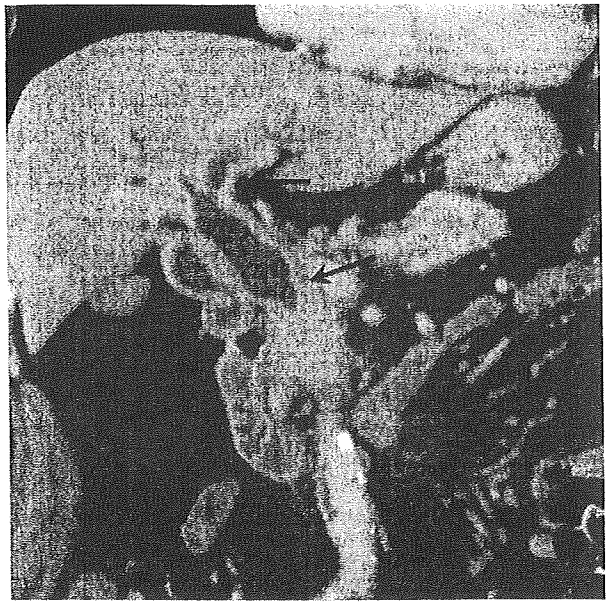

Fig. 4. Bile duct lesions in a 50-year-old woman. Coronal reformation of contrast-enhanced CT shows marked bile duct wall thickening (arrows).

3. Results

Among 90 patients, 83 (92.2%) had various extra-pancreatic lesions associated with AIP. We summarize the distribution and frequency of the extra-pancreatic lesions in Table 1, and describe the details of each type of lesion in the following sections.

3.1. Lachrymal and salivary gland lesions

Among 80 patients that underwent Ga-67 scintigraphy, 38 (47.5%) had lachrymal or salivary gland lesions (Table 1). Two submandibular lesions and two lachrymal lesions were histopathologically proven by biopsy, and other lesions were clinically diagnosed. Ga-67 scintigraphy showed increased uptake in either the lachrymal or salivary gland in 36 of these 38 patients (95%) (Fig. 1a). The submandibular gland was involved in 29 patients (76%), the lachrymal gland in 27 (75%), the parotid gland in 5 (13%), and the sublingual gland in 2 (5%). Increased uptakes were symmetrical in all except three of the patients; one showed right-side-dominant uptake at the lachrymal gland, one showed left-side-dominant uptake at the lachrymal gland, and one showed right-side-dominant uptake at the submandibular gland. After corticosteroid therapy, increased uptake of all lesions was disappeared.

Neck MRI was performed in 40 patients in whom increased uptake was seen on Ga-67 scintigraphy or clinical symptoms were described. The MRIs showed a bilateral homogeneous swelling of the glands without a discernable mass (Fig. 1b) in 14 patients, unilateral swelling in 2 patients and normal findings in 24 patients. Biopsies performed in two patients showed that the swelling submandibular glands contained abundant IgG4-bearing plasma cells. No lesions showed the salt-and-pepper appearance characteristic of Sjoegren's syndrome [39].

3.2. Hilar lymphadenopathy

Hilar lymphadenopathy was revealed in 54 of 69 patients (78%) undergoing thoracic CT (Table 1), and contrast-enhanced CT was used to improve the visualization of bilateral hilar lymphadenopathy (Fig. 2a). Ga-67 scintigraphy also showed marked bilateral hilar uptake in 60 of 80 cases (75%) (Fig. 2b) (Table 1). After corticosteroid therapy, bilateral hilar lymphadenopathy disappeared in all cases. No biopsies were taken. There was no unilateral lymphadenopathy found by CT or Ga-67 scintigraphy.

3.3. Pulmonary abnormalities

Lung lesions were revealed in 25 of 46 patients (54%) undergoing thin-slice CT (Table 1). Five patients were histopathologically

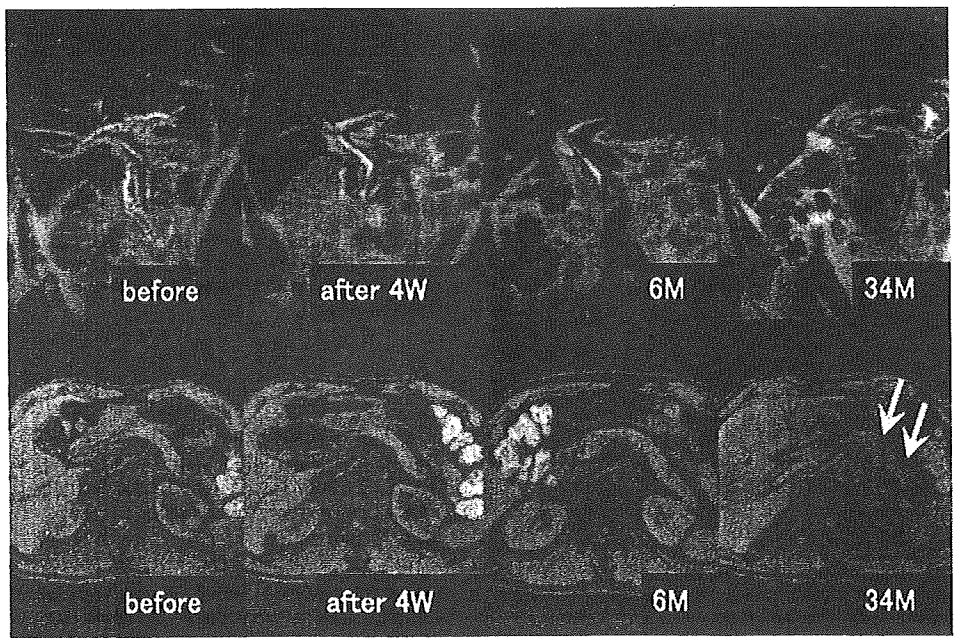

Fig. 5. Bile duct lesions in a 69-year-old man. Oblique coronal T2-weighted images (upper column) before and after corticosteroid therapy images show gradual improvement of the bile duct lesion. Thirty-four months after corticosteroid therapy, the bile duct lesion had relapsed. Axial fat saturated T1-weighted images (lower column) show a new pancreatic lesion (white arrows) accompanied by relapsed bile duct lesion.

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proven by biopsy and 20 were clinically diagnosed. There were four types of CT findings, including nodular lesions, bronchial thickening, interlobular thickening, and consolidation. Nodular lesions (3–26 mm in diameter) were found in 18 (39%) (Fig. 3a), bronchial thickening in 14 (30%) (Fig. 3b), interlobular thickening in 7 (15%) (Fig. 3c), and consolidations in 2 (4%) (Fig. 3d). Almost all the nodular lesions were located adjacent to the pleura. Two or more types of lesions coexisted in 14 cases (Fig. 3e). All lesions diminished or disappeared after corticosteroid therapy.

3.4. Bile duct abnormalities

CT or MRI showed extra-pancreatic bile duct lesions in 63 of 81 patients (78%) (Table 1); nine patients were excluded due to unavailable CT or MR images, or if the detailed analysis of bile ducts was difficult due to occlusion by drainage tubes. Biopsies were performed in 19 cases, and abundant IgG4-bearing plasma cell infiltration was evident. Other 44 cases were clinically diagnosed. Almost all bile duct abnormalities in these 63 patients included extensive wall thickening, with occlusion of the intrahepatic bile duct in 23 (28%) and of the common hepatic or intra-pancreatic common bile duct in 37 (46%) (Fig. 4). MRI clearly demonstrated these lesions (Fig. 5), and showed prominent wall thickening with a laminar structure (Fig. 6) in some cases and focal wall thickening of the common bile duct in 5 (6%). Magnetic resonance cholangiopancreatography (MRCP) was performed in 66 patients and showed intrahepatic bile duct stenosis, which mimicked primary sclerosing cholangitis (PSC) in 6 (9%). Severe bile duct dilatation was observed when the pancreas head was swollen.

3.5. Peri-pancreatic and para-aortic lymphadenopathy

CT or MRI showed peri-pancreatic or para-aortic lymphadenopathy in 51 of 90 cases (57%) (Table 1). All lesions were clinically diagnosed. CT clearly demonstrated lymph node swelling that produced high signal intensities on diffusion-weighted MRI (Fig. 7a, b). No biopsies were taken, but corticosteroid therapy was effective for all lesions.

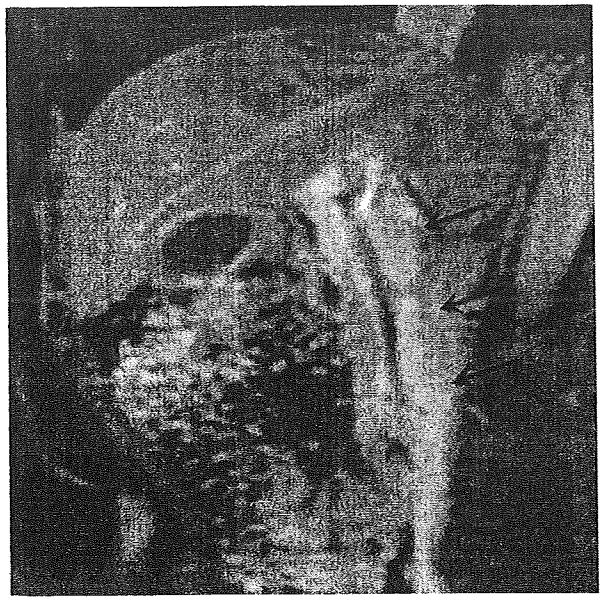

Fig. 6. Bile duct lesions in a 50-year-old woman. Coronal contrast-enhanced MRI shows prominent wall thickening of the bile duct with significant laminar structure (arrows).

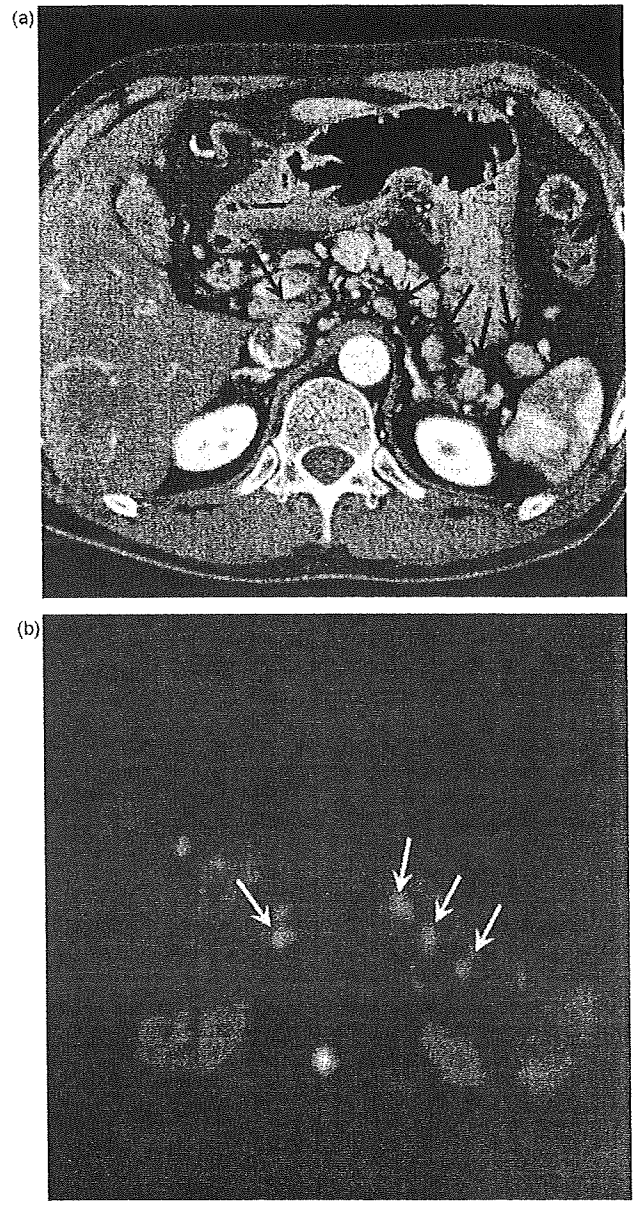

Fig. 7. Peri-pancreatic and para-aortic lymphadenopathy in a 55-year-old man. (a) Contrast-enhanced CT shows multiple peri-pancreatic lymphadenopathy (arrows). (b) On diffusion-weighted images, these lesions are detected as high intensity signals (white arrows).

3.6. Renal lesions

CT or MRI showed renal lesions in 13 of 90 cases (14%), including parenchymal lesions in 10 and hilar lesions in 3 (Fig. 2c) (Table 1). Four renal parenchymal lesions and 1 hilar lesion were diagnosed by biopsies and other 12 cases were clinically diagnosed. All 10 parenchymal lesion cases had multiple bilateral lesions, and 2 had unilateral renal atrophy. Contrast-enhanced CT showed renal parenchymal lesions as slightly enhanced wedge- or node-shaped lesions. Multiphase dynamic contrast-enhanced CT were performed in 5 of 10 patients and revealed that the shapes changed over time and were ill-defined in the delayed phase (Fig. 8) in all cases. Renal lesions were unclear on T1-weighted MR images, but

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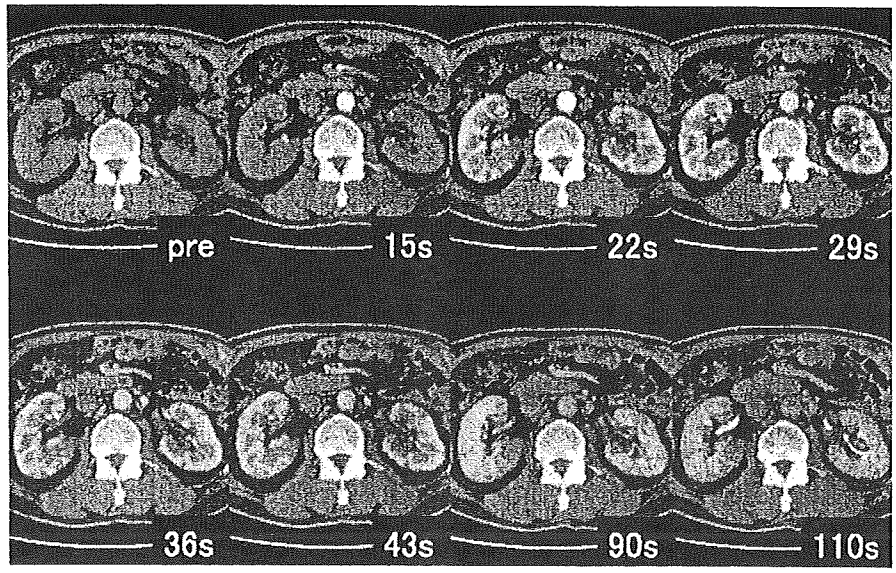

Fig. 8. Renal lesions in a 67-year-old man (same case as in Fig. 1). Multiphase dynamic contrast-enhanced CT shows multiple poorly enhanced lesions in both kidneys, and sequential changes in the lesions over time.

had slightly high intensity on T2-weighted MR images, and high intensity on diffusion-weighted images. These lesions were wedge- or node-shaped on dynamic multiphase T1-weighted images. CT and MRI showed prominent and diffuse renal pelvic wall thickening in renal hilus lesions. Ga-67 scintigraphy showed increase uptake in all renal lesions.

3.7. Retroperitoneal lesions

CT showed retroperitoneal fibrosis in 17 of the 86 cases for which findings were available (20%) (Table 1), including a soft tissue density around the aorta in 11 (Fig. 9a), soft tissue masses around both ureters in 3 (Fig. 9b), paravertebral masses in 2, a pelvic retroperitoneal mass in 1, and increased fat density around the superior mesenteric artery in 1 (Fig. 9c). The biopsy specimens from 3 para-aortic lesion cases and 2 peri-ureteral lesion cases showed abundant IgG4 plasma cell infiltration. A paravertebral mass and pelvic retroperitoneal mass were observed together in the same patient (Fig. 2d, e). Three of 11 cases with para-aortic lesions had aortic aneurysms. One of 3 cases with peri-ureteral lesions had hydronephrosis.

3.8. Other lesions

CT or MRI showed *ligamentum teres* lesions in 2 of the 90 cases (2%) (Table 1). Both lesions were clinically diagnosed and no biopsy was taken. These lesions showed a spindle-shaped soft tissue mass on CT, hypointense mass on T1-weighted image, and hyperintense mass on T2-weighted images (Fig. 10).

Ga-67 scintigraphy exhibited Ga-67 accumulation in the prostate in 8 of 80 cases (10%) (Table 1). Biopsy specimens from two patients showed abundant IgG4-positive plasma cells and lymphocyte infiltration. Other six patients were clinically diagnosed. Diffusion-weighted MRI showed prostatic swelling with high intensity signals (Fig. 11), which mimicked prostate cancer or prostatitis.

4. Discussion

The present study demonstrated some characteristics found in images of various extra-pancreatic lesions associated with AIP. Because they favorably respond to corticosteroid therapy, it is important to differentiate these IgG4-related extra-pancreatic lesions from other diseases that might affect the same organs, including Sjögren's syndrome, sarcoidosis, and primary sclerosing cholangitis. In general, differentiation has previously been based on histological findings and the response to corticosteroid therapy. In this study, we aimed to identify characteristics in images that would be useful for the diagnosis and differentiation of AIP-related lesions with noninvasive imaging.

The major causes of lachrymal and salivary gland swelling include Sjögren's syndrome, Mikulicz disease, or Küttner tumors. Recent reports have shown that high serum IgG4 concentrations and abundant IgG4-bearing plasma cell infiltration were associated with Mikulicz disease and Küttner tumors, but not Sjögren's syndrome [40–42]. In our series, lachrymal and salivary gland lesions responded well to corticosteroid therapy, and thus were similar to those described in Mikulicz disease and Küttner tumors. For this reason, we speculate that the lachrymal and salivary gland lesions associated with AIP may arise from the same or similar systemic conditions as those associated with Mikulicz disease and Küttner tumors.

In our series, 95% of the patients presented with lachrymal or submandibular gland enlargement and only five patients (12.5%) presented with parotid gland enlargement. By contrast, patients with Sjögren's syndrome frequently present with parotid gland enlargement. This difference in the distribution of lesions might be important for the differentiation of AIP-associated lesions and Sjögren's syndrome lesions. In addition, a salt-and-pepper appearance is typically found on the MRIs in Sjögren's syndrome [38]. By contrast, the salivary gland lesions in our series always exhibited homogeneous swelling. This difference in imaging characteristics should be helpful for the differentiation between the two diagnoses. Furthermore, we found that increased Ga-67 uptake by the pancreas and the presence of other extra-pancreatic lesions are indicative of an AIP diagnosis.

Hilar Ga-67 accumulation has been reported in other diseases, including sarcoidosis or primary biliary cirrhosis. Thus, it is necessary to differentiate between these diseases and AIP. Saegusa et al. reported that hilar and pancreatic Ga-67 accumulations are characteristic features of AIP during the active stage of the disease [26].

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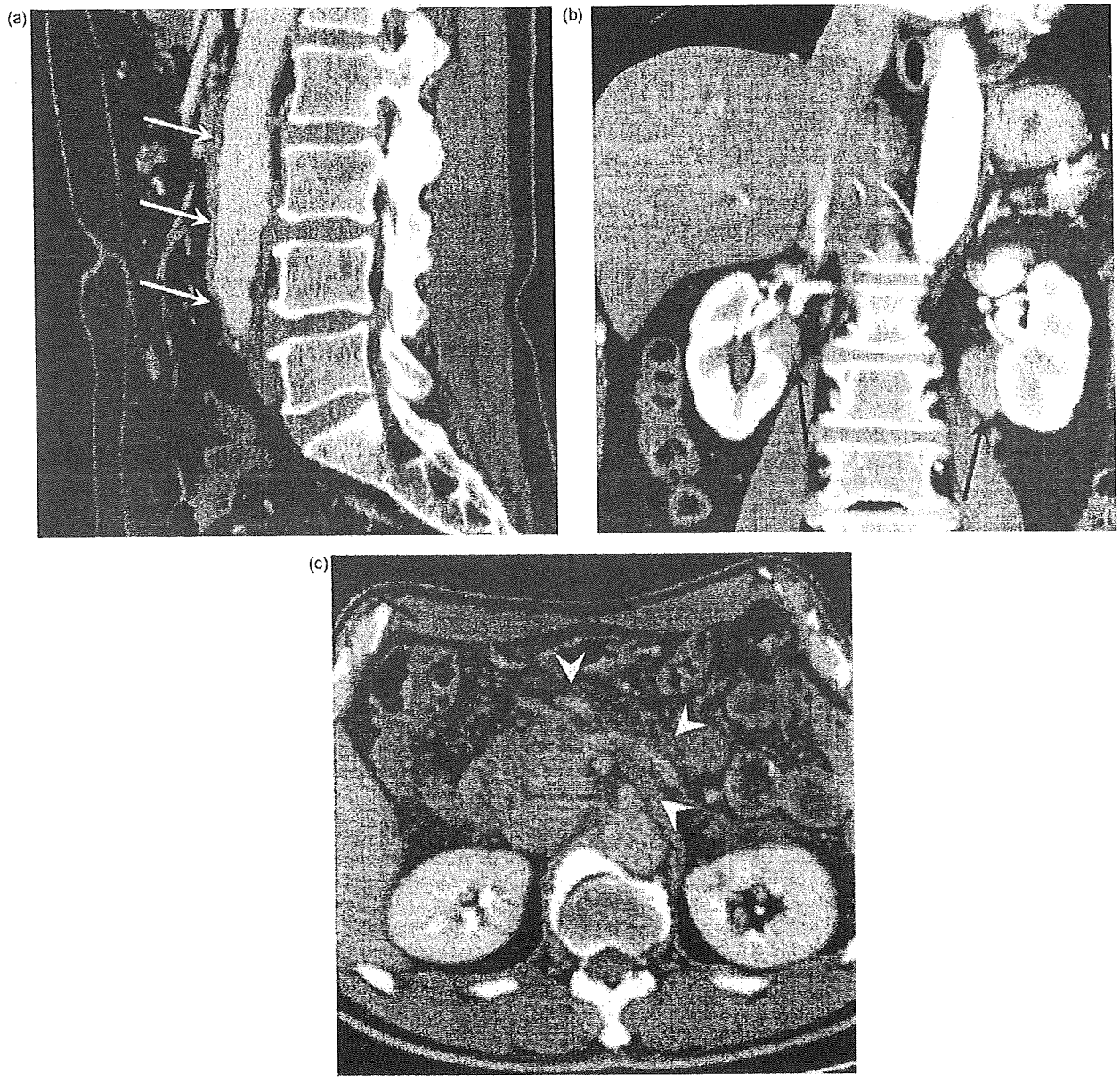

Fig. 9. Retroperitoneal lesions. (a) Sagittal reformation of contrast-enhanced CT shows a soft tissue mass along the aorta (white arrows). (b) Coronal reformation of contrast-enhanced CT shows masses around both ureters (arrows). No hydronephrosis is observed. (c) Axial contrast-enhanced CT shows increased fat density around superior mesenteric artery and splenic vein (white arrow heads).

In our series, almost all patients had Ga-67 accumulation in the pancreas and, concomitantly, in extra-pancreatic lesions, including the lachrymal gland, salivary gland, lung, retroperitoneum and/or prostate. Systemic Ga-67 accumulation was helpful for diagnosing AIP-associated hilar lymphadenopathy. In addition, a normal serum ACE value might be helpful for the exclusion of sarcoidosis.

Radiologically, lung lesions associated with AIP were similar to those indicative of nonspecific inflammatory nodules, chronic bronchitis, chronic bronchiolitis, nonspecific interstitial pneumonia (NSIP), and lymphocytic interstitial pneumonia (LIP). All these lesions had a tendency to spread in the interstitial space of the lung, including the lymphoid channel. At present, we have no clear understanding of the relationship between AIP-related lung lesions and other lung lesions; this remains an issue for future study.

Bile duct abnormality was the most common abdominal finding associated with AIP in the present study, though Shoe et al. reported that retroperitoneal fibrosis was most frequently seen among abdominal lesions in AIP patients [36]. We suspect that differences in the case numbers and study methods were the cause of this disparity. The bile duct wall thickening found in AIP-associated lesions has not been reported in primary sclerosing cholangitis or bile duct carcinoma. In the absence of swelling of the pancreas head, intrahepatic bile duct dilatation was mild, regardless of the bile duct wall thickness. These findings were helpful in differentiating between AIP-associated lesions and those associated with PSC or bile duct carcinoma. In addition, a favorable response to corticosteroid therapy was a distinguishing factor between AIP- and PSC-related lesions, although some sclerosing cholangitis can be

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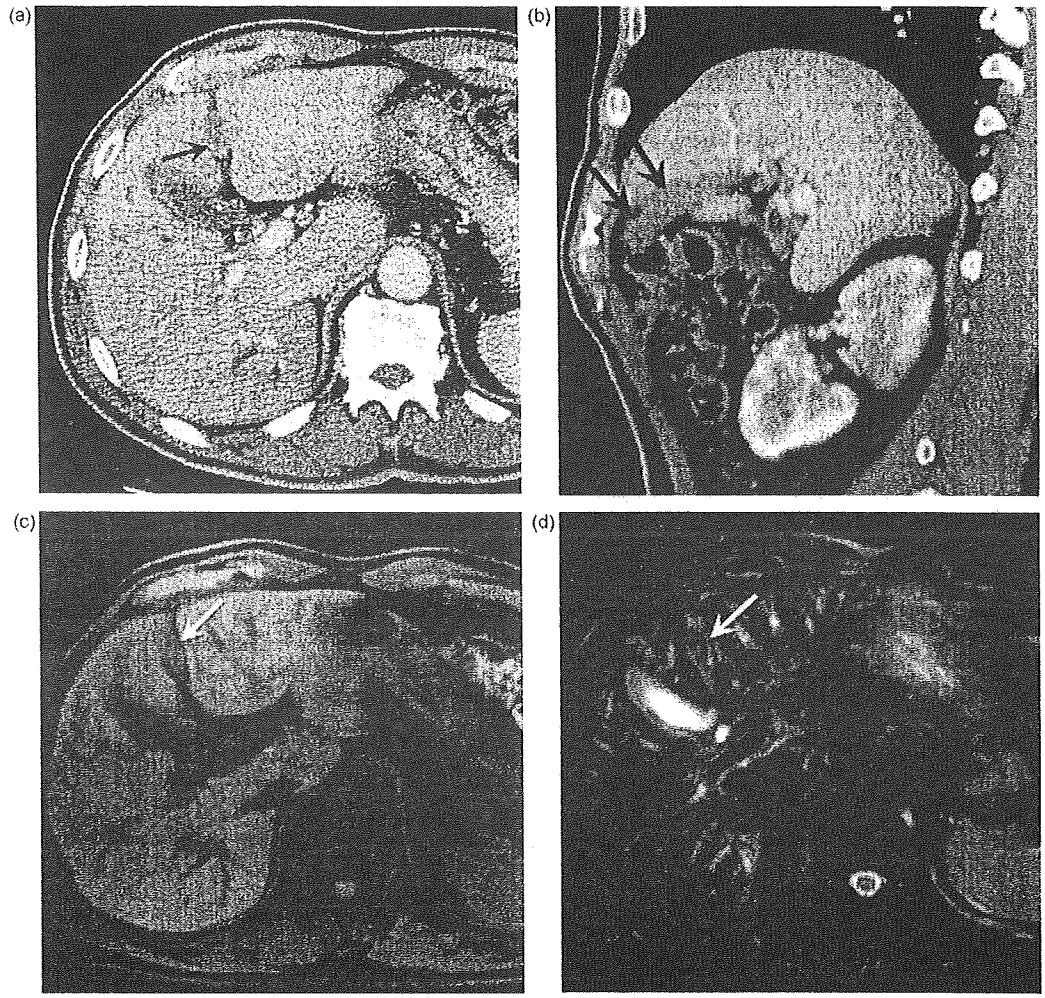

Fig. 10. Lesions in the *ligamentum teres*. (a) Contrast-enhanced CT shows soft tissues mass in the *ligamentum teres* (arrow). (b) Saggital image of contrast-enhanced CT shows the spindle-shape mass along *ligamentum teres* (large arrows). (c–d) MR images show hypointense mass on fat saturated T1-weighted images and hyperintense mass on fat saturated T2-weighted images (white arrows).

cured by steroid therapy [7,17,20,43,44]. However, pancreatic and bile duct lesions had similar pathologies [27,45–47]; thus, when diagnosis with imaging is difficult, a biopsy should be performed. Endoscopic retrograde cholangiopancreatography (ERCP) has also been reported to be useful for differentiating between AIP- and PSC-associated lesions [48].

AIP-associated peri-pancreatic or para-aortic lymphadenopathy is not easily distinguished from inflammatory lymph node swelling and malignant lymphomas. We found that the coexistence of lymphadenopathies and pancreatic lesions is the only distinguishing feature of AIP.

Poorly enhanced multiple wedge-shaped or round lesions associated with AIP are not easily differentiated from those associated with pyelonephritis or infarctions [49,50]. We found graduated enhancement on dynamic contrast-enhanced CTs or MRIs and increased Ga-67 uptake; these characteristics might be specific for AIP-associated renal lesions. Renal hilus lesions are often difficult to differentiate from urothelial tumors, retroperitoneal fibrosis, and lymphomas. Our results suggested that the observation of a combination of pancreatic and extra-pancreatic lesions would be indicative of AIP.

There are only a few reports that describe para-aortic soft tissue masses associated with AIP [12,36,51,52]. Results from those studies were consistent with our findings of a broad homogeneous soft tissue mass along the aorta. This finding should be useful for differentiating AIP from other inflammatory conditions and retroperitoneal tumors. However, the diagnosis is often difficult when a slightly increased density of fat is observed. Kasashima et al. reported that one type of inflammatory abdominal aortic aneurysm was IgG4-related and may be the result of IgG4-related peri-aortitis or retroperitoneal fibrosis [53]. In our series, 3 of 11 patients with para-aortic soft tissue thickening were diagnosed with aortic aneurysms. Hence, IgG4-related para-aortic soft tissue thickening might increase the risk of developing an aortic aneurysm.

This was the first report of AIP-associated *ligamentum teres* lesions. They were rare (2%) but characteristic finding, and both lesions disappeared after corticosteroid therapy. If the Ga-67 scintigraphy shows increased uptake in these lesions, extra-pancreatic lesions associated with AIP can be easily differentiated from other tumors or tumor-like lesions, such as metastatic tumors, leiomyoma, leiomyosarcoma, and extramedullary hematopoiesis because Ga-67 accumulation is very rare in these tumors.

We found prostatic lesions by Ga-67 scintigraphy and diffusion-weighted MRIs. These were often difficult to differentiate from prostate cancer and prostatitis. We found that biopsy was manda-

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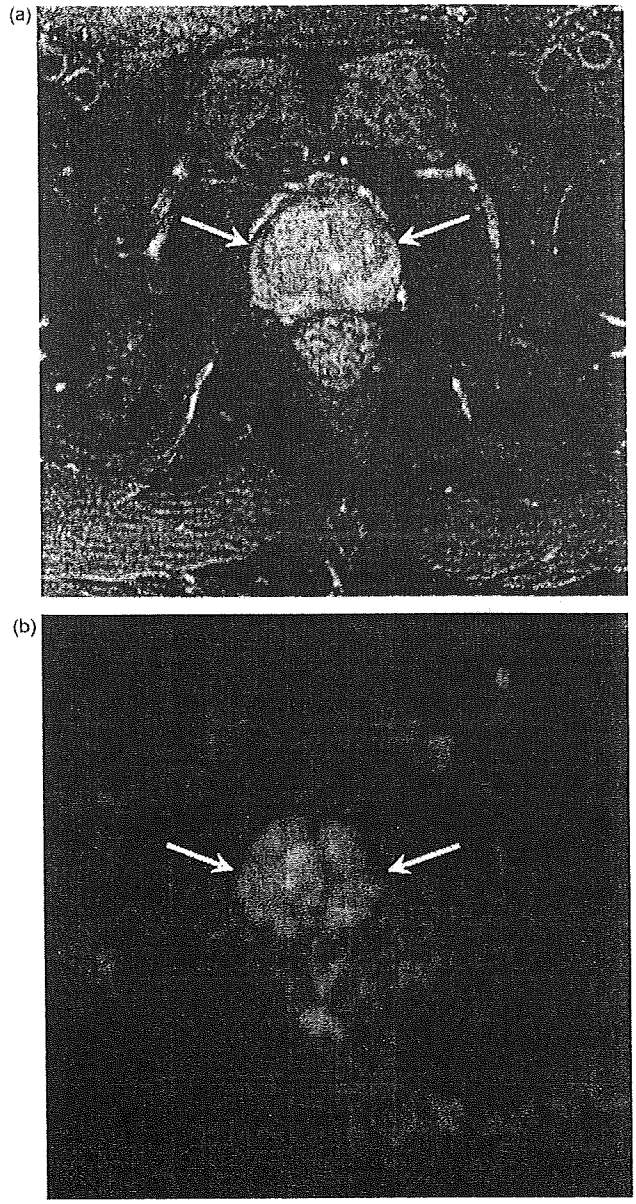

Fig. 11. Prostatic lesions in a 66-year-old man (same case as in Fig. 9a). (a) Axial T2-weighted image with fat saturation shows prostate swelling (white arrows). No mass lesions can be detected. (b) Diffusion-weighted image shows diffuse high intensity signal in the prostate (white arrows).

tory for confirming abundant IgG4-positive plasma cells and lymphocyte infiltration; in addition, a favorable response to corticosteroid therapy was helpful in identifying AIP. To date, a few AIP-associated prostate lesions have been reported, but detailed image findings have not been previously described [54,55].

In this study, we described variable findings of multiple AIP-associated soft tissue masses in the kidney, around the ureter, aorta, paravertebral region, *ligamentum teres*, and orbit. These lesions resembled inflammatory pseudotumors because they frequently contained abundant plasma cell infiltration. Diagnostic imaging played an important role in detecting these lesions, and the characteristic finding of multiple lesions may provide a useful tool for the correct diagnosis of AIP-associated lesions.

AIP and AIP-associated extra-pancreatic lesions are frequently found simultaneously; thus, it is important to examine all other organs when a pancreatic lesion is found. However, these lesions are not always synchronous; thus, successive diagnostic imaging should be mandatory for the detection of newly occurring AIP-associated lesions.

Our study had some limitations, mainly due to using variable models as well as variable scan protocols of CT, MRI and gamma camera because of the length of period reviewed in our retrospective study. Furthermore, all patients were not checked by every image test. Another limitation was that many extra-pancreatic lesions were not histopathologically proven. Therefore, there is a problem that some of the lesions improved by corticosteroid therapy might not be always AIP related extra-pancreatic lesions. Conversely, some of the lesions that were not improved by corticosteroid therapy might include AIP related extra-pancreatic lesions. This problem was not analyzed in this study.

5. Conclusion

AIP is accompanied by various extra-pancreatic lesions and characterized by a variety of image findings. Diagnostic imaging plays an important role in the comprehensive evaluation of these lesions. Radiologists and physicians should keep in mind that multiple lesions may or may not be synchronous under different conditions.

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Long-Term Follow-Up of Autoimmune Pancreatitis: Characteristics of Chronic Disease and Recurrence

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Autoimmune pancreatitis is a unique disease, characterized by lymphoplasmacytic inflammation in the acute stages. However, the active clinical features are unlikely to persist for long periods. Through long-term follow-up, we investigated the disease course in 51 patients with autoimmune pancreatitis. We found recurrence in 21 (41%) patients and pancreatic stone formation in 9 (18%) patients. Pancreatic stone formation was significantly more frequent in the recurrence group (7/21, 33%), compared with the nonrecurrence group (2/30, 7%). Moreover, we found high serum immunoglobulin G4 concentrations in 13 of 175 (7.4%) patients with ordinary chronic pancreatitis. This suggested that pancreatic stone formation is closely associated with recurrence and that autoimmune pancreatitis might transform into ordinary chronic pancreatitis after several recurrences. We found that the immune complex level, with a cutoff value of 10 μg/dL, served as a good predictor of recurrence, with high sensitivity (61.9%), specificity (70.0%), and efficacy (66.7%). We also confirmed that HLA and cytotoxic T-lymphocyte antigen-4 polymorphisms were useful predictors for AIP recurrence.

Autoimmune pancreatitis (AIP) has been characterized by irregular narrowing of the main pancreatic duct (MPD) and sonolucent swelling of the parenchyma, both as a result of lymphoplasmacytic inflammation during the active stage of the disease. AIP has also been characterized by the absence of pancreatic stone formation.[1] These findings suggest that AIP is clearly different from chronic pancreatitis, including alcohol-induced chronic pancreatitis, and has a distinctive disease profile. However, these pathologic features are found in the acute stage of the disease; thus, it is unlikely that the inflammatory characteristic of this condition persists for long periods. In a chronic form of the disease, we might find different features from those now generally recognized. Furthermore, AIP is generally found in older people with suppressed immune surveillance systems; consequently, these patients might be susceptible to various malignant diseases.

To clarify these issues, we conducted long-term follow-ups of AIP, and we investigated 4 topics: (1) outcome, recurrence, and pancreatic stone formation; (2) the ability of AIP to transform into chronic pancreatitis; (3) prediction of recurrence by serum markers; and (4) association of AIP with malignancies.

Outcome, Recurrence, and Pancreatic Stone Formation

We performed long-term follow-ups for 51 patients with AIP to assess outcomes.[2] The observation periods were 24-178 months (mean, 72 months). Corticosteroid therapy was administered to 42 patients. During the long-term follow-up, 21 patients (41%) showed recurrences that required a second course of corticosteroid therapy (Figure 1).

We previously reported that a high serum IgG4 concentration was frequently and specifically found in AIP, representing disease activity.[3] For these 51 patients, we found that the serum IgG4 concentration remained slightly high in more than 60% of patients, although they were in a clinically inactive state after corticosteroid therapy.[4] This suggested that the active inflammatory process might persist even when patients are in a clinically inactive state; these conditions might facilitate recurrences.

We found pancreatic stone formation in 9 of 51 patients (18%). Among the 21 patients with recurrence, 7 (33%) exhibited pancreatic stone formation; in contrast, pancreatic stones were found in only 2 of the 30 (7%) patients in the nonrecurrence group (Figure 1). Accordingly, pancreatic stone formation was judged to be closely associated with recurrence.[2] Previous studies investigated the recurrence of AIP, but they reported lower recurrence rates, ranging from 6%-23%.[5-7] Although the exact reasons for these discrepancies are unclear, they might be related to the number of patients, the follow-up periods, and the type of corticosteroid therapy.

Although a previous study reported that the absence of pancreatic stones is a characteristic of AIP,[1] the potential for forming pancreatic stones is not absent in AIP.[2,6] Incomplete obstruction of the MPD system and the stasis of pancreatic juice might give rise to the formation of pancreatic stones. The finding of irregular narrowing or stricture of the MPD in patients with AIP provided further support for this potential mechanism. In addition, recurrent attacks might have intensified incomplete obstruction of the duct system and caused pancreatic juice stasis, which could have facilitated stone formation; however, we lack evidence to confirm this hypothesis.

One patient with AIP exhibited a pancreatic stone after several recurrences. In June 1996, a 55-year-old man was admitted to our hospital with epigastralgia. His serum amylase level was elevated to 3000 U/L, and he was diagnosed with acute

Abbreviations used in this paper: AIP, autoimmune pancreatitis; CTLA4, cytotoxic T-lymphocyte antigen 4; ERP, endoscopic retrograde pancreatography; IC, immune complex; MPD, main pancreatic duct; mRF, monoclonal rheumatoid factor; MRI, magnetic resonance imaging; SNP, single nucleotide polymorphism.

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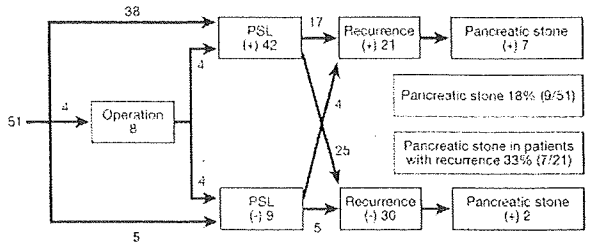

Figure 1. Outcome of long-term follow-up for 51 patients with AIP. Observation periods lasted for 24–178 months (mean, 72 months).

pancreatitis. Next, obstructive jaundice appeared. His serum IgG4 concentration had increased to 486 mg/dL, and endoscopic retrograde pancreatography (ERP) showed irregular narrowing in the pancreatic head region (Figure 2*A*). He was diagnosed with AIP, and steroid therapy was administered. This resulted in amelioration of the ERP finding and lowered the IgG4 levels to 213 mg/dL. In August 1998, the patient showed jaundice and obstruction of the common bile duct by endoscopic retrograde cholangiopancreatography, but there was no swelling of the pancreatic parenchyma or irregular narrowing of the MPD. Serum IgG4 was dramatically elevated to 1135 mg/dL. He was diagnosed with recurrence, primarily in the bile duct lesion. Steroid therapy was administered, and this resulted in the amelioration of the bile duct obstruction. Ten years after the onset, in February 2006, the patient complained of epigastralgia, and ERP showed a narrowing in the body of the MPD and dilatation in the tail region (Figure 2*B*). At that time, he was prescribed prednisolone at a maintenance dose of 2.5 mg. An increase of prednisolone to 20 mg ameliorated the MPD narrowing and dilatation. However, after a reduction of the prednisolone dose, a pancreatic stone appeared in the body and tail regions of the MPD (Figure 2*C*). This case supported the hypothesis that multiple recurrences and the resulting MPD stenosis and pancreatic juice stasis might induce pancreatic stone formation. It also showed that high IgG4 concentrations correspond to recurrence and clearly indicated the active stage of AIP.

On the other hand, we also examined a patient with AIP who had low serum IgG4 concentrations and no recurrences during 10 years of follow-up. In November 1997, a 65-year-old woman was admitted to an affiliated hospital, presenting with epigastralgia, obstructive jaundice, and pancreatic head swelling. She was diagnosed with pancreatic cancer. However, endoscopic retrograde cholangiopancreatography showed diffuse irregular narrowing of the MPD, suggesting a diagnosis of AIP (Figure 2*D*). Her serum IgG4 value was 42 mg/dL, and her serum antinuclear antibody level was ×160. She was diagnosed with AIP and given corticosteroid therapy, which ameliorated these findings. During the 10-year follow-up, she showed no serum elevation of IgG4 and no abnormal image findings including pancreatic swelling that would have suggested recurrences.

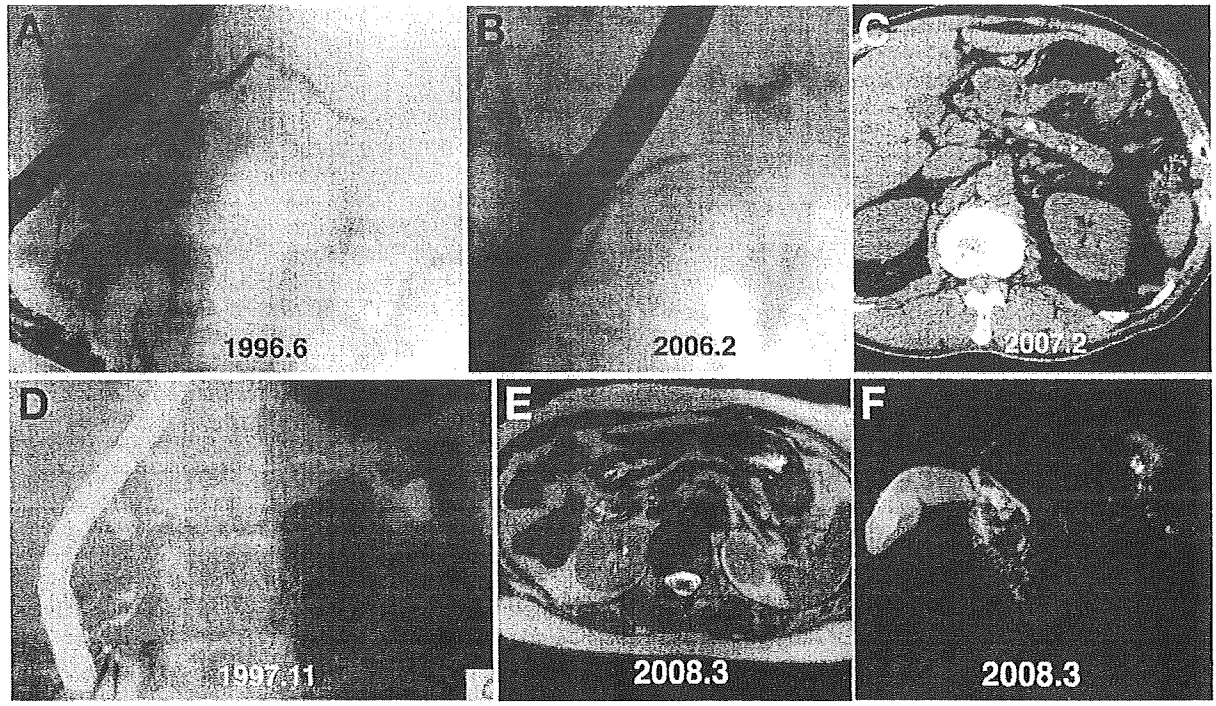

Figure 2. Image findings from 55-year-old man with AIP who had high serum IgG4 concentrations. A pancreatic stone appeared after several recurrences during 10-year follow-up. (*A*) ERP at onset showed irregular narrowing of the head region of the pancreas; (*B*) 10 years after onset, ERP image of MPD showed narrowing in the body and dilatation in tail region. (*C*) Pancreatic stone appeared in the body and tail regions of MPD after reduction in prednisolone dose. For comparison, *lower panels* show image findings for 65-year-old woman with AIP who had normal serum IgG4 concentrations during 10 years of follow-up. (*D*) ERP performed at onset shows diffuse irregular narrowing of MPD; (*E*) MRI and (*F*) MRI of pancreas showed no progression or duct changes in 10 years of follow-up.

Magnetic resonance imaging (MRI) (Figure 2*E*) and MRI of the pancreas (Figure 2*F*) also showed no progression or duct changes during those 10 years. Furthermore, she exhibited no pancreatic stone formation. Accordingly, this case suggested that a normal IgG4 concentration during long-term follow-up accurately represented an inactive state and no disease progression. This also suggested that low serum IgG4 concentrations might be considered an indication for the cessation of maintenance corticosteroid therapy.

Autoimmune Pancreatitis Can Transform Into Chronic Pancreatitis

The results of this long-term follow-up suggested that some patients with AIP could develop pancreatic stones after several recurrent attacks. Further, this suggests that some cases of AIP might transform into ordinary chronic pancreatitis. If true, the next question was whether AIP was a precursor of ordinary chronic pancreatitis. We considered serum IgG4 elevation to be a serologic marker of AIP, even at chronic or advanced stages, because more than 60% of patients with AIP maintained high serum IgG4 concentrations after the clinical symptoms had resolved.[4] To investigate whether AIP might result in ordinary chronic pancreatitis, we measured serum levels of IgG4 in 175 patients with chronic pancreatitis that had been diagnosed before 1995, the year that AIP was first described.[1] We found high serum IgG4 concentrations in 13 of 175 patients with ordinary chronic pancreatitis (7.4%) (12 men and 1 woman; mean age, 56 years; 9 alcoholic and 4 idiopathic). Of the 13 patients, 3 had been diagnosed for the first time with pancreatic cancer, and 1 had been recently diagnosed with AIP. The remaining 9 patients showed typical findings of ordinary chronic pancreatitis, including pancreatic stones or irregular dilation of the MPD (Figure 3). This suggested that an advanced stage of AIP might result in ordinary chronic pancreatitis. It did not rule out the possibility that AIP might represent an early stage of ordinary chronic pancreatitis.[8] Consistent with our results, a previous study found that serum IgG4 was elevated in the sera of 11.9% of patients with ordinary chronic pancreatitis.[9]

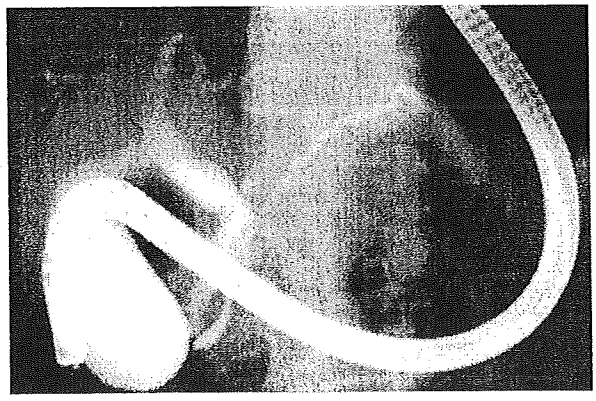

Figure 3. Endoscopic retrograde cholangiopancreatography of 84-year-old man with alcoholic chronic pancreatitis who had high serum IgG4 concentration (585 mg/dL).

Prediction of Recurrence by Serum Markers

Various serum markers and genetic markers have been reported to be associated with the recurrence of AIP. We found that for monitoring AIP, both IgG4 and the immune complex (IC), determined by the monoclonal rheumatoid factor (mRF) method, were useful markers.

In the clinical course of a 69-year-old woman, we found 2 recurrences in which serum elevations of IC and IgG4 preceded the overt appearance of clinical recurrence by several months. This indicated that, in addition to IgG4, IC can sensitively predict recurrence and represent disease activity.[8] We then investigated various serum markers for their efficacy in predicting recurrences by comparing the levels of various markers in recurrence and nonrecurrence groups. We found that the IC value, as determined by the monoclonal rheumatoid method (IC-mRF), was significantly higher at onset in the recurrence group compared with the nonrecurrence group. With a cutoff value of 10 μg/dL, IC-mRF performed well in predicting recurrence, with good sensitivity (61.9%), specificity (70.0%), and efficacy (66.7%). The probability of recurrence was 60% when IC-mRF >10 mg/dL and 30% when IC-mRF <10 mg/dL.

Complement factors C3 and C4 have also been reported as useful markers for monitoring disease activity or tissue damage. We found decreased serum C3 or C4 levels in 35% and 37% of AIP patients, respectively. This suggested that complement activation might play a role in the pathogenesis of AIP.[10] We compared the serum levels of C3 and C4 in patients with high serum IC and those with normal IC levels. Serum C4 was significantly lower and serum C3 tended to be lower in the high IC group compared with the normal IC group.[10] These results, together with the IC data, suggested that a classical pathway might be operating in some AIP patients. In turn, this suggested that high serum IC might be useful for predicting both tissue damage and the probability of recurrence. A report by Kubota et al[11] showed that high serum IgG, diffuse pancreatic swelling, and low bile duct stenosis were frequently observed in patients who had relapsed. A logistic regression analysis showed that diffuse pancreatic swelling was a predictor of recurrence.

Specific HLA polymorphisms were also reported to predict the recurrence of AIP and acted as primary determinants of autoimmune hepatitis susceptibility or relapse.[12] Furthermore, substitution of an aspartic acid at position 57 of the HLA designated DQβ1 (DQβ1 57) was reported to affect the recurrence of AIP. Thus, Park et al[12] examined associations between the onset of AIP recurrence and the density of nonaspartic acids at DQβ1 57. They observed in patients who experienced a recurrence that homozygosity of nonaspartic acids was associated with a significantly earlier onset of recurrence compared with heterozygosity of nonaspartic acids.

The cytotoxic T-lymphocyte antigen 4 (CTLA4) polymorphism has been reported to be another predictor of AIP recurrence.[13] CTLA4 is an inhibitory receptor expressed on the cell surface of activated-memory T cells and on regulatory T cells; it acts largely as a negative regulator of T-cell responses by modulating positive T-cell costimulatory signals on antigen-presenting cells. Single nucleotide polymorphisms (SNPs) in CTLA4, termed +49A/G, have been specifically associated with susceptibility to autoimmune diseases, including type 1 diabetes, autoimmune thyroid disease, autoimmune hepatitis, and primary biliary cirrhosis. To investigate whether CRLA4 was associated

with AIP pathogenesis in our cohort, we determined the presence of 4 CTLA4 gene SNPs in patients with AIP and healthy controls. We detected SNP +6230G/G significantly more frequently in patients with AIP than in healthy subjects. In addition, we found that the +49A/A and +6230A/A genotypes were associated with an enhanced risk of recurrence.[13]

Autoimmune Pancreatitis and Malignancies

AIP is generally found in older individuals with suppressed immune surveillance systems who consequently have increased susceptibility to various malignant diseases. To date, among 51 AIP patients, we found 11 malignant lesions in 9 patients (17.6%); these included malignant lymphoma, lung cancer, hepatocellular carcinoma, renal carcinoma, breast cancer, duodenal cancer, colon cancer, prostate cancer, and ovarian cancer. These findings were consistent with other reports that found various malignant lesions complicated with AIP.[6,14-16] Some malignancies might occur during or after maintenance therapy with corticosteroid, suggesting that a steroid-induced immunosuppressive state could induce malignant lesions. Because we had no age-matched controls, we could not determine whether AIP represented a significantly higher risk for malignant diseases. Incidentally, it is important to differentiate between AIP and pancreatobiliary malignancies. Bile duct cancer was reported to be associated with AIP[16]; thus, the co-occurrence of bile duct cancer should be investigated, even with a confirmed diagnosis of AIP.

Twelve cases of pancreatic cancer associated with AIP have been previously reported.[17-21] Among the 12 cases, 5 occurred concurrently with AIP, and 7 occurred from 3–5 years after the diagnosis of AIP. It has been estimated that, generally, two thirds of pancreatic cancers occur in the head region. Surprisingly, 9 of the 12 tumors (75%) associated with AIP were located in the body and tail regions of the pancreas, including 3 in the head, 5 in the body, 3 in the tail, and 1 in the body and tail regions. Accordingly, the occurrence of a tumor in the body and tail of the pancreas might be a characteristic finding of pancreatic cancer as a complication of AIP.[22] In addition to the immunosuppressive state, a chronic inflammatory process similar to ordinary chronic pancreatitis might evoke pancreatic malignancy.[23] We believe that, although the exact cancer prevalence is unknown, a careful follow-up with tumor markers is mandatory.

In conclusion, our findings showed that 40% of patients with AIP had recurrences during long-term follow-up. Many patients had been treated with prednisolone at onset because of the highly active state of the disease. We observed that although the disease appeared to be clinically inactive during or after maintenance therapy, relapse was likely to occur, and that long-term therapy was required. To prevent clinical relapse, corticosteroid therapy should be restarted at an early subclinical stage of relapse, and reliable parameters must be identified for predicting relapse. Among several possible serum markers, we found that the IC-mRF value showed significant elevation in the recurrence group, suggesting that IC might be useful for predicting the recurrence of AIP. Furthermore, a normal IgG4 value represented the inactive state and might be an indicator for the cessation of maintenance corticosteroid therapy. Some patients with AIP exhibited complications with pancreatic stones after several attacks of recurrence. Thus, AIP appeared to transform into a form of chronic pancreatitis. AIP appears to be associated with a variety of malignant lesions. To date, 12 cases of pancreatic cancer associated with AIP have been reported, with a preponderance in body and tail regions of the pancreas. Although AIP should be carefully differentiated from pancreatic cancer, we recommend monitoring for the co-occurrence of pancreatic cancer even when a diagnosis of AIP is confirmed.

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Conflicts of interest
The authors disclose no conflicts.

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A Novel Heterophilic Antibody Interaction Involves IgG4

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Abstract

IgG4 has been implicated in a diverse set of complex pathologies – e.g. autoimmune pancreatitis (AIP), idiopathic membranous nephropathy – and carries unique features including lack of activation of the classical complement pathway and a dynamic Fab-arm exchange. We recently showed that the rheumatoid factor (RF)-like activity of IgG4 is achieved through a hitherto unknown, Fc–Fc (and not Fab–Fc as is the case in classical RF; CRF) interaction; hence the name, novel RF (NRF). Here, we further explore the resemblance/difference between CRF and NRF. As heterophilic interactions of human IgM RF (CRF) are well known, we checked whether this is the case for IgG4. Human IgG4 showed variable reactivity to animal IgGs: reacting intensely with rabbit and mouse IgGs, but weakly with others. The binding to rabbit IgG was not through the Fab (as in CRF) but via the Fc piece, as was recently shown for human IgG (NRF). This binding correlates with the IgG4 concentration *per se* and could therefore be of diagnostic usage and incidentally explain some observed interferences in biological assays. In conclusion, here is defined a novel heterophilic antibody interaction and is established the universality of the unique Fc–Fc binding, both involving IgG4.

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Introduction

A unique form of chronic pancreatitis displaying hypergammaglobulinemia, pancreatic lymphoplasmacytic inflammation and favourable response to corticosteroids has been recently dubbed 'autoimmune pancreatitis' (AIP) [1, 2]. Available genetic studies have further linked AIP susceptibility to the *HLA-DRB1*0405-DQB1*0401* haplotype, and with polymorphisms in Fc Receptor-Like 3 (*FcRL3*) and *CTLA4* genes [3–6]. An outstanding finding in AIP is the existence of a high serum concentration for IgG4 which has been documented in 90% of patients [7]. This occurs in parallel to an abundant IgG4 positive plasma cell infiltration in the pancreatic tissue [8] collectively suggesting that IgG4 may have a decisive role in AIP pathogenesis. At present, AIP is considered to be the pancreatic expression of a broader IgG4-associated systemic disease [9].

The role of IgG4 in immune response and autoimmunity has not yet been fully elucidated, despite recent elegant studies implicating it in defined organ-specific autoimmunity, e.g. idiopathic membranous nephropathy [10]. The biology of IgG4 is equally interesting. Indeed, this Ig subclass is unique to the fact that it is unable to activate the classical pathway of complement and possesses a 'dynamic lifestyle' given a continuous process of half-molecule exchange, referred to 'Fab-arm exchange' [11, 12]. Moreover, IgG4 can exist in sera of healthy subjects as anti-IgG antibody, alike the rheumatoid factor (RF) [13–15]. This is exacerbated in patients with AIP where the rise in IgG4 concentration has helped us to understand the topology of IgG4–IgG interaction. To

our surprise, this interaction was not through the classical Fab–Fc recognition in the RF (hence called classical RF; CRF) but via an unprecedented Fc–Fc binding allowing to dub this interaction as 'novel rheumatoid factor' (NRF) versus CRF (the IgM RF) [16]. This has been since validated by at least another group [17]. Because the CRF is well known to react with the Fc piece of animal IgG [18] and this has been indeed part of its historical identity, at times used in diagnostics, we set to further asses if the human IgG4 may follow a similar interaction. If this was indeed the case, the question shall arise as to if this is done through the same Fc–Fc interaction.

Results

Human IgG4 binds to IgG from various animal species

IgG4 remains a peculiar subclass of human IgG given several unique characteristics mentioned earlier. These include both its implication in unique pathophysiological situations as well as its inherent physicochemical properties. Paramount among the latter are perhaps its dynamic Fab-arm exchange [12] and the more recently identified RF activity which is executed through a unique Fc–Fc interaction unlike the classical Fab–Fc recognition of the IgM anti-IgG RF [16, 17]. Here, we aim to take an incremental step in further characterizing human IgG4 RF (NRF) and its resemblance/dis-resemblance to CRF. One main characteristic of CRF is its reactivity to animal IgG [19]. This was used at times in diagnostic tests and has been a source of biological interference in clinical assays [20].

Animal IgGs showed a range of reactivity to human IgG4 purified from patients with AIP. Mouse and rabbit IgGs had strong reactivity to human IgG4, guinea pig, bovine, goat and dog had intermediate reactivity, and finally sheep, horse and rat IgG scarcely reacted at all (Fig. 1). Because mouse IgG had such strong reactivity to human IgG4, we checked the reactivity of its different subclasses. There was intense reactivity of mouse IgG2a with human IgG4 but weak reactivity with the other 3 mouse IgG subclasses (Fig. 2).

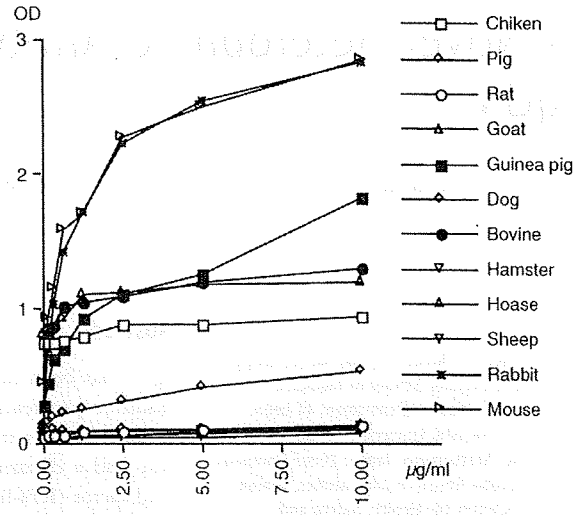

Figure 1 ELISA showing human IgG4 reactivity for various animal IgGs. Various animal IgG was coated onto a microplate and then reacted with horseradish peroxidase-human IgG4. Human IgG4 shows a wide variety of reactivity to animal IgGs.

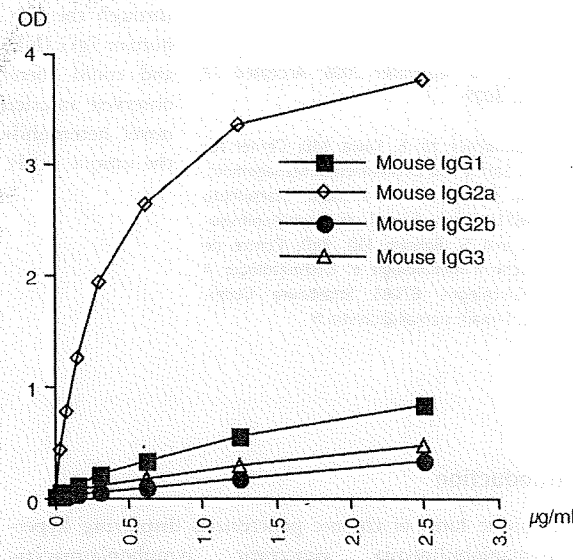

Figure 2 ELISA showing human IgG4 reactivity for mouse IgG subclasses. Each mouse IgG subclass was coated onto a microplate and then reacted with horseradish peroxidase-human IgG4. Human IgG4 shows strong reactivity to mouse IgG2a.

Human IgG4 binds animal IgG via an Fc–Fc interaction

Because rabbit (like mouse) IgG had strong reactivity to human IgG4, among the various animal IgGs tested, we assessed the topology of rabbit IgG reactivity to human IgG4 by Western blot (WB) and ELISA. The identity of each IgG4, IgG4 F(ab')2 and IgG4 Fc sample was confirmed by the reactivity with the corresponding horseradish peroxidase (HRP)-labelled antibody: HRP-labelled anti-IgG4 Fc antibody reacted to purified IgG4 and IgG4 Fc, but not to IgG4 F(ab')2; HRP-labelled anti-human κ antibody reacted to IgG4 F(ab')2, but not to IgG4 Fc. We further checked whether the IgG4 Fc bound to rabbit IgG or not. A preparation of rabbit IgG was resolved on a 10% sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE) under reducing conditions and then transferred onto a polyvinylidene difluoride (PVDF) membrane. HRP-labelled anti-IgG4

Fc antibody and HRP-labelled anti-human κantibody had no reactivity to rabbit IgG (Fig. 3, lanes 1 and 2). HRP-labelled anti-IgG4 Fc antibody showed strong reactivity to rabbit IgG after lanes were incubated with purified IgG4 (Fig. 3, lane 3) or IgG4 Fc (Fig. 3, lane 5). However, HRP-labelled anti-human κantibody showed no reactivity to rabbit IgG after the lane was incubated with IgG4 F(ab')2 (Fig. 3, lane 4). These results indicated that it was IgG4 Fc rather than the Fab piece that bound to rabbit IgG, as for human IgG, and that therefore IgG4 binding to rabbit IgG is not because of antibody (Fab) activity.

In vivo assessment

The binding of serum IgG4 to rabbit IgG was negligible in 130 healthy controls, as well as in patients with other autoimmune diseases including autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), systemic lupus erythematosus (SLE) and Sjögren's syndrome, as well as those affected with other pancreatic diseases, i.e. chronic pancreatitis and pancreatic cancer. This was in contrast to the properties of serum IgG4 in a cohort of patients with AIP in whom there were significantly elevated concentration of IgG4 that bound to rabbit IgG (Fig. 4).

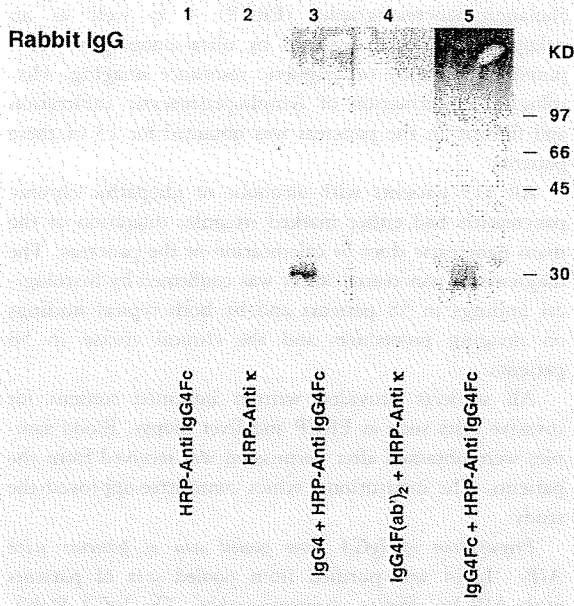

Figure 3 The topology of IgG4–rabbit IgG interaction. Western blotting was used to establish whether the Fc or the Fab portion(s) of IgG4 reacted to rabbit IgG. Rabbit IgG was blotted on each lane. Horseradish peroxidase (HRP) labelled anti-human IgG4 Fc or HRP-labelled anti-human κlight-chain showed no reactivity to rabbit IgG (lanes 1 and 2). HRP labelled anti-IgG4 Fc antibody reacted in lanes 3 and 5, which were previously incubated with purified IgG4 and IgG4 Fc, respectively. HRP labelled anti-human κ light-chain had no reactivity to lane 4, which was incubated with IgG4 F(ab')2 (lane 4). These results indicated that IgG4 binds to rabbit IgG by its own Fc, and not by Fab as a classical rheumatoid factor. Experiments were repeated three times with identical results.

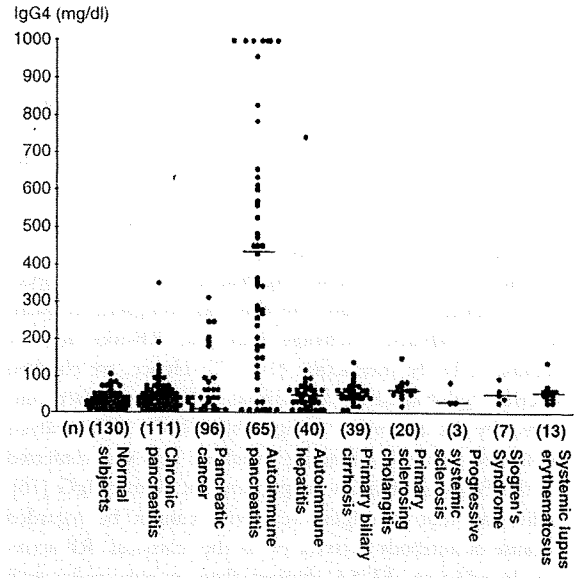

Figure 4 Scattergram of serum levels of IgG4 bound to rabbit IgG. Serum concentrations of IgG4 that bound to rabbit IgG were significantly elevated in patients with autoimmune pancreatitis, whereas those in other conditions were remained in lower levels.

In patients with AIP, serum levels of IgG4 bound to rabbit IgG were correlated well with the serum IgG4 level itself (correlation coefficient 0.899, $P < 0.0001$), but not with serum RF levels (correlation coefficient −0.0723, $P = 0.580$). For these patients, high serum IgG4 concentrations (>135 mg/dl) were found in 50 of 65 (76.9%), and the median IgG4 value was 711 mg/dl.

Discussions

The present study demonstrated that human IgG4 obtained from patients with AIP bound to a variety of animal IgGs, albeit with different affinities. High affinity was found for mouse and rabbit IgG, whereas weak or minimal affinity was found for sheep, horse and rat IgG. It is well established for human IgM RF (CRF) to have high affinity for rabbit IgG; indeed this is used in clinical testing for detection of human RF in rheumatoid arthritis for instance [19]. Accordingly, the reactivity of human IgG4 for animal IgG resembles that of RF in regard to the affinity of reactivity for rabbit IgG. We have meagre data to explain the difference in affinity of human IgG4 for various animal IgGs. In our study, human IgG4 reacted intensely to the mouse IgG2a subclass, but minimally to the other 3 mouse IgG subclasses, indicating that human IgG4 has different affinities for

each subclass of an individual animal IgG. The low affinity of human IgG4 for some animal IgGs may be because of a low-affinity IgG subclass being purified from the whole IgG of animal by protein A or G affinity chromatography and available commercially. Alternatively, evolutionary changes in IgG structure may account for the difference. Clearly, further experiments are needed to outline the mechanism behind the strong variation in IgG4 binding to IgG from different species.

There are several interesting features for human IgG4 [11]: inability to activate the classical complement pathway [21], Fab-arm exchange [12] and RF-like activity because of Fc–Fc interaction [16, 17]. Hence, we checked whether IgG4 binding for animal IgG was in fact conferred by its Fc piece, using rabbit IgG. WB analysis confirmed that IgG4 binding to rabbit IgG was conferred by the Fc piece of IgG4, as pertains for human IgG [16], indicating that this IgG4 reactivity cannot be regarded because of antibody activity or to the 'classical' RF activity. In addition, ELISA showed that in our study, each patient with AIP with a high serum IgG4 concentration had a high serum level of IgG4 that bound to rabbit IgG, and the serum level of IgG4 bound to rabbit IgG closely correlated with the actual serum IgG4 concentration but not with serum RF levels. Hence, human IgG4 showed intense reactivity to animal IgGs, with an Fc–Fc interaction similar to that seen with human IgG subclasses. Furthermore, serum RF seems to have little effect to this interaction (data not shown).

The present assay system using rabbit IgG should provide several promising utilities. Incidentally, it will provide an alternative assay system for measuring serum IgG4 levels at low cost, because it needs no IgG4 capture antibody. Second, it can combine with a RF assay system: thus because RF also binds to rabbit IgG, both IgG4 and RF activities can be measured together, when the respective HRP-labelled anti-IgG4 and anti-IgG or anti-IgM are used as tracer antibodies in a single assay system. However, in contrast, a high affinity of IgG4 for rabbit or mouse IgG may interfere in various assay systems in which rabbit or mouse antibody are used for capture, when samples with high serum IgG4 concentration are assayed.

Given that IgG4 binding to rabbit IgG mimics the (classical) RF, the role of IgG4 may mimic that of RF. In general and to date, it remains to be determined whether RF activity is beneficial or detrimental [22], i.e. Fc–Fc interactions, like low-affinity polyreactive IgM RFs may aid in the clearance of immune complexes by forming larger ones that are more effectively cleared [23] but conversely, and like high-affinity IgG and IgA RF, they may have harmful effects such as deposition in blood vessels causing vasculitis or nephritis [24, 25].

In conclusion, the distinctly elevated serum IgG4 found in AIP can bind various animal IgGs, as does CRF. This unique, novel and at presently universal heterophilic interaction – IgG4-Fc binding to the Fc piece of animal IgG – may have several promising utilities and/or cause/explain interference in some biological assays but more generally helps further to define the NRF versus CRF classification.

Subjects and methods

Subjects. Serum samples were obtained from 65 patients with AIP – 54 men and 11 women, aged 38–79 (median age 62.4 years) – 111 patients with alcoholic or idiopathic chronic pancreatitis, 96 with pancreatic cancer, 40 with AIH, 39 with PBC, 20 with PSC, 13 with SLE and seven with Sjögren's syndrome. One hundred and thirty normal subjects were also included in the study. Serum samples were stored at −20 °C before use.

All 65 patients with AIP fulfilled the revised diagnostic criteria proposed by Japan's Pancreas Society [26]. These include the following biological and radiological findings: elevated serum immunoglobulin including IgG4 and/or positive auto-antibodies, e.g. anti-nuclear antibody and RF, the irregular narrowing of the main pancreatic duct – as evidenced by endoscopic retrograde cholangio-pancreatography (ERCP) – as well as an enlarged pancreas as assessed by ultra-sonography, computed tomography or magnetic resonance imaging. Histological confirmation of lymphoplasmacytic infiltration and fibrosis in the pancreas was obtained for 13 of these patients.

All 111 patients with alcoholic or idiopathic chronic pancreatitis had either marked irregular dilatation of the main pancreatic duct or calcification of the pancreas. The diagnosis of pancreatic cancer was confirmed by histological findings in 38 patients and by both typical findings on imaging procedures and the clinical course in 58 patients.

All subjects provided written informed consent for invasive tests such as ERCP and liver biopsy. Blood samples were obtained after permission was received from the patients. The institutional ethics committee approved the study.

Preparation of IgG4 from pooled sera of patients with AIP. IgG4 was purified from pooled sera of patients with AIP by affinity chromatography. The IgG4 $F(ab')_2$ fraction and IgG4 Fc fractions were derived by digesting purified IgG4 with pepsin and papaïn, respectively [16]. HRP conjugation of purified IgG4 was performed using peroxidase labelling kit-NH2 (DMT LK11; Dojindo Molecular Technologies, Inc., MD, USA) according to the manufacturer's instructions. The peroxidase-conjugated mouse anti-IgG4 monoclonal antibody reacted to the Fc portion of IgG4 (South Biotech 9200-05). Goat anti-humanκ light chain (Bethyl Laboratories, Inc, A80115P) was used to detect IgG4 $F(ab')_2$.

