A non-invasive screening technique for type 1 autoimmune pancreatitis

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Videosurgery Miniinv 2014; 9 (4): 517–522
DOI: 10.5114/wiitm.2014.44290

Abstract

Introduction: *Type 1 autoimmune pancreatitis (AIP) is the pancreatic manifestation of a systemic fibroinflammatory IgG4-related disease. Accurate diagnosis of AIP can avoid major hepatobiliary and pancreatic surgery as it respond dramatically to corticosteroid therapy.*
Aim: *This research investigated the feasibility of using peripheral blood cell immunohistochemistry, serum IgG4, T-cell receptor (TCR) and serum isoelectric focusing electrophoresis in the screening of type 1 autoimmune pancreatitis (AIP).*
Material and methods: *The peripheral blood from 3 type 1 AIP patients, 10 pancreatic cancer patients and 40 normal controls was collected. Sediment smears were jointly incubated with anti-IgG4 and anti-IgG. The percentage of IgG4/IgG positive cells was counted and serum TCR and IgG4 were detected through the whole process. After serum isoelectric focusing electrophoresis, anti-IgG4 and anti-IgG were used to confirm the components of serum.*
Results: *In the serum isoelectric focusing electrophoresis, IgG4 and IgG strips showed mirrored distribution in type 1 AIP patients, while there were no strips in the normal controls and pancreatic cancer. Compared with pancreatic tumor patients and healthy controls, serum TCR was significant increased in AIP. The percentage of IgG4/IgG positive cells of peripheral blood cell immunohistochemistry was related to serum IgG4 and hormone therapy reactions.*
Conclusions: *Peripheral blood cell immunohistochemistry, serum IgG4, TCR and serum isoelectric focusing electrophoresis is suitable for the screening of type 1 AIP and monitoring its response assessment.*

Key words: *type 1 autoimmune pancreatitis, peripheral blood cell immunohistochemistry, serum IgG4, TCR, serum isoelectric focusing electrophoresis.*

Introduction

According to the literature, the incidence of autoimmune pancreatitis (AIP) accounts for 2% to 11% of all chronic pancreatitis cases. The nationwide epidemiological survey of Japan found that the AIP incidence rate was 2.2/100,000 and the number is rapidly increasing with an annual incidence of 0.9/100,000 [1, 2]. Among them, most are type 1 AIP [3]. It can be inferred that there are at least tens of thousands of AIP patients in China, and most AIP may be misdiagnosed or missed from diagnosis [4, 5]. Currently, the diagnosis and differential diagnosis of AIP are basically dependent on the pathology of pancreatic tissue. However, pancreatic biopsy may cause considerable trauma, pancreatic leakage and other risks, and the treatment efficacy cannot be monitored. Hence, the pathology of pancreatic tissue is known as an imperfect "golden rule" to

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differentiate AIP from pancreatic cancer [6]. With the progress of non-invasive diagnosis techniques, especially serum markers and imaging examination [7], the definite cases of AIP are increasing, and unnecessary operations are avoided. Meanwhile, the postoperative effects of patients have been greatly improved. Therefore, a simpler and more precise non-invasive approach with good repeatability to replace biopsy is the key to improve the diagnosis rate of AIP.

Aim

This research investigated the feasibility of using peripheral blood cell immunohistochemistry, serum IgG4, TCR and serum isoelectric focusing electrophoresis or indicators in the screening of type 1 autoimmune pancreatitis.

Material and methods

Study subjects

Three patients with type 1 AIP (2 men and 1 woman), 10 with pancreatic cancer, and 40 normal controls were examined and treated at Fujian University Affiliated Hospital between August 2009 and May 2013. Type 1 AIP diagnosis was based on the Asian Diagnostic Criteria for Autoimmune Pancreatitis. The criteria consist of the following radiological, serological, and histopathological items: (1) radiological imaging showing narrowing of the main pancreatic duct and enlargement of the pancreas, which are characteristic of the disease; (2) laboratory data showing abnormally elevated levels of serum γ-globulin, IgG or IgG4, or the presence of autoantibodies; (3) histopathological examination of the pancreas demonstrating marked fibrosis and prominent infiltration of lymphocytes and plasma cells, which is called lymphoplasmacytic sclerosing pancreatitis (LPSP). For a diagnosis of AIP, criterion 1 must be present, together with criterion 2 and/or criterion 3. Blood samples were taken from 1–3 days before treatment and the monthly follow-up after treatment. At the same time, we randomly selected 40 healthy controls. This study was approved by the Fujian Medical University Ethics Committee and the ethics committee supervises the process of the experiment.

Criteria of curative effect

Clinical remission can be identified if the clinical symptoms and signs are remitted, results from the laboratory tests are normal and imaging showed the recovery of swelling organs. Clinical improvement can be identified if the clinical performance, results of laboratory tests and imaging are obviously improved (≥ 50% compared with pre-treatment). If the above-described results cannot be reached, the treatment is ineffective.

Histology and immunostaining

Pancreatic tissue and peripheral blood cells of patients were stained with hematoxylin and eosin (HE). Immunostaining for infiltrating plasma cells was performed using monoclonal antibody against human IgG4 (Zymed Laboratory, San Francisco, CA, USA, or The Binding Site, Birmingham, UK), and antihuman IgG (Dako, Glostrup, Denmark). IgG4 positive plasma cells were counted in five different high power fields (hpf), and the average IgG4 positive plasma cell count was calculated. The average IgG4+/IgG+ plasma cell ratio of at least two different hpf (2–5 hpf) was calculated. Peripheral blood cells were damage of red blood cells with 20% glacial acetic acid, smear, staining, and microscopy.

Isoelectric focusing electrophoresis

Serum samples were diluted 500 times. Isoelectric focusing analysis of serum was run in agarose gels detected after immunofixation with rabbit antiserum (IgG, IgG4, and trypsin).

Clinical features

The clinical data including allergic symptoms and those resulting from other organ involve of AIP was noted. Serum TCR determined using an enzyme-linked immunosorbent assay (ELISA) kit (RD systems, Minneapolis, MN, USA) prior to and after glucocorticoid therapy in patients with AIP, pancreatic cancer, and normal control.

Results

Clinical and laboratory features of type 1 AIP

The patients were 2 men and 1 woman. Tables I and II show the clinical and laboratory features of the patients with AIP. Serum IgG4 level was significantly increased (range: 12.335–34.400 g/l), but autoantibodies such as antinuclear antibody (ANA) and anti-extractable nuclear antigen (ENA) were

Table I. Imaging features and specificity pathology of patients

Case	Gender	Age	Organ involvement and performance	Imaging features	Specificity pathology	Dispose	Lapse
1	Male	54	Chronic pancreatitis, chronic cholecystitis, jaundice	Atrophy of pancreas, pancreatic uncinate multiple calcification, dilated pancreatic duct	(Pancreatic tissue) chronic pancreatitis, lymph, infiltration of plasma cells 45%	Operation, postoperative prednisolone 35 mg/day	Improvement
2	Male	43	Autoimmune pancreatitis, jaundice	Pancreatic duct beaded enlargement	(Pancreatic puncture) lymph, infiltration of plasma cells, IgG4 positive cells 45%	Prednisolone 40 mg/day	Improvement
3	Female	38	Interstitial pneumonia, chronic pancreatitis, cholangitis, jaundice	Diffuse enlargement of the pancreas, nodular cirrhosis, cholecystitis	(Pancreatic puncture) infiltration of plasma cells, IgG4 positive cells 52%	Prednisolone 45 mg/day	Relieve

Table II. Laboratory examination results of patients

Case	Weight loss (kg/12 months)	ANA (< 1.0)	IgG (0–16)	IgG4 (0.08–1.4 g/l)	IgE (0–100 IU/ml)	CRP (0–8 mg/l)	Ca125 (0–35 U/ml)	Ca199 (0–34 U/ml)
1	6	0.25	28.63	12.335	985	10.5	22.45	22.15
2	5	0.13	52.36	34.400	1250	5.6	15.11	10.25
3	8	0.96	21.40	16.852	377	15.6	38.85	12.36

negative. The most frequent clinical manifestations was painless obstructive jaundice.

Results of serum samples by isoelectric focusing electrophoresis

The strips of IgG4 and IgG in type I AIP patients showed symmetry distribution in the serum isoelectric focusing electrophoresis (Photos 1 A, B), while the pancreatic cancer patients were negative (Photo 1 C).

IgG and IgG4 stain of peripheral blood cell

Staining of IgG and IgG4 in peripheral blood cell smear with EDTA anticoagulation showed very few lymphocytes (0-2/HP) and serious interference. The morphology of IgG and IgG4 staining of lymphocytes was irregular and it cannot be identified whether this was nonspecific adsorption, leading to difficult interpretation (Photos 2 A, B). After the treatment with 20% glacial acetic acid, the density of lymphocytes was significantly increased (20–60/HP), and IgG and IgG4 had specific binding in the cytoplasm of the plasma cells. In the light background of damaged red cells, the outlines of lymph and plasma cells were very clear (Photos 2 C, D).

The relationship of serum TCR and AIP course

Serum IgG4/IgG in all AIP patients was > 0.4, and it decreased after application of glucocorticoid. Sim-

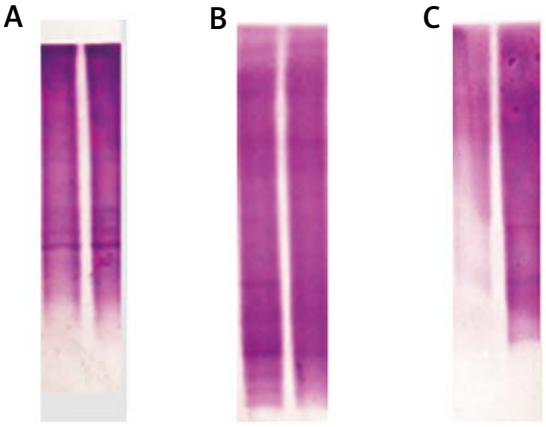

Photo 1. Serum isoelectric focusing electrophoresis of AIP and pancreatic cancer. **A** – IgG4 and IgG staining of no. 1 patients with AIP (left: IgG4, right IgG staining), a mirror image of distribution. **B** – IgG4 and IgG staining of no. 2 patients with AIP (left: IgG4, right IgG staining), roughly mirroring distribution. **C** – IgG4 staining of patients with pancreatic cancer (left) and no. 3 patient with type 1 AIP (right)

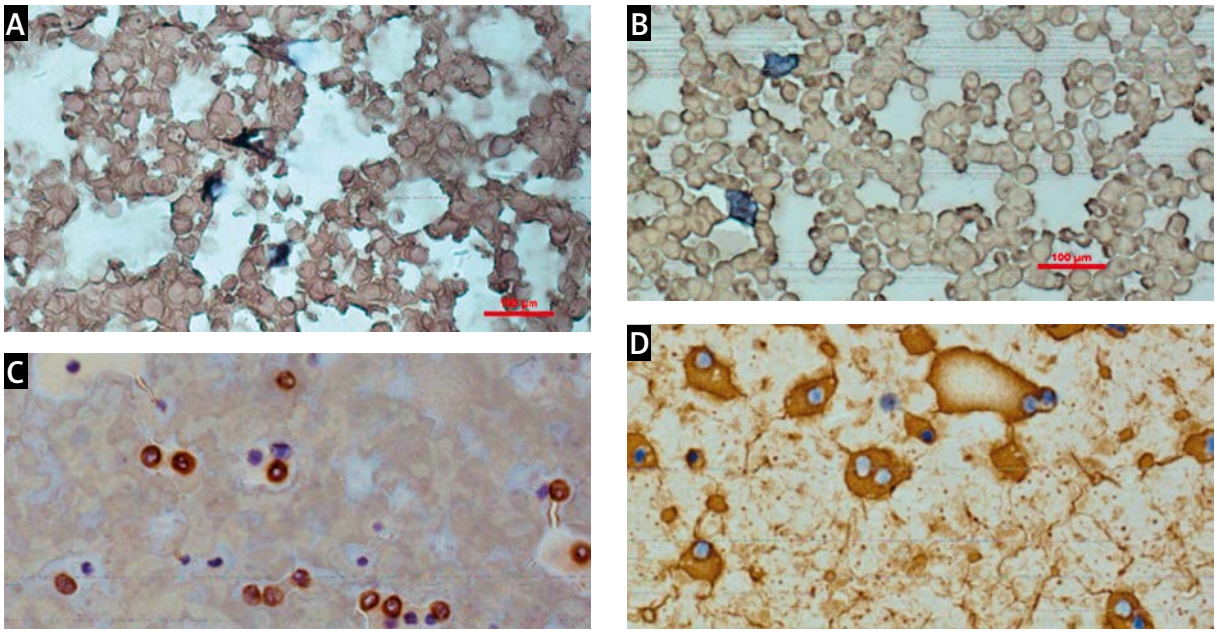

Photo 2. IgG and IgG4 stain of peripheral blood cell. **A** – IgG4 stain of blood cell with direct application of EDTA anticoagulation. **B** – IgG stain of blood cell with direct application of EDTA anticoagulation. **C** – IgG4 stain of blood cell after treatment with 20% glacial acetic acid. **D** – IgG stain of blood cell after treatment with 20% glacial acetic acid

ilarly, the percentage of IgG4/IgG positive cells in peripheral blood cells also decreased after treatment. Serum TCR levels in AIP patients were significantly higher than those in normal controls and the pancreatic cancer group (Figure 1).

Discussion

The AIP misdiagnosis rate is very high. Because of its obstructive jaundice performance combined with swelling pancreas, it is hard to distinguish it from pancreatic cancer and AIP patients often receive unnecessary surgery. According to statistics, 21% to 28% of AIP patients underwent pancreaticoduodenectomy due to suspicious pancreaticobiliary malignancies, which seriously affected patients' life quality. The AIP may happen to people in any age group. Reports of a 10-year-old AIP patient are found [8–10]. But AIP is often observed among elderly men which overlapping the high-incidence age and gender of pancreatic cancer. Studies from Japan, Korea, China and other Asian countries [11–14] reported the AIP pathological features as type 1, while European ones described more type 2 pathological patterns [5, 15–17]. The sensitivity and specificity of AIP and common chronic pancreatitis reached 91.2% and 98.0% respectively according to pancreatic histology [18]. For type 2 AIP, neutrophil infiltration in duct and acinus cannot be fully explained by autoimmunity. Thus, Japanese experts believe that it is still controversial whether type 2 AIP is an autoimmune-mediated inflammatory process [19, 20]. Common symptoms of patients include jaundice, abdominal pain and weight loss, and a small proportion of patients have acute pancreatitis, and some may have pancreatic duct stones [21, 22]. Three cases in our research presented painless jaundice and weight loss. One case had concurrent pancreatic duct stones. All cases in this report were misdiagnosed preoperatively as pancreatic cancer.

The serum IgG4 level is the most commonly used marker for AIP. Recently, a nationwide case analysis in Japan showed that 87.6% of 546 AIP patients had increasing serum IgG4. In distinguishing AIP and pancreatic cancer, the sensitivity and specificity of IgG4 were 80% to 92% and 98% respectively [23–26]. However, the serum IgG4 level of 10% of pancreatic cancer patients also rose, leading to the challenged clinical value of serum IgG4 [27, 28]. In our research, serum IgG4 level was higher than the normal upper limit by 8 times, and all had concurrent organ damage outside the pancreas. Recent studies

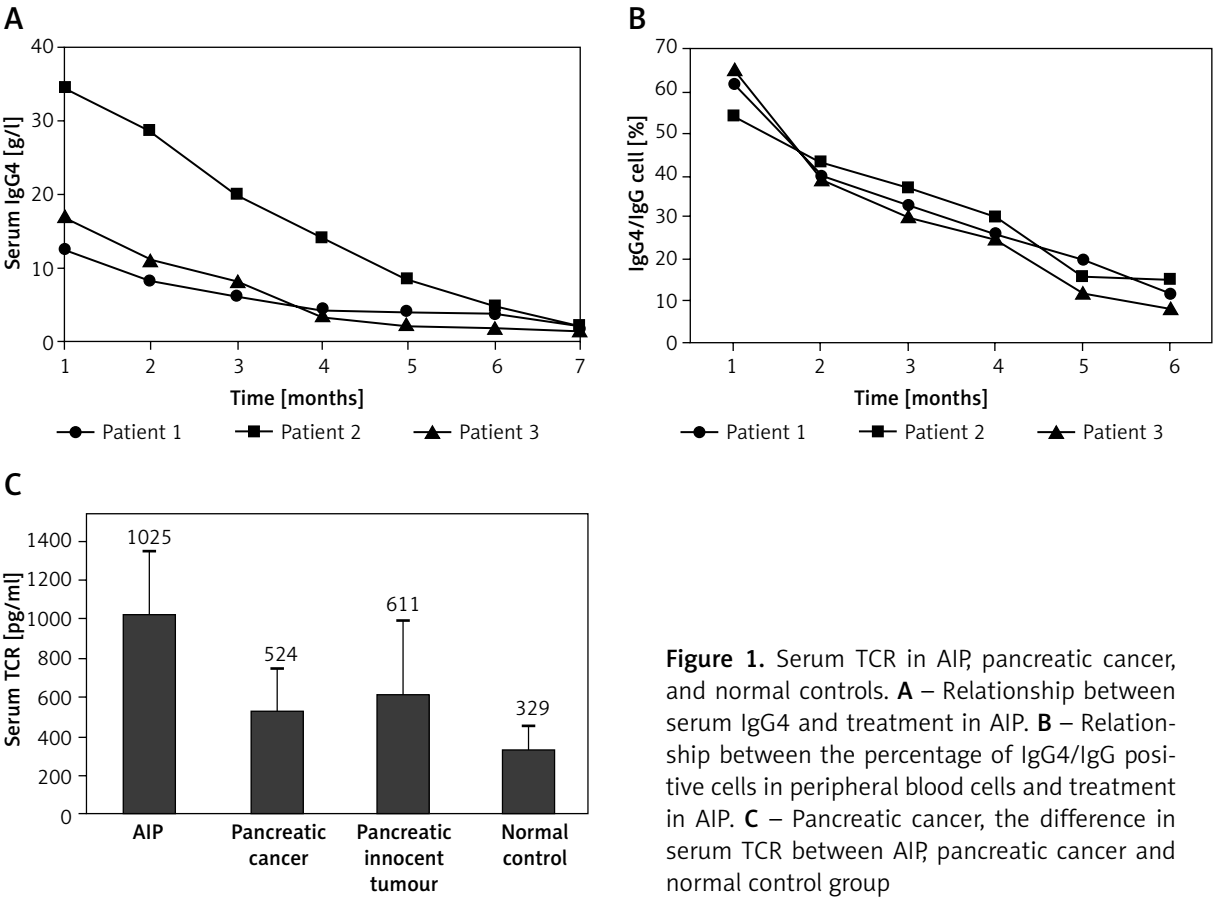

Figure 1. Serum TCR in AIP, pancreatic cancer, and normal controls. **A** – Relationship between serum IgG4 and treatment in AIP. **B** – Relationship between the percentage of IgG4/IgG positive cells in peripheral blood cells and treatment in AIP. **C** – Pancreatic cancer, the difference in serum TCR between AIP, pancreatic cancer and normal control group

have reported that IgG4-related disease sometimes involves the systemic lymph nodes and it is undeniable that TCR is active in the autoimmune process [29]. In this study, we found that serum TCR in AIP was significantly higher than pancreatic cancer and the normal control. Therefore, serum TCR is expected to become a diagnostic marker of AIP with high resolution and good repeatability. IgG and IgG4 concentration strips were detected among 3 patients, which showed the mirror distribution. This indicated that AIP patients had lymphocytic monoclonal proliferation, which mainly focused on plasmocyte proliferation that secreted IgG4.

The pathology of pancreatic tissue is known as an imperfect "golden rule" to differentiate AIP, but pancreatic biopsy may cause considerable trauma, pancreatic leakage, tumor ectopia and implantation and other risks. Thus, this is not suitable for clinically general survey and illness monitoring. As AIP-invaded plasma cells and lymphocytes are from the blood, we made an attempt to replace pancreatic tissue with peripheral blood anticoagulated by EDTA and monitor the distribution and ratio of IgG and IgG4 positive cells. However, the ratio of lymphocytes in peripheral blood was 1/500 relative to red blood cells, and the test tended to have background interference from red blood cells. Additionally, the morphology of IgG and IgG4 staining of lymphocytes was irregular, leading to difficult interpretation (Figures 1 A, B). After the treatment with 20% glacial acetic acid, the density of lymphocytes was significantly increased (20–60/HP), and IgG and IgG4 had specific binding in the cytoplasm of the plasma cells. In the light background of damaged red cells, the outlines of lymph and plasma cells were very clear (Figure 1 C).

Conclusions

Peripheral blood has advantages such as being non-invasive and easy to obtain specimens. Thus, it can provide a sound foundation for clinical diagnosis and prognosis, and become a reliable source of tissues for non-invasive screening. Detection results of peripheral blood cell immunohistochemistry and serum isoelectric focusing electrophoresis are con-

sistent with the pathological results of pancreatic tissues. Therefore, these techniques are suitable for screening and response assessment of AIP, and better reflect the humanistic care for patients.

Acknowledgments

Financially supported by the project of Natural Science Foundation of Fujian Province (2013J01302), National Natural Science Foundation of China (81201362, 81201590, 21275028), Fujian Medical Innovations (2012-CXB-21), Foundation of Fujian Education Department (JA10143, JA12143), National High Technology Investigation Project Foundation of China (2012AA022604) and Outstanding Youth Foundation of Fujian Provincial Higher Education (JA12133).

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Received: 11.03.2014, **accepted:** 10.04.2014.