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Association of immunoglobulin G4 and free light chain with idiopathic pleural effusion

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Summary

The cause of pleural effusion remains uncertain in approximately 15% of patients despite exhaustive evaluation. As recently described immunoglobulin (Ig)G4-related disease is a fibroinflammatory disorder that can affect various organs, including the lungs, we investigate whether idiopathic pleural effusion includes IgG4-associated etiology. Between 2000 and 2012, we collected 830 pleural fluid samples and reviewed 35 patients with pleural effusions undiagnosed after pleural biopsy at Yamaguchi-Ube Medical Center. Importantly, IgG4 immunostaining revealed infiltration of IgG4-positive plasma cells in the pleura of 12 patients (34%, $IgG4^+$ group). The median effusion IgG4 level was 41 mg/dl in the $IgG4^+$ group and 27 mg/dl in the $IgG4^-$ group ($P < 0{\cdot}01$). The light and heavy chains of effusion IgG4 antibodies of patients in the $IgG4^+$ group were heterogeneous by two-dimensional electrophoresis, indicating the absence of clonality of the IgG4 antibodies. Interestingly, the κ light chains were more heterogeneous than the λ light chains. The measurement of the κ and λ free light chain (FLC) levels in the pleural fluids showed significantly different κ FLC levels (median: 28·0 *versus* 9·1 mg/dl, $P < 0{\cdot}01$) and κ/λ ratios (median: 2·0 *versus* 1·2, $P < 0{\cdot}001$) between the $IgG4^+$ and $IgG4^-$ groups. Furthermore, the κ/λ ratios were correlated with the $IgG4^+/IgG^+$ plasma cell ratios in the pleura of the $IgG4^+$ group. Taken together, these results demonstrate the involvement of IgG4 in certain idiopathic pleural effusions and provide insights into the diagnosis, pathogenesis and therapeutic opportunities of IgG4-associated pleural effusion.

Keywords: fibrinous pleuritis, free light chain, IgG4-related disease, pleural effusion

Introduction

Pleural effusion remains common, originating from a wide range of pathologies including congestive heart failure, pneumonia and cancer [1]. A diagnostic algorithm for the differentiation of a pleural effusion proposed by Light *et al.* has been widely accepted [2] and recommended in the British Thoracic Society pleural disease guideline [3]. Nonetheless, the cause of the pleural effusion remains unclear in a substantial percentage of patients with persistently exudative effusions after the history, physical examination and biochemical and cytological tests of pleural fluid [4–6]. No diagnosis has been established for up to 15% of patients, despite invasive procedures such as thoracoscopy or open pleural biopsy [4,6,7]. Therefore, a new approach is needed to detect the cause(s) of undiagnosed pleural effusions [8–11]. Because immunoglobulin G4 (IgG4)-related disease is recognized as a fibroinflammatory condition of unknown cause that can affect multiple organs including the lungs and pleura [12,13], IgG4 might be related to certain idiopathic pleural effusions.

Hamano *et al.* originally reported elevated serum IgG4 concentrations in patients with autoimmune pancreatitis [14], and IgG4-related autoimmune disease has been proposed as a new clinicopathological entity characterized by $IgG4^+$ plasma cell infiltration [15]. High serum IgG4 levels and infiltration of $IgG4^+$ plasma cells have also been reported in other organs, including salivary and lacrimal glands [15–18]. Although the criteria for diagnosis of

IgG4-related disease in the lung have not been established, elevated serum IgG4 concentrations and histopathological examinations, such as marked lymphoplasmacytic infiltration including $IgG4^+$ cells and fibrosis, have been recommended [19–21]. Taniguchi *et al.* have reported interstitial pneumonia associated with autoimmune pancreatitis and marked infiltration of $IgG4^+$ plasma cells in the pulmonary alveolar septum [22]. Common radiological findings of IgG4-related lung disease include hilar and mediastinal lymphadenopathy, thickening of perilymphatic interstitium with or without subpleural and/or peribronchovascular consolidation, and the pathological examination reveals lymphoplasmacytic infiltration with fibrosis, which correlates well with the radiological manifestations [23].

It has been reported that pleural effusion may occur in association with systemic IgG4-related disease [16,24–30]. Conversely, there have been a few case reports on isolated IgG4-related pleural effusion [26,31,32], but little is known about the involvement of IgG4 in the pleural effusion. In this study, we hypothesize that idiopathic pleural effusions include IgG4-associated aetiology and demonstrate pleural infiltration of $IgG4^+$ plasma cells in a substantial percentage of patients with idiopathic pleural effusion.

Methods

Patients

Idiopathic pleural effusion was defined as any persistent, exudative pleural effusion that remains undiagnosed after the history and physical examination, biochemical and cytological studies of pleural fluid, radiographic examinations and histopathological analysis of biopsied specimens [4,6]. Diagnosis of idiopathic pleural effusion was made after a minimum of 1-year follow-up (range = 1–10 years), with detailed exploration including computed tomographic (CT) scanning to exclude other causes of effusion such as malignant pleural mesothelioma and carcinomatous pleuritis, according to previous studies that mainly performed follow-up of 1–2 years [4,7,33–36]. In this retrospective study, we accumulated 830 pleural fluid samples at Yamaguchi-Ube Medical Center between 2000 and 2012 and reviewed 35 patients with undiagnosed pleural effusions who underwent thoracoscopy and pleural biopsy, after excluding three patients who had a malignancy during follow-up. Biochemical data were obtained for the sera and pleural fluids when thoracentesis was conducted. Biological and bacterial analyses of sera and pleural fluids, CT scan, cytological and histological examination did not demonstrate malignancy or infectious disease in patients with pleural effusions. The patients' pleural fluids were stored at −80°C until use. This study was approved by the institutional review board of NHO Yamaguchi-Ube Medical Center (Approval no. 26–2). Written informed consent was obtained from each patient or their family for the use of data and samples.

IgG4 immunohistochemistry

The parietal pleura were obtained from biopsy specimens. Immunostaining for IgG or IgG4 was performed by activation of the pleura with 0·1% trypsin, incubation with rabbit polyclonal anti-human IgG antibody (Dako, Glostrup, Denmark; cat. no. A0423) or biotinylated mouse monoclonal anti-human IgG4 antibody (clone HP-6025; Sigma B3648; Sigma, St Louis, MO, USA) [37,38] and HistoFine® Simple Stain™ Max PO Multi (Nichirei Biosciences, Tokyo, Japan; cat. no. 724152), and development with 3,3'-diaminobenzidine (Nichirei Bioscience; cat. no. 715301). The average number of $IgG4^+$ plasma cells within three high-power fields (HPFs) was calculated, and patients with the presence of > 10 $IgG4^+$ plasma cells/HPF and an $IgG4^+/IgG^+$ cell ratio of $> 40\%$, as described for biopsy specimens [23,38,39], were assigned to the $IgG4^+$ group.

Immunoglobulin analysis of pleural fluids

IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE in pleural fluids were quantitated with Bio-Plex Pro Assays Human Isotyping 7-Plex (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. Bio-Plex Suspension Array System was operated with Bio-Plex Manager (version 6·0).

Purification of IgG4 antibodies from pleural fluids

IgG4 was purified from pleural fluids by diethylaminoethyl (DEAE)-cellulose ion exchange chromatography and subsequently by affinity chromatography on anti-IgG4 antibody-coupled Sepharose-4. Pleural fluid was dialyzed against 0·01 M phosphate buffer (pH 7·0). DEAE-cellulose (DE52; Whatman Biosystems, Chalfont St Giles, UK) in a column ($\phi 1 \times 30$ cm) was equilibrated with 0·01 M phosphate buffer (pH 7·0). The dialyzed pleural fluid (20 ml) was passed onto the DEAE column and the fall-through fractions containing IgG were collected. IgG4 in the IgG of the fall-through fractions was purified with anti-IgG4-coupled Sepharose-4 that had been prepared by coupling monoclonal anti-IgG4 antibody (clone HP-6025; Sigma) to CNBr-activated Sepharose-4 (GE) according to the manufacturer's instructions. The bound IgG4 was eluted with 0·1 M glycine-HCl (pH 2·7) and neutralized immediately with 1 M Tris.

Two-dimensional electrophoresis (2-DE) of effusion IgG4 antibodies

2-DE of purified IgG4 was performed as described previously [40,41]. Briefly, isoelectric focusing (IEF) gel solution contained 8·5 M urea, acrylamide/Bis (5% T, 3% C), 10% glycerol, 1·3 mM lysine and a mixture of Pharmalyte pH 3–10 (1·25%) and pH 5–8 (1·25%) (GE), which was degassed and polymerized by adding ammonium persulphate and TEMED

to concentrations of 0·05 and 0·1%, respectively. Purified IgG4 was reduced in the presence of 5% β-mercaptoethanol at room temperature for 1 h, and urea was added to a concentration of 8·5 M immediately before loading onto the capillary IEF gel (ϕ1 mm × 5 cm). IEF was run in Mini-PROTEAN 2-D Electrophoresis Cell (Bio-Rad) at 200 V for 15 min, 400 V for 15 min and 750 V for 2·5 h. After IEF, capillary gel was equilibrated with sodium dodecyl sulphate (SDS) sample buffer containing 5% β-mercaptoethanol for 30 min. After washing the capillary gel with SDS running buffer, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed at 25 mA constant per gel. After transferring the proteins onto PVDF membranes, γ4, κ and λ chains on the blots were probed with biotinylated anti-IgG4 (Sigma; cat. no. B3648)/horseradish peroxidase-conjugated Extavidin (Sigma; cat. no. E2886), peroxidase-conjugated anti-κ and anti-λ light chain antibodies (Bio-Rad; cat. nos STAR127P and STAR129P), respectively.

Free light chain (FLC) analysis of pleural fluids

The levels of κ and λ FLCs in pleural fluids were measured by latex-based immunoassay using Freelite kappa kit and Freelite lambda kit (The Binding Site, Birmingham, UK). The measurement with Freelite was performed by The BN II System (Siemens, Munich, Germany) at a qualified clinical laboratory of SRL Inc. (Tokyo, Japan). The diagnostic ranges for serum κ FLC, λ FLC and κ/λ FLC ratio are 3·3–19·4 mg/l, 5·7–26·3 mg/l and 0·26–1·65, respectively [42].

Statistical analysis

The Mann–Whitney *U*-test was used to assess differences in the laboratory data, pleural fluid immunoglobulin levels and FLC levels between the $IgG4^+$ and $IgG4^-$ groups. The immunoglobulin data are expressed as median and interquartile range (IQR) unless stated otherwise. A correlation coefficient was obtained using Pearson's equation. $P < 0{\cdot}05$ was considered statistically significant. All statistical analyses were conducted using IBM SPSS statistics (version 22·0; IBM, Armonk, NY, USA).

Results

Characteristics of patients' pleural fluids and pleura

Clinical and demographic information of 35 patients with idiopathic pleural effusion was obtained from the medical records (Tables 1 and 2). Biopsies from the parietal pleura of these patients demonstrated diffuse sclerosing inflammation, but no malignant cells were identified. Fibrosis was pronounced on the side of the pleural cavity, while storiform fibrosis was not seen. Diffuse lymphoplasmacytic infiltration was observed in 16 of 35 patients (Fig. 1a,b). No obliterative phlebitis was observed. IgG4 immunostaining was performed to examine whether $IgG4^+$ plasma cells were present in the pleura of the 35 patients (Fig. 1c–h). $IgG4^+$ plasma cells were variably detected, and the cut-off for IgG4 positivity was set to 10 $IgG4^+$ plasma cell counts per HPF, as proposed for biopsy specimens [23,39]. Of 35 patients, 12 patients showing $>$ 10 $IgG4^+$ plasma cells/HPF were assigned to the $IgG4^+$ group (median = 31; range = 20–70; Tables 1 and 2) and 23 patients with $\leq$ 10 $IgG4^+$ plasma cells/HPF to the $IgG4^-$ group (median = 0·3; range = 0–5). All patients in the $IgG4^+$ group showed $IgG4^+/IgG^+$ cell ratios greater than 40% (Table 2). The patients in the $IgG4^+$ group were older men with a median age of 76 years, and biochemical analysis showed lower median effusion LDH and CRP levels for this group (Table 1).

Table 1. Clinical characteristics of patients with pleural effusions of unknown cause

	$IgG4^-$ group ($n = 23$)	$IgG4^+$ group ($n = 12$)	P
Age (years)	70 (63–78)	76 (73–80)	0·092
Sex (male/female)	22/1	12/0	0·851
Serum			
Total protein (g/dl)	7·1 (6·4–7·3)	7·2 (6·7–7·6)	0·420
Albumin/globulin	1·00 (0·80–1·30)	1·05 (0·85–1·20)	0·932
LDH (IU/l)	186 (164–235)	181 (164–227)	0·797
CRP (mg/dl)	1·48 (0·75–2·14)	0·83 (0·27–1·83)	0·161
Pleural fluid			
Total protein (g/dl)	4·3 (3·9–5·0)	4·5 (3·6–5·2)	1·000
LDH (IU/l)	356 (197–577)	221 (145–340)	0·049
CRP (mg/dl)	0·89 (0·51–1·24)	0·34 (0·17–1·31)	0·085
ADA (U/l)	21·7 (16·4–23·5)	21·5 (15·9–29·5)	0·719
LDH in pleural fluid/serum	1·60 (1·29–3·00)	1·19 (0·77–1·95)	0·079

Data are presented as median (interquartile range). LDH = lactate dehydrogenase; CRP = C-reactive protein; ADA = adenosine deaminase. The normal ranges for total protein, albumin/globulin ratio, LDH and CRP in serum are 6·0–8·3 g/dl, 1·0–2·0, 120–240 IU/l and $<$ 0·3 mg/dl, respectively. The normal range for pleural fluid ADA is $<$ 30 U/l [2].

Immunoglobulin analysis of pleural fluids

Effusion IgG4 levels were significantly higher in the $IgG4^+$ group than in the $IgG4^-$ group (median = 41 *versus* 27 mg/dl, $P < 0{\cdot}01$, Fig. 2d). The proportion of IgG4 to the total IgG was also higher in the $IgG4^+$ group than in the $IgG4^-$ group (median = 3·2 *versus* 1·9%, $P < 0{\cdot}01$, Fig. 2h), which confirms higher IgG4 production in the pleura of the former group. The pleural fluid IgA levels were also elevated in the $IgG4^+$ group (median = 403 *versus* 193 mg/dl, Fig. 2f), in contrast to those of IgG1, IgG2, IgG3, IgM and IgE (Fig. 2a–c,e,g).

Clonality of the IgG4 antibodies of patients in the $IgG4^+$ group

To exclude the possibility of malignant lymphoma and multiple myeloma, the clonality of the effusion IgG4

Table 2. Clinical features of patients in the immunoglobulin (Ig)G4$^+$ group.

					CT scan findings				
n	Age/sex	Effusion IgG4, mg/dl	IgG4$^+$ PC counts/HPF*	IgG4$^+$/IgG$^+$ PC ratio (%)	Respiratory symptom	Pleura	Pulmonary lesion	Mediastinal lymphadenopathy	Extrapulmonary lesion	Follow-up period (years)
1	75M	1133·1	48	85	Dyspnoea on effort	(–)	(–)	(–)	(–)	6
2	81M	20·3	70	93	Abnormality of X-ray	Pleural plaque	(–)	(–)	(–)	2
3	68M	239·6	45	75	Dyspnoea on effort	Pleural plaque and thickening	Ground glass attenuation	(–)	(–)	5
4	76M	44·1	31	48	Abnormality of X-ray	Pleural thickening	Fibrosis	(–)	(–)	3
5	73M	60·1	24	65	Cough	Pleural thickening	Consolidation	(–)	(–)	9
6	73M	37·6	20	75	Abnormality of X-ray	Pleural plaque	Round atelectasis	(–)	(–)	10
7	73M	36·7	22	70	Abnormality of X-ray	(–)	Pneumoconiosis nodule	(–)	(–)	9
8	84M	36·3	26	81	Abnormality of X-ray	Pleural plaque and thickening	(–)	(–)	(–)	3
9	77M	36·5	31	69	Abnormality of X-ray	(–)	Consolidation	(–)	(–)	3
10	78M	36·3	29	54	Abnormality of X-ray	Pleural plaque	(–)	(–)	(–)	5
11	67M	50·7	35	79	Dyspnoea on effort	Pleural plaque	(–)	(–)	(–)	6
12	81M	50·6	57	51	Abnormality of X-ray	(–)	(–)	(–)	(–)	1

*PC = plasma cell; HPF = high-power field (×400); CT = computed tomography.

antibodies of patients in the IgG4$^+$ group was examined by 2-DE (Fig. 3). The control human IgG4, κ myeloma protein, was separated into four γ4 H chain spots with different isoelectric points and one κ L chain spot, but no λ L chain spot (Fig. 3a–c). In contrast, the H and L chains of the IgG4 antibodies of the patients were more heterogeneous in terms of isoelectric point and molecular weight (Fig. 3d–i). In particular, both κ and λ L chains were detected, indicating that their IgG4 antibodies are polyclonal. The numbers of the spots of the κ and λ chains were 10 and 5–6, respectively, which suggests that the κ chain predominates in the effusion IgG4 antibodies of the patients.

Free light chains (FLC) analysis of pleural fluids

As the κ chain was associated predominantly with IgG4 H chain in the patients (Fig. 3), it was presumed that the κ-type was also predominant in the FLCs of the pleural fluids of the IgG4$^+$ group. FLCs are produced in excess of the H chains during immunoglobulin synthesis and secreted into the circulation. The FLC assay was developed originally to support the diagnosis of L chain multiple myeloma, and has been used to assess the excess of one L chain isotype over another by using κ/λ ratio as a surrogate for clonal expansion [43–45]. Interestingly, the κ FLC levels were higher in the patients of the IgG4$^+$ group than in the IgG4$^-$ group (median = 30·1 *versus* 9·1 mg/dl, $P < 0{\cdot}01$) (Fig. 4a), whereas the median λ FLC levels were not significantly different (Fig. 4b). Importantly, the median κ/λ FLC ratio was above the normal range and significantly higher in the IgG4$^+$ group than in the IgG4$^-$ group (2·0 *versus* 1·2, $P < 0{\cdot}001$) (Fig. 4c). In a comparison of patients between the IgG4$^-$ and IgG4$^+$ groups, the receiver operating characteristic (ROC) curve for the κ/λ FLC ratio had a sensitivity of 0·87, a specificity of 0·83 at a cut-off value of 1·42 with an area under the curve (AUC) of 0·88 (Fig. 5). In addition, the κ/λ FLC ratios were found to be correlated with the IgG4$^+$ plasma cell counts/HPF and the IgG4$^+$/IgG$^+$ cell ratios in the pleura of the IgG4$^+$ group (Fig. 6). These results are consistent with the predominance of the κ chain in the 2-DE patterns of the effusion IgG4 antibodies (Fig. 3).

Discussion

The aetiology of exudative pleural effusion sometimes remains unknown, despite thoracoscopy and histological examination of pleural biopsy specimens. In this study, we have shown that 34% of patients with idiopathic pleural effusion are associated with IgG4. To our knowledge, this study is the first to investigate the incidence of IgG4-associated pleural effusion in patients with idiopathic pleural effusion. A pleural marker that might be related to this pleural effusion is also discussed.

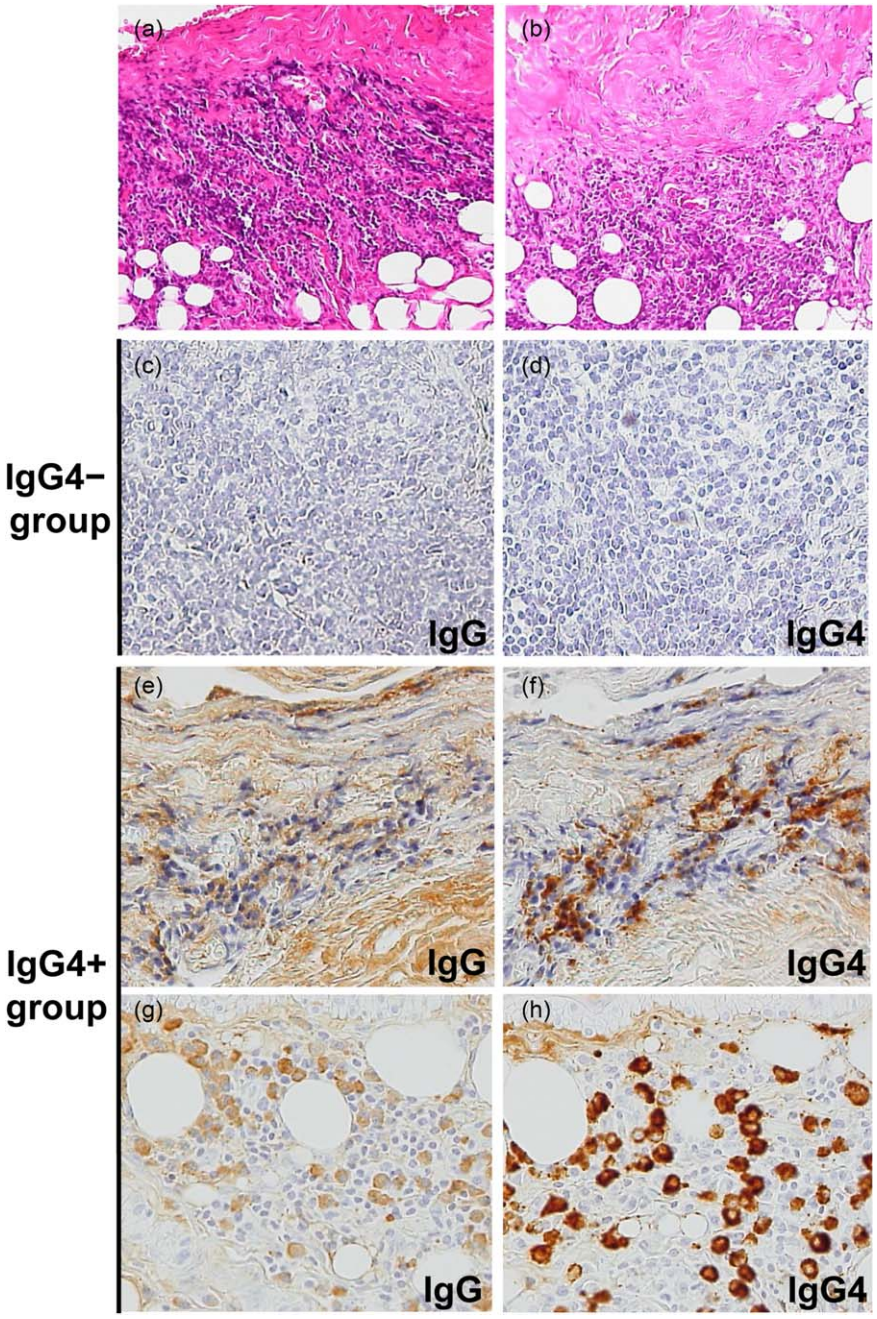

Fig. 1. Histopathological features of the parietal pleura of patients with idiopathic pleural effusion. (a,b) Fibrous thickening of the pleura and prominent lymphoplasmacytic infiltrate in the subpleural fibrous and adipose tissue (haematoxylin and eosin staining, magnification ×100). (c–h) Immunostaining for immunoglobulin (Ig)G or IgG4, magnification ×200. (a,e,f) Case 1, effusion IgG4: 1133·1 mg/dl. (b,g,h). Case 2, effusion IgG4: 20·3 mg/dl (Table 2).

Pleural effusions are manifested in some systemic IgG4-related disease. In previous case reports on IgG4-related disease, pleural effusion occurred as one of the symptoms of the systemic disease involving pancreas, salivary glands, etc. Nodular lesions and bronchovascular involvement are the most common pulmonary manifestations, and various combinations of pulmonary abnormalities are often found in the same patients [23,46,47]. In contrast, the patients in this study did not show multi-organ system involvement other than pleuritis. Considering the elevated effusion IgG4 levels, tissue $IgG4^+$ plasma cell numbers and $IgG4^+/IgG^+$ plasma cell ratios (Table 2), however, the IgG4-associated aetiology is evident for the patients in the $IgG4^+$ group of this study. The patients in the $IgG4^+$ group were elderly men and less inflammatory compared with those in the $IgG4^-$ group (Table 1), which is in agreement with characteristics of IgG4-related disease that affects predominantly older men and progresses slowly with relatively weak inflammation signs [48]. IgG4 antibodies are considered generally to be anti-inflammatory [49,50], but their pathogenic effects have also been reported [51,52]. At present, it is unclear whether the increased production of IgG4 antibody is a causative factor of the pleural effusion or a bystander phenomenon associated with chronic inflammatory reactions.

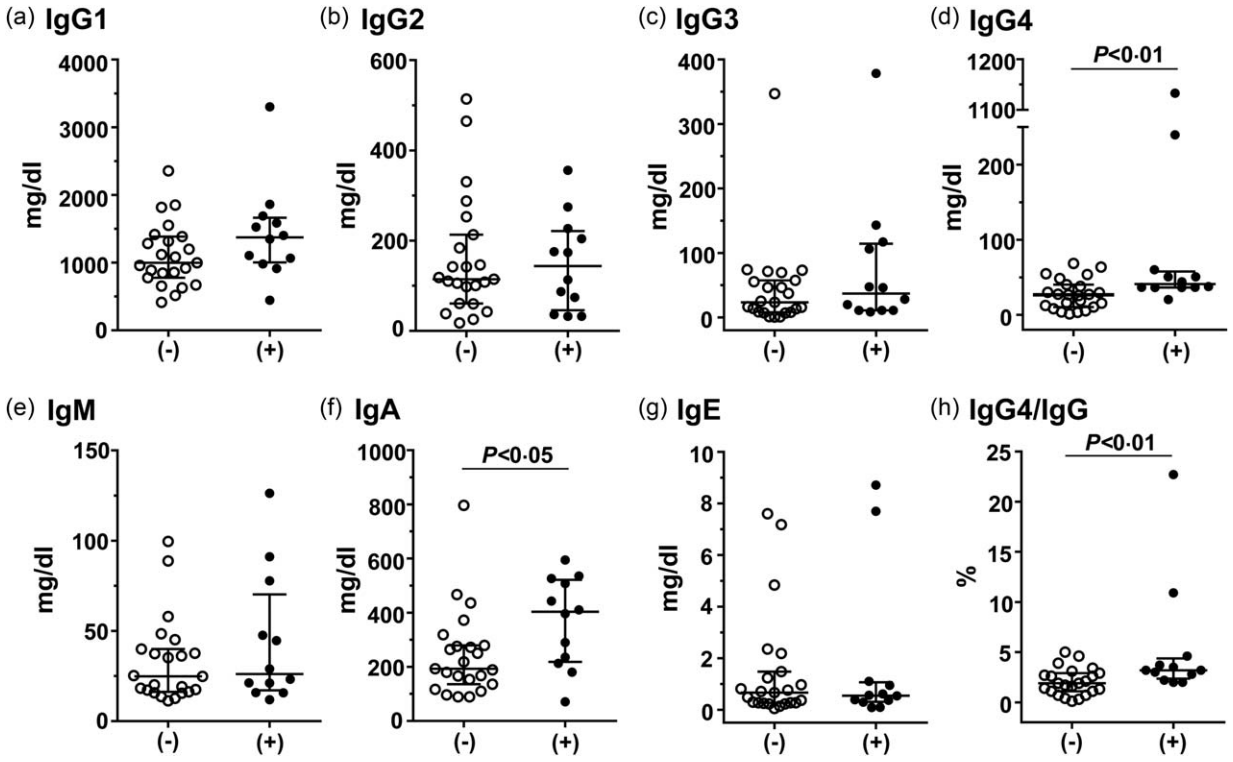

Fig. 2. Comparison of pleural fluid levels of immunoglobulins between the immunoglobulin (Ig)G4$^-$ and IgG4$^+$ groups. (−), IgG4$^-$ group; (+), IgG4$^+$ group. Median and interquartile ranges are shown.

In differential diagnosis, sarcoidosis and multi-centric Castleman's disease were ruled out by clinical and radiological findings, including lack of mediastinal/hilar or extrapulmonary lymphadenopathy in the chest X-ray and CT scan examinations (Table 2). Although lymphoma/myeloma needs to be suspected when the IgG4 levels are markedly high, in this study the IgG4 antibodies were polyclonal and no patient developed malignancy during at least 1-year follow-up (median 5 years, Table 2). Interestingly, the effusion IgG4 levels did not correlate with the extent of pleural infiltration of IgG4$^+$ plasma cells (cases 1 and 2, Fig. 1, Table 2). Although the effusion IgG4 level of case 2

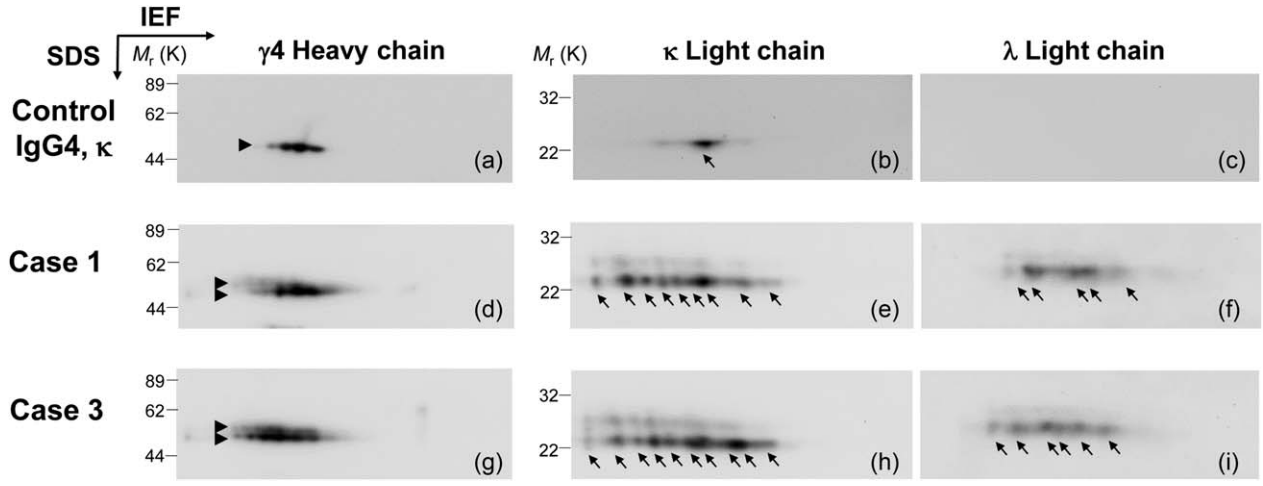

Fig. 3. Analysis of the clonality of the effusion immunoglobulin (Ig)G4 antibodies of patients in the IgG4$^+$ group by two-dimensional electrophoresis (2-DE). Control IgG4 κ myeloma protein from Sigma (cat no. I4639) (a –c). Effusion IgG4 antibodies of representative cases with abnormal IgG4 levels (d–i). The H and L chains were probed with anti-IgG4-Fc (left), anti-κ chain (middle) and anti-λ chain (right) antibodies.

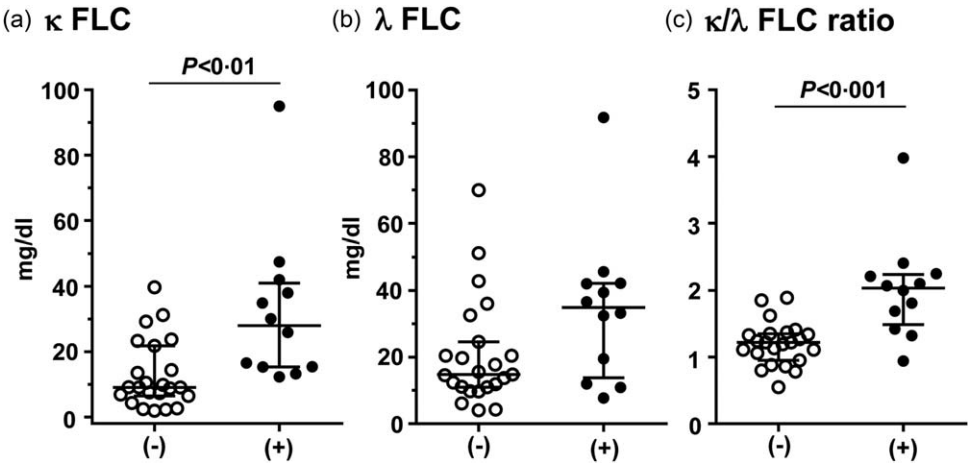

Fig. 4. Comparison of pleural fluid levels of the κ and λ free L chains (FLC) (a,b) and κ/λ ratio (c). Median and interquartile ranges are shown. (−), Immunoglobulin (Ig)G4$^-$ group; (+), IgG4$^+$ group.

in the IgG4$^+$ group was as low as 20·3 mg/dl, IgG4 immunostaining exhibited dense infiltration of IgG4$^+$ plasma cells in the pleura (Fig. 1h). The discrepancy between serum IgG4 concentrations and immunohistochemical findings has also been noted in IgG4-related disease [53]. Therefore, a biomarker other than IgG4 is needed to support the diagnosis of this pleural effusion. The measurements of FLC in the pleural fluid may be considered.

Elevated FLC levels (Fig. 4a,b) are likely to reflect the activation of polyclonal B cells that infiltrate in the pleura. Higher κ FLC levels and κ/λ ratios in the IgG4$^+$ group than in the IgG4$^-$ group may be useful to discriminate the IgG4-associated pleural effusion (Fig. 4). Over-production of serum FLC and high serum κ/λ ratios have been shown recently to correlate with the disease activity of systemic lupus erythematosus [54], rheumatoid arthritis, primary Sjőgren's syndrome [55] and IgG4-related disease [56]. However, the reason for the high κ/λ ratios in these diseases is not known. One possibility to explain the high κ/λ ratios in the IgG4$^+$ group is dominant selection of Vκ genes by possible antigen(s) that elicit pleuritis. It has been reported that the κ/λ ratios of granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibodies in the sera of patients with autoimmune pulmonary alveolar proteinosis are correlated with disease severity [57]. The study suggests the occurrence of selective expansion of λ-type anti-GM-CSF antibody-positive B cell clones in the peripheral lymphatic tissues. However, neither autoantigens nor disease-specific IgG4 autoantibodies have been identified in IgG4-related disease [12]. A second possibility is a preferential association of the κ L chains with the γ4 and α H chains because the levels of IgG4 and IgA were elevated in the pleural fluids (Fig. 2). The κ L chains have been shown to be associated preferentially with IgG4 and IgA H chains from the analyses of subclass distribution in 659 IgG myeloma sera [58] and 176 IgG and 62 IgA myeloma proteins [59]. These studies show the mean κ/λ ratios for the IgG4 myeloma proteins as 3·0 ($n = 24$ [58]) and 2·7 ($n = 11$ [59]) and that for IgA as 2·1 ($n = 42$ [59]). The correlations of the effusion κ/λ FLC ratios with the IgG4$^+$ cell counts and IgG4$^+$/IgG$^+$ cell ratios in the pleura are in agreement with this notion (Fig. 6). It has been reported that FLC can confer mast cell-dependent hypersensitivity in mice and that increased κ FLC monomer and dimer levels and high κ/λ ratios are often found in the cerebrospinal fluid of patients with multiple sclerosis [60,61]. The pleural effusions in the IgG4$^+$ group may be attributable to the accumulation of κ FLCs.

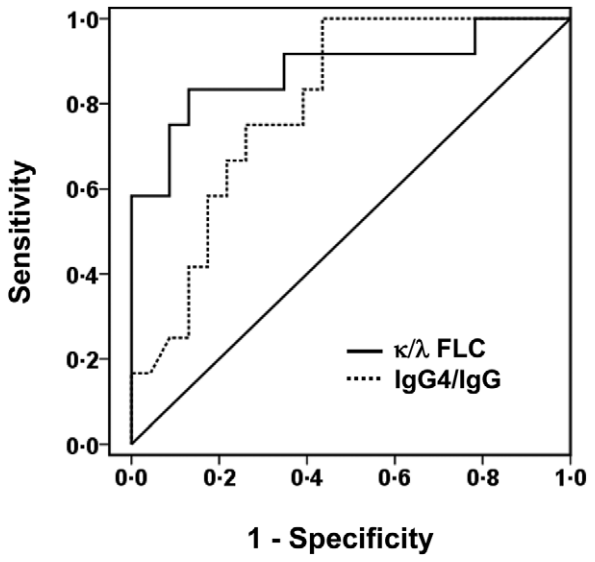

Fig. 5. Receiver operating characteristic (ROC) analysis on diagnostic utility of immunoglobulin (Ig)G4 and κ/λ ratio for distinguishing patients between the IgG4$^-$ and IgG$^+$ groups. Cut-off value for κ/λ ratio, 1·42; sensitivity, 0·87; specificity, 0·83. Area under the curve (AUC), 0·88; 95% confidence interval (CI) for the AUC, 0·74 – 1·00. Cut-off value for IgG4/IgG ratio, 2·75%; sensitivity, 0·75; specificity, 0·74; AUC, 0·80; 95% CI for the AUC, 0·66–0·94.

IgG4-related pleural lesions are reported to be steroid-responsive [26,32,62]. Considering B cell activation as a

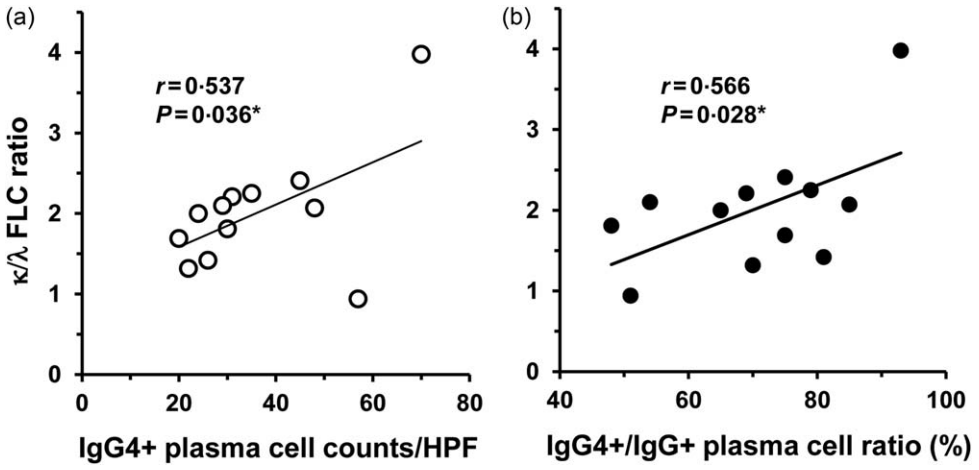

Fig. 6. Correlation of the effusion κ/λ free L chains (FLC) ratio with immunoglobulin (Ig)G4$^+$ plasma cell counts (a) and IgG4$^+$/IgG$^+$ plasma cell ratio (b) in the pleura of patients in the IgG4$^+$ group. *One-tailed *P*-value.

possible mechanism for the pleural effusion in our study, the same therapeutic strategy with immunosuppressive agents may be applicable to our cases. One patient in the IgG4$^+$ group who had suffered recurrent pleural effusions (Case 1, Table 2) received corticosteroids, which ameliorated the pleural effusion. Conversely, pleural effusions resolved spontaneously in a subset of patients, and so watchful waiting may be appropriate in some patients with mild pleural effusion or asymptomatic pleuritis, as described for IgG4-related disease in other organs [63]. However, criteria to justify treatment of IgG4-related pleural lesions need to be established by future prospective studies.

This study has several limitations. This is a retrospective study with a small number of patients. One patient was followed-up for 1 year, although more than 1-year follow-up is recommended for detection of occult pleural malignancy [7,33,36]. It was not possible in all patients to assess the development of an extra-pleural IgG4-related lesion during follow-up. As serum samples were not available, the serum levels of IgG4, κ and λ FLCs could not be analysed. Immunoglobulins including IgG4 were quantitated by a capture sandwich immunoassay, which is different from nephelometry used in the literature, and so the IgG4 concentrations in this study cannot be compared directly with those in the previous reports on IgG4-related disease.

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Disclosure

The authors declare no conflicts of interest.

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