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Clinicopathological features of immunoglobulin G4-related pleural lesions and diagnostic utility of pleural effusion cytology

Satomi Kasashima^{1,2,3}  | Atsuhiro Kawashima^{2,3} | Satoru Ozaki¹ | Toshiyuki Kita⁴ | Tomoyuki Araya⁴ | Yasuhiko Ohta⁵ | Mitsutaka Suzuki⁵

¹Department of Clinical Laboratory Science, Kanazawa University, Kanazawa, Japan

²Department of Pathology, Kanazawa Medical Center, Kanazawa, Japan

³Department of Clinical Laboratory, Kanazawa Medical Center, Kanazawa, Japan

⁴Department of Respiratory medicine, Kanazawa Medical Center, Kanazawa, Japan

⁵Department Thoracic surgery of National Hospital Organization, Kanazawa Medical Center, Kanazawa, Japan

Correspondence

Satomi Kasashima, Department of Clinical Laboratory Science, Kanazawa University, Kanazawa, Japan.

Email: sato-kasa@staff.kanazawa-u.ac.jp

Abstract

Objective: Immunoglobulin (Ig)G4-related disease is a recently described systemic immune-mediated fibro-inflammatory disease that frequently occurs in tumorous form. Herein, we elucidated the clinicopathological and cytological characteristics of IgG4-related pleural lesions (PLs).

Patients and methods: Among 22 patients with fibro-inflammatory PLs of idiopathic aetiology, eight cases were diagnosed as IgG4-PL and the remaining 14 as non-IgG4-PL according to comprehensive diagnostic criteria for IgG4-related disease. Cell block examination of pleural effusion (CBPE) was performed in five patients with IgG4-PL and in six with non-IgG4-PL. Both groups were compared in terms of clinical presentation, laboratory data, histopathological features of resected pleura, and cytological features of pleural effusion (PE).

Results: PE was the most common (six patients, 75%) clinical presentation of IgG4-PL. IgG4-PL comparatively showed significantly more frequent concomitant allergic disease ($P = .021$), higher serum IgE levels ($P = .012$), higher adenosine deaminase levels in pleural fluid ($P = .005$), and rare spontaneous recovery without treatment ($P = .046$). The IgG4-PL group was histologically characterised by thicker fibrous pleura, storiform fibrosis, and infiltration of regulatory T cells, eosinophils and basophils. Using CBPE, IgG4-PL was cytologically distinct with numerous IgG4+ cells and eosinophils. The cytology of CBPE positively correlated with the histology of pleural tissue in the number of IgG4+ cells and eosinophils ($R = .769$ and $.803$, respectively).

Conclusion: IgG4-PL frequently presents with PE and is histologically and cytologically characterised by abundant infiltration of IgG4+ cells and eosinophils. We believe that CBPE with immunohistochemistry/special staining could assist in the auxiliary diagnosis of IgG4-PL.

KEYWORDS

cell block examination, chronic pleuritis, immunoglobulin G4-related disease, immunoglobulin G4-related pleural lesion, pleural effusion

1 | INTRODUCTION

Immunoglobulin (Ig)G4-related disease (IgG4-RD), a chronic, immune-mediated fibrous inflammatory disease, can affect almost all organ systems in a synchronous or metachronous manner.¹ The general clinical peculiarities of IgG4-RD are predominance in older men, high serum IgG4 concentration, tumorous swelling of affected organs and dramatic response to steroid therapy.¹ Histological features of IgG4-RD are described as massive lymphoplasmacytic infiltration with abundant IgG4+ plasma cells, storiform fibrosis and obliterative phlebitis.¹

In the lung, IgG4-RD is characterised by diverse histological features such as interstitial pneumonia, peribronchial/periarterial nodules, and inflammatory pseudotumours.²⁻⁴ Diagnostic criteria for IgG4-RD occurring in the lung were recently established.⁵ In contrast, the understanding of clinicopathological characteristics of IgG4-related pleural lesions (IgG4-PL) in serial case studies is limited.² Therefore, in the present study, we retrospectively investigated clinicopathological features of IgG4-PL in a single-centre case series.

Although histological examination of the affected organ is recommended for the definitive diagnosis of IgG4-RD,¹ obtaining tissue samples from lungs and pleura is rather difficult and sample volume is frequently too small to render an accurate diagnosis.^{3,4} It is noteworthy that compared with pleural tissue resection, pleural effusion (PE) cytology is relatively easy and minimally invasive in addition to being cost-effective.^{6,7} Therefore, we also evaluated the utility of PE cytology in the diagnosis of IgG4-PL.

2 | PATIENTS AND METHODS

2.1 | Ethics committee approval

The present study was approved by the Human Investigation Review Committee of Kanazawa Medical Center (NO. 28-109) and conformed to the principles outlined in the Declaration of Helsinki. The patients gave informed consent prior to participation in the study.

2.2 | Patient selection for IgG4-related pleural lesions

From January 2008 to December 2015, 148 patients underwent thoracoscopy or open surgical biopsy at the Department of Thoracic Surgery, Kanazawa Medical Center. On the basis of clinicopathological findings, we excluded patients with obvious aetiologies including malignant tumours (eg, metastatic cancer, leukemia, lymphoma and mesothelioma), noticeable infections (ie, granulomatous pleuritis due to tuberculosis or mycosis, abscess due to bacterial infection and lymphomatoid granulomatosis due to Epstein-Barr virus infection), autoimmune diseases, specific immune diseases (ie, rheumatoid arthritis, systemic lupus erythematosus and necrotising vasculitis due to granulomatosis with

polyangiitis) and/or cardiovascular diseases (ie, heart failure, arrhythmia, ischaemic heart disease and valve heart disease). The remaining 22 patients with fibrous pleuritis or fibro-inflammatory pleuritis of unknown origin⁷ were classified into a IgG4-PL or non-IgG4-PL group based on the comprehensive pathological diagnostic criteria for IgG4-RD as follows: (a) dense lymphoplasmacytic infiltration and fibrosis; (b) dense infiltration of IgG4+ plasmacytes (>50/high power field [hpf], 10 × eyepiece, and 40 × lens); and (c) IgG4+/IgG+ plasmacytes ratio of >40%,⁸ and in addition high serum IgG4 concentrations (>135 mg/dL). Thus, in total, eight patients with IgG4-PL and 14 with non-IgG4-PL were included in this study.

For all patients, the PE sample was examined using ordinary cytology smears using Giemsa and/or Papanicolaou stains. Regarding the cell block examination of PE (CBPE), the PE sample was placed into a syringe and centrifuged and its supernatant discarded. The sediment at the bottom of the syringe was fixed in neutral 10% formalin and embedded in paraffin. At the Kanazawa Medical Center, CBPE was initiated at the beginning of January 2013. Samples from five patients with IgG4-PL and six with non-IgG4-PL were analysed using CBPE.

2.3 | Data collection

On the basis of electronic medical records of the patients, we retrospectively investigated clinical features including age, sex, location, clinical outcome, complications, medical history, medication, laboratory data such as serum IgG, IgG4, IgE, C-reactive protein levels, white blood cell count, and thoracentesis pleural fluid data including total protein, glucose, lactate dehydrogenase, adenosine deaminase (ADA), hyaluronic acid, pH, specific gravity and cell count.

Histological examination of the resected pleural tissue (HEP) using haematoxylin-eosin and Elastica van Gieson stains was performed. Certain features, including the presence of hyalinising fibrosis, storiform fibrosis, lymphoid follicles, obliterative phlebitis, neutrophil infiltration and mesothelial hyperplasia, were noted. Pleural thickness was estimated via microscopic measurement at the thickest part of the specimen. Immunohistochemical examination of HEP and CBPE was performed using antibodies to detect IgG (DakoCytomation, Glostrup, Denmark; polyclonal, ×1000), IgG4 (Nichirei Bioscience Inc., Tokyo, Japan; clone HP6025, ×2), c-kit (CD117; DakoCytomation; polyclonal, clone A4502, ×800), IgE (DakoCytomation; clone A0094, ×4000) and forkhead box P3 (FOXP3; Abcam, Cambridge, UK; mouse monoclonal, clone ab20034, 236A/E7, ×100). The c-kit, IgE, and FOXP3 antibodies were used to detect mast cells, basophils, and regulatory T cells (Tregs), respectively.⁹ Immunoreactivities of all the antibodies were confirmed with positive and negative controls. For each antibody, the number of immunopositive cells in prominently inflamed areas was counted in five different hpf; the average number of immunopositive cells per hpf was calculated.⁹ Special staining for eosinophils (eosinophil-mast cell kit; ScyTek Laboratories Inc., Logan, UT, USA) was used to

detect eosinophils; the method of cell counting for these cells was identical to that for the immunopositive cells.

2.4 | Statistical analysis

To compare data between IgG4-PL and non-IgG4-PL groups, median analysis was used for continuous values and chi-square test of variance was used to compare positive rates. Spearman's correlation coefficient was used to test for associations between continuous variables. All analyses were performed with the software SPSS version 20 (International Business Machines Corporation, Armonk, NY, USA). A *P*-value of $<.05$ was considered to indicate statistical significance.

3 | RESULTS

3.1 | Clinical features

Among the 148 patients who underwent histological examination of pleural tissue, 22 had idiopathic pleural lesions; eight of these patients (36.3%) fulfilled criteria for IgG4-PL. Regarding age at onset, sex and location, there were no significant differences between patients in the IgG4-PL and non-IgG4-PL groups (Tables 1 and 2). Case 6 was previously reported.¹⁰ Regarding the clinical presentation at first onset in IgG4-PL patients, massive and/or chronic PE was the most frequent (six patients, 75%; Figure 1), whereas pleural thickening or nodules happened more rarely (three patients, 37.5%). Several months later, pleural thickening or nodules occurred in half of the patients in the IgG4-PL group who originally presented with PE. A past medical history of allergic diseases (eg, bronchial asthma, chronic bronchitis and eosinophilic rhinitis) was detected in five patients in the IgG4-PL group, with a significant difference in comparison with the number of patients in the non-IgG4-PL group ($n = 0$; $P = .021$). IgG4-RD in other regions was detected in half of the patients in the IgG4-PL group. Asbestos exposure was seen in two patients in the IgG4-PL group and in one patient in the non-IgG4-PL group.

With regard to treatment, five patients in the IgG4-PL group and all patients in the non-IgG4-PL group did not receive treatment. Subsequently, half of the patients in the non-IgG4-PL group recovered spontaneously after 6-17 months. In contrast, one IgG4-PL patient without treatment suffered persistent PE during a period of 5 years (Case 1). Moreover, the other four patients in the IgG4-PL group without medical care had suffered a progressive increase in the volume of the PE during a period of 8-24 months (Cases 2-5); in two of them, respiratory function gradually deteriorated because of encapsulated PE (Cases 4 and 5). Two patients in the IgG4-PL group received steroids; although one patient recovered from both massive PE and eosinophilic pneumonia after 6 months (Case 7), the other patient showed a gradual increase in PE and developed respiratory failure due to encapsulated PE 10 months later (Case 6). Another IgG4-PL patient received a folk remedy of immunotherapy based on her own request, and PE was found to be in remission approximately 3 years later (Case 8).

3.2 | Laboratory data

Laboratory findings at presentation in the two groups are summarised in Table 3. Serum C-reactive protein and white blood cell counts did not differ between the two groups, whereas the median serum IgE level was significantly higher in the IgG4-PL group than that in the non-IgG4-PL group (764 IU/mL vs 185 IU/mL; $P = .012$).

Regarding the data of thoracentesis for PE, all cases in both groups showed exudative pleural fluid according to Light's criteria.¹¹ In terms of total protein, glucose, lactate dehydrogenase, hyaluronic acid, pH and specific gravity of PE, no significant difference was found between the two groups, but ADA level was significantly higher in the IgG4-PL group than in the other group (32.2 U/L vs 18.8 U/L; $P = .005$). On examination of the cellular constituents of the PE samples, the total cell count and the percentage of eosinophils were significantly higher in the IgG4-PL group than in the non-IgG4-PL group (2297/ μ L vs 336/ μ L; $P = .003$; 22% vs 0.5%; $P = .003$). Remarkably, both groups showed a higher percentage of lymphocytes and a lower percentage of neutrophils in the PE samples.

3.3 | Histopathological findings in resected pleura

Histopathological findings in the two groups are summarised in Table 4 and Figures 2 and 3. The median pleural thickness in the IgG4-PL group (2.5 mm) was significantly greater than that in the non-IgG4-PL group (1.4 mm; $P = .011$). Storiform fibrosis was significantly more frequent in the IgG4-PL group (six patients, 75.6%) than in the non-IgG4-PL group (three patients, 21.4%; $P = .045$). Lymphoplasmacytic infiltration was almost identical between the two groups. In the IgG4-PL group, obstructive phlebitis (two patients, 25%) and lymphoid follicles (three patients, 37.5%) were common features; however, there was no significant difference in these findings between the two groups. In the non-IgG4-PL group, hyalinising fibrosis (five patients, 35.7%) and mesothelial hyperplasia (three patients, 21.4%) were rather frequently detected.

The IgG4-PL group showed significantly higher numbers of basophils (median 23/hpf vs 1.5/hpf; $P = .002$) and eosinophils (median 18.3/hpf vs 1.1/hpf; $P = .001$) than the non-IgG4-PL group. Tregs infiltration was higher in the IgG4-PL group (median 36.1/hpf) than in the non-IgG4-PL group (median 17.2/hpf; $P = .078$). Very few mast cells (range, 0-5/hpf) were found in both groups.

3.4 | Cytological findings in PE

Cytological findings in the two groups are summarised in Table 5 and Figure 4. In ordinary cytology smears of the PE samples, several plasmacytes, lymphocytes and eosinophils were sparsely distributed and mixed with histiocytes and mesothelial cells. In the haematoxylin-eosin- or Giemsa-stained CBPE, plasmacytes and lymphocytes

TABLE 1 Clinicopathological features of the patients with immunoglobulin (Ig)G4-related pleural lesions

Clinical features					Histopathological features									
	Age	Sex	Clinical features at presentation	Additional respiratory features	Complications	Other IgG4-RD	Therapy for pleural lesion	Clinical outcome	Serum IgG4 (mg/dL)	Numbers of IgG4+ cells (hpf)	Pleural thickness (mm)	Storiform fibrosis	Lymphoid follicle	Obliterative phlebitis
Case 1	78	M	Right PE	None	DM, renal failure, asbestos exposure	None	No treatment	5 y later, complete remission of PE, 7 y later, died of pneumonia	180	106	3.4	+		
Case 2	83	M	Bilateral PE, IP, pleural nodule	2 mo later, PT	Membranous nephropathy, AMI	None	No treatment	20 mo later, no change of PE	169	64	2.1	+		
Case 3	56	M	Right PE	12 mo later, PT	Asthma, DM, renal failure	Retroperitoneum	No treatment	8 mo later, slight progression of PE	145	80	2.1	+		+
Case 4	76	F	Bilateral PE	2 mo later, multiple pulmonary nodules	Urinary bladder carcinoma	Lung	No treatment	18 mo later, progression of PE and encapsulated PE	232	154	1.8		+	
Case 5	80	M	Pulmonary hilar tumorous lesion	1 mo later, PE	Chronic bronchitis, COPD, AAA, asbestos exposure	None	No treatment	24 mo later, progression of PE and encapsulated PE	221	85	1.8		+	+
Case 6	65	M	Bilateral PE, pneumonia	2 mo later, PT	Chronic bronchitis, DM	Pancreas, kidney	Low-dose steroid	Gradual progression of PE, 10 mo later, respiratory failure due to collapse	164	50	2.6	+		
Case 7	65	M	Chronic pneumonia	3 mo later, PT	Eosinophilic rhinitis	Lymph node	Low-dose steroid, steroid pulse therapy	6 mo later, remission of PE	328	104	2.3	+		
Case 8	81	F	Left PT	3 mo later, PE, 2 y later, IP	Asthma	None	Immune therapy	34 mo later, remission of PE	234	77	4.7	+	+	

IgG4-RD, IgG4-related disease; M, male; F, female; PE, pleural effusion; PT, pleural thickening; IP, interstitial pneumonia; hpf, high power field; DM, diabetic mellitus; AMI, acute myocardial infarction; COPD, chronic obstructive pulmonary disease; AAA, aortic abdominal aneurysm.

TABLE 2 Comparison of the clinical features between the patients of immunoglobulin (Ig)G4-related and non-IgG4-related pleural lesions

	IgG4-related 8 cases	Non-IgG4-related 14 cases	P-value
Clinical findings			
Age (y)	65 (55-83)	66 (53-85)	.732
Sex (male/female)	6/2	10/4	.856
Location (right/left/bilateral)	2/1/5	4/6/4	.825
Clinical presentation at first consult			
Pleural effusion	6 (75%)	7 (50.0%)	.191
Pleural thickness	1 (12.5%)	3 (14.3%)	.601
Pleural nodule/tumorous lesion	2 (25%)	3 (21.4%)	.736
Pleuritis/pneumonia	2 (25%)	4 (28.6%)	.751
Risk factors associated with IgG4-RD			
Other lesions of IgG4-RD	4 (50%)	0	.019
Allergic disease	5 (62.5%)	0	.021
Smoking habit	5 (62.5%)	6 (42.9%)	.782
Clinical outcome			
Medication (steroid/immune-therapy)	3 (37.5%)	0	.068
No treatment and remission	0/5 (0%)	7/14 (50%)	.046

Age shows median value (range).

were evenly distributed. It was occasionally difficult to determine the exact cell types. However, using immunohistochemistry or special staining in CBPE, it was possible to count each cell type per hpf. Cytologically, the IgG4-PL group had significantly higher numbers of IgG4+ cells (median 52.8/hpf vs 1.1/hpf; $P = .014$) and eosinophils (median 60.5/hpf vs 16.8/hpf; $P = .027$) than the non-IgG4-PL group. In both groups, numbers of basophils and mast cells were similarly few (both, range 0-11/hpf). The number of Tregs was higher in the IgG4-PL group (median 145/hpf); nevertheless, the difference in

values between the IgG4-PL and non-IgG4-PL (median 78/hpf) groups was not significant ($P = .078$).

3.5 | Correlation between number of positive cells in pleural tissue and PE

Evaluation using HEP and CBPE was possible in 11 patients. Among them, there was a significant positive correlation between HEP and CBPE in the number of IgG4-immunopositive cells ($R = .769$, $P = .016$) and the number of eosinophils ($R = .803$, $P = .009$). Serum IgG4 correlated with the number of IgG4-positive cells on both HEP ($R = .769$, $P = .016$) and CBPE ($R = .761$, $P = .037$).

4 | DISCUSSION

In previous reports, pleural involvement was observed in 21% of patients with IgG4-RD affecting the thorax¹² or 30% of patients with IgG4-RD of the lung.^{3,4} A recent serial study reported that in 34% (12 of 35) of patients with idiopathic PE, the condition was associated with IgG4-RD.¹² Likewise, we report that IgG4-RD was detected in 36% of pleural lesions of unknown origin.

Nodular pleural thickening or tumour formation has been often reported as the main clinical presentation of IgG4-PL, whereas PE has been considered a rare feature of IgG4-PL.^{13,14} However, a recent review article reported that PE was one of the most common clinical features in IgG4-PL patients,¹⁵ similar to our results. Indeed, our results showed that PE often appeared initially in IgG4-PL patients and pleural thickening occurred subsequently. In general, diffuse pleuritis is a cause of progression to exudative PE. Therefore, we inferred that IgG4-PL could clinically arise as PE probably caused by lymphoplasmacytic pleuritis at an early stage and then shift partially to nodular or tumorous lesions.

Note that under a nontreatment state, the natural course in the two groups was different; no patient in the IgG4-PL group improved completely, and half of the patients in the non-IgG4-PL group

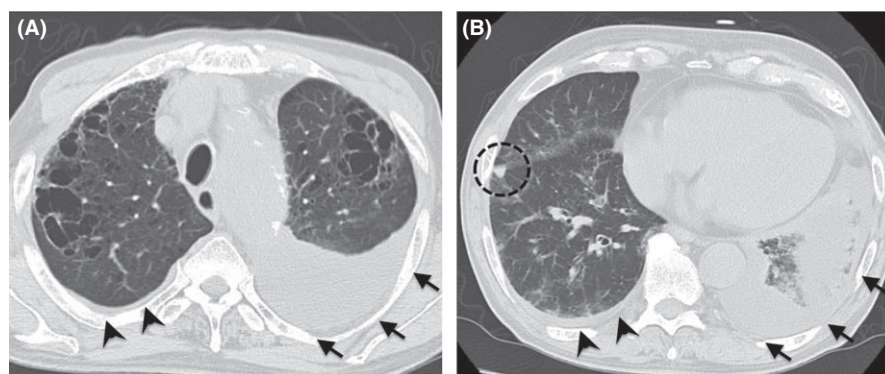


FIGURE 1 Radiological findings of immunoglobulin G4-related pleural lesion (Case 2). A large amount of pleural fluid was observed in the left thoracic cavity (arrows), and a small amount of pleural fluid was observed in the right thoracic cavity (arrowheads). (A) Emphysematous changes were detected in the upper lobe of both lungs. (B) A small nodule was detected in the intralobular pleura of the right lung (dotted circle). Multiple peribronchial and/or periarterial abnormalities with ground glass appearance were distributed in both lungs

TABLE 3 Comparison of the laboratory findings between the patients of immunoglobulin (Ig)G4-related and non-IgG4-related pleural lesions

	IgG4-related 8 cases	Non-IgG4-related 14 cases	P-value
Blood laboratory data			
Serology			
Serum IgG4 (mg/dL)	245 (164-324)	48 (33-64)	.002
Serum IgE (IU/mL)	724 (110-1440)	185 (20-340)	.012
C-reactive protein (mg/dL)	0.87 (0.56-1.12)	1.12 (0.75-2.22)	.234
Biochemistry			
Total protein (g/dL)	7.0 (5.3-8.1)	6.4 (5.3-7.9)	.0547
Lactate dehydrogenase (U/L)	186 (161-400)	183 (119-250)	.548
Blood cell counts			
White blood cells (/ μ L)	8700 (4500-12 000)	8900 (3800-13 500)	.855
Neutrophils (%)	67.1 (64.1-69.1)	67.8 (56.4-84.0)	.541
Lymphocytes (%)	17.2 (11.0-25.0)	16.7 (8.0-28.5)	.978
Monocytes (%)	6.5 (5.1-9.0)	8.2 (2.0-12.1)	.266
Eosinophils (%)	7.2 (1.5-13.9)	2.5 (0.6-7.4)	.002
Histiocytes (%)	0.4 (0.0-0.6)	0.4 (0.0-1.3)	.737
Thoracentesis fluid data			
Biochemistry			
Total protein (g/dL)	4.9 (4.6-5.7)	4.6 (2.8-6.3)	.332
Glucose (mg/dL)	121 (98-154)	94 (79-128)	.072
Lactate dehydrogenase (U/L)	211 (106-1396)	300 (113-1146)	.688
Adenosine deaminase (U/L)	32.2 (26.6-50.1)	18.8 (13.2-41.2)	.005
Hyaluronic acid	120 000 (823-69 500)	16 500 (6210-73 700)	.496
pH	7.48 (7.38-7.93)	7.59 (7.31-7.90)	.894
Specific gravity	1.03 (1.03-1.04)	1.03 (1.01-1.05)	.094
Pleural fluid cell counts			
Cell counts (/ μ L)	2297 (1213-5312)	336 (31-2147)	.003
Neutrophils (%)	3 (0-18)	4 (0-74)	.821
Lymphocytes (%)	66 (2-87)	88 (0-98)	.132
Eosinophils (%)	22 (10-86)	0.5 (0-24)	.003
Histiocytes (%)	3 (1-9)	1.5 (0-8)	.711

All data show median value (range).

recovered spontaneously. Several reported cases and one patient (Case 7) of the IgG4-PL group with massive PE responded well to steroids.^{12,16-19} However, despite steroid administration to one patient in the IgG4-PL group (Case 6), PE and respiratory failure deteriorated. We thought that the lack of treatment response in this patient could be caused by the inappropriate amount and/or length of the steroid management.¹ In addition, this case histologically showed thicker and more fibrous pleura, which may have affected the efficacy of steroid treatment; in the advanced stage of IgG4-RD, most patients become refractory to steroids due to irreversible organ disorders or dysfunction caused by maximally dense fibrosis.¹⁹ Therefore, we feel that it is important to consider IgG4-PL in differential diagnosis of idiopathic PL at the early stage, in order to provide suitable therapy.

The level of ADA, a biomedical marker, in pleural fluid is most frequently used in the subsidiary diagnosis of tuberculosis pleuritis. Nevertheless, a few IgG4-PL cases with a high ADA levels have recently been reported.^{16,19} Likewise, the IgG4-PL group showed significantly increased ADA levels in pleural fluid compared with the non-IgG4-PL group; however, the underlying mechanism associated with elevated ADA level in IgG4-PL is still unknown. ADA is predominantly produced by monocytes or macrophages via the activation of cell-mediated immunity, eg, initially triggered by tubercle bacilli infection. Nagayasu et al. augured that T cells in association with B cells may induce the activation of cell-mediated immunity in IgG4-PL.¹⁹ The predominant increase of lymphocyte and eosinophil levels by cell count in the pleural fluid was also a characteristic finding in IgG4-PL, which reflects the histological features of massive

TABLE 4 Comparison of the histological features of resected pleura between the patients of immunoglobulin (Ig)G4-related and non-IgG4-related pleural lesions

	IgG4-related 8 cases	Non-IgG4- related 14 cases	P- value
Histological findings			
Fibrous thickness (mm)	2.5 (1.8-4.7)	1.4 (0.6-3.2)	.011
Lymphoplasmacytic infiltration	7 (87.5%)	8 (57.1%)	.319
Storiform fibrosis	6 (75.6%)	3 (21.4%)	.045
Hyalinisation	1 (12.5%)	5 (35.7%)	.497
Lymphoid follicle	3 (37.5%)	4 (28.6%)	.965
Obstructive phlebitis	2 (25%)	1 (7.1%)	.597
Neutrophil infiltration	1 (12.5%)	1 (7.1%)	.721
Mesothelial hyperplasia	1 (12.5%)	3 (21.4%)	.909
Immunohistochemistry			
IgG+ cells (/hpf)	90.5 (78-188)	75.5 (2-201)	.375
IgG4 + cells (/hpf)	88 (50-154)	20 (0-32)	.001
IgG4/IgG (%)	63.3 (50-90)	26.5 (0-35)	.001
Regulatory T cells (/hpf)	36 (20-58)	17 (1-36)	.078
Basophils (/hpf)	23 (7-40)	1.5 (0-2)	.002
Mast cells (/hpf)	3 (0-4)	1.3 (0-5)	.333
Special staining			
Eosinophils (/hpf)	18.3 (7-23)	1.1 (0-2)	.001

Fibrous thickness, immunohistochemical and special staining data show median value (range); hpf, high power field.

infiltration of lymphoplasmacytes with eosinophils in the pleural tissue of IgG4-PL.

The pathogenesis of IgG4-RD remains poorly understood; however, multiple immune-mediated mechanisms, which can be triggered by autoimmunity and infectious agents, might contribute to the fibro-inflammatory process. Type 2 helper T cells and Tregs overexpress cytokines including interleukins (IL-4, IL-5, IL-10, and IL-13) and transforming growth factor β , promoting eosinophilia and leading to increased serum IgG4 and IgE, and progression of fibrosis.^{1,9} We demonstrated that approximately half of the IgG4-PL patients had an underlying allergic disease. Interestingly, two patients with IgG4-PL had a history of asbestos exposure. Similarly, two recent reports have indicated asbestos-induced immune dysregulation as one possible aetiology of IgG4-RD; one case showed rounded atelectasis,²¹ and the other formed pleural plaque.²² The relationship between asbestos exposure and IgG4-PL is still hypothetical. Further studies with a higher number of IgG4-PL cases are warranted.

As in the condition of IgG4-RD occurring in other organs,¹ IgG4+ cell infiltration and storiform fibrosis were the most characteristic histological findings in IgG4-PL. Likewise, Treg, eosinophil and basophil infiltrations were histological characteristics of IgG4-PL. Accordingly, we propose that special staining of eosinophils as well as immunohistochemistry might be a valuable additional method for IgG4-PL screening. Pathological pleural fibrous thickening was also a frequent feature of IgG4-PL, but it might not be sufficient to be radiologically detected. The presence of lymphoid follicles was not specific to IgG4-PL, probably because lymphoid follicles represent reactive changes secondary to chronic PE in both groups.

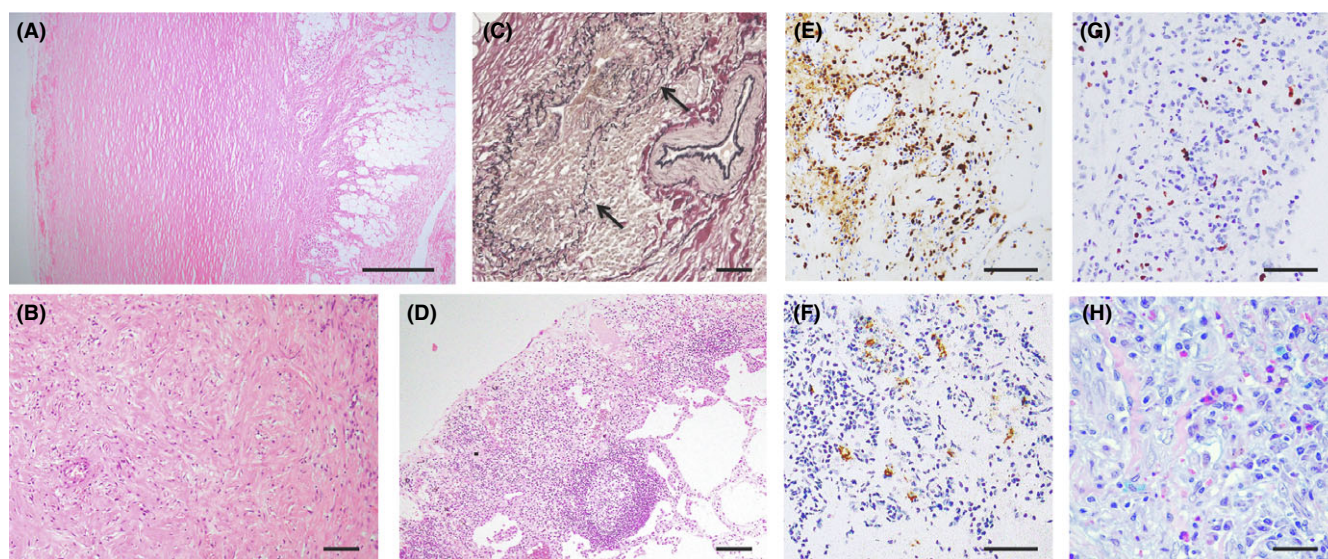


FIGURE 2 Histopathological findings of the resected pleura of immunoglobulin (Ig)G4-related pleural lesion (Case 2). Fibrous thickening of pleura (2.1 mm; A; original magnification $\times 10$; scale bar: 500 μ m), storiform fibrosis (B; original magnification $\times 40$; scale bar: 200 μ m), obstructive phlebitis (arrows; C; Elastic van Gieson stain, original magnification $\times 40$; scale bar: 200 μ m), and lymphoplasmacytic infiltration with lymphoid follicles (D; original magnification $\times 20$; scale bar: 100 μ m) were characteristically observed. There were numerous immunopositive cells for IgG4 (E), IgE (F), and FOXP3 (G; original magnification $\times 80$; scale bar: 25 μ m). Using eosinophil staining, eosinophils were stained with pink cytoplasm and mast cells were stained with pale blue cytoplasm. Many eosinophils were detected, and a small number of mast cells were sparsely distributed (H; original magnification $\times 100$; scale bar: 50 μ m)

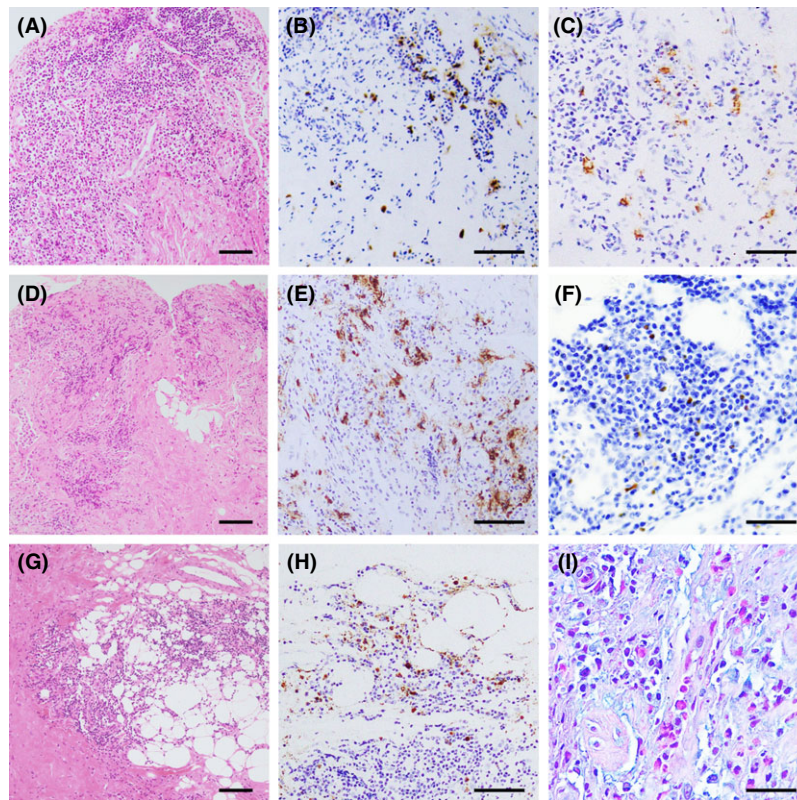


FIGURE 3 Histopathological findings of the resected pleura of immunoglobulin (Ig)G4-related pleural lesion (A–C, Case 6; D–F, Case 5; and G–I, Case 8). Lymphoplasmacytic infiltration with fibrosis (A, D, and G; original magnification $\times 40$; scale bar: 200 μm) and numerous infiltration of IgG4+ cells (B, E, and H; original magnification $\times 80$; scale bar: 25 μm) were characteristically detected. There were numerous immunopositive cells for IgE (C) and FOXP3 (F; original magnification $\times 100$; scale bar: 50 μm). Using special staining, many eosinophils were detected (I; original magnification $\times 100$; scale bar: 50 μm)

Obstructive phlebitis was observed at a lower frequency in IgG4-PL than in IgG4-RD of other organs,¹ possibly due to the rather sparse distribution of veins in the pleura.

Several studies have indicated that increased serum IgG4 alone was insufficient for a diagnosis of IgG4-PL.^{22–24} Even though histological examination is important for the diagnosis of IgG4-PL, safe and adequate pleural tissue sampling has not always been feasible.²⁴

TABLE 5 Comparison of pleural effusion cytological findings between the patients of immunoglobulin (Ig)G4-related and non-IgG4-related pleural lesions

	IgG4-related 5 cases	Non-IgG4-related 6 cases	P-value
IgG+ cells (/hpf)	85.5 (30–155)	16 (7–20)	.127
IgG4 + cells (/hpf)	52.8 (17–67)	1.1 (0–5)	.014
IgG4/IgG (%)	51.6 (43–62)	18.1 (16–20)	.014
Regulatory T cells (/hpf)	145 (126–167)	78 (34–148)	.180
Basophils (/hpf)	3 (0–11)	3.8 (2–6)	.801
Mast cells (/hpf)	3.5 (1–11)	2.4 (1–3)	.323
Eosinophils (/hpf)	60.5 (11–80)	16.8 (2–32)	.027

Immunohistochemical and special staining data show median value (range); hpf, high power field.

Although cytopathology has attracted attention as an alternative method to biopsy, several investigations described that it was difficult to diagnose IgG4-RD using fine needle aspiration cytology of tumorous lesions in IgG4-RD because of a wide variety of nonspecific cytological features.^{26,27} By contrast, CBPE enables immunostaining and special staining of pleural fluid cells and measurement of the number of each cell type per hpf.⁶ Therefore, even considering the fact that tissue structure and fibrosis cannot be evaluated in CBPE, we identified the utility of CBPE in supporting the diagnosis of IgG4-PL; in particular, the presence of numerous IgG4+ cells in CBPE correlated with the presence of IgG4+ cells in HEP and with serum IgG4 level. A recent case report of IgG4-PL similarly described the presence of IgG4 + cells and 20%–60% of IgG4+/IgG+ cell ratio using CBPE.¹⁹

Recently, several cases with multiple serosal (pericardium and peritoneum) involvement in IgG4-RD have been reported.^{15,28–30} It is noteworthy that approximately half of the patients with pericardial involvement in IgG4-RD presented with pericardial effusion.¹⁵ Considering the utility of CBPE in the diagnosis of IgG4-PL, the serosal fluid cell block has the potential to be a less invasive and more convenient tool to diagnose serosal involvement in IgG4-RD.³⁰

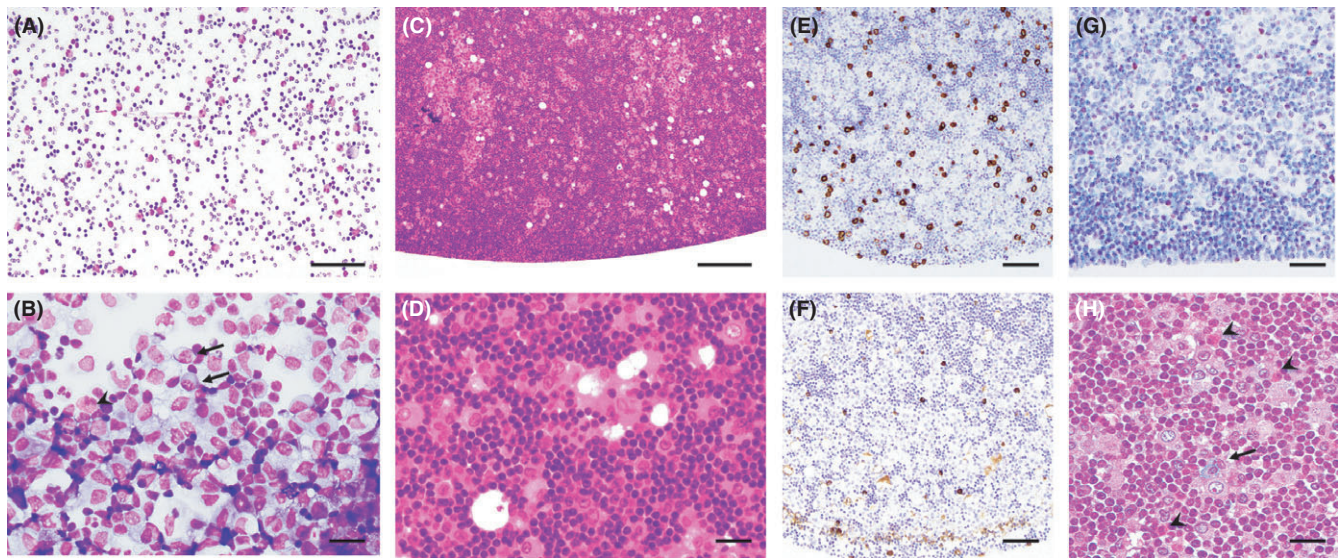


FIGURE 4 Cytological findings in the pleural effusion of immunoglobulin (Ig)G4-related pleural lesion (Case 2). In ordinary cytology smears with Giemsa staining, a few plasmacytes (arrows), lymphocytes, and eosinophils (arrowheads) were sparsely distributed, with a mixture of histiocytes and mesothelial cells (A, B). In a cell block using haematoxylin-eosin staining, plasmacytes and lymphocytes were evenly distributed (C, D; A, C; original magnification $\times 10$; scale bar: 500 μm , B, D; original magnification $\times 200$; scale bar: 100 μm). Numerous cells immunopositive for IgG4 (E) and FOXP3 (G), and a few cells immunopositive for IgE (F) were detected in the pleural fluid (E, F; original magnification $\times 40$; scale bar: 50 μm , G original magnification $\times 80$; scale bar: 100 μm). There were several eosinophils (arrowheads) and a few mast cells (arrow; H; original magnification $\times 200$; scale bar: 30 μm)

Our study had several limitations. It was a retrospective study focused on a small number of patients, particularly those who underwent CBPE. We could not examine the IgG4 level in pleural fluid, which might be helpful in the diagnosis of IgG4-PL. The relation between serum and pleural fluid levels of IgG4 would be worth investigating. Further multicentre studies examining more patients with IgG4-PL are likely to provide more definitive data in the future.

5 | CONCLUSION

In this study, we found IgG4-PL to be more frequent than previously thought and that it commonly presents with PE. It is necessary to consider IgG4-PL as one of the causes of idiopathic PE. We believe that IgG4-PL should be included as one of the causes of a high ADA level in pleural fluid. Although histological examination of pleural tissue is necessary to accurately diagnose IgG4-PL, CBPE with immunohistochemistry/special staining would assist in diagnosing IgG4-PL.

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ORCID

Satomi Kasashima  <http://orcid.org/0000-0002-9158-9898>

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