Serum soluble interleukin-2 receptor level and immunophenotype are prognostic factors for patients with diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma is the most common form of non-Hodgkin lymphoma. Although many studies have attempted to identify prognostic factors, most have focused on conventionally treated patients. The influence of anti-CD20 antibody (rituximab) should be considered now. We evaluated the prognostic significance of serum soluble interleukin-2 receptor levels and germinal center B-cell-like or non-germinal center B-cell like subgroups in 80 patients with diffuse large B-cell lymphoma, who had been treated with rituximab. Serum soluble interleukin-2 receptor levels ranged from 322 to 39900 U/mL (median 1365 U/mL). Sixteen (20%) were germinal center B-cell-like subgroups, and the remainder (80%) non-germinal center B-cell-like. Survival analysis associated lower serum soluble interleukin-2 receptor level and germinal center B-cell-like phenotype with better overall survival ($P = 0.015$), whereas multivariate analysis, including International Prognostic Index factors, revealed that only higher performance status score and higher serum lactate dehydrogenase levels significantly affected survival. However, serum soluble interleukin-2 receptor levels were elevated in patients with higher International Prognostic Index scores as well as in the non-germinal center B-cell-like subgroup. Serum soluble interleukin-2 receptor levels, International Prognostic Index, and subphenotypes were strongly correlated with each other. Our study showed that soluble interleukin-2 receptor is quite useful and may serve as a substitute for the International Prognostic Index, especially for patients undergoing treatment. Moreover, the differentiation between the germinal center B-cell-like and non-germinal center B-cell-like phenotypes is also useful for predicting patients with diffuse large B-cell lymphoma, even among those treated with rituximab. (*Cancer Sci* 2009; 100: 1255–1260)

Diffuse large B-cell lymphoma (DLBCL) is one of the most common subtypes of non-Hodgkin lymphoma (NHL), representing 30% to 40% of adult cases of NHL, and its incidence has increased in the past few decades. DLBCL is recognized clinically and biologically as a heterogeneous group of tumors.[1] Recently, therapeutic methods have also improved. In particular, anti-CD20 antibody (rituximab) is now the most powerful tool for B-cell malignancies.

Many investigators have reported on the prognostic factors of NHL, and the most powerful one we have recognized is the International Prognostic Index (IPI).[2] The IPI contains patients' age, performance status (PS), Ann Arbor clinical stage (CS), serum lactate dehydrogenase (LDH) activity, and the number of extranodal lesions. In addition, in the rituximab era, a revised IPI has been proposed, which is better than the standard IPI.[3] Several reports have revealed that serum soluble interleukin-2 receptor (sIL-2R) level may also be a prognostic factor for NHL.[4–6] IL-2R is expressed not only on the surface of activated T or B lymphocytes, but also on parts of lymphoid malignancies. sIL-2R is released from the cell membrane by cleavage of IL-2R, and its serum level rises in patients with some malignancies, including malignant lymphoma.[7,8]

Recently, by using a cDNA microarray profiling method, DLBCL was divided into two groups: a germinal center B-cell-like type (GCB-type) and an activated B-cell like type (ABC-type).[9] The GCB-type DLBCL shows overexpression of the genes that characterize normal germinal center (GC) cells, whereas the ABC subgroup overexpresses a group of genes that are highly transcribed by *in vitro*–activated peripheral blood B-cells.

Some reports have shown that GCB-type DLBCL has a better prognosis than ABC-type DLBCL.[9–11] At present, however, a DNA microarray profiling method is not available for all DLBCL patients in most diagnostic pathology laboratories. Instead, immunohistochemistry is a clinically useful prognostic tool. Hans *et al.*[12] showed that immunohistochemistry staining patterns for CD10, Bcl-6, and MUM-1 can be used to classify DLBCL into GCB and non-GCB subgroups that correlate prognostically with the groups defined by the cDNA microarray method.

As far as we know, there is only one report which has clarified the prognostic factors after rituximab,[13] and no report has analyzed both subgroups (GCB *versus* non-GCB) and serum sIL-2R simultaneously. We sought to compare both subtypes and serum sIL-2R levels in terms of patient survival, and to evaluate the prognostic factors in patients who were treated with rituximab.

Materials and Methods

Patient characteristics. Formalin-fixed, paraffin-embedded tissue blocks of 107 primary biopsies of DLBCL were retrieved from the files of the Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital, where the diagnoses were made between 1997 and 2007. Among these 107 patients, 80 patients were treated with rituximab containing chemotherapy as described below, and we analyzed these 80 cases (rituximab group). Informed consent for retrospective analysis and for additional immunophenotypic analysis was obtained according to the Declaration of Helsinki.

Cases were reclassified according to the World Health Organization classification.[1] Patients were selected only on the basis of availability of clinical information and histological material. Histology was reviewed by three pathologists (TY, YS, TM), and only patients with DLBCL, not otherwise specified (NOS) were included.

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All patients were previously untreated, and were treated according to standard primary anthracycline-containing combination chemotherapy (predominantly cyclophosphamide, doxorubicine, vincristine, and predonisolone (CHOP) regimen). Seventy-seven cases who started their first chemotherapy after September 2003 were treated by an anthracycline-containing chemotherapy combination with rituximab, an anti-CD20 monoclonal antibody, except for one case that was negative for CD20. Three cases that were diagnosed before 2003 received primary chemotherapy without rituximab. These cases relapsed after 27, 89, and 91 months, respectively, and then received secondary chemotherapy with rituximab. The median follow-up period of the rituximab group was 22 months.

Immunohistochemistry. The panel of monoclonal antibodies included antibodies against the following antigens: CD20 (L26, 1:100; Novocastra, Newcastle upon Tyne, UK), CD3 epsilon (PS-1, 1:50; Novocastra), CD5 (4C7, 1:100; Novocastra), CD10 (56C6, 1:50; Novocastra), Bcl-2 (3.1, 1:200; Novocastra), Bcl-6 (PG-B6p, 1:50; Dako, Carpinteria, CA, USA), MUM1 (MUM1p, 1:50; Dako), Ki-67 (MIB-1, 1:1000; Novocastra), and p53 (Pab1801, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Three-micrometer paraffin sections from formalin-fixed material were dehydrated and deparaffinized according to standard procedures. A previous step of heat-induced antigen retrieval was used for all antigens in all cases. After incubation with the primary antibodies, the immunoreaction was developed using the Dako Envision_System peroxidase technique (Dako) with diaminobenzidine (DAB) as chromogen. The slides were counterstained with Mayer's hematoxylin. Cases were considered positive if 30% or more of the tumor cells were stained with an antibody, as the previous reports recommended.[12,14]

Clinicopathological assessment. All cases were stratified into GCB and non-GCB subgroups. The procedure to classify subgroups was that of Hans *et al.*[12]

The clinical parameters we assessed were International Prognostic Index (IPI) risk, gender, serum sIL-2R level, B symptoms, bone marrow invasions, bulky masses, and survival rate. IPI scoring includes age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), clinical stage, serum LDH level, and the number of extranodal sites. The survival rate was calculated from the time of diagnosis to the time of last follow-up.

Statistical analysis. Survival curves were estimated by the Kaplan–Meier method.[15] The significance of survival differences was determined by the log-rank and generalized Wilcoxon tests. A difference was considered significant when either of these two tests found it to be so. Differences between groups were evaluated by the Mann–Whitney *U*-test (non-parametric analysis) or Fisher's exact test.

Cox multivariate analysis[16] was carried out to estimate the prognostic impacts of the biomarkers and IPI risk factors. The level of significance was set at $P < 0.05$. Data were analyzed using SPSS software version 14.0 for Windows (SPSS, Chicago, IL, USA).

Results

Patient characteristics. The median age was 66.0 years (range, 29–87 years). Forty-one (51.3%) were male and 39 (48.8%) were female. The median serum sIL-2R level was 1365 U/mL (range, 322–39900 U/mL). Other patient characteristics are outlined in Table 1. Sixty-nine patients (86.3%) achieved complete response (CR) and four patients showed partial remission (PR), for an overall response rate of 91.3%.

Immunophenotype of DLBCL. Following the criteria described by Hans *et al.*[12] 16 cases (20%) were categorized in the GCB subgroup and 64 cases (80%) in the non-GCB subgroup (Fig. 1). The GCB : non-GCB ratio was 1:4. CD5 was expressed in 13.8% (11/80) of the tumors, Bcl-2 in 67.5% (54/80), and p53 in 13.8% (11/80).

Table 1. Main initial characteristics of 80 patients with diffuse large B-cell lymphoma

Characteristics	No. (%)
Performance status (ECOG 2)	20 (25)
B symptoms (n = 60)†	24 (40)
Ann Arbor stage	
I	8 (10)
II	18 (22)
III	19 (24)
IV	35 (44)
Bulky disease (n = 76)†	22 (29)
Bone marrow invasion (n = 77)†	17 (22)
High serum LDH levels (> normal)	40 (50)
Revised International Prognostic Index	
Very good risk	11 (14)
Good risk	32 (40)
Poor risk	37 (46)
High serum sIL-2R levels (>1500 U/mL)	37 (46)
Primary extranodal origin (excluding Waldeyer's ring)	34 (43)
Complete remission (CR) rate	69 (86)
Overall response rate (CR + PR)	73 (91)
Number of relapse	14 (18)
Treated with hematopoietic stem cell transplantation	5 (6)

The median age of the patients was 66 years (range, 29–87 years); 41 were male and 39 were female.
†The n shown reflects the number of patients for whom data were available.
ECOG, Eastern Cooperative Oncology Group scale; LDH, lactate dehydrogenase; PR, partial remission; sIL-2R, soluble interleukin-2 receptor.

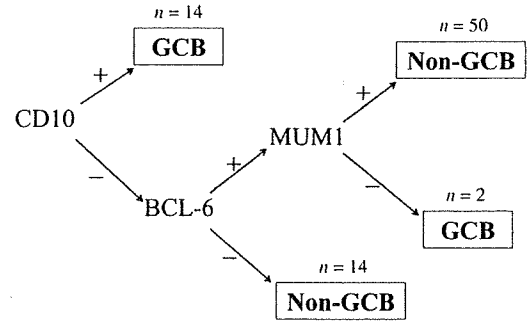

Fig. 1. Classification of germinal center B-cell-like (GCB) and non-GCB-type diffuse large B-cell lymphomas according to Hans *et al.*[12]

Survival analysis. According to the Kaplan–Meier method, the 5-year-overall survival rate was 78.4% (Fig. 2a). When the cases were divided into revised IPI risk groups, the very-good risk group had the highest survival rate while the poor-risk group had the lowest, and the survival rates differed significantly among groups (Fig. 2b). When the cases were divided into GCB and non-GCB subgroups, patients who were in the GCB subgroup were all alive, while the 5-year-overall survival rate of the patients who were in the non-GCB subgroup was 73.3%, and the median survival between subgroups differed significantly (Fig. 3a). We set a serum sIL-2R cut-off level at 1500 U/mL because this was close to the median sIL-2R level, 1365 U/mL. The patients' 4-year survival rates for the high (>1500 U/mL) and low (! 1500 U/mL) sIL-2R groups were 65.8% and 89.3%, respectively (P = 0.010, Fig. 3b). A three-group classification, in which GCB and non-GCB subgroups were divided between higher and lower sIL-2R levels, showed that the GCB subgroup had the best survival rate, while the non-GCB subgroup with a

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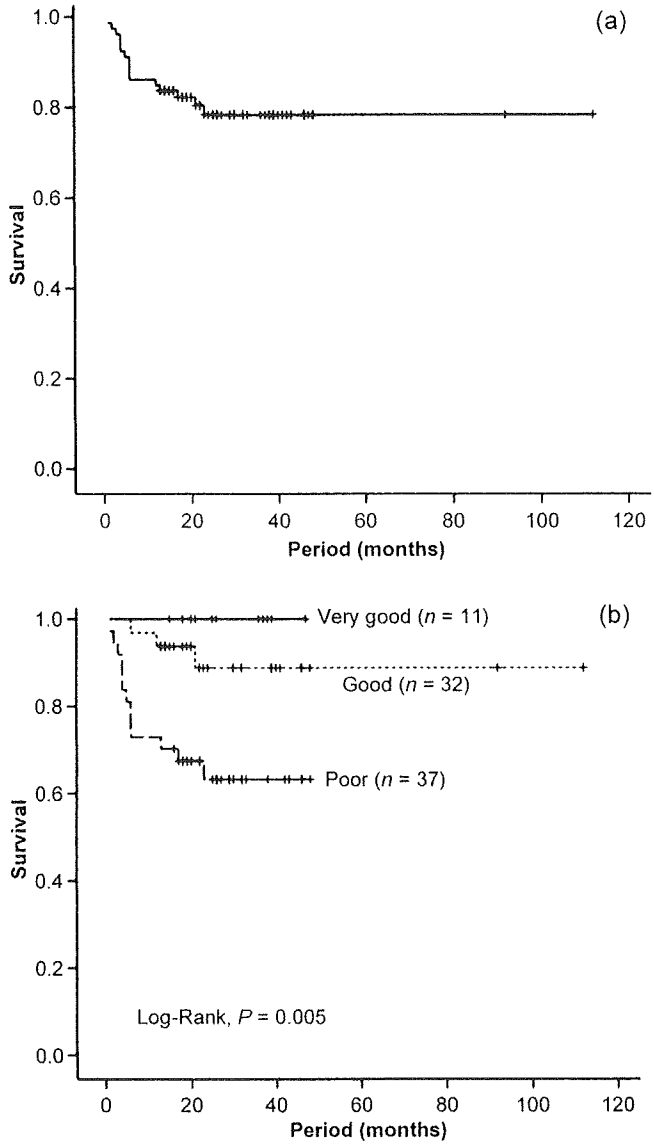

Fig. 2. Overall survival of the study population. (a) Overall survival of 80 patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab. (b) Overall survival according to the revised International Prognostic Index risk groups.

higher sIL-2R level (>1500 U/mL) had the worst; this confirmed significant differences among the groups (Fig. 3c).

Univariate and multivariate analyses of overall survival. As shown in Table 2, univariate analysis showed that the overall survival was significantly worse for patients aged 60 years or older, patients with a PS score above 2, patients who had B symptoms, bulky masses, or bone marrow invasion, patients with elevated serum LDH levels, and patients with tumors expressing CD5. Multivariate analysis showed that higher PS score (score 2 or over) and above-normal serum LDH levels were independent prognostic factors for overall survival (Table 3).

Effects of rituximab on patients' prognoses. As shown in the patients' characteristics, we firstly chose 107 cases: 80 were in the rituximab group and the other 27 were treated without rituximab (non-rituximab group). The results of patients' prognoses were shown in Fig. 4. The overall survival curve of the rituximab group showed a better tendency than the non-rituximab group, but there were no statistically significant differences.

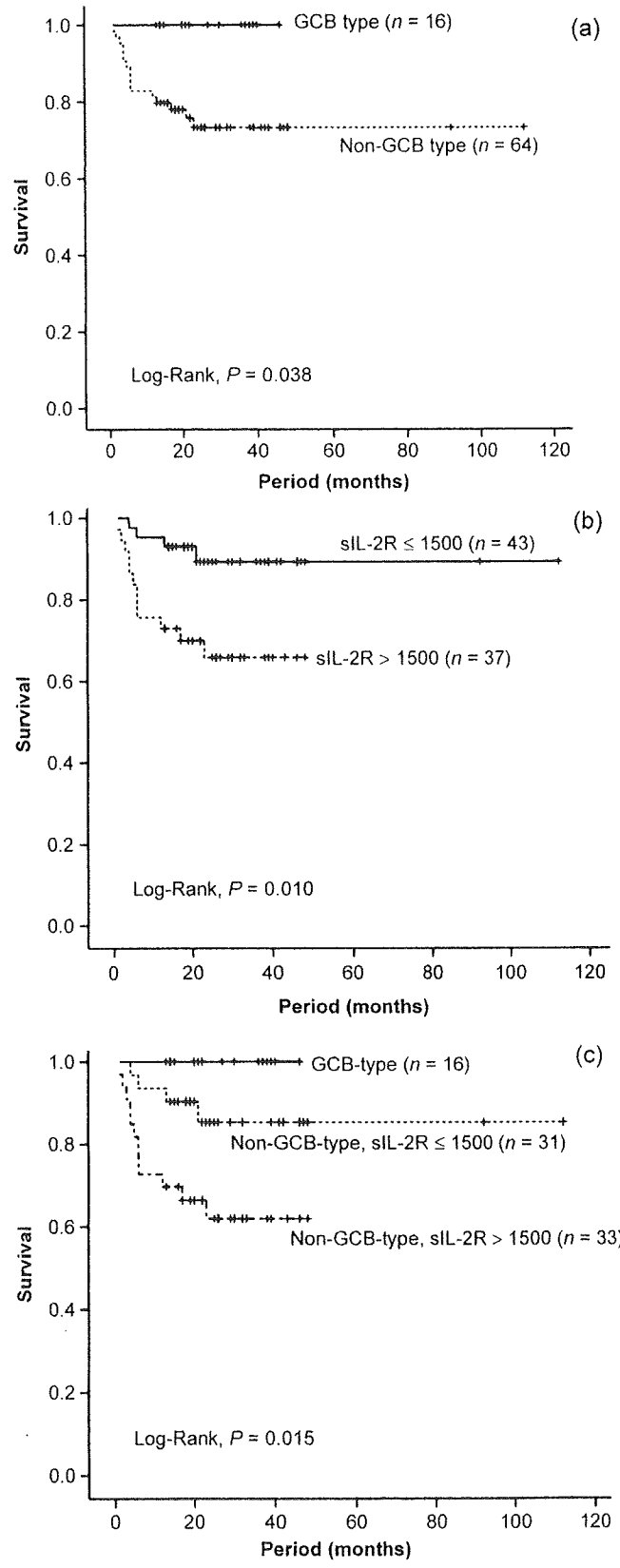

Fig. 3. Overall survival according to (a) germinal center B-cell-like (GCB) and non-GCB subtypes, (b) serum soluble interleukin-2 receptor (sIL-2R) level is higher than 1500 U/mL or lower, and (c) both subphenotype and serum soluble interleukin-2 receptor (sIL-2R) levels.

Table 2. Prognostic factors in univariate analysis

		Numbers	P-values	
			Log-rank	G-Wilcoxon
CD5	Positive	11	0.002	0.012
	Negative	69		
Bcl-2	Positive	54	0.954	0.848
	Negative	26		
p53	Positive	11	0.1	0.078
	Negative	69		
Age	! 60 years	30	0.005	0.007
	>60 years	50		
Gender	Male	41	0.109	0.074
	Female	39		
Performance status	0, 1	60	<0.001	<0.001
	2, 3, 4	20		
LDH	Normal	40	0.001	0.001
	Higher	40		
Stage	I or II	26	0.054	0.062
	III or IV	54		
No. of extranodal sites (n = 74)†	! 1	49	0.46	0.475
	>1	25		
B symptom (n = 60)†	Present	24	0.003	0.003
	Absent	36		
Bulky disease (n = 76)†	Present	22	<0.001	<0.001
	Absent	54		
Bone marrow invasion (n = 77)†	Present	17	0.004	0.002
	Absent	60		

†The n shown reflects the number of patients for whom data were available.
LDH, lactate dehydrogenase.

Table 3. Cox multivariate analysis for overall survival

Variable	Unfavorable	HR	95% CI	P-values
Age	>60 year	7.954	0.991–63.811	0.051
PS	2	2.962	1.014–8.653	0.047
LDH	>normal	6.531	1.105–38.594	0.038
sIL-2R	>1500 U/mL	0.82	0.213–3.152	0.773

CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; PS, performance status; sIL-2R, soluble interleukin-2 receptor.

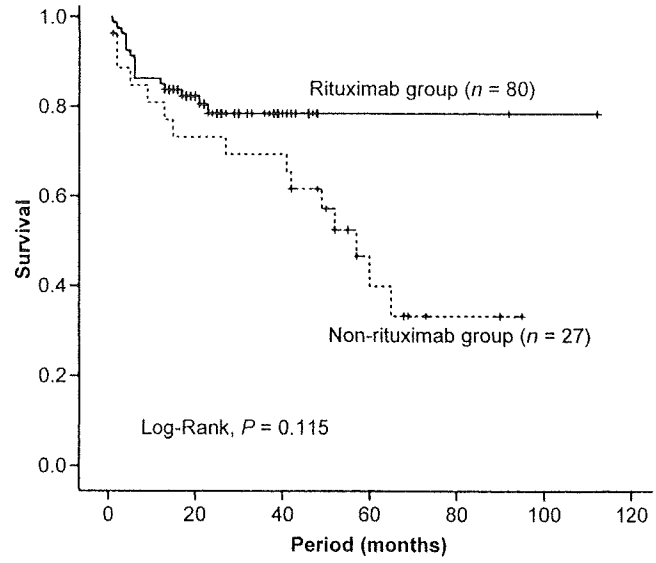

Fig. 4. Overall survival according to whether patients received rituximab-containing chemotherapy or not.

Discussion

Survival analysis in the present study showed that GCB-type patients had better prognoses, whereas patients with elevated sIL-2R levels or who were in the non-GCB subgroup had poorer prognoses in DLBCL. In multivariate analysis, however, a higher sIL-2R level was not an independent prognostic factor. We wondered if this was attributable to the strong correlations between IPI risk groups and sIL-2R levels. Indeed, as shown in Figure 5, the serum sIL-2R level was closely correlated with revised-IPI risk groups. As the figure shows, the worse the revised-IPI risk becomes, the higher the sIL-2R level is, and there are significant differences among all revised-IPI risk groups. Many reports indicate that the determination of the IPI risk group at the initial point before treatment is a very powerful prognostic tool. Our data showed a similar result. However, such determination is not appropriate when the patients are followed-up after treatment, or at a time of tumor relapse. This is because the IPI risk groups include several criteria. On the other hand, serum sIL-2R level is a continuous variable and is suitable for following the measured levels continuously. Our data suggest that sIL-2R may be prognostically useful with DLBCL patients after their treatments. Serum sIL-2R levels can be elevated not only in malignant lymphomas but also in non-hematopoietic disorders such as collagen diseases[17] or other malignancies.[18] Thus, we should consider these other factors, too.

Interestingly, there are mutual relationships between the IPI poor-risk group and the non-GCB subgroup (by Fisher's exact test, $P = 0.023$). And the serum sIL-2R levels are higher in patients in the non-GCB subgroup than in those of the GCB subgroup (by Mann–Whitney U-test, $P = 0.007$, Fig. 6). When we classified cases into GCB and non-GCB subgroups, the GCB : non-GCB ratio was 1:4. Shiozawa *et al.*[19] reported that

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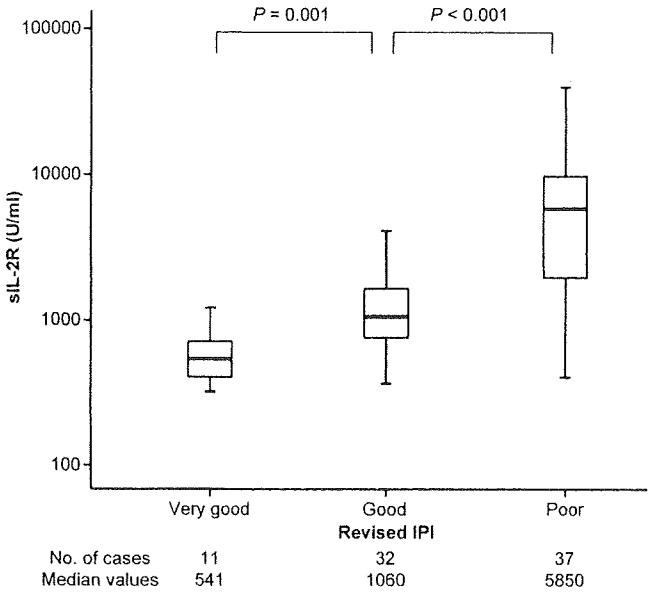

Fig. 5. Serum soluble interleukin-2 receptor (sIL-2R) levels according to revised International Prognostic Index (IPI) risk group.

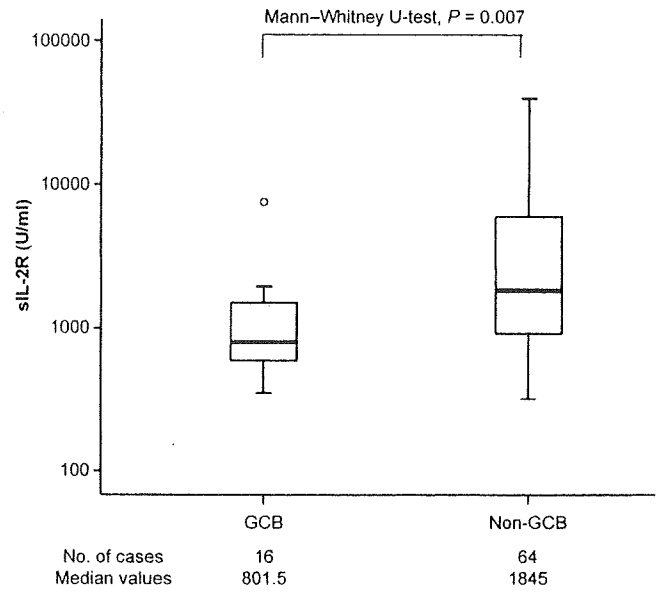

Fig. 6. Serum soluble interleukin-2 receptor (sIL-2R) levels according to subphenotype.

the GCB subgroup was less frequent in Asian countries; the approximate GCB : non-GCB ratio was 1:1.5–1:2.5. They described that non-GCB dominance in Asian countries is different from that in Western countries, where almost no differences between these two groups.[20–22]

Several reports indicate that the GCB-type of DLBCL has a better prognosis than the non-GCB type.[9–11] But most of those reports concerned the administration of chemotherapy without rituximab. Nowadays, anthracycline-containing combination chemotherapy with rituximab is a standard treatment protocol for patients with DLBCL. Several investigators have reported that in patients with DLBCL who were treated with rituximab-containing chemotherapy, there were no significant differences in outcomes between GCB and non-GCB subgroups.[23,24] Costa *et al.*[25] reported that GCB-type and non-GCB-type DLBCL had similar outcomes following autologous hematopoietic stem cell transplantation. Our present data, however, showed that there were significant differences in survival between GCB and non-GCB subgroups (Fig. 3a). This finding is similar to that reported by Fu *et al.*[13] Although the reasons for the different conclusions between our data and those in previous reports remain unclear, Fu *et al.*[13] referred that one of the reasons was a technical problem. Significantly greater numbers of cases need to be examined in order to reach a clearer conclusion. Even so, our clinical data came from a single institute whose treatment strategy is quite uniform, and we believe that classifying DLBCL patients into GCB *versus* non-GCB subgroups probably is also prognostically useful.

We also analyzed CD5 and p53 expression by immunohistochemistry. Both are prognostic biomarkers in DLBCL.[26–28] Our data showed that 11 cases of DLBCL were positive for CD5 and 11 cases of DLBCL had overexpression of p53. In univariate analysis, the cases that were positive for CD5 showed poorer prognoses than the negative ones ($P < 0.05$), whereas p53 did not show significant differences in survival. Multivariate analysis compared with IPI showed significant differences for CD5 (CD5: relative risk, 3.26 [95% confidence interval: 1.17–9.10], $P = 0.024$; p53: relative risk, 2.11 [95% confidence interval: 0.67–6.69], $P = 0.205$), so we confirmed that CD5 is an independent prognostic marker. The report from Chang *et al.*[28] is the one in which patients received chemotherapy without rituximab; thus p53 may not be a prognostic biomarker in the rituximab era.

In conclusion, it is useful to distinguish between GCB and non-GCB subgroups and to measure serum sIL-2R among DLBCL patients treated with rituximab in order to evaluate the risk, tumor activity, and prognosis more accurately. CD5 are also useful biomarkers.

Acknowledgments

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ORIGINAL PAPER

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Chronic sclerosing pyelitis with an increased number of IgG4-positive plasma cells

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Abstract IgG4-related disease has been recently described. This disease occurs in various anatomic locations including pancreas, biliary tract, liver, retroperitoneum, kidney, breast, lung, thyroid gland, prostate, salivary gland, lacrimal gland, and lymph node. In this article, we report the first case of IgG4-related disease arising in the renal pelvis. A 49-year-old Japanese woman was found to show left hydronephrosis by a medical checkup. Histological examination of the renal pelvic tumor showed IgG4-related disease. Her postoperative serum IgG4 was elevated, and this was compatible with IgG4-related disease. Systemic examination showed swelling of major and minor salivary glands and the lacrimal glands, and biopsy of the minor salivary gland revealed the finding of IgG4-related disease. Finally, pathologists and clinicians should be aware of the possibility that the renal pelvis may be involved in IgG4-related systemic disease.

Key words IgG4-related disease · Renal pelvis · Salivary gland

Introduction

To date, immunoglobulin (IgG)4-related disease is a systemic disorder, probably autoimmune in nature, and has been reported in various sites including pancreas, biliary tract, liver, retroperitoneum, kidney, breast, lung, thyroid gland, prostate, salivary gland, lacrimal gland, and lymph nodes.[1–12] Additionally, this disease usually has a good response to steroid therapy.[1,2] However, there are no descriptions of IgG4-related disease arising in the renal pelvis. In this article, we report the first case of IgG4-related disease arising in the renal pelvis.

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Case report

A 49-year-old Japanese woman was found to show left hydronephrosis by a medical check-up. She had no history of hematuria. Her urinary cytology showed negative findings for neoplasm. A subsequent computed tomography scan examination disclosed left hydronephrosis with marked thickness of the left renal pelvic wall. Surgical resection of the tissue extending from the left kidney to left upper ureter was performed. The lesion measured 3.5 cm in maximum diameter. Histological examination disclosed IgG4-related disease of the renal pelvis. Subsequently, the IgG subclass serum value was determined. Her postoperative serum level of IgG4 was elevated (555 mg/dl), and the rate of IgG4 value to total IgG value showed an increase to 20.46%. After establishment of the diagnosis of IgG4-related disease involving the renal pelvis, she was systematically examined, particularly in the digestive tract and cervical area. As a result, the clinician found multiple swellings of her submandibular gland, minor salivary gland, and lacrimal glands. A biopsy specimen of the minor salivary gland revealed findings consistent with IgG4-related disease. No lesions were observed in retroperitoneum, pancreas, and biliary tract. Systemic steroid therapy was not performed because her symptom was mild. However, she now receives periodic physical examination.

Tissues from left nephroureterectomy were fixed in 10% formalin and embedded in paraffin. Sections 3 μm thick were stained with hematoxylin and eosin and Masson trichrome. Additionally, immunohistochemical staining was performed using a Histofine Simple stain-PO (multi) kit (Nichirei, Tokyo, Japan), as previously described.[13–15] In the

present study, we employed antibodies against CD20cy (L26, 1:200; DAKO, Glostrup, Denmark), CD79acy (HM57, 1:50; DAKO), CD3 (F7.2.38, 1:50; DAKO), CD45RO (UCHL1, 1:200; DAKO). CD5 (4C7, prediluted; Nichirei), CD10 (56C6, prediluted; Novocastra Laboratories, Newcastle, UK), IgG (polyclonal, 1:3200; Novocastra), and IgG4 (HP6025, 1:640; Zymed Laboratory, San Francisco, CA, USA). All human specimens were obtained after approval from patients.

Pathological findings

Macroscopic findings of the renal pelvic lesion

Grossly, the cut surface of the renal pelvic lesion showed a whitish color with a solid consistency (Fig. 1).

Microscopic findings of the renal pelvic lesion

Histologically, lymphocytes had markedly infiltrated beneath the urothelial epithelium of the renal pelvis (Fig. 2a). Many lymphoid follicles were evident, and prominent sclerotic fibrosis was seen in interfollicular areas (Fig. 2b). Additionally, numerous plasma cells were identified in the interfollicular area (Fig. 2c). Infiltrating lymphocytes and plasma cells did not show significant cytological atypia.

Immunohistochemical findings of the renal pelvic lesion

Most B (CD20cy/CD79acy-positive cells) and T lymphocytes (CD3/CD45RO-positive cells) were independently distributed in follicular and interfollicular areas, respectively. The diagnosis of malignant lymphoma was excluded. More than 50% of plasma cells positive for IgG were positive for IgG4 (Fig. 2d).

Discussion

IgG4-related disease has been recently discovered. Although many investigators consider IgG4-reated disease as a distinct disease entity, the pathogenesis of this disease remains unknown. To date, on the one hand, many investigators have previously reported that various organs including pancreas, biliary tract, liver, retroperitoneum, kidney, breast, lung, thyroid gland, prostate, salivary gland, lacrimal gland,

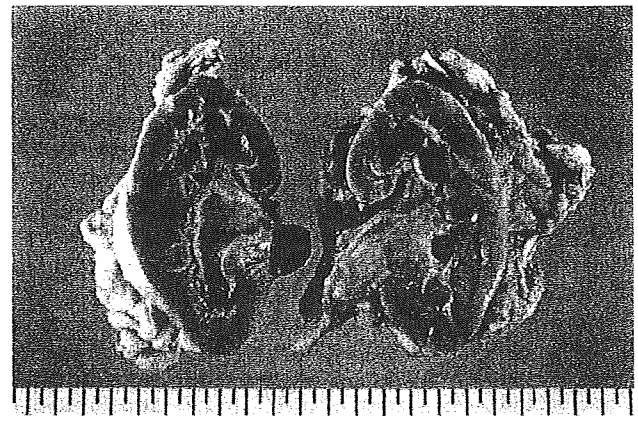

Fig. 1. Macroscopic findings of renal pelvic lesion. A whitish thick lesion is observed along the renal pelvic wall

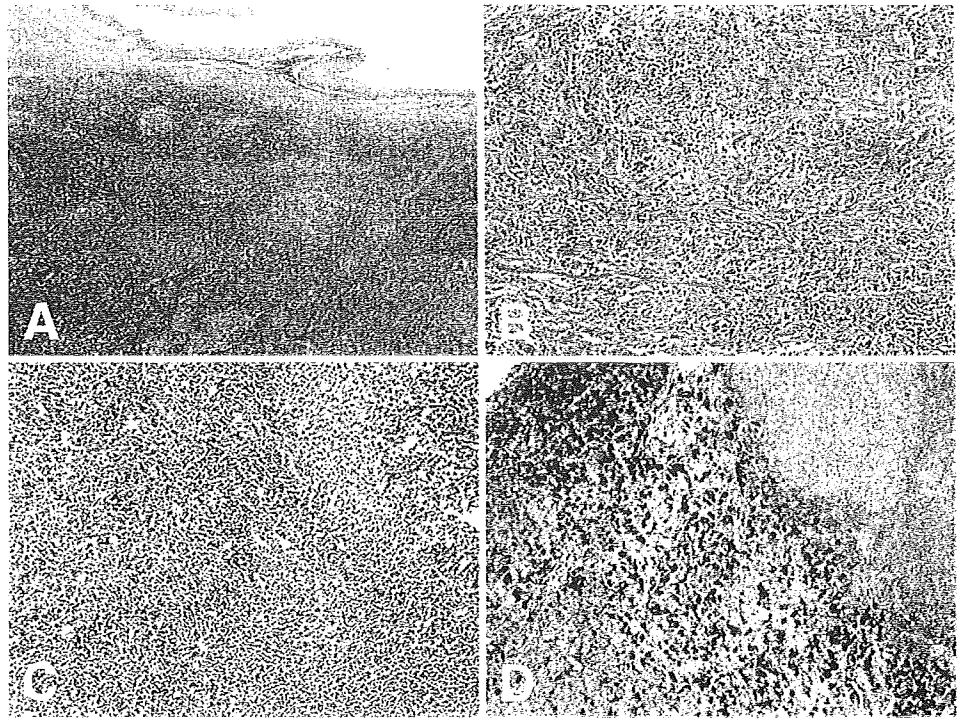

Fig. 2. Microscopic findings of renal pelvic lesion. **A** Lymphoproliferative lesion is seen under urothelial epithelium of the renal pelvis. Hematoxylin and eosin (H&E). ×40. **B** In the interfollicular area, a sclerotic lesion consisting of dense fibrosis can be identified. H&E. ×100. **C** In the interfollicular area, infiltration of many plasma cells is observed. H&E. ×100. **D** Immunohistochemical result of IgG4. Many plasma cells are reactive for IgG4. ×100

and lymph node may be involved in IgG4-related systemic disease.[1-12] In this case, we found many IgG4-positive cells among IgG-positive cells. Therefore, I certainly believe that chronic sclerosing pyelitis reminiscent of inflammatory pseudotumor may constitute a part of IgG4-related systemic disease. On the other hand, some inflammatory pseudotumors arising in the renal pelvis have been previously reported.[16] However, there are no descriptions of IgG4 in that article because IgG4-related disease has been recently discovered.[1,2] To the best of our knowledge, there are no descriptions of IgG4-related disease that forms a lymphoproliferative lesion in the renal pelvis. Therefore, this is the first report of IgG4-related disease arising in the renal pelvis. However, it is possible that previously reported inflammatory pseudotumors may be actually IgG4-related disease if the relationship between these lesions and IgG4 is examined in detail. Further examination is needed to clarify IgG4-related disease arising in the renal pelvis.

Finally, pathologists should be aware of the possibility that the renal pelvis may be involved in IgG4-related systemic disease.

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CASE REPORT

Primary sclerosing cholangitis with elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells

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Abstract Immunoglobin G4-related sclerosing cholangitis (IgG4-SC) is recognized as one of the systemic sclerosing diseases characterized by abundant IgG4-positive plasma cells with effective steroid therapy. On the other hand, primary sclerosing cholangitis (PSC), recognized as a sclerosing cholangitis of unknown origin without steroid efficacy, has been often clinically confused with IgG4-SC. To date, the prognosis of IgG4-SC is unclear, while the prognosis of PSC is well known to be poor. Therefore, it is clinically very important to be able to distinguish IgG4-SC from PSC. However, at the present time it still remains unclear whether PSC may sometimes be misdiagnosed as IgG4-SC or not. Herein, we report three rare cases of PSC with elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in the liver: a young male with ulcerative colitis (UC), and elderly female and a young female, each with elevated serum IgG4 levels. The first two patients showed infiltration of abundant IgG4-positive plasma cells in the portal area of the liver without response to steroid therapy. From our experiences, we emphasize that some patients with PSC, who do not respond to steroid therapy, show elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells, although the mechanism still remains unclear.

Keywords Primary sclerosing cholangitis · IgG4 · Autoimmune pancreatitis

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Introduction

Recently, autoimmune pancreatitis (AIP) has been accepted worldwide as a unique, distinctive disease, in which histopathological findings show abundant infiltration of IgG4-positive plasma cells and fibrosis, known as lymphoplasmacytic sclerosing pancreatitis (LPSP), and clinical manifestations that dramatically respond to steroid therapy. In addition to pancreatic lesions, patients with AIP have occasional extrapancreatic lesions such as sclerosing cholangitis (SC), sclerosing sialoadenitis, and retroperitoneal fibrosis similar to LPSP. Among the extrapancreatic lesions, the bile duct is the most commonly involved organ, manifesting as a sclerosing cholangitis which results in obstructive jaundice. AIP is recognized as the pancreatic manifestation of a novel systemic disease referred to as IgG4-related sclerosing disease [1].

IgG4-related sclerosing cholangitis (IgG4-SC) is a recently recognized disease entity characterized by microscopic findings of sclerosing inflammation with an infiltration of abundant IgG4-positive plasma cells, and AIP is associated in most cases. Before establishing the concept of AIP, IgG4-SC used to be misdiagnosed as primary sclerosing cholangitis (PSC) complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important. The cholangiographic findings in IgG4-SC and PSC are similar [2, 3]. Elevation

of serum IgG4 is frequently observed in patients with IgG4-SC, which responds dramatically to steroid therapy [4]. In contrast, even if the patients with PSC are medicated, it remains a progressive disease that involves the intra- and extra-hepatic bile ducts and leads to biliary cirrhosis. The effects of steroid therapy for PSC have been reported to be skeptical [5, 6] and liver transplantation is the only effective therapy. Histopathologically, lymphoplasmacytic and eosinophilic infiltration with mild fibrosis are seen in both IgG4-SC and PSC; and recent studies based on immunohistochemical findings of liver biopsy specimens report that IgG4-positive plasma cell infiltration is significantly more severe in IgG4-SC than in PSC [4, 7–11]. However, herein, we report 3 cases of PSC with an infiltration of abundant IgG4-positive plasma cells and ineffective steroid therapy.

Case report

Case 1

A 32-year-old man with elevated serum levels of hepatobiliary enzymes was admitted to our hospital. At the age of 22 years, the patient was diagnosed as PSC in other hospitals, and he had been treated with ursodeoxycholic acid. At the age of 24 years, he was found to have ulcerative colitis (UC). Physical examination at the time of admission revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 5700/μl (3500–8500); peripheral eosinocyte count, 251/μl (18–510); C-reactive protein, 1.1 mg/dl (<0.3); total bilirubin, 10.9 mg/dl (0.2–1.2); alkaline phosphatase, 2929 U/l (107–340); γ-glutamyl transpeptidase, 413 U/l (11–64); asparate aminotransferase, 133 U/l (13–35); alanine aminotransferase, 192 U/l (5–35); amylase, 127 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgG4, IgA, and IgM levels were 2104 mg/dl (870–1700), 96 mg/dl (4.8–105), 291 mg/dl (110–410), and 157 mg/dl (33–190), respectively. Rheumatoid factor was negative. Antinuclear antibody was positive. Among tumor markers, CEA was 1.8 ng/ml (<5.0) and CA19-9 was 237.2 U/ml (<37). Magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) revealed strictures of both the hepatic hilar region and the distal common bile duct and no narrowing of the main pancreatic duct (Fig. 1a, b). Cytology of bile juice was negative for malignancy. Histopathological examination by liver biopsy showed moderate lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 1c). Duct and ductular proliferation was conspicuous. Fibrous cholangitis (onion-skin fibrosis) was observed. These findings were compatible with PSC. The numbers of immunohistochemically identified IgG4-positive plasma cells were counted under five different high-power fields (hpf). Immunostaining study showed typical inflammation with abundant IgG4-positive plasma cells (126 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC. His liver dysfunction was serious, with progressive ascites and jaundice, therefore it was determined that liver transplantation might be necessary.

Although oral steroid therapy requires a long period for drug tapering, steroid pulse therapy is a well-recognized alternative for refractory autoimmune pancreatitis without steroid tapering, as previously reported [12]. Therefore, we twice administrated steroid pulse therapy with 500 mg/day of methylprednisolone for 3 days/week. The hepato-biliary enzymes improved a little after steroid therapy, but MRCP revealed no improvements of strictures of the hilar and distal common bile ducts. Therefore, we strongly suspected PSC. Two months later, we decided on liver transplantation with consent of the patient and his family. Histopathological findings of the liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 2a, b). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed, which suggested typical advanced PSC findings. Histopathological findings of the pancreas biopsy during the operation showed infiltration of mononuclear cells around the pancreatic duct (Fig. 2c) with an infiltration of abundant IgG4-positive plasma cells (Fig. 2d), but did not show LPSP.

Case 2

A 74-year-old woman was admitted to our hospital with liver dysfunction. Laboratory examinations showed the following values (normal range): peripheral white cell count, 8900/μl (3500–8500); C-reactive protein, 2.19 mg/dl (<0.3); total bilirubin, 0.6 mg/dl (0.2–1.2); alkaline phosphatase, 1544 U/l (107–340); γ-glutamyl transpeptidase, 1030 U/l (11–64); asparate aminotransferase, 130 U/l (13–35); alanine aminotransferase, 140 U/l (5–35); amylase, 44 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgM, and IgE levels were 1960 mg/dl (870–1700), 77 mg/dl (33–190), and 370 (0–320), respectively. Antinuclear antibody was positive. Antimitochondrial antibody was negative. Among tumor markers, CEA, CA19-9, and soluble interleukin 2 receptor (sIL-2R) were 2.1 ng/ml (<5.0), 18.5 U/ml (<37), and 611 U/ml (<650), respectively. Abdominal computed tomography (CT) showed dilatation of common bile duct (Fig. 3a) and no significant pancreatic lesions (Fig. 3b). ERCP revealed irregular narrowing of the

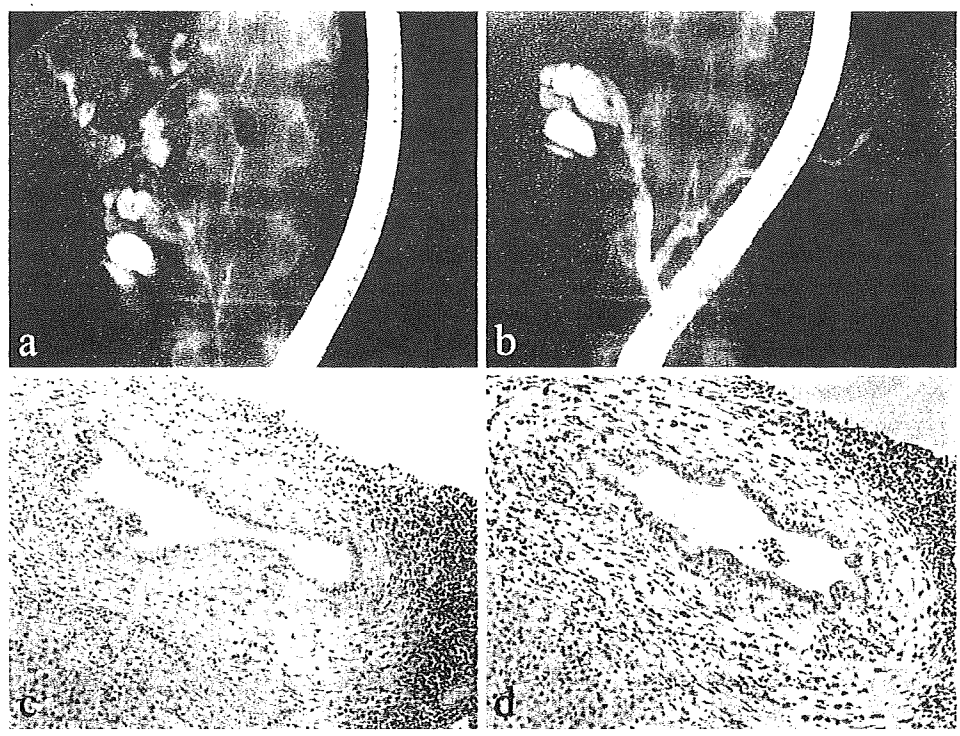


Fig. 1 ERCP images and histopathological findings of case 1 on clinical onset. ERCP revealed strictures of the hepatic hilar area (**a**) and the distal common bile duct, and no narrowing of the main pancreatic duct (**b**). Histopathological findings of the liver biopsy showed a moderate lymphoplasmacytic and eosinophil infiltration with fibrosis, and fibrous cholangitis (onion-skin fibrosis) (H&E staining ×200, **c**). IgG4-immunostaining of the liver biopsy showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

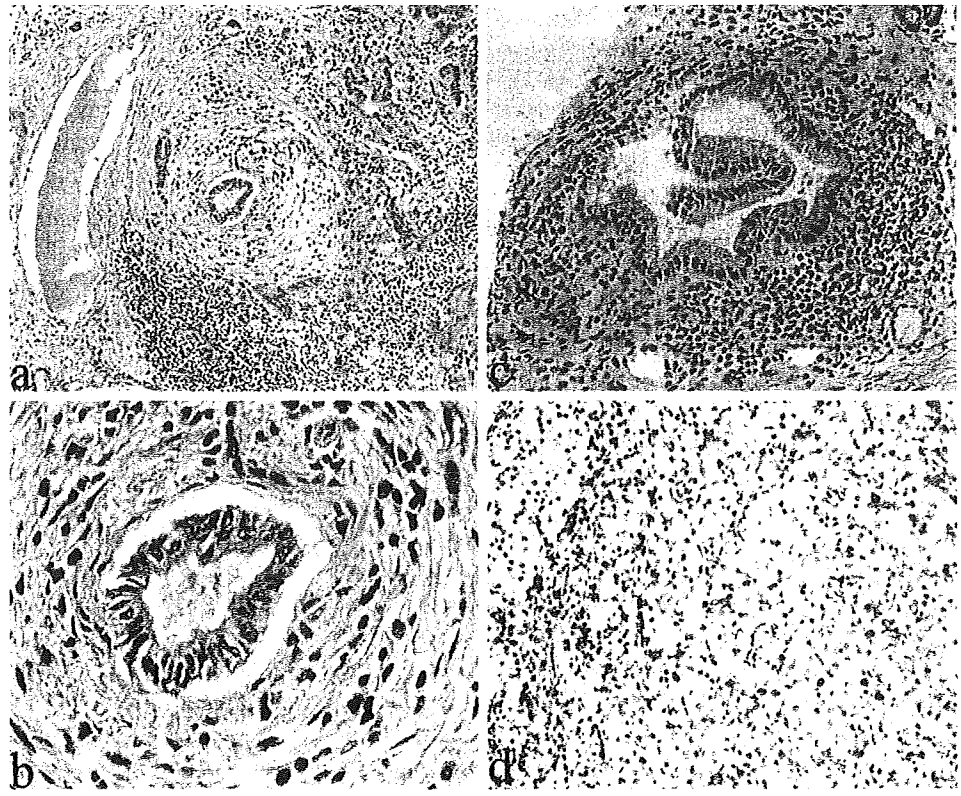


Fig. 2 Histopathological findings of case 1 on transplantation. Histopathological findings of liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (H&E staining ×100, **a**; ×400, **b**). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed. Histopathological findings of the pancreas biopsy during operation showed that infiltration of mononuclear cells around pancreatic duct (H&E staining ×200, **c**). IgG4-immunostaining of the pancreas biopsy during operation showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

intrahepatic bile ducts (Fig. 3c) and no narrowing of the main pancreatic duct (Fig. 3d). Intraductal ultrasonography (IDUS) detected wall thickness of the intrahepatic and common bile ducts. Cytology of bile juice was negative for malignancy. She was diagnosed with PSC and treated with ursodeoxycholic acid.

Four months after clinical onset, the patient was referred to our hospital for further evaluation of recurrent

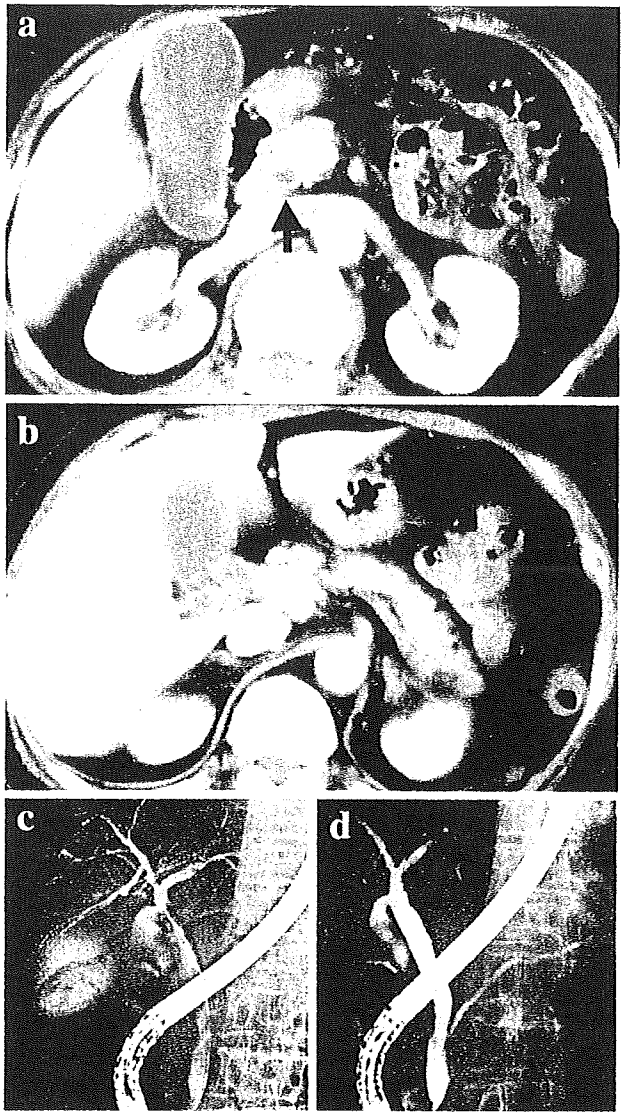


Fig. 3 Abdominal computed tomography (CT) images of case 2 on clinical onset. Abdominal CT showed a dilatation of common bile duct (**a**) and no significant pancreatic lesions (**b**), such as swelling of the pancreas. ERCP revealed irregular narrowing of the intrahepatic bile duct (**c**), and no narrowing of the main pancreatic duct (**d**)

obstructive jaundice. Laboratory tests showed elevations of IgG4 to 206 mg/dl (4.8–105). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area, which is compatible with PSC (Fig. 4a). However, an inflammation with abundant IgG4-positive plasma cells (16 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC, was also found. Then, we suspected IgG4-SC, and steroid therapy was initiated at a dose of 30 mg/day. The dose of steroid was reduced by 5 mg/week until it reached 10 mg/day. MRCP revealed no improvements of the irregular narrowing of the intrahepatic lesion and the common bile duct after steroid therapy. One year later, her liver dysfunction developed into liver cirrhosis.

Case 3

The patient was a 23-year-old woman who was admitted to our hospital with the complaint of jaundice. ERCP revealed stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (Fig. 5a). Endoscopic naso-biliary drainage (ENBD) was performed. The pancreatic-duct image showed no narrowing of the main pancreatic duct. Cytology of bile juice was negative for malignancy. Physical examination revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 10100/μl (3000–8500); peripheral eosinocyte count, 91/μl; C-reactive protein, 0.09 mg/dl (<0.3); total bilirubin, 5.0 mg/dl (0.2–1.2); alkaline phosphatase, 1750 U/l (107–323); γ-glutamyl transpeptidase, 211 U/l (8–45); asparate aminotransferase, 90 U/l (12–31); alanine aminotransferase, 101 U/l (6–24); amylase, 37 U/l (32–112). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgA, and IgM levels were 2570 mg/dl (1092–1577), 208 mg/dl (134–287), and 363 mg/dl (60–161), respectively. Rheumatoid factor, antinuclear antibody, and antimitochondrial antibody were negative. The irregular dilatation of bile ducts improved 5 months after a drainage procedure with a biliary plastic stent, but irregular narrowing of the intrahepatic bile ducts persisted (Fig. 5b). She was diagnosed with PSC and treated with ursodeoxycholic acid.

Three years after clinical onset, the patient was referred to our hospital for further evaluation of recurrent obstructive jaundice. Histopathological examination by liver biopsy showed an infiltration of lymphocytes and ductular proliferation in the portal area (Fig. 6a), and a few IgG4-positive plasma cells (1 cell/hpf) were detected (Fig. 6b). Laboratory tests showed elevations of IgG4 to 313 mg/dl (4.8–105). Therefore, we suspected IgG4-SC and steroid therapy was initiated at the dose of 30 mg/day. The dose of steroid was reduced by 5 mg/day biweekly until it reached 10 mg/day. MRCP revealed no improvements of strictures of the intrahepatic and the common bile ducts after steroid therapy.

Discussion

Sarles et al. [13] observed the first case of pancreatitis with hypergammaglobulinemia, and Yoshida et al. first proposed the concept of autoimmune pancreatitis (AIP) in 1995 [14], in which patients show diffusely enlarged

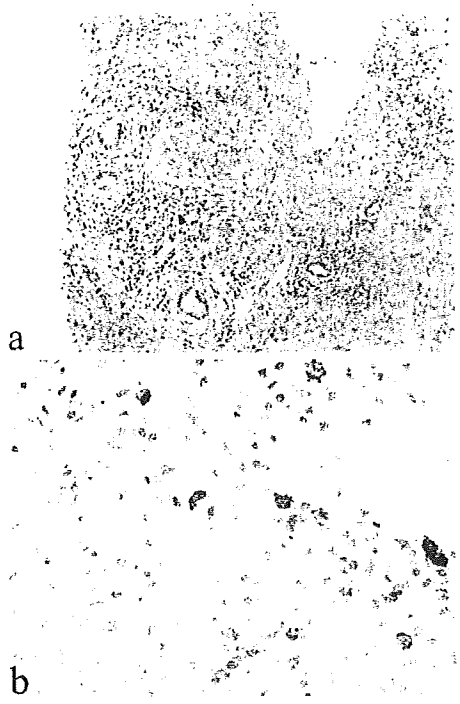
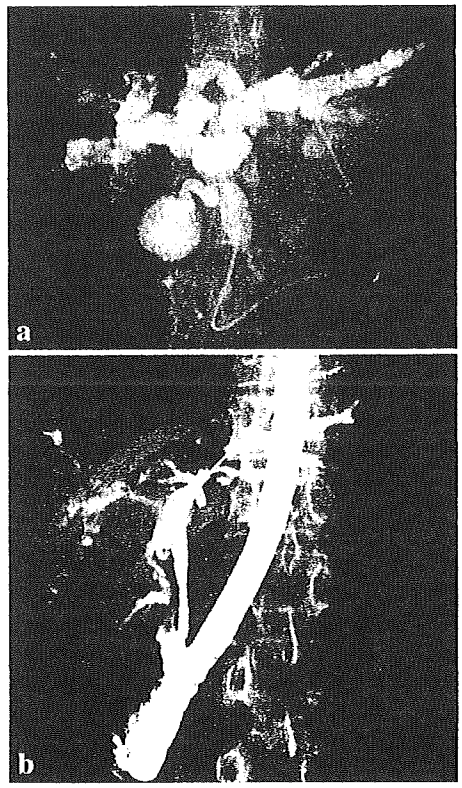


Fig. 4 Histopathological findings of the liver (case 2). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area (H&E staining ×100, **a**). IgG4-immunostaining of the liver specimens showed infiltration of abundant IgG4-positive plasma cells (×400, **b**)



◀ **Fig. 5** Endoscopic nasobiliary drainage (ENBD) cholangiogram and ERCP image of case 3 on clinical onset. Cholangiography through an ENBD tube revealed a stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (**a**). ERCP after a drainage procedure with a biliary plastic stent revealed improvement of bile duct dilatation, but irregular narrowing of the intrahepatic bile ducts persisted (**b**)

pancreas, narrowing pancreatogram, increased serum IgG, presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal efficacy. Thereafter, many AIP cases have been reported by Japanese investigators, and AIP has been accepted as a new clinical entity [15–17]. Patients with AIP often show discomfort in the epigastrium, obstructive jaundice due to bile-duct stricture, and diabetes mellitus. AIP is more common in middle-aged and elderly men. Patients with AIP often also have extrapancreatic lesions such as biliary lesions, sialoadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [18–23], which suggests that AIP may be a systemic disorder. In 2001, Hamano et al. [24] reported that patients with AIP have high serum IgG4 concentrations. Kamisawa et al. [1] proposed IgG4-related sclerosing disease. Recently, IgG4-SC was recognized as a disease entity characterized by

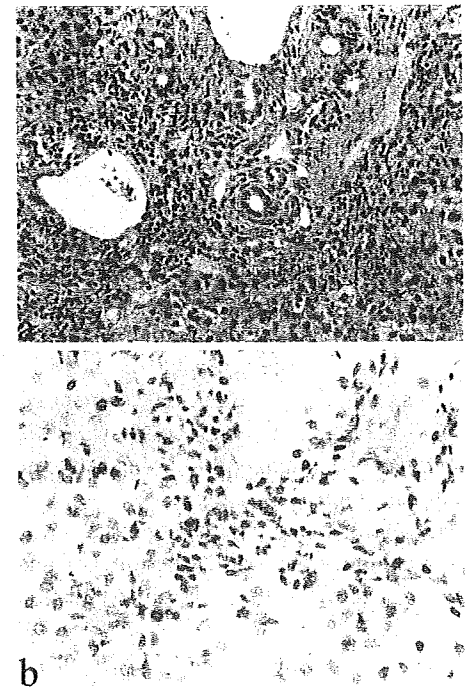


Fig. 6 Histopathological findings of the liver (case 3). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration and ductular proliferation in the enlarged portal area (H&E staining ×200, **a**). IgG4-immunostaining of the liver specimens showed infiltration of few IgG4-positive plasma cells (×400, **b**)

sclerosing inflammation with an infiltration of abundant IgG4-positive plasma cells, and AIP was associated in most cases. Before establishing the concept of AIP, IgG4-SC used to be misdiagnosed as PSC complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important, because the effective treatments and the prognoses are different. Although IgG4-SC is usually associated with pancreatic lesions, a few patients with IgG4-SC have shown little pancreatic change or other organ involvement [25, 26]. The correct diagnosis of such cases is difficult.

In this study, we presented 3 PSC cases with elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells in the liver, which usually support the diagnosis of IgG4-SC. In the 3 cases presented, abdominal ultrasound and abdominal CT scan did not show inflammatory swelling of the whole pancreas, and ERCP did not show strictures over one-third of the main pancreatic duct (MPD), which is characteristic of AIP [27]. Cholangiography in these 3 patients showed strictures of the intrahepatic and common bile ducts, and no narrowing of the MPD. After steroid therapy, strictures of the intrahepatic and common bile ducts were not improved on MRCP images. These findings supported the diagnosis of PSC. In these 3 patients, however, there were findings atypical for PSC. First, the serum IgG4 concentrations in cases 2 and 3 were elevated. The Japanese criteria of AIP contain three approaches: pancreatic imaging, laboratory data, and histopathology [18]: (1) Pancreatic image examinations show the narrowing of the main pancreatic duct and enlargement of pancreas which are characteristic of the disease; (2) Laboratory data show the presence of autoantibodies, or elevated levels of serum gammaglobulin, IgG, or IgG4; (3) Histopathological examinations of the pancreas show fibrosis and pronounced infiltration of cells, mainly lymphocytes and plasmacytes. For a diagnosis, criterion (1) must be present, together with criterion (2) and/or (3). However, it is necessary to exclude malignant diseases such as pancreatic or biliary cancers. In the diagnostic criteria of Korea [28] and Asia [29], apparent pancreatic lesions comparable with AIP must be present for a diagnosis of AIP. Two patients (cases 2 and 3) did not fulfill the diagnostic Japanese, Korean, and Asian criteria, because they had no apparent pancreatic lesions comparable with AIP. Secondly, infiltration of abundant IgG4-positive plasma cells in the liver specimens was found in cases 1 and 2. IgG4 immunostaining showing >10 IgG4-positive plasma cells/hpf is suggestive of AIP in the HISORt criteria by the Mayo Clinic [30] and Korean criteria [28]. The presence of IgG4-SC in the HISORt criteria can be diagnosed in patients with effective steroid therapy. Two patients (cases 1 and 2) did not fulfill the HISORt criteria because they had no response to steroid therapy.

The role of IgG4 in patients with PSC has been used to differentiate clinical syndromes of atypical PSC cases. In 1991, Kawaguchi et al. [31] first described clinical and pathological features of variant cases of PSC, which were later known as sclerosing cholangitis complicated with autoimmune pancreatitis (AIP). In 1995, Takikawa et al. [32] analyzed 192 cases of Japanese PSC and found two peaks in the age distribution. Some cases in elderly patients were complicated with chronic pancreatitis, which was regarded as sclerosing cholangitis complicated with autoimmune pancreatitis. The patients in cases 1 and 3 were young, and case 2 was an elderly woman. In 2004, Takikawa et al. [33] analyzed 269 additional cases of Japanese PSC and showed that 7% of these cases had AIP. In a recent study, Mendes et al. [34] have reported that 12 (9%) of 127 PSC patients had elevated serum IgG4 levels. These patients also had significantly higher levels of ALP and total bilirubin, and higher PSC Mayo risk scores. Mendes's study also reveals that IgG4-SC may have been included among PSC cases in the United States. There may possibly be disease entities such as overlap syndrome. In our cases, it is difficult to differentiate IgG4-SC from PSC on cholangiographic and immunohistochemical findings. The findings of elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in the liver usually support the diagnosis of IgG4-SC. On the other hand, the patients of cases 1 and 3 were younger, and the patient of case 1 was associated with UC. These clinical characteristics may be compatible with PSC. In a recent study, Kawabe et al. have reported an advanced state of biliary cirrhosis and atrophic pancreas but did not reveal typical imaging findings of AIP and AIP-related sclerosing cholangitis [35]. Hamano et al. have reported 3 patients with IgG4-SC who had no apparent pancreatic lesions comparable with AIP [26]. These cases were improved only by steroid therapy or drainage. In this study, however, our 3 patients with sclerosing cholangitis who had no apparent pancreatic lesions comparable with AIP did not respond to steroid therapy. Some AIP patients may develop pancreatic stones and the conventional type of chronic pancreatitis [36, 37]. Though there may be a possibility that far advanced stages of AIP with sclerosing cholangitis who had no pancreatic lesions might not respond to steroid therapy, the long-term untreated prognosis of AIP still remains unclear. Therefore, further studies are necessary. Finally, we diagnosed our 3 patients as PSC according to the commonly used diagnostic criteria for PSC [38]. They did not fulfill all criteria, and histopathological finding of the pancreas in case 1 did not show so-called LPSP. Here, our cases showed elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in patients with PSC, which do not respond to steroid therapy. Therefore, it is necessary to be aware of the possibility of

PSC with these findings to correctly differentiate PSC from IgG4-SC. The mechanisms of increased serum IgG4 and the role of the infiltrated IgG4-positive plasma cells in the portal area still remain unclear at this time. Recent studies of immune tolerance and allergy show that high dose antigen exposures cause immune deviation both of Th2 response in favor of Th0/Th1, and in the generation of IL-10- and TGF-β-producing regulatory T cells [39], though our 3 patients were not found to have allergic disease. Additionally, IL-10 induces preferential switching of B cell response in favor of producing IgG4 antibodies, and possibly IgA antibodies under the influence of TGF-β [40]. Our previous data [41] and others [11] showed that IL-10 secreted from increased inducible peripheral regulatory T cells may be involved in switching B cells to produce IgG4-positive cells and increased serum IgG4 in IgG4-related sclerosing pancreatitis (autoimmune pancreatitis) or IgG4-related sclerosing cholangitis, but not in PSC [11]. These findings suggested that increased IgG4 may be reactive and involved in the pathophysiology of IgG4-related diseases, but not in the pathogenesis. Further studies are necessary to clarify the role of IgG4.

In conclusion, some of the patients with PSC show elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells, and do not respond to steroid therapy.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term outcome of autoimmune pancreatitis

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Abstract
Purpose Autoimmune pancreatitis (AIP) is a unique form of pancreatitis and can be complicated with various extrapancreatic lesions. Little is known about the long-term clinical course of AIP. Here we aimed to document the clinical course of AIP.
Methods For this study, we recruited 21 patients, averaging 66.5 years in age (range, 19–84 years) and observed them at a mean interval of 40.8 months (range, 18–130 months). Three of the patients were also diagnosed with retroperitoneal fibrosis, 3 had sialoadenitis, 2 had chronic thyroiditis, 1 had interstitial nephritis, and 1 had interstitial pneumonia. Three of the patients underwent surgical therapy, 12 patients received methylprednisolone (PSL) treatment, and the 6 remaining patients received no treatment.
Results Enlargement of the pancreas was attenuated in all the PSL-treated patients. Seven of the 21 patients showed pancreatic atrophy, of whom 2 were non-PSL-treated patients. Three patients developed chronic pancreatitis. One patient was diagnosed with pancreatic cancer after 50 months of PSL therapy.
Conclusions As with chronic pancreatitis patients, AIP patients should be observed closely for abnormality in pancreatic function.

Keywords Autoimmune pancreatitis · Chronic pancreatitis · Pancreatic cancer

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Introduction

Chronic pancreatitis (CP), of which about 30–40% of cases are idiopathic [1], is a disorder characterized by chronic inflammation and fibrosis of the pancreas that leads to irreversible pancreatic dysfunction and finally to pancreatic insufficiency. Sarles et al. [2] observed the first case of pancreatitis with hypergammaglobulinemia. In 1995, Yoshida et al. [3] first proposed the concept of "autoimmune pancreatitis (AIP)," in which patients show diffusely enlarged pancreas, narrowing pancreatogram, increased serum IgG, the presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and the efficacy of steroid treatment. Since that time, many AIP cases have been reported by Japanese gastroenterologists, and AIP has been accepted as a new clinical entity [4–6]. Many reports have shown that steroid treatment was very effective against AIP with increased immunoglobulin (Ig) G, IgG4, or autoantibodies. Recent studies have clarified that extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialoadenitis, interstitial nephritis, and retroperitoneal fibrosis, are often observed in AIP, suggesting that AIP may not be a discrete entity [7–9]. Imaging studies of patients with AIP show characteristically diffuse enlargement of the pancreas and irregular narrowing of the main pancreatic duct [10, 11]. Typical immunological abnormalities include increased levels of serum gammaglobulin, IgG, and IgG4,

and the presence of autoantibodies. Histopathological findings show lymphoplasmacytic sclerosing pancreatitis, which is a fibrotic change entailing dense infiltration of lymphocytes and IgG4-positive plasmacytes [12]. However, little is known about the prognosis of AIP patients. In the present study, we report the outcome and clinical features of our experiences with patients with AIP under long-term observation.

Methods

We diagnosed 52 patients with AIP at Kansai Medical University and affiliated hospitals. All of these patients were diagnosed according to the clinical diagnostic criteria for AIP proposed by the Research Committee of Intractable Diseases of the Pancreas supported by the Japanese Ministry of Health, Labor, and Welfare, and the Japan Pancreas Society [13]. All 52 patients fulfilled diagnostic criteria 1 and 2. In brief, they exhibited diffuse enlargement of the pancreas and diffuse narrowing of the main pancreatic duct with an irregular wall. They also showed high levels of serum gammaglobulin (normal range, > g/dl), IgG (normal range, >1800 mg/dl), or IgG4 (normal range, >135 mg/dl). However, histological findings of the pancreas were not confirmed in any of them.

Of the 52 patients, 21 (14 male, 7 female, averaging 66.5 years in age; range, 19–84 years) were followed up for 18 months or more (mean period, 40.8 months; range, 18–130 months). Extrapancreatic lesions were observed in 10 patients: retroperitoneal fibrosis was diagnosed in 3 patients, sialoadenitis in 3, thyroiditis in 2, interstitial nephritis in 1, and interstitial pneumonia in 1. Four patients had associated diabetes mellitus (DM), and 1 had nonalcoholic steatohepatitis (NASH; Table 1).

Three patients underwent surgical treatment: these patients underwent left-lobe hepatectomy, pancreatoduodenectomy, and bilio-jejunostomy when AIP or associated conditions were misdiagnosed as cholangioductal cancer, pancreatic cancer, and mass-forming pancreatitis, respectively. Twelve patients were treated with oral steroid (methylprednisolone; PSL), and 6 patients were observed without treatment. The initial dose of oral PSL was 30 mg/day, decreasing by 5 mg/day every 2 weeks. The maintenance doses of PSL were 10 mg/day in three patients, 7.5 mg/day in two, and 5 mg/day in five; maintenance

Table 1 Clinical profiles of 21 patients

Patient number	Onset age (years)	Sex	Treatment	Follow-up period (months)	Associated diseases
1	64	M	PSL	20	Thyroiditis, sialoadenitis, DM
2	77	M	PSL	50	Pancreatic cancer
3	71	M	PSL	31	
4	66	M	PSL	79	DM
5	66	F	Bilio-jejunostomy; steroid pulse	130	Retroperitoneal fibrosis Hashimoto's disease
6	62	F	None	32	Sialoadenitis
7	63	M	PSL	34	
8	71	M	PSL	27	Retroperitoneal fibrosis
9	71	F	None	21	NASH
10	77	F	PD	48	
11	54	M	None	39	Inflammatory pseudotumor (liver)
12	70	M	None	21	
13	19	F	None	57	
14	73	M	Hepatectomy	27	Retroperitoneal fibrosis
15	65	M	PSL	23	
16	74	M	PSL	83	DM
17	65	M	PSL	23	DM
18	84	F	PSL	19	
19	81	M	PSL	47	
20	73	M	None	35	
21	51	F	PSL	18	Interstitial pneumonia Mikulicz's disease

PSL treatment with methylprednisolone, *PD* pancreatoduodenectomy, *DM* diabetes mellitus, *NASH* nonalcoholic steatohepatitis

therapy with oral PSL was discontinued in two of the patients. We observed six patients without administering oral steroid therapy, because one patient had NASH and five patients showed only pancreatic body and/or tail enlargement without extrapancreatic lesions. No patients had any complications during the follow-up period.

To evaluate clinical courses, levels of pancreatic enzymes, the glycosylated hemoglobin value (HbA1c), and morphological changes as indicated by computed tomography (CT) were studied. Pancreatic size was evaluated by CT according to the method of Heuck et al. [14]. The width of the pancreas along its longest axis was measured on CT images and compared with the transverse diameter of the vertebral body. The pancreatic size on the first CT image was defined as 100%. Pancreatic atrophy was defined according to the criteria of of Heuck et al. [14]. In brief, the vertebral body diameter was regarded as 100% and from this the corresponding pancreatic diameter was calculated. Pancreatic atrophy was defined as a ratio of the vertebral body to the pancreatic body of 20% or less.

Results

Morphological changes of the pancreas

In all 12 patients treated with oral PSL, the enlargement of the pancreas improved. After 18 months, the average pancreatic size had decreased to 51.3% of that before treatment (Fig. 1a). Pancreatic atrophy developed in 5 of the 12 patients treated with PSL and in 2 of the 6 patients without medication. The pancreatic size was not changed in 2 of the patients without PSL treatment (Fig. 1b). The incidence of pancreatic atrophy in the steroid-treated group was higher than that in the nontreated group. Pancreatic calcification was observed in 1 patient after 12 months of PSL therapy. Chronic pancreatitis with dilation of the main pancreatic duct (MPD) developed in 2 patients after PSL therapy; in 1 after 12 months and in 1 after 19 months. Pancreatic cyst occurred in 1 patient after 24 months of PSL therapy.

Pancreatic enzymes

Serum trypsin levels (100–550 ng/ml) were monitored periodically in 10 of the 21 observed patients. In 1 of these 10 patients, serum trypsin levels decreased after PSL therapy, while in 1 patient, serum trypsin levels increased after PSL therapy. In 3 of the 10 patients, serum trypsin levels decreased to below the normal limit, while in 1 patient, serum trypsin levels decreased after PSL therapy (Fig. 2a). Elastase I levels (<400 ng/dl) were monitored in 12 of the 21 patients. In 4 of these 12 patients, elastase I

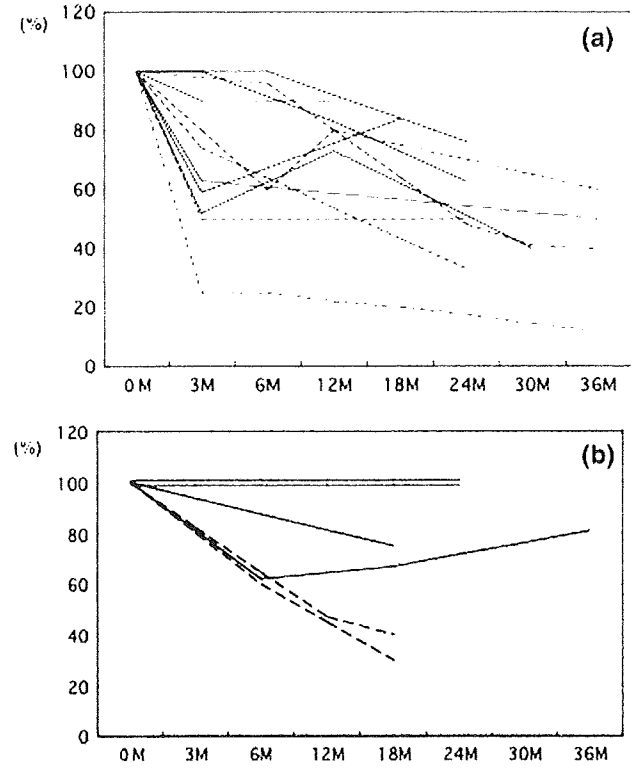

Fig. 1 Follow up of pancreatic enlargement in patients with steroid therapy (**a**) and without steroid therapy (**b**). Pancreatic enlargement was evaluated by computed tomography (CT). The width of the pancreas along its maximum longitudinal axis was measured on CT images and compared with the transverse diameter of the vertebral body. The pancreatic size on the first CT image was defined as 100%. In all 12 PSL-treated patients (**a**), the enlargement of the pancreas was attenuated; after 24 months, the average pancreatic size had decreased to 51.3% of the pancreatic size before treatment. Pancreatic atrophy developed in 5 of these patients (*dashed lines*). In 2 of the patients without PSL treatment, there were no changes in pancreatic size. Two of the patients without PSL treatment showed pancreatic atrophy (*dashed lines*). *M* months

levels fell to within normal limits: in 3 patients after PSL therapy and in 1 patient naturally without PSL therapy (Fig. 2b).

Pancreatic exocrine function

In our short-term follow-up series, after 6 months with steroid treatment, pancreatic exocrine function showed improvement in 11 patients, as determined by the urine exocrine *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA) test (73.1–90.1%) (Fig. 3). In the long-term follow-up series, pancreatic exocrine function was monitored by the BT-PABA test in 10 of the 21 patients (Fig. 4). Four of them showed improvement of pancreatic exocrine function by steroid therapy, while 6 (3 with steroid and 3 without steroid therapy) showed progressive dysfunction.