ORIGINAL ARTICLE

# Prospective multicenter study on the usefulness of EUS-guided FNA biopsy for the diagnosis of autoimmune pancreatitis

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**Background and Aims:** In the International Consensus Diagnostic Criteria (ICDC), autoimmune pancreatitis (AIP) is classified into types 1 and 2, and its definite histopathology diagnosis can be made based only on surgical or core biopsy specimens. Although EUS-guided FNA (EUS-FNA) biopsy is a safe technique for the collection of pancreatic tissue, no consensus viewpoint has been reached with regard to the role of EUS-FNA biopsy in the diagnosis of AIP. This study investigated the utility of pancreatic tissue collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle in the diagnosis of AIP.

**Methods:** Patients with suspected AIP were prospectively enrolled in Nagoya University Hospital and Nagoya University–affiliated institutions. Pancreatic tissue was collected from each by EUS-FNA biopsy with a standard 22-gauge aspiration needle.

**Results:** Fifty patients were registered, including 45 with a final diagnosis of AIP. Lymphoplasmacytic infiltration and abundant immunoglobulin $G_4$–positive plasmacyte infiltration (>10/high-power field) were detected in 36 (72%) and 27 (54%) patients, respectively. Obliterative phlebitis and storiform fibrosis were not detected in our study. Granulocytic epithelial lesions (GEL) were observed in 3 patients. The sensitivity, specificity, positive predictive value, and negative predictive value of EUS-FNA biopsy to definitively diagnose AIP were 7.9% (3/38), 100% (12/12), 100% (3/3), and 25.5% (12/47), respectively. Pathology evaluation of pancreatic tissue collected by EUS-FNA biopsy improved the diagnostic accuracy in 8 (16%) of the 50 patients.

**Conclusions:** In this study, EUS-FNA biopsy by using a standard 22-gauge aspiration needle is not an effective diagnostic method for most patients with AIP. The combination of level 2 histology diagnosis of AIP with other findings specified in the ICDC slightly improved the diagnostic accuracy, although it still remains insufficiently accurate for routine clinical use.(Clinical trial registration number: 000006297.) (Gastrointest Endosc 2016;■:1-8.)

Autoimmune pancreatitis (AIP) was initially reported by Yoshida et al[1] in 1995. In this form of pancreatitis, an autoimmune mechanism is involved in the initial pathology, which leads to swelling of the pancreas and narrowing of the pancreatic duct. In the International Consensus Diagnostic Criteria (ICDC),[2] published in 2011, AIP is histopathologically classified as type 1, which manifests as the characteristic lymphoplasmacytic sclerosing pancreatitis (LPSP),[3,4] and type 2 AIP, which manifests as idiopathic duct-centric chronic pancreatitis

*Abbreviations: AIP, autoimmune pancreatitis; EUS-FNA, EUS-guided FNA; GEL, granulocytic epithelial lesion; H&E, hematoxylin and eosin; HPF, high-power field; ICDC, International Consensus Diagnostic Criteria; IDCP, idiopathic duct-centric chronic pancreatitis; IgG, immunoglobulin G; LPSP, lymphoplasmacytic sclerosing pancreatitis; NPV, negative predictive value; PPV, positive predictive value.*

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(IDCP).[3-6] Because type 1 AIP often occurs with concomitant diverse extrapancreatic lesions with immunoglobulin $G_4$ ($IgG_4$)-positive plasma cell infiltration in the pancreas and extrapancreatic lesions, it is therefore considered to be a pancreatic lesion that occurs as a consequence of systemic $IgG_4$-related disease.[7-10] On the other hand, type 2 AIP develops more frequently in young persons, and it is often complicated by inflammatory bowel disease. In general, in type 2 AIP, the serum $IgG_4$ levels are normal, and no $IgG_4$-positive plasma cells are observed histopathologically.[2] However, many points remain unclear with regard to the pathology. In the consensus from the American Pancreatic Association/Japan Pancreas Society meeting, which was held in Hawaii in 2009, and the ICDC, the definite histopathology diagnosis of AIP including diagnosis of each type can be made based only on surgical specimens or tissue collected by core biopsy.[2,11] EUS-FNA biopsy is a safe, established technique that is widely used to collect pancreatic tissue,[12-17] and the usefulness of core biopsy[18-20] and EUS-FNA biopsy by using a 19-gauge needle[21] for diagnosis of AIP has been reported. However, the diagnosis of AIP based on tissue collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle, which is widely used in clinical practice, has been investigated only in a small number of retrospective case studies.[22-24]

We performed a prospective, multicenter study to investigate whether or not pancreatic tissue collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle can contribute to the diagnosis of AIP.

## MATERIALS AND METHODS

### Study design

This was a prospective, multicenter study involving the Nagoya University Hospital and the Nagoya University–affiliated institutions (18 hospitals). Written informed consent was obtained from each patient, and the study was performed after approval of the ethics committees of each institution. The study was registered at the University Hospital Medical Information Network Clinical Trials Registry (no. 000006297) on June 9, 2011. All authors had access to the study data and have reviewed and approved the final manuscript.

### Patients

Patients who met the pancreatic parenchyma findings, as specified in the ICDC (ie, diffuse enlargement with delayed enhancement, sometimes associated with rim-like enhancement, or segmental and/or focal enlargement with delayed enhancement), pancreatic duct findings (long [>1/3 length of the main pancreatic duct] or multiple strictures without marked upstream dilatation or segmental and/or focal narrowing without marked upstream dilatation [duct size <5 mm]) and who underwent EUS-FNA biopsy by using a standard 22-gauge aspiration needle were enrolled between July 1, 2011 and December 31, 2014. The enrollment criteria were as follows: (1) patients between 20 and 80 years old at the time of enrollment, (2) performance status of 0 to 1, (3) preservation of main organ functions at the time of diagnosis, and (4) provision of written consent to participate in the study. The exclusion criteria were as follows: (1) steroid administration within 3 months before the initiation of treatment, (2) poorly controlled infection (including active tuberculosis), (3) serious drug allergy, (4) difficulty in observation of the course by endoscopy, (5) malignant tumor, (6) serious adverse events, (7) pregnant and lactating women, women who were possibly pregnant, and women who might want to become pregnant, (8) severe mental disorder, and (9) judgment as being ineligible by the principal or subinvestigator.

### EUS-FNA biopsy procedure

While the patient was under conscious sedation, a curved linear array echoendoscope was inserted orally, and the lesion was punctured with a standard 22-gauge aspiration needle (25-gauge when application was difficult), this was an end-hole needle, via the gastric or intestinal wall for the collection of pancreatic tissue. EUS-FNA biopsy was performed in the involved gland in the cases with focal pancreatic enlargement. Because it was easy to perform EUS-FNA biopsy, we sampled from the pancreatic body and tail in cases with diffuse involvement of the gland. We attempted to avoid the visible main pancreatic duct. A stylet was set, and the puncture was applied while the stylet was in place. After puncture with the needle and stylet, the stylet was removed from the needle, and the needle was moved to-and-fro 10 to 20 times. Two specimens were collected under a 20-mL negative pressure (the make and model of curved linear array echoendoscope and puncture needle were not specified because they varied between institutions). The collected specimens were immediately placed in formalin solution to prevent drying of the tissue, and the presence of a whitish tissue component with a tube-like solid appearance was confirmed visually. When no whitish component was observed, an additional specimen was collected (the mean [± standard deviation {SD}] number of EUS-FNA biopsy procedures was 2.02 ± 0.48, range 1-4). This study was conducted to confirm whether a standard 22-gauge aspiration needle could be used to collect pancreatic biopsy specimens. After collection, a hemostat was applied, and antibiotics were administered by drip infusion to prevent hemorrhage and infection, respectively.

### Pathology sample preparation

After formalin fixation, the specimens were embedded in paraffin, sectioned, and subjected to hematoxylin-eosin (H&E) staining and immunostaining (staining for IgG and $IgG_4$). When the quality of the immunostaining was poor,

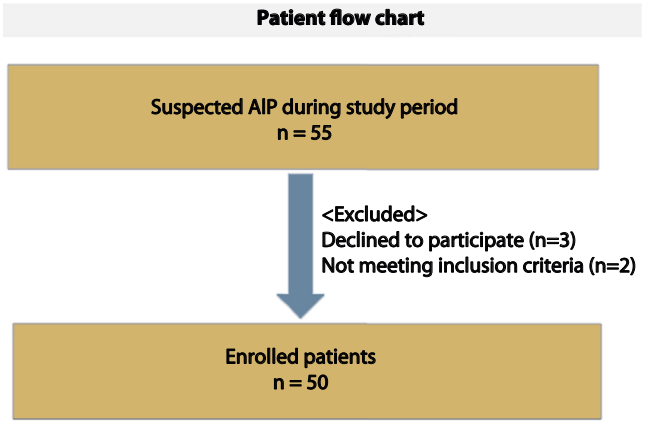


**Figure 1.** Flowchart of enrolled patients. Three patients declined to participate, and 2 patients were excluded due to steroid administration within 3 months before the initiation of treatment. *AIP,* autoimmune pancreatitis.

unstained samples were stained by the Nagoya University Hospital Department of Pathology and Laboratory Medicine.

## Pathology evaluation

Two specialists (Y.S., A.S.) at the Nagoya University Hospital Department of Pathology and Laboratory Medicine evaluated the histopathology of the preparations including the LPSP findings (marked lymphocyte and plasma cell infiltration, obliterative phlebitis, storiform fibrosis, $IgG_4$-positive plasma cell infiltration >10/high-power field [HPF]) and the IDCP findings (granulocytic epithelial lesion [GEL], neutrophil and lymphocyte infiltration in pancreatic acini, and $IgG_4$-positive plasma cell infiltration ≤10/HPF). According to the ICDC, the level-1 criteria of LPSP were positive for 3 or more of the 4 LPSP findings, and the level-2 criteria were positive for 2 of the 4 items. Similarly, the level-1 criteria of IDCP were positive for both GEL and $IgG_4$-positive plasma cell infiltration ≤10 HPF, and the level-2 criteria were positive for both neutrophil and lymphocyte infiltration in the pancreatic acini and $IgG_4$-positive plasma cell infiltration ≤10/HPF. When the diagnosis was inconsistent, the 2 pathologists consulted and agreed on the final pathology diagnosis. Each pathologist was blinded to the clinical details and made evaluations without knowledge of the assessment made by the other in a blinded manner, in order to avoid errors of pathology evaluation.

## Study outcome

In the ICDC, the diagnosis of definitive AIP could be made based on the pathology examination alone by using tissue samples meeting level-1 histology diagnosis. As the primary endpoint, we evaluated the diagnostic performance for each type of AIP based on the specimens collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle. In order to evaluate the ability to obtain a definite (level 1) histology diagnosis of AIP including subtype, we provide the descriptive statistics, that is, sensitivity, specificity, positive predictive value

**TABLE 1. Baseline characteristics, n = 50**

| | |
|---|---|
| Men:women | 39:11 |
| Age, mean (range), y | 64.4 (22-80) |
| No. of needle passes, mean ± SD (range) | 2.02 ± 0.48 (1-4) |
| Diagnosis after EUS and before FNA biopsy, no. | |
| AIP | 45 |
| Chronic pancreatitis | 3 |
| Pancreatic neoplasm | 2 |
| Diagnosis according to ICDC at the time of enrollment, no. | |
| Definitive AIP type 1 | 27 |
| Nonspecified diagnosis | 23 |
| Final diagnosis, no. | |
| Definitive AIP type 1 | 35 |
| Probable AIP type 1 | 6 |
| Definitive AIP type 2 | 3 |
| Probable AIP type 2 | 1 |
| Not otherwise specified (NOS) | 3 |
| Nonspecified diagnosis | 2 |
| Parenchymal imaging, no. | |
| Typical* | 30 |
| Indeterminate† | 20 |
| Ductal imaging, no. | |
| Long or multiple strictures | 30 |
| Segmental and/or focal narrowing | 20 |
| Serum $IgG_4$ level (mg/dL)‡ | |
| $IgG_4$ <135 | 14 |
| 135 ≤ $IgG_4$ ≦270 | 9 |
| 270 < $IgG_4$ | 27 |
| Other organ involvement, no. | |
| Sclerosing cholangitis | 11 |
| Retroperitoneal fibrosis | 5 |
| Symmetrically enlarged salivary/lachrymal glands | 3 |
| $IgG_4$-related nephritis | 4 |
| Ulcerative colitis | 1 |
| $IgG_4$-related disease, no. | |
| New diagnosis | 41 |
| History | 0 |

*SD,* Standard deviation; *AIP,* autoimmune pancreatitis; *IDCP,* idiopathic duct-centric chronic pancreatitis; *IgG4,* immunoglobulin $G_4$.
*Typical imaging findings: diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement).
†Indeterminate imaging findings: segmental/focal enlargement with delayed enhancement.
‡Normal range for serum $IgG_4$ level is <135 mg/dL.

**TABLE 2. Histology findings according to all cases, type 1 and type 2 AIP**

| Histology findings | All n = 50 | | Type 1 AIP* n = 41 | | Definitive AIP type 1 n = 35 | | Type 2 AIP† n = 4 | | Definitive AIP type 2 n = 3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | No. | % | No. | % | No. | % | No. | % | No. |
| Lymphoplasmacytic infiltration | 72 | 36/50 | 87.8 | 36/41 | 85.7 | 30/35 | 0 | 0/4 | 0 | 0/3 |
| Obliterative phlebitis | 0 | 0/50 | 0 | 0/41 | 0 | 0/35 | 0 | 0/4 | 0 | 0/3 |
| Storiform fibrosis | 0 | 0/50 | 0 | 0/41 | 0 | 0/35 | 0 | 0/4 | 0 | 0/3 |
| Abundant $IgG_4$-positive plasmacyte infiltration >10 HPF | 54 | 27/50 | 65.8 | 27/41 | 62.9 | 22/35 | 0 | 0/4 | 0 | 0/3 |
| $IgG_4$/IgG ratio >40% | 48 | 24/50 | 58.5 | 24/41 | 51.4 | 18/35 | 0 | 0/4 | 0 | 0/3 |
| Granulocytic infiltration of the duct wall | 6 | 3/50 | 0 | 0/41 | 0 | 0/35 | 75 | 3/4 | 100 | 3/3 |
| Granulocytic acinar infiltrate | 8 | 4/50 | 0 | 0/41 | 0 | 0/35 | 100 | 4/4 | 100 | 3/3 |
| Absent or scant (0-10 cells/HPF) $IgG_4$-positive cells | 8 | 4/50 | 0 | 0/41 | 0 | 0/35 | 100 | 4/4 | 100 | 3/3 |

*AIP,* Autoimmune pancreatitis; *$IgG_4$,* immunoglobulin $G_4$; *HPF,* high-power field.
*Type 1 AIP: 35 definitive AIP type 1 + 6 probable AIP type 1.
†Type 2 AIP: 3 definitive AIP type 2 + 1 probable AIP type 2.

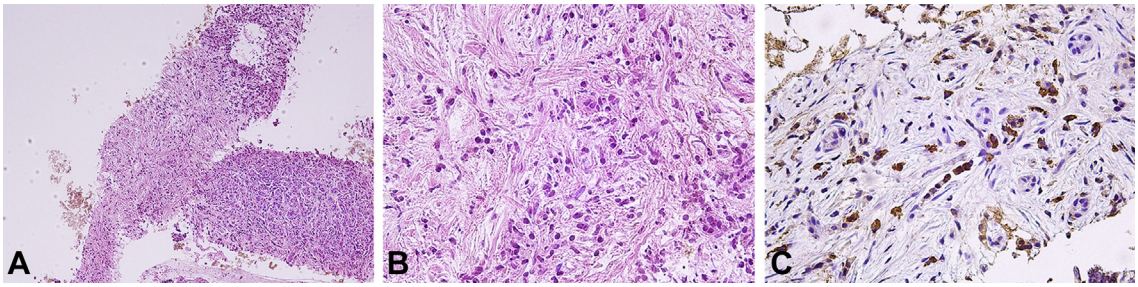


**Figure 2.** Histopathology of lymphoplasmacytic sclerosing pancreatitis. **A,** H&E staining of tissue obtained by EUS-guided FNA biopsy by using a standard 22-gauge aspiration needle (H&E, orig. mag. ×100). **B,** The findings demonstrate replacement of the acinar structure by lymphoplasmacytic infiltration and fibrosis (H&E, orig. mag. ×400). **C,** Abundant immunoglobulin $G_4$–positive cells are found in the high-power field ($IgG_4$, orig. mag. ×400).

(PPV) and negative predictive value (NPV). On the other hand, the combination of the level-2 histology diagnosis with the other findings (ie, parenchymal imaging, ductal imaging, serology, other organ involvement, response to steroid therapy) enable the diagnosis of definitive AIP. The secondary endpoint was clarification of the histology findings of AIP by using EUS-FNA biopsy with a standard 22-gauge aspiration needle and the associated other findings specified in the ICDC. The final diagnoses were made based on the ICDC. Definitive AIP type 1 and probable AIP type 1 were designated as type 1 AIP, and definitive AIP type 2 and probable AIP type 2 was designated as type 2 AIP.

## RESULTS

### Patients

Fifty-five patients suspected of having AIP were enrolled between July 1, 2011 and December 31, 2014. Three patients declined to participate, and 2 patients were excluded because of steroid administration within 3 months before the initiation of treatment. In total, 50 patients were registered in this study (Fig. 1).

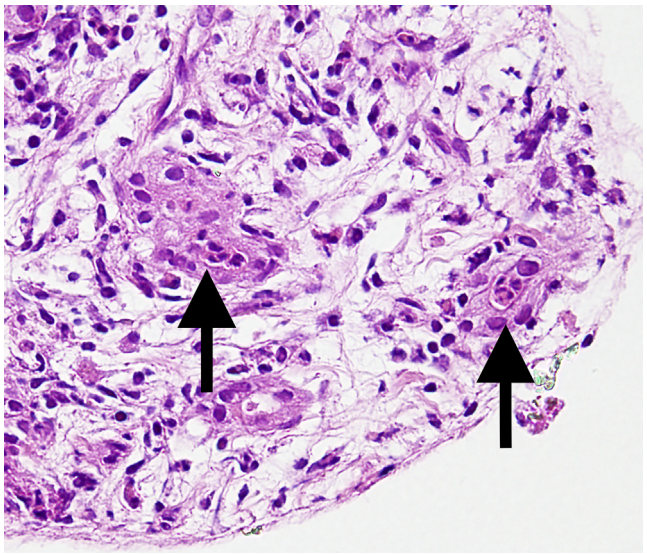

**Figure 3.** Histopathology of idiopathic duct-centric chronic pancreatitis. The pancreatic duct contains a granulocyte epithelial lesion (*arrows*) (H& E, orig. mag. ×400).

The patient characteristics are shown in Table 1. Thirty-nine patients were men, accounting for a large proportion, and the mean age was 64.4 years. Diagnosis after EUS and

**TABLE 3. Histology diagnosis based on the ICDC**

| | LPSP | | | |
|---|---|---|---|---|
| | Level 1 | | Level 2 | |
| | % | No. | % | No. |
| Type 1 | 0 | 0/41 | 65.8 | 27/41 |
| Parenchymal imaging | | | | |
| Typical* | 0 | 0/24 | 54.2 | 13/24 |
| Indeterminate† | 0 | 0/17 | 82.3 | 14/17 |
| Ductal imaging | | | | |
| Long or multiple strictures | 0 | 0/24 | 66.7 | 16/24 |
| Segmental/focal narrowing | 0 | 0/17 | 64.7 | 11/17 |
| Serum $IgG_4$ level‡ | | | | |
| $IgG_4$ <135 | 0 | 0/8 | 87.5 | 7/8 |
| 135 ≦$IgG_4$ ≤270 | 0 | 0/8 | 75 | 6/8 |
| 270 < $IgG_4$ | 0 | 0/25 | 56 | 14/25 |
| Other organ involvement | | | | |
| Sclerosing cholangitis | 0 | 0/11 | 54.5 | 6/11 |
| Retroperitoneal fibrosis | 0 | 0/5 | 40 | 2/5 |
| Symmetrically enlarged salivary/lachrymal glands | 0 | 0/3 | 66.7 | 2/3 |
| $IgG_4$-related nephritis | 0 | 0/4 | 25 | 1/4 |
| | **IDCP** | | | |
| | **Level 1** | | **Level 2** | |
| | **%** | **No.** | **%** | **No.** |
| Type 2 | 75 | 3/4 | 25 | 1/4 |
| Other organ involvement | | | | |
| Ulcerative colitis | 25 | 1/4 | 0 | 0/4 |

*ICDC,* International Consensus Diagnostic Criteria; *IDCP,* idiopathic duct-centric chronic pancreatitis; *LPSP,* lymphoplasmacytic sclerosing pancreatitis; *IgG4,* immunoglobulin $G_4$.
*Typical imaging findings: diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement).
†Indeterminate imaging findings: segmental/focal enlargement with delayed enhancement.
‡Normal range for serum $IgG_4$ level is <135 mg/dL.

before FNA biopsy was 45 AIP, 3 chronic pancreatitis, and 2 pancreatic neoplasms. The diagnoses at the time of enrollment according to ICDC were 27 definitive AIP type 1 and 23 nonspecified diagnosis cases. Fourteen cases exhibited normal serum $IgG_4$ levels. Forty-one cases were newly diagnosed as $IgG_4$-related disease.

## Histology characteristics

The histology findings of LPSP and IDCP detected by EUS-FNA biopsy are shown in Table 2. Lymphoplasmacytic infiltration and abundant $IgG_4$-positive plasmacyte infiltration >10/HPF in type 1 AIP were detected in 36 (72%) and 27 (54%) of 50 patients, respectively (Fig. 2). Obliterative phlebitis and storiform fibrosis were not detected in our study. The characteristic findings of IDCP and GEL were noted in 3 patients (Fig. 3), and granulocytic acinar infiltration and the absence of or scant (0-10 cells/HPF) $IgG_4$-positive cells were noted in 4 patients.

## Histology diagnosis based on the ICDC by EUS-FNA biopsy

The histology was evaluated based on the ICDC. None of the patients met the level-1 criteria of LPSP, and 27 patients (68%, 27/41) met the level-2 criteria (Table 3). There were no associations between the increases in the serum $IgG_4$ levels and the pathology evaluation. Three patients (75%, 3/4) met the level-1 criteria of IDCP (ie, positive for both GEL and $IgG_4$-positive plasma cell infiltration ≤10/HPF), and 1 patient (25%, 1/4) met the level-2 criteria (positive for both neutrophil and lymphocyte infiltration in the pancreatic acini and $IgG_4$-positive plasma cell infiltration ≤10/HPF). The sensitivity, specificity, PPV, and NPV of EUS-FNA biopsy for level-1 histology diagnosis of AIP were 7.9% (3/38), 100% (12/12), 100% (3/3), and 25.5% (12/47), respectively. And the sensitivity, specificity, PPV, and NPV of EUS-FNA biopsy for level-2 histology diagnosis of AIP were 57.9% (22/38), 50% (6/12), 78.6% (22/28), and 27.3% (6/22), respectively.

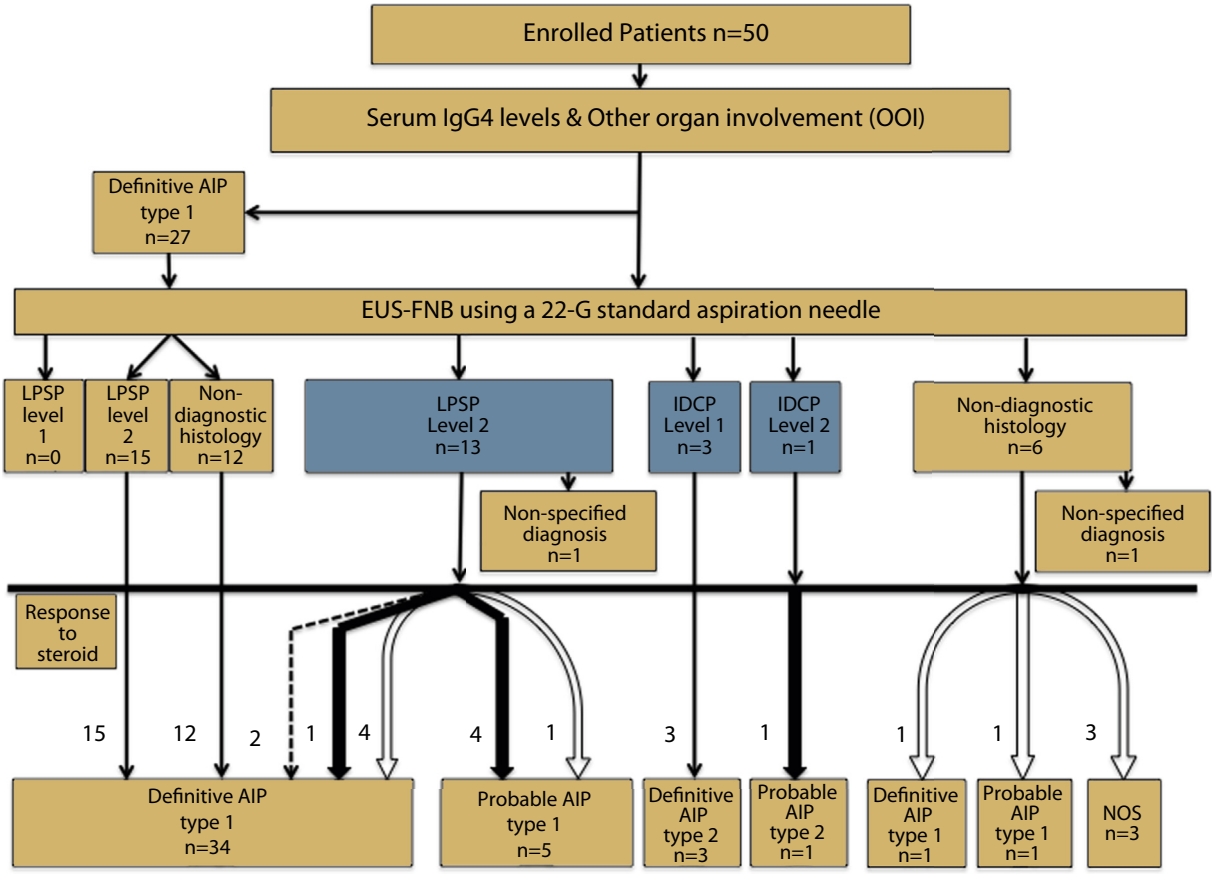


**Figure 4.** Contribution of EUS-FNA biopsy to the diagnosis of autoimmune pancreatitis (AIP). This figure shows all enrolled patients' diagnostic procedures. A *dashed arrow* shows the cases diagnosed as AIP without a steroid trial. *Black thick arrows* show the cases diagnosed as AIP that needed both histology findings and information on response to steroid therapy. *White arrows* show the cases diagnosed as AIP with information on response to steroid therapy without histology findings. The addition of pathologic evaluation of pancreatic tissue collected by EUS-FNA biopsy without response to steroid therapy improved the diagnostic accuracy in 8 of the 50 patients (16%). Two patients who had no symptoms did not receive a steroid trial at their requests. *IgG,* immunoglobulin G; *AIP,* autoimmune pancreatitis; *LPSP,* lymphoplasmacytic sclerosing pancreatitis; *NOS,* not otherwise specified.

## Role of EUS-FNA biopsy in the diagnosis of AIP

The final diagnoses according to ICDC are shown in Figure 4. The addition of pathologic evaluation of pancreatic tissue collected by EUS-FNA biopsy improved the diagnostic accuracy in 8 of the 50 patients (16%). When the diagnosis of type 1 AIP was compared with the clinical findings, the diagnostic accuracy was improved by the addition of the pathology diagnosis in patients with segmental and/or focal pancreatic enlargement and in those with normal serum $IgG_4$ levels (Table 4). In type 2 AIP, the pathology findings essential for the diagnosis of type 2 AIP were observed in 4 patients.

No procedure-related adverse events developed during this study.

## DISCUSSION

Although it was difficult to observe pathology findings meeting 3 or more items of the LPSP in pancreatic tissues collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle, it was possible to observe findings meeting 2 of these criteria. The combination of the level-2 findings of LPSP with the other findings specified in the ICDC (ie, parenchymal imaging, ductal imaging, serology, other organ involvement, response to steroid therapy) enabled the diagnosis of AIP in 7 patients (17%, 7/41). In type 2 AIP, tissue samples meeting the IDCP level-1 criteria were collected, and the diagnosis could be made based on the pathology examination alone. However, we believe that EUS-FNA biopsy alone is not helpful in differentiating between type 1 and type 2 AIP, because the ability of EUS-FNA biopsy to definitively diagnose type 1 and type 2 AIP was poor (ie, sensitivity, specificity, PPV, and NPV of EUS-FNA biopsy for a level-1 histology diagnosis of AIP were 7.9% [3/38], 100% [12/12], 100% [3/3], and 25.5% [12/47], respectively). The addition of pathology findings observed in specimens collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle improved the diagnostic accuracy in 8 patients (16%, 8/50).

There have been several reports on the diagnostic performance of EUS-FNA biopsy for the pathologic

**TABLE 4. Contribution of EUS-FNA biopsy to the final diagnosis**

| | No. of diagnosed patients | | | |
|---|---|---|---|---|
| | Without EUS-FNA biopsy | With EUS-FNA biopsy | Increase | % |
| AIP* | 35 | 45 | 11 | 24.4 |
| Type 1 | 34 | 41 | 7 | 17.1 |
| Parenchymal imaging | | | | |
| Typical† | 22 | 24 | 2 | 8.3 |
| Indeterminate‡ | 12 | 17 | 5 | 29.4 |
| Ductal imaging | | | | |
| Long or multiple strictures | 22 | 25 | 3 | 12 |
| Segmental/focal narrowing | 12 | 16 | 4 | 25 |
| Serum $IgG_4$ level§ | | | | |
| $IgG_4$ <135 | 3 | 8 | 5 | 62.5 |
| 135 ≦$IgG_4$ ≤270 | 6 | 8 | 2 | 25 |
| $IgG_4$ 270 < | 25 | 25 | 0 | 0 |
| Other organ involvement | | | | |
| Sclerosing cholangitis | 11 | 11 | 0 | 0 |
| Retroperitoneal fibrosis | 5 | 5 | 0 | 0 |
| Symmetrically enlarged salivary/lachrymal glands | 3 | 3 | 0 | 0 |
| $IgG_4$-related nephritis | 4 | 4 | 0 | 0 |
| Type 2 | 1 | 4 | 4 | 100 |
| Other organ involvement | | | | |
| Ulcerative colitis | 1 | 1 | 0 | 0 |

*EUS-FNA,* EUS-guided FNA; *AIP,* autoimmune pancreatitis; *$IgG_4$,* immunoglobulin $G_4$.
*All AIP cases were diagnosed according to ICDC criteria.
†Typical imaging findings: diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement).
‡Indeterminate imaging findings: segmental/focal enlargement with delayed enhancement.
§Normal range for serum immunoglobulin $G_4$ (IgG4) level is <135 mg/dL.

diagnosis of AIP.[18-24] Mizuno et al[18] evaluated the diagnostic performance of EUS-FNA biopsy by using a 19-gauge Tru-Cut needle (QuickCore, Wilson-Cook, Winston-Salem, NC), in which lymphocyte and plasma cell infiltration, obliterative phlebitis, storiform fibrosis, and $IgG_4$-positive plasma cell infiltration >10/HPF were observed in 100%, 36%, 93%, and 64% of patients, respectively.[18] In a study of EUS-FNA biopsy reported by Kanno et al[22] that mainly used a spring-equipped 22-gauge needle, not a core biopsy, marked lymphocyte and plasma cell infiltration, obliterative phlebitis, storiform fibrosis, and $IgG_4$-positive plasma cell infiltration >10/HPF were observed in 92%, 16%, 80%, and 36% of patients, respectively.[22] However, in a retrospective study performed by Imai et al[24] on the diagnosis of AIP by using EUS-FNA biopsy with a standard 22-gauge aspiration needle (EchoTip, Wilson Cook, Bloomington, Minn and NA-11J-KB, Olympus, Tokyo, Japan), which is widely used in routine clinical practice, they were able to collect a sufficient amount of tissue for differentiation from pancreatic cancer. It was insufficient to make a qualitative diagnosis of AIP, and they could not confirm $IgG_4$-positive plasma cell infiltration >10/HPF in any patient.[24]

In our study, which used a standard 22-gauge aspiration needle (Expect and Expect Slimline, Boston Scientific, Natick, Mass, EchoTip, Wilson Cook, Bloomington, Minn, and NA-11J-KB, Olympus, Tokyo, Japan), marked lymphocyte and plasma cell infiltration were observed in 72%, and $IgG_4$-positive plasma cell infiltration >10/HPF was detectable in 54% of patients, which were comparable with those observed in samples collected by core biopsy in previous reports. However, obliterative phlebitis and storiform fibrosis could not be diagnosed in the tissues collected from any of the patients. Because the amount of sample collected by using a standard 22-gauge aspiration needle is smaller than that obtained by core biopsy by using a 19-gauge needle, this difference in the absolute amount of sample may have resulted in the difference in detectability. However, in a study by Kanno et al,[22] using a spring-equipped 22-gauge needle, storiform fibrosis was detected at a rate of 80%, suggesting that rapid movement to and fro is important to collect a sufficient amount of tissue using a standard 22-gauge aspiration needle. With respect to obliterative phlebitis, Miyabe et al[25] reported that diagnosing with small amounts of sample could be improved by adding Elastica van Gieson staining to H&E staining. The use of immunostaining also may improve the detection rate in samples collected using a standard 22-gauge aspiration needle.

In our analysis, pathologic evaluation of tissues collected by EUS-FNA biopsy by using a standard 22-gauge aspiration needle was not necessary to diagnose type 1 AIP for many cases with diffuse enlargement of the pancreas and seropositive cases because these are typical clinical findings of AIP. On the other hand, for cases with segmental and/or focal enlargement of the pancreas and seronegative cases, differentiation from pancreatic cancer is a problem in actual clinical practice. Pathologic evaluation of the tissue collected by EUS-FNA biopsy could be distinguished from pancreatic cancer and be used to diagnose type 1 AIP along with the other findings specified in the ICDC.

Regarding the pathology diagnosis of type 2 AIP by using EUS-FNA biopsy, only a small number of cases have been reported previously. In our study, 4 patients were diagnosed as having type 2 AIP, and GEL, 1 of the criteria of level-1 IDCP, was confirmed in 3 of them. One patient was suspected of having type 2 AIP with inflammatory bowel disease and ulcerative colitis, which is a typical complication of type 2 AIP. In the remaining 2 patients, the serum $IgG_4$ levels were normal, and only diffuse enlargement of the pancreas was noted on imaging. The additional pathologic evaluation of tissue collected by EUS-FNA biopsy was necessary to diagnose type 2 AIP in these cases.

No incidents occurred because of the EUS-FNA biopsy technique in any patient, and EUS-FNA biopsy was applied safely in all cases.

One limitation of this study is the small number of patients.

In this study, we demonstrated the role of EUS-FNA biopsy using a standard 22-gauge aspiration needle. EUS-FNA biopsy by using a 22-gauge standard needle is not an effective diagnostic method for most patients with AIP. The combination of level 2 histologic diagnosis of AIP with other findings specified in the ICDC slightly improved the diagnostic accuracy, although it still remains insufficiently accurate for routine clinical use. It may be necessary to accumulate more cases and confirm these findings in a study involving a larger patient population.

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