BMJ open

Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004407
Article Type:	Research
Date Submitted by the Author:	05-Nov-2013
Complete List of Authors:	Takada, Toshinori; Niigata University, Division of Respiratory Medicine Tanaka, Junichi; Niigata University, Division of Respiratory Medicine Moriyama, Hiroshi; Niigata University, Division of Respiratory Medicine Terada, Masaki; Niigata University, Division of Respiratory Medicine Suzuki, Eiichi Narita, Ichiei; Niigata University, Division of Respiratory Medicine Kawabata, Yoshinori Yamaguchi, Tetsuo Hebisawa, Akira Sakai, Fumikazu Arakawa, Hiroaki
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Occupational and environmental medicine
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, Tropical medicine < INTERNAL MEDICINE, Interstitial lung disease < THORACIC MEDICINE

Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

[1]Junichi Tanaka, MD, [1]Toshinori Takada, [1]Hiroshi Moriyama, MD, [1]Masaki Terada, MD, MD, [2]Eiichi Suzuki, MD, [1]Ichiei Narita, MD, [3]Yoshinori Kawabata, MD, [3]Tetsuo Yamaguchi, MD, [3]Akira Hebisawa, MD, [3]Fumikazu Sakai, MD, and [3]Hiroaki Arakawa, MD,

[1]Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan, [3]Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Tokyo, Japan

Corresponding author: Toshinori Takada, M.D., PhD

Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University

1-757 Asahimachi-dori, Chuo-ku, Niigata, 951-8510, Japan

Tel; +81-25-227-2200, Fax; +81-25-227-0775, Email; ttakada@med.niigata-u.ac.jp

Keywords: hard metal, pulmonary fibrosis, electron probe microanalysis

Word count: 2,620

ABSTRACT

Background: Hard metal lung disease has a variety of pathological patterns including giant cell interstitial pneumonia (GIP) and usual interstitial pneumonia (UIP). Although UIP pattern is considered the prominent feature in advanced disease, it is unknown whether GIP finally progresses to UIP pattern or not.

Objective: To clarify clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Methods: We obtained the clinical records, chest CT, and lung tissue from nineteen cases diagnosed as hard metal lung disease. Lung tissue was elementally analyzed by electron probe microanalyser. We classified the patients into two groups according to the pathological findings and statistically compared clinical data.

Results: Fourteen cases were pathologically diagnosed as GIP or centrilobular inflammation/fibrosing. The other five cases were UIP pattern or upper lobe fibrosis. Elemental analyses of lung specimens of GIP showed tungsten throughout the centrilobular fibrotic areas. In UIP pattern, tungsten was detected in periarteriolar area and subpleural fibrosis in no association with centrilobular fibrosis or inflammatory cell infiltration. The GIP group was younger ($p<0.01$), had shorter exposure duration ($p<0.01$), lower serum KL-6 ($p<0.05$), and higher lymphocyte percentage in bronchoalveolar lavage fluid ($p<0.05$) than the fibrosis group.

Conclusions UIP pattern or upper lobe fibrosis is remarkably different from GIP in distribution of hard metal elements, associated interstitial inflammation and fibrosis, and clinical features. In hard metal lung disease, UIP pattern or upper lobe fibrosis

may not be an advanced form of GIP.

Strengths and limitations of this study

1, Nineteen cases of hard metal lung disease, a rare occupational lung disease, were collected and their clinical features were documented.

2, Lung tissue from all the patients was elementally analyzed by a patented technique, an improved element analysis using electron probe microanalyzers with wavelength dispersive spectrometer.

3, Since the relative frequencies of incidence of hard metal lung disease and IPF, the probability that someone with hard metal exposure will develop idiopathic UIP/IPF cannot be inferred.

INTRODUCTION

Hard metal is a synthetic compound that combines tungsten carbide with cobalt. Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, and interstitial lung disease which is recognized as hard metal lung disease.[1-3] In many cases with hard metal lung disease, multinucleated giant cells with centrilobular fibrosis is prominent resulting in a pattern of giant cell interstitial pneumonia (GIP).[4-6] We demonstrated that hard metal accumulated in the centrilobular area may trigger the inflammation in cooperation with $CD163^+$ monocyte-macrophages and $CD8^+$ lymphocytes using electron probe microanalyzers with wavelength dispersive spectrometer (EPMA-WDS).[7] In addition to classical GIP, hard metal lung disease has a variety of pathological patterns, desquamative interstitial pneumonia, obliterative bronchiolitis, and usual interstitial pneumonia (UIP) pattern.[4, 8] The lesions of classical GIP are usually centered on the centrilobular areas. On the other hand, the key histologic features of the UIP are predominantly distributed at the periphery of the acinus or lobule.[9, 10] Hard metal lung disease has pathological patterns of both GIP and UIP, and the UIP pattern is thought to be the prominent feature in advanced cases of the disease. The key question is whether UIP pattern is an advanced form of GIP or not. In order to elucidate relationship between GIP and lung fibrosis with detection of hard metal elements, we collected cases with tungsten in lung tissue and reviewed their clinical records. We then elementally reexamined lung specimens by EPMA-WDS. We finally classified the patients into two groups according to the histological findings and statistically

compared their clinical features. Pathological and elemental analyses in the study suggest that UIP pattern or upper lobe fibrosis may be different from an end-stage form of GIP.

METHODS

Patient population

We performed a nationwide survey by announcing inquiry for cases of hard metal lung disease to the major medical institutes and hospitals all over the country for the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases. Nineteen patients were finally diagnosed as hard metal lung disease because of presence of tungsten in lung specimens detected by EPMA-WDS. We obtained information of patient profile such as age, gender, duration of hard metal exposure, history of pneumothorax, history of allergy, symptoms, physical findings, serum levels of Krebs von den Lungen-6 (KL-6) and SP-D, arterial blood gas data, pulmonary function tests, bronchoalveolar lavage (BAL) cell profiles and treatment and prognosis in order to make a data base. We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

HRCT scan findings

All patients with hard metal lung disease except one underwent high-resolution computed tomography (HRCT) scanning. Two radiologists (observers) who were blinded to clinical, laboratory, or pulmonary function test results evaluated CT scan findings. The observers judged each CT scan for the presence or absence of three main features of centrilobular nodules, ground glass opacity, and pneumothorax. They also noted other remarkable findings; traction bronchiectasis, reticular pattern, subpleural linear opacity, consolidation, bulla, centrilobular emphysema, atelectasis, and bronchial wall thickening and entered these results into a data sheet independently. After evaluation, disagreement on the results between the observers for some HRCT scans was resolved by discussion and consensus.

Sample preparation and pathological study

Each tissue sample was serially cut into 3 μm-thickness sections and subjected to pathological study and EPMA-WDS analysis. For pathological study, formalin-fixed 3 μm serial sections were stained with hematoxylin-eosine and Elastica van Gieson method. Two pathologists (observers), who were blinded to clinical, laboratory, or pulmonary function test results, evaluated pathological findings. After evaluation, disagreement on the pathological diagnoses between the observers for some specimens was resolved by discussion and consensus.

Electron probe microanalysis

Examination of tissue sections with EMPA-WDS was performed according to

procedures previously described.[11] X-ray data were obtained with an EPMA-WDS (EPMA 8705, EPMA-1610, Shimadzu Ltd, Kyoto, Japan). For qualitative element analysis, three areas of 5 x 5 μm to 10 x 10 μm in the centrilobular legion of GIP or fibrosing lesion of interstitial lung diseases were screened. The distribution of amino nitrogen corresponding to the pathological image was also mapped for each sample.

Statistical analysis

Comparisons of categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by Mann-Whitney's U-test. A p Value <0.05 was considered significant.

RESULTS

Characteristics of subject

Clinical features are summarized in Table 1 and 2. Demographic findings in 8 of these patients have been reported previously.[7] All the subjects had an occupational history of hard metal industry for 1 to 36 years. One patient (case 15) was doing deskwork in an insufficiently ventilated room of a hard metal grinding company. Five patients had occupational history of hard metal industry but were not exposed at the diagnosis of hard metal lung disease. Five patients (case 2, 5, 7, 8, and 15) had an allergic history and were patch tested for Co, Ni, Cr, Hg, Au, Zn, Mn, Ag, Pd, Pt, Sn, Cu, Fe, Al, In, Ir, Ti. 4 of 5 patients (case 2, 5, 7, and 15) were found to be positive for cobalt. Pulmonary function tests revealed restrictive lung defect characterized by reduced vital

capacity and lung diffusing capacity. BAL findings showed increased total cell counts, increased lymphocytes and eosinophils, with normal CD4/CD8 ratio. Bizarre multinucleated giant cells were not noted in BAL.

Table 1. Demographic features of subjects

Patient	Age	Sex	Smoking history	Occupational history (hard metal exposure)	Exposure duration (months)	Exposure at diagnosis
1	39	M	non	Hard metal shaping/drilling	12	No
2	53	M	ex	Hard metal shaping/drilling	30	No
3	21	M	non	Metal grinding	32	Yes
4	42	M	ex	Hard metal shaping/drilling	36	Yes
5	48	M	non	Metal grinding	48	NA
6	45	M	non	Hard metal shaping/drilling	60	Yes
7	32	F	non	Metal grinding	60	Yes
8	32	F	non	Metal grinding	72	No
9	44	F	non	Hard metal shaping/drilling	72	Yes
10	62	M	non	Metal grinding	72	No
11	40	F	non	Hard metal shaping/drilling	96	NA
12	48	M	non	Metal grinding	120	NA
13	49	F	non	Hard metal shaping/drilling	120	Yes
14	65	F	non	Metal grinding	144	No
15	50	F	non	Desk worker in hard metal factory	168	Yes
16	53	M	non	Quality control of hard metals	264	NA
17	60	M	ex	Hard metal shaping/drilling	276	Yes
18	53	M	non	Hard metal shaping/drilling	372	Yes
19	65	M	non	Hard metal shaping/drilling	444	Yes

Abbreviation; NA, not available

Table 2. Clinical characteristics of Patients with Hard metal lung disease

		Value
Mean age at diagnosis (yrs)		46.4 ± 14.1 (21 - 65)
Gender M/F		12/7
Smoking history	Cur/Ex/Never	0/3/16
Chief complaints	dry cough	13/19
	breath shortness	8/19
Pneumothorax	Yes	8/19
Allergic history	Yes	5/19
Patch test to cobalt	positive	4/5
Mean exposure duration (yrs)		10.7 ± 10.3 (1 - 36)
Physical findings	rales on auscultation	11/19
	fine crackles	8/19
	finger clubbing	4/18
	edema of leg	1/16
Laboratory tests	KL-6	502.7 ± 267.5 U/ml
	SP-D	216.1 ± 192.4 ng/ml
Pulmonary function tests		
	%VC	64.8 ± 25.3 %
	FEV_1%	85.6 ± 10.7 %
	%DLco	53.4 ± 17.0 %
Bronchoalveolar lavage		
	Total cell count	3.13 ± 2.11 $\times 10^5$ /ml
	Lymphocytes	24.3 ± 22.3 %
	Neutrophils	3.07 ± 2.86 %
	Eosinophils	3.01 ± 5.03 %
	CD4/8 ratio	1.65 ± 2.96

The mean numbers ± standard deviations and ranges in parentheses are shown.

Radiological findings

HRCT of all patients except one with hard metal lung disease were available for review

of radiological findings. Conventional CT findings of case 12 were added to the table (Table 3). Centrilobular nodules (Fig 1 A, B) and ground glass opacity were identified in chest CT of 16 patients. In some patients, reticular opacities, traction bronchiectasis, and subpleural curvilnear opacities were also present (Fig 1 C, D). Although centrilobular micronodular opacities were noted in those patients, they were unremarkable.

Table 3. Radiologic findings of patients with hard metal lung disease

	CT features			
Patient	centrilobular nodules	ground-glass opacities	pneumothorax	other findings
1	+	-	-	bronchial wall thickening
2	+	+	-	reticular opacities
3	+	+	+	
4	+	-	+	subpleural curvilnear opacities
5	+	+	-	
6	-	+	-	reticular opacities, consolidation
7	+	+	+	
8	+	+	-	traction bronchiectasis
9	+	+	-	
10	+	+	-	reticular opacities, traction bronchiectasis
11	+	-	+	
12	+	+	+	subpleural curvilnear opacities
13	+	+	-	
14	+	+	-	traction bronchiectasis, apical cap
15	+	+	+	traction bronchiectasis
16	-	+	+	subpleural/peribronchovascular consolidation atelectasis, bulla
17	+	+	-	bulla, centrilobular emphysema
18	-	+	-	reticular opacities
19	+	+	-	reticular opacities

Pathological findings and elemental analysis

Pathological findings and detected elements in lung tissue of 19 cases were summarized in Table 4. Four major histological features noted in this study were as follows: GIP characterized with centrilobular fibrosis (Fig 2 A, B) and characteristic giant cells showing cannibalism (Fig 2 C), centrilobular inflammation/fibrosis similar to GIP but without giant cells, UIP pattern characterized with patchy distribution and temporal heterogeneity, and dense fibrosis with fibroblastic foci (Fig 3 A, B, D, E, F) [12], upper lobe fibrosis characterized with apical scar/cap type fibrosis mainly in the upper lobe.[13]

Elemental analyses of lung specimens of GIP and centrilobular inflammation/fibrosis demonstrated that tungsten was mapped almost throughout the centrilobular fibrotic areas (Fig 2 D, E). Analyses of lung specimens of UIP pattern by EPMA-WDS revealed that tungsten and tantalum were distributed in periarteriolar area (Fig 4, D, E) and in subpleural fibrosis with dense acellular collagen (Fig 4 G, H, J, K). However, these elements were not accompanied by centrilobular inflammation/fibrosis (Fig 4, A, B). Lung histopathology in one case showed apical cap-like fibrosis with tungsten deposits detected in the fibrotic region but without GIP.[14] In total, elemental analysis by EPMA-WDS detected tungsten but no cobalt or tantalum in 10 patients, tungsten and cobalt in 5 patients, and tungsten and tantalum in 4 patients (Table 4).

Table 4. Pathological findings and elemental analysis of patients with hard metal lung disease

			elements detected		
Patient	sampling method	pathological findings	W	Co	Ta
1	VATS	centrilobular inflammation/fibrosis	+	-	-
2	VATS	GIP	+	-	-
3	TBB, VATS	GIP	+	-	-
4	VATS	centrilobular inflammation/fibrosis	+	-	-
5	VATS	GIP	+	-	-
6	Autopsy	GIP, DAD	+	-	-
7	VATS	centrilobular inflammation/fibrosis	+	+	-
8	VATS	GIP	+	-	+
9	VATS	GIP	+	+	-
10	VATS	UIP	+	-	+
11	VATS	GIP	+	+	-
12	Autopsy	GIP, DAD	+	-	-
13	VATS	GIP	+	-	-
14	VATS	GIP, UIP/NSIP?	+	-	+
15	VATS	GIP	+	+	-
16	VATS, Autopsy	upper lobe fibrosis	+	-	-
17	TBB, Lobectomy	UIP	+	-	-
18	VATS	UIP	+	+	-
19	VATS	UIP, centrilobular fibrosis	+	-	+

Abbreviation; TBB, trans-bronchial biopsy; VATS, video-assisted thoracic surgery; GIP, giant cell interstitial pneumonia; DAD, diffuse alveolar damage; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia

Comparison of clinical features

We then classified the patients with hard metal lung disease into two groups according to their pathological findings. We grouped GIP and centrilobular inflammation/fibrosis together, because the latter pattern was considered to be a variant

of GIP due to the similar distribution of lesions. One patient was pathologically diagnosed as upper lobe fibrosis. It has such characteristic findings of subpleural, zonal, rather well defined fibrosis with small cysts and honeycomb lesions similar to that of UIP pattern that we grouped UIP pattern and upper lobe fibrosis together and named them the fibrosis group. We then compared clinical features between the GIP group and the fibrosis group. The GIP group was younger, had shorter exposure duration, lower serum KL-6, and higher lymphocyte percentage in BAL fluid compared with the fibrosis group (Table 5).

Table 5. Comparison of clinical features between GIP group and fibrosis group

	GIP group (n=14)	Fibrosis group (n=5)	p-value
Age (yrs)	43.1 ± 10.8	58.6 ± 5.41	0.0071
Gender (M/F)	7/7	5/0	0.1060
Exposure duration (months)	73.0 ± 48.8	285.6 ± 140.3	0.0072
Pneumothorax (+/-)	6/8	2/3	1.0000
KL-6 (U/ml)	398.7 ± 189.4	710.8 ± 297.7	0.0233
SP-D (ng/ml)	260.3 ± 257.5	161.0 ± 54.75	0.9025
PaO_2 (Torr)	84.3 ± 14.3	84.4 ± 11.2	0.9215
$PaCO_2$ (Torr)	42.8 ± 2.75	56.0 ± 34.6	0.6572
%VC (%)	64.4 ± 27.1	65.5 ± 24.1	0.7340
FEV_1% (%)	85.4 ± 12.9	86.1 ± 2.62	0.9097
%DLco (%)	50.8 ± 16.7	57.2 ± 18.8	0.3709
Bronchoalveolar lavage			
Total cell count ($\times 10^5$/ml)	3.52 ± 2.41	2.26 ± 0.96	0.3952
Lymphocytes (%)	31.5 ± 23.0	8.40 ± 9.08	0.0148
CD4/8 ratio	0.76 ± 0.51	3.22 ± 4.85	0.2975

DISCUSSION

Pathological features of GIP are interstitial pneumonia with centrilobular fibrosis with multinucleated giant cells in the airspaces.[15] Sometimes centrilobular inflammation/fibrosis is only noted with few giant cells. EPMA-WDS analysis of lung tissue of hard metal lung disease demonstrated that tungsten was distributed in a relatively high concentration almost throughout the centrilobular fibrosis and in giant cells.[7] Comparison of distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements were associated with centrilobular inflammation/fibrosis by $CD163^+$ macrophages in cooperation with $CD8^+$ lymphocytes. Thus, centrilobular inflammation/fibrosis without giant cells should also be a variant of hard metal lung disease. GIP was also found in Belgian diamond polishers exposed not to hard metal dust, but to cobalt-containing dust, which confirmed that cobalt plays a dominant role in hard metal lung disease.[16] Cobalt is a well-known skin sensitizer, causing allergic contact dermatitis, and it can also cause occupational asthma.[17] Four patients were positive for patch testing for cobalt. Although such patch testing has been claimed to carry some risk of aggravation of disease in the situation with beryllium, cobalt is included in the routine metal allergy test panel and caused no worsening of hard metal lung disease suggesting allergic inflammation should be different between hard metal lung disease and berylliosis.

Respiratory symptoms of hard metal lung diseases sometimes improve on holidays and exacerbate during workdays, which resemble those of hypersensitivity pneumonitis. Histopathology findings in hypersensitivity pneumonitis may also include centrilobular

fibrosis in association with isolated giant cells.[18] However, they do not show cannibalism as those in hard metal lung disease. BAL is the most sensitive tool to detect hypersensitivity pneumonitis: a marked lymphocytosis with decreased CD4/8 ratio is characteristic of BAL findings.[19] BAL findings of patients with hard metal lung disease show increased total cell counts with increased lymphocytes and decreased CD4/CD8 ratio.[4, 20-22] Reduced CD4/8 ratio is consistent with the findings of immunohistochemistry in the previous study.[7] In this study, we found that lymphocyte percentage in BAL fluid was increased with rather low CD4/8 ratio in the GIP group, but they were not recognized in fibrosis group.

UIP pattern is the pathological abnormality essential to the diagnosis of idiopathic pulmonary fibrosis (IPF). Interstitial inflammation and fibrosis in UIP pattern does not usually involve centrilobular area and peribronchioles. Three cases who were pathologically diagnosed as UIP pattern also had centriolobular micronodular opacities in HRCT findings. One patient was pathologically diagnosed as UIP pattern and centrilobular fibrosis. Elemental analysis by EPMA-WDS of lung specimens of UIP pattern demonstrated that tungsten accumulated in periarteriolar area and subpleural fibrosis. However, tungsten in periarteriolar area was hardly associated with any fibrosis or inflammatory cells. These results suggest that inhaled hard metal elements in UIP pattern may not trigger as much inflammation as in GIP. Patients develop hard metal lung disease usually after mean exposure duration of more than 10 years. Although most studies have found no relation between disease occurrence and length of occupational exposure, individuals with increased susceptibility may develop hard

metal lung disease after relatively short and low levels of exposure. The GIP group was younger and had shorter exposure duration suggesting that those who had UIP pattern were individuals with decreased susceptibility. Upper lobe fibrosis was pathologically diagnosed in one patient. Although it is significantly different from UIP pattern, tungsten in the fibrosis was not associated with inflammation around the element, either. With regard to the relationship between hard metal elements and surrounding inflammation, upper lobe fibrosis looks similar to UIP pattern in the other cases.

Liebow first described GIP as a form of idiopathic interstitial pneumonia.[23] It is now recognized that GIP is pathognomonic for hard metal lung disease.[24] Since tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals, the presence of tungsten and/or cobalt in BAL fluid or lung specimens leads to a definite diagnosis of hard metal lung disease. According to the results of elemental analyses in this study, five cases with UIP pattern or upper lobe fibrosis should be diagnosed as hard metal lung disease. However, the pathological findings of UIP pattern demonstrated no microscopic connection between centrilobular fibrosis and the UIP area, dense fibrosis with fibroblastic foci. EPMA-WDS analyses of lung specimens of UIP pattern revealed that tungsten and tantalum in periarteriolar area were not accompanied by centrilobular inflammation/fibrosis as seen in typical GIP. In addition, clinical features of the fibrosis group were different from those of the GIP group. We identified tungsten in subpleural fibrosis with dense acellular collagen from UIP pattern and in the fibrotic region from apical cap-like fibrosis. Fibrotic reactions

of these patients could have caused accumulation of hard metal particles as the scars contract and cut off lymphatic drainage. Those who are not sensitive to hard metal elements, particularly cobalt, might simply have idiopathic UIP or upper lobe fibrosis by accident as everyone with interstitial lung disease and a history of asbestos exposure does not have asbestosis.[25] However, microscopic findings of the lung specimen of UIP pattern included mild centrilobular inflammation and multinucleated giant cells with cannibalism, which could never been seen in idiopathic UIP/IPF. If we knew the relative frequencies of incidence of the two diseases, hard metal lung disease and IPF, the likelihood of someone with hard metal exposure developing idiopathic UIP/IPF could be inferred.

Hard metal lung disease is caused by exposure to cobalt and tungsten carbide. Toxicity stems from reactive oxygen species generation in a mechanism involving both elements in mutual contact.[26] Inhaled cobalt and tungsten carbides may cause lung toxicity even in those who are less sensitive to those elements, which can result in lung fibrosis with GIP features. Qualitative elemental analysis of fibrosing lesion in GIP also demonstrated the presence of miscellaneous elements: Al, Si, Ti, Cr, and Fe, in addition to tungsten, cobalt, and/or Ta.[7] Several sources of evidence suggest that environmental agents may have an etiologic role in IPF. A meta-analysis of six case-control studies demonstrated that six exposures including cigarette smoking, agriculture/farming, livestock, wood dust, metal dust, and stone/sand were significantly associated with IPF.[27] Metal dust must contain various metal elements. In an EPMA analysis field of the lung biopsy specimen from upper lobe fibrosis, we found

tungsten scattered throughout the fibrosis as well as aluminum, silicon, and titanium.[14] Miscellaneous metal dust inhaled in addition to tungsten and cobalt may cause UIP pattern in less sensitive individuals.

Acknowledgement

The authors thank the following doctors for the supply of cases: Dr. T. Endo from Nagaoka Chuo General Hospital, Dr. M. Amano and Dr. S. Aoki from Showa General Hospital, Dr. T. Ishiguro from Gifu Municipal Hospital, Dr. M. Sakai from Saga Social Insurance Hospital, Dr. M. Tajiri from Kurume University, Dr T. Ishida from Niigata Prefectural Central Hospital, Dr. K. Koreeda from Minami Kyusyu National Hospital, Dr. K. Okuno from Kasai City Hospital, Dr. Y. Shimaoka from Nagaoka Red Cross Hospital, Dr. K. Kashiwada from Nippon Medical School, Dr. T. Sawada and Dr. A. Shiihara from Kanagawa Cardiovascular and Respiratory Center, Dr. K. Tachibana from National Hospital Organization Kinki-chuo Chest Medical Center, Dr. T. Azuma from Shinshu University, Dr. K. Hara and Dr. T. Ishihara from NTT east corporation Kanto Medical Center, Dr. Y. Waseda from Kanazawa University, Dr. H. Ishii from Oita University, Dr. H. Matsuoka from Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dr. A Hara from Nagasaki University, Dr. O. Hisata from Tohoku University, and Dr. H. Tokuda from Social Insurance Chuo General Hospital. The authors also would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens.

Statements

a. contributorship,

JT and HM, elemental analysis; ES, IN, and TY, interpretation of the results; MT, ES, YK, AH, pathological study; JT and TT, manuscript preparation; and FS and

HA, radiological examination.

b. funding,

This research received no specific funding.

c. ethics,

We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

d. data sharing,

There are no data shared in the study.

References

1. Nemery B. Metal toxicity and the respiratory tract. *Eur Respir J*. 1990;**3**:202-19.

2. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environmental health perspectives*. 2000;**108 Suppl 4**:685-96.

3. Takada T, Moriyama H. Hard Metal Lung Disease. In: Huang Y-CT, Ghio AJ, Maier LA, editors. A Clinical Guide to Occupational and Environmental Lung Diseases Respiratory Medicine. New York: Springer; 2012. p. 217-30.

4. Davison AG, Haslam PL, Corrin B et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;**38**:119-28.

5. Cugell DW. The hard metal diseases. *Clin Chest Med*. 1992;**13**:269-79.

6. Anttila S, Sutinen S, Paananen M et al. Hard metal lung disease: a clinical, histological, ultrastructural and X-ray microanalytical study. *Eur J Respir Dis*. 1986;**69**:83-94.

7. Moriyama H, Kobayashi M, Takada T et al. Two-dimensional analysis of elements and mononuclear cells in hard metal lung disease. *Am J Respir Crit Care Med*. 2007;**176**:70-7.

8. Ohori NP, Sciurba FC, Owens GR et al. Giant-cell interstitial pneumonia and hard-metal pneumoconiosis. A clinicopathologic study of four cases and review of the literature. *Am J Surg Pathol*. 1989;**13**:581-7.

9. Travis WD, Matsui K, Moss J et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with

usual interstitial pneumonia and desquamative interstitial pneumonia. *Am J Surg Pathol*. 2000;**24**:19-33.

10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;**157**:1301-15.

11. Moriyama H, Yamamoto T, Takatsuka H et al. Expression of macrophage colony-stimulating factor and its receptor in hepatic granulomas of Kupffer-cell-depleted mice. *Am J Pathol*. 1997;**150**:2047-60.

12. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000;**161**:646-64.

13. Shiota S, Shimizu K, Suzuki M et al. [Seven cases of marked pulmonary fibrosis in the upper lobe]. *Nihon Kokyuki Gakkai Zasshi*. 1999;**37**:87-96.

14. Kaneko Y, Kikuchi N, Ishii Y et al. Upper lobe-dominant pulmonary fibrosis showing deposits of hard metal component in the fibrotic lesions. *Intern Med*. 2010;**49**:2143-5.

15. Naqvi AH, Hunt A, Burnett BR et al. Pathologic spectrum and lung dust burden in giant cell interstitial pneumonia (hard metal disease/cobalt pneumonitis): review of 100 cases. *Arch Environ Occup Health*. 2008;**63**:51-70.

16. Demedts M, Gheysens B, Nagels J et al. Cobalt lung in diamond polishers. *The American review of respiratory disease*. 1984;**130**:130-5.

17. Nemery B, Verbeken EK, Demedts M. Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung). *Semin Respir Crit Care Med*. 2001;**22**:435-48.

18. Churg A, Muller NL, Flint J et al. Chronic hypersensitivity pneumonitis. *Am J Surg Pathol*. 2006;**30**:201-8.

19. D'Ippolito R, Chetta A, Foresi A et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med*. 2004;**98**:977-83.

20. Okuno K, Kobayashi K, Kotani Y et al. A case of hard metal lung disease resembling a hypersensitive pneumonia in radiological images. *Intern Med*. 2010;**49**:1185-9.

21. Kakugawa T, Mukae H, Nagata T et al. Giant cell interstitial pneumonia in a 15-year-old boy. *Intern Med*. 2002;**41**:1007-12.

22. Forni A. Bronchoalveolar lavage in the diagnosis of hard metal disease. *Sci Total Environ*. 1994;**150**:69-76.

23. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. *Prog Respir Res*. 1975:1-33.

24. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc*. 1991;**5**:95-104; discussion 5-8.

25. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. *The American review of respiratory disease*. 1991;**144**:689-96.

26. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environmental health perspectives*. 1997;**105 Suppl 5**:1013-20.

27. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease?

Proc Am Thorac Soc. 2006;**3**:293-8.

FIGURE LEGENDS

Figure 1

High-resolution computed tomography of the chest illustrating differences in the radiographic appearance of the lungs in giant cell interstitial pneumonia (GIP) and in usual interstitial pneumonia (UIP) pattern. (A, B) In GIP of case 9, centriolobular micronodular opacities pathologically correspond to centrilobular fibrosis and giant cell accumulation within the alveolar space. (C, D) In UIP pattern of case 10, reticular opacities and traction bronchiectasis are present with centriolobular micronodular opacities.

Figure 2

Representative images of light microscopic findings and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of S6 specimen from case 9 pathologically diagnosed as giant cell interstitial pneumonia. (A, B, and C) The black square area in centrilobular fibrosis is stepwise magnified to show multinucleated giant cells with cannibalism. (A, D) The green square area in subpleural zone is elementally analyzed by EPMA-WDS to show (E) many orange spots corresponding to tungsten. A qualitative colored image of tungsten distribution is superimposed onto a lung tissue image of amino nitrogen colored green. Note that tungsten is widely distributed in centrilobular fibrosis as well as surrounding alveolar walls. Original magnification, (A) panoramic view, (B) x 4, (C) x 60, and (D) x 8.

Figure 3

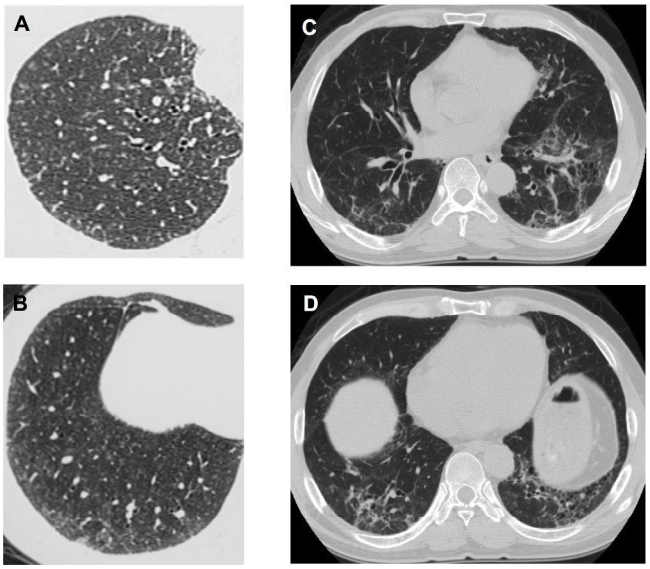
Representative images of light microscopic findings of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern. (A, B) A low magnification view of left S1+2 specimen demonstrates a combination of patchy interstitial fibrosis with alternating areas of normal lung and architectural alteration due to chronic scarring or honeycomb change. Note that there are several small bronchioles with mild centrilobular inflammation (blue arrows). (B, C) Multinucleated giant cells with cannibalism are also shown in a stepwise-magnified black square area located in subpleural fibrosis. (D, E, F) Left S10 specimen from the same patient also shows characteristic fibroblastic foci (black arrows) in the background of dense acellular collagen in a stepwise-magnified square area located in subpleural fibrosis. Original magnification, (A, D) panoramic view, (B) x 2, (C) x 40, (E) x 4 and (F) x 20.

Figure 4

Representative images of light micrographs and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern (A). (B, C) An arteriole and its surrounding interstitium (orange square) are elementally analyzed by EPMA-WDS to demonstrate that (D) tungsten and (E) tantalum are distributed in periarteriolar area with little fibrosis. Elemental analysis by EPMA-WDS of subpleural fibrosis with dense acellular collagen (green square in B, F,

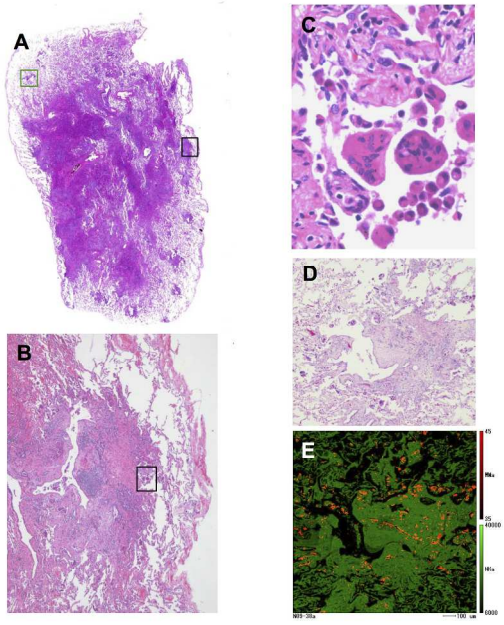
I) also shows (G, J) tungsten and (H, K) tantalum almost randomly distributed in magnified images (yellow squares in G and H are magnified to show (J) tungsten and (K) tantalum). Note that the distribution of tungsten is not completely the same as that of tantalum. Original magnification, (A) panoramic view and (B) x 4.

Fig 1



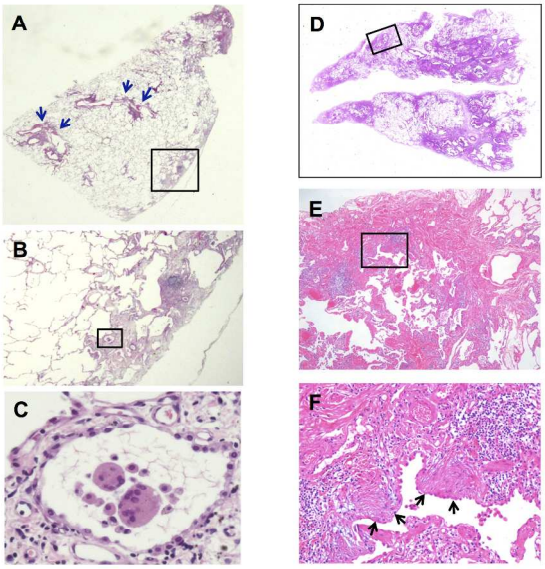
297x209mm (300 x 300 DPI)

Fig 2



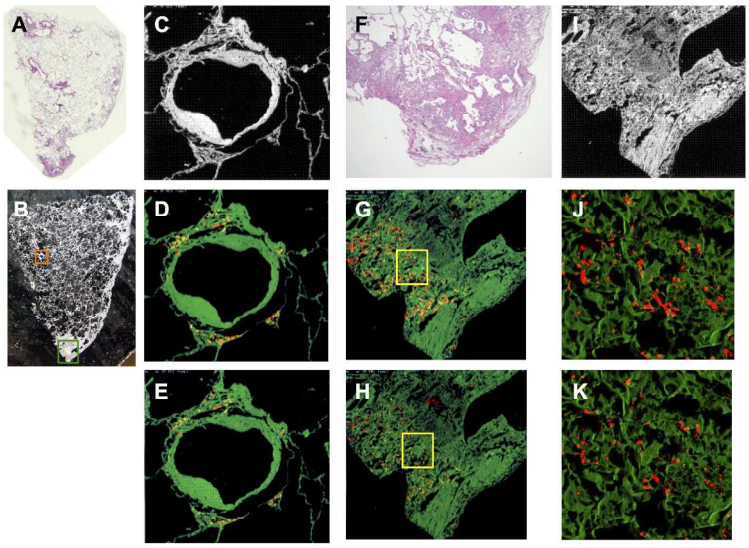
297x209mm (300 x 300 DPI)

Fig 3



297x209mm (300 x 300 DPI)

Fig 4



297x209mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract **p. 1, 3-4**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale p. 5	2	Explain the scientific background and rationale for the investigation being reported
Objectives, p. 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design, p.6	4	Present key elements of study design early in the paper
Setting, p.6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants, p.6	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables, p.6	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement, p.6-8	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias, p.6	9	Describe any efforts to address potential sources of bias
Study size, p. 8, 9	10	Explain how the study size was arrived at
Quantitative variables, p. 18	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods, p. 8	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants, p. 8, 9	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data, p. 10	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data, p. 12	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results, p. 13, 14	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses, p. 18	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results, p. 15, 16	18	Summarise key results with reference to study objectives
Limitations, p. 18	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation, p.17, 18	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability, p 18	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding NA	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004407.R1
Article Type:	Research
Date Submitted by the Author:	30-Jan-2014
Complete List of Authors:	Takada, Toshinori; Niigata University, Division of Respiratory Medicine Tanaka, Junichi; Niigata University, Division of Respiratory Medicine Moriyama, Hiroshi; Niigata University, Division of Respiratory Medicine Terada, Masaki; Niigata University, Division of Respiratory Medicine Suzuki, Eiichi Narita, Ichiei; Niigata University, Division of Respiratory Medicine Kawabata, Yoshinori Yamaguchi, Tetsuo Hebisawa, Akira Sakai, Fumikazu Arakawa, Hiroaki
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Occupational and environmental medicine
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, Thoracic medicine < INTERNAL MEDICINE, Interstitial lung disease < THORACIC MEDICINE

An Observational Study of Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

[1]Junichi Tanaka, MD, [1]Hiroshi Moriyama, MD, [1]Masaki Terada, MD, [1]Toshinori Takada, MD, [2]Eiichi Suzuki, MD, [1]Ichiei Narita, MD, [3]Yoshinori Kawabata, MD, [3]Tetsuo Yamaguchi, MD, [3]Akira Hebisawa, MD, [3]Fumikazu Sakai, MD, and [3]Hiroaki Arakawa, MD,

[1]Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan, [3]Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Tokyo, Japan

Corresponding author: Toshinori Takada, M.D., PhD

Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University

1-757 Asahimachi-dori, Chuo-ku, Niigata, 951-8510, Japan

Tel; +81-25-227-2200, Fax; +81-25-227-0775, Email; ttakada@med.niigata-u.ac.jp

Keywords: hard metal, pulmonary fibrosis, electron probe microanalysis

Word count: 2,910

Statements

a. contributorship,

JT and HM, elemental analysis; ES, IN, and TY, interpretation of the results; MT, ES, YK, AH, pathological study; JT and TT, manuscript preparation; and FS and HA, radiological examination.

b. funding,

This research received no specific funding.

c. ethics,

We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

d. data sharing,

There are no data shared in the study.

ABSTRACT

Background: Hard metal lung disease has pathological patterns including giant cell interstitial pneumonia (GIP) and usual interstitial pneumonia (UIP). Although UIP pattern is considered the prominent feature in advanced disease, it is unknown whether GIP finally progresses to UIP pattern.

Objective: To clarify clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Methods: We obtained the clinical records, chest CT, and lung tissue from nineteen cases diagnosed as hard metal lung disease. Lung tissue was elementally analyzed by electron probe microanalyser. We classified the patients into two groups according to the pathological findings and statistically compared clinical data.

Results: Fourteen cases were pathologically diagnosed as GIP or centrilobular inflammation/fibrosing. The other five cases were UIP pattern or upper lobe fibrosis. Elemental analyses of lung specimens of GIP showed tungsten throughout the centrilobular fibrotic areas. In UIP pattern, tungsten was detected in periarteriolar area and subpleural fibrosis in no association with centrilobular fibrosis or inflammatory cell infiltration. The GIP group was younger (43.1 vs 58.6 yrs) with shorter exposure duration (73 vs 285 months) ($p<0.01$), lower serum KL-6 (398 vs 710 U/ml), and higher lymphocyte percentage in bronchoalveolar lavage fluid (31.5 vs 3.22 %) ($p<0.05$) than the fibrosis group.

Conclusions UIP pattern or upper lobe fibrosis is remarkably different from GIP in distribution of hard metal elements, associated interstitial inflammation and fibrosis,

and clinical features. In hard metal lung disease, UIP pattern or upper lobe fibrosis may not be an advanced form of GIP.

Strengths and limitations of this study

1, Nineteen cases of hard metal lung disease, a rare occupational lung disease, were collected and their clinical features were documented.

2, Lung tissue from all the patients was elementally analyzed by a patented technique, an improved element analysis using electron probe microanalyzers with wavelength dispersive spectrometer.

3, Since the relative frequencies of incidence of hard metal lung disease and IPF, the probability that someone with hard metal exposure will develop idiopathic UIP/IPF cannot be inferred.

INTRODUCTION

Hard metal is a synthetic compound that combines tungsten carbide with cobalt. Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, or interstitial lung disease which is recognized as hard metal lung disease.[1-3] In many cases with hard metal lung disease, multinucleated giant cells with centrilobular fibrosis are prominent resulting in a pattern of giant cell interstitial pneumonia (GIP).[4-6] We demonstrated that hard metal accumulated in the centrilobular area may trigger the inflammation in cooperation with $CD163^+$ monocyte-macrophages and $CD8^+$ lymphocytes using electron probe microanalyzers with wavelength dispersive spectrometer (EPMA-WDS).[7] In addition to classical GIP, hard metal lung disease has a variety of pathological patterns, desquamative interstitial pneumonia, obliterative bronchiolitis, and usual interstitial pneumonia (UIP) pattern.[4, 8] The lesions of classical GIP are usually centered on the centrilobular areas. On the other hand, the key histologic features of UIP are predominantly distributed at the periphery of the acinus or lobule.[9, 10] Hard metal lung disease has pathological patterns of both GIP and UIP, and the UIP pattern is thought to be the prominent feature in advanced cases of the disease.[8] The key question is whether UIP pattern is an advanced form of GIP or not. In order to elucidate relationship between GIP and lung fibrosis with detection of hard metal elements, we collected cases with tungsten in lung tissue and reviewed their clinical records. We then elementally reexamined lung specimens by EPMA-WDS. We finally classified the patients into two groups according to the histological findings and

statistically compared their clinical features. Pathological and elemental analyses in the study suggest that UIP pattern or upper lobe fibrosis may be different from an end-stage form of GIP.

METHODS

Patient population

We collected patients by announcing inquiry for cases of hard metal lung disease to the major medical institutes and hospitals all over Japan for the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases. We obtained information of patient profile such as age, gender, duration of hard metal exposure, history of pneumothorax, history of allergy, symptoms, physical findings, serum levels of Krebs von den Lungen-6 (KL-6) and SP-D, arterial blood gas data, pulmonary function tests, bronchoalveolar lavage (BAL) cell profiles and treatment and prognosis in order to make a data base. We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

HRCT scan findings

All patients with hard metal lung disease except one had undergone high-resolution computed tomography (HRCT) scanning. Two radiologists (observers) who were blinded to clinical, laboratory, or pulmonary function test results evaluated CT scan

findings. The observers judged each CT scan for the presence or absence of three main features of centrilobular nodules, ground glass opacity, and pneumothorax. They also noted other remarkable findings; traction bronchiectasis, reticular pattern, subpleural linear opacity, consolidation, bulla, centrilobular emphysema, atelectasis, and bronchial wall thickening and entered these results into a data sheet independently. After evaluation, disagreement on the results between the observers for some HRCT scans was resolved by discussion and consensus.

Sample preparation and pathological study

Each tissue sample was serially cut into 3 μm-thickness sections and subjected to pathological study and EPMA-WDS analysis. For pathological study, formalin-fixed 3 μm serial sections were stained with hematoxylin-eosine and Elastica van Gieson method. Two pathologists (observers), who were blinded to clinical, laboratory, or pulmonary function test results, evaluated pathological findings. After evaluation, disagreement on the pathological diagnoses between the observers for some specimens was resolved by discussion and consensus.

Electron probe microanalysis

Examination of tissue sections with EMPA-WDS was performed according to procedures previously described.[11] X-ray data were

obtained with an EPMA-WDS (EPMA 8705, EPMA-1610, Shimadzu Ltd, Kyoto, Japan). In order to have representative element maps, we at first microscopically

scanned tissue specimens and looked for lesions of centrilobular fibrosis with low magnification because hard metal related elements, tungsten/cobalt were always found around centrilobular areas according to our experiences. For EMPA analysis, we at first screened areas of about 1.5 mm x 1.5 mm at largest covering centrilobular lesions or fibrosing lesion of interstitial lung diseases observed by pathological study to make rough element maps. Then we focused into areas from 5x5 to 10x10 μm at smallest to draw fine maps for elements. Each pixel in the focused areas in the tissue was scanned by three wavelength dispersive crystals; RAP, PET, and LiF for screening elements of Al, K, RAP; Si, K, PET; Ti, K, LiF; Cr, K, LiF; Fe, K, LiF; Co, K, LiF; Ta, M, PET; W, M, PET, and Zn, L, RAP. Since generated X-ray signals from each pixel were the smallest part of a distribution map, we simultaneously obtained element maps with qualitative analyses of pixels in the focused area. The distribution of amino nitrogen corresponding to the pathological image was also mapped for each sample.

Statistical analysis

Comparisons of categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by Mann-Whitney's U-test. A p Value <0.05 was considered significant.

RESULTS

Characteristics of subject

When we held the Tokyo ILD Meeting, 22 cases were collected and suspected to be

hard metal lung diseases due to occupational history and pathological findings, but 3 cases were excluded because tungsten or cobalt were not detected in the lung tissue. Nineteen patients were finally diagnosed as hard metal lung disease because of presence of tungsten in lung specimens detected by EPMA-WDS. In 4 of 19 patients, the presence of tungsten, cobalt, or tantalum was not known in the first place and proved by element analysis at the meeting.

Occupational history and clinical features are summarized in Table 1 and 2. Demographic findings in 6 of these patients have been reported previously (case 2, 5, 7, 8, 10, and 16 corresponding to case 1, 3, 5, 6, 14, and 16 in 2007 report, respectively).[7] All the subjects had an occupational history of hard metal industry for 1 to 36 years. One patient (case 15) was doing deskwork in an insufficiently ventilated room of a hard metal grinding company. Five patients had occupational history of hard metal industry but were not exposed at the diagnosis of hard metal lung disease. The delay between cessation of exposure and biopsy in the patients were 5 years, 4 months, 2 months, and 6 months for case 1, 2, 8, and 14, respectively. Case 10 had worked as a metal grinder for 6 years and then as a chimney cleaner at a copper mine for 32 years. He visited a hospital complaining of dry cough after 32-year work as a chimney cleaner and was finally diagnosed as hard metal lung diseases 4 years later by surgical biopsy. Five patients (case 2, 5, 7, 8, and 15) had an allergic history and were patch tested for Co, Ni, Cr, Hg, Au, Zn, Mn, Ag, Pd, Pt, Sn, Cu, Fe, Al, In, Ir, Ti. 4 of 5 patients who had undergone patch testing (case 2, 5, 7, and 15) were found to be positive for cobalt. Pulmonary function tests revealed restrictive lung defect

characterized by reduced vital capacity and lung diffusing capacity. BAL findings showed increased total cell counts, increased lymphocytes and eosinophils, with normal CD4/CD8 ratio. Bizarre multinucleated giant cells were noted in 3 patients.

Table 1. Demographic features of subjects

Case	Age	Sex	Smoking history	Occupational history (hard metal exposure)	Exposure (y/m) start/duration	Bx year	Exposure at Dx
1	39	M	non	Hard metal shaping/drilling	2000/12	2006	No
2	53	M	ex	Hard metal shaping/drilling	2002/30	2002	No
3	21	M	non	Metal grinding	2005/32	2008	Yes
4	42	M	ex	Hard metal shaping/drilling	2005/36	2009	Yes
5	48	M	non	Metal grinding	2000/48	2004	NA
6	45	M	non	Hard metal shaping/drilling	1982/60	1987	Yes
7	32	F	non	Metal grinding	1988/60	1993	Yes
8	32	F	non	Metal grinding	1997/72	2003	No
9	44	F	non	Hard metal shaping/drilling	1990/72	1996	Yes
10	62	M	non	Metal grinding	1963/72	2003	No
11	40	F	non	Hard metal shaping/drilling	1997/96	2005	NA
12	48	M	non	Metal grinding	1981/120	1992	NA
13	49	F	non	Hard metal shaping/drilling	1999/120	2009	Yes
14	65	F	non	Metal grinding	1988/144	2000	No
15	50	F	non	Desk worker in hard metal factory	1985/168	1996	Yes
16	53	M	non	Quality control of hard metals	1974/264	2001	NA
17	60	M	ex	Hard metal shaping/drilling	1972/276	1995	Yes
18	53	M	non	Hard metal shaping/drilling	1971/372	2005	Yes
19	65	M	non	Hard metal shaping/drilling	1963/444	2008	Yes

Abbreviation; Bx, biopsy; Dx, diagnosis; NA, not available.

Table 2. Clinical characteristics of Patients with Hard metal lung disease

		Value
Mean age at diagnosis (yrs)		46.4 ± 14.1 (21 - 65)
Gender	M/F	12/7
Smoking history	Cur/Ex/Never	0/3/16
Chief complaints	dry cough	13/19
	breath shortness	8/19
Pneumothorax	Yes	8/19
Allergic history	Yes	5/19
Patch test to cobalt	positive	4/5
Mean exposure duration (yrs)		10.7 ± 10.3 (1 - 36)
Physical findings	rales on auscultation	11/19
	fine crackles	8/19
	finger clubbing	4/18
	edema of leg	1/16
Laboratory tests	KL-6	502.7 ± 267.5 U/ml
	SP-D	216.1 ± 192.4 ng/ml
Pulmonary function tests		
	%VC	64.8 ± 25.3 %
	FEV_1%	85.6 ± 10.7 %
	%DLco	53.4 ± 17.0 %
Bronchoalveolar lavage		
	Total cell count	3.13 ± 2.11 $\times 10^5$ /ml
	Lymphocytes	24.3 ± 22.3 %
	Neutrophils	3.07 ± 2.86 %
	Eosinophils	3.01 ± 5.03 %
	CD4/8 ratio	1.65 ± 2.96

The mean numbers ± standard deviations and ranges in parentheses are shown.

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

Radiological findings

HRCT of all patients except one with hard metal lung disease were available for review of radiological findings. Conventional CT findings of case 12 were added to the table (Table 3). Centrilobular nodules (Fig 1 A, B) and ground glass opacity were identified in chest CT of 16 patients. In some patients, reticular opacities, traction bronchiectasis, and subpleural curvilinear opacities were also present (Fig 1 C, D). Although centrilobular micronodular opacities were noted in those patients, they were not predominant.

Table 3. Radiologic findings of patients with hard metal lung disease

	CT features				
Case	CL nodules	GGO	PTx	other findings	radiological diagnosis
1	+	-	-	bronchial wall thickening	bronchitis (DPB like)
2	+	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
3	+	+	+		subacute HP
4	+	-	+	subpleural curvilinear opacities	subacute HP
5	+	+	-		subacute HP
6	-	+	-	reticular opacities, consolidation	Interstitial pneumonia NOS
7	+	+	+		subacute HP
8	+	+	-	traction bronchiectasis	subacute HP
9	+	+	-		subacute HP
10	+	+	-	reticular opacities traction bronchiectasis	UIP
11	+	-	+		subacute HP
12	+	+	+	subpleural curvilinear opacities	chronic HP
13	+	+	-		subacute HP
14	+	+	-	traction bronchiectasis, apical cap	chronic HP
15	+	+	+	traction bronchiectasis	subacute HP
16	-	+	+	subpleural/peribronchovascular consolidation, atelectasis, bulla	upper lobe predominant IP or chronic IP NOS
17	+	+	-	bulla, centrilobular emphysema	UIP
18	-	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
19	+	+	-	reticular opacities	chronic HP

Abbreviation; CL, centrilobular; GGO, ground-glass opacities; PTx, pneumothorax; DPB, diffuse panbronchiolitis; IP, interstitial pneumonia; NOS, not otherwise specified; NSIP, non-specific interstitial pneumonia; UIP, usual interstitial pneumonia; HP, hypersensitivity pneumonitis

Pathological findings and elemental analysis

Pathological findings and detected elements in lung tissue of 19 cases were summarized in Table 4. Four major histological features noted in this study were as follows: GIP

characterized with centrilobular fibrosis (Fig 2 A, B) and characteristic giant cells showing cannibalism (Fig 2 C), centrilobular inflammation/fibrosis similar to GIP but without giant cells, UIP pattern characterized with patchy distribution and temporal heterogeneity, and dense fibrosis with fibroblastic foci (Fig 3 A, B, D, E, F) [12], upper lobe fibrosis characterized with apical scar/cap type fibrosis mainly in the upper lobe.[13] In the case of upper lobe fibrosis, biopsy specimen contained apical cap-like subpleural dense fibrosis which was composed of airspace fibrosis (intraluminar organization) with collapse and increased elastic framework. In autopsy taken 4 years later, we recognized remarkable subpleural elastosis with a few of cannibalistic giant cells.

Elemental analyses of lung specimens of GIP and centrilobular inflammation/fibrosis demonstrated that tungsten was mapped almost throughout the centrilobular fibrotic areas (Fig 2 D, E). Analyses of lung specimens of UIP pattern by EPMA-WDS revealed that tungsten and tantalum were distributed in periarteriolar area (Fig 4, D, E) and in subpleural fibrosis with dense acellular collagen (Fig 4 G, H, J, K). However, these elements were not accompanied by centrilobular inflammation/fibrosis (Fig 4, A, B). Lung histopathology in one case showed apical cap-like fibrosis with tungsten deposits detected in the fibrotic region but without GIP.[14] In total, elemental analysis by EPMA-WDS detected tungsten but no cobalt or tantalum in 10 patients, tungsten and cobalt in 5 patients, and tungsten and tantalum in 4 patients (Table 4).

Table 4. Pathological findings and elemental analysis of patients with hard metal lung disease

	sampling			elements detected		
Case	method	site(s)	pathological findings	W	Co	Ta
1	VATS	rt. S5/S8	centrilobular inflammation/fibrosis	+	-	-
2	VATS	lt. S2/S9	GIP	+	-	-
3	TBB/VATS	rt. apex	GIP	+	-	-
4	VATS	rt. S9	centrilobular inflammation/fibrosis	+	-	-
5	VATS	rt. S4/S9	GIP	+	-	-
6	Autopsy	NA	GIP, DAD	+	-	-
7	VATS	rt. S8	centrilobular inflammation/fibrosis	+	+	-
8	VATS	rt. S4/S6	GIP	+	-	+
9	VATS	rt. S2/S6	GIP	+	+	-
10	VATS	lt. S1+2/S10	UIP, GIP	+	-	+
11	VATS	lt. S1+2/S9	GIP	+	+	-
12	Autopsy	NA	GIP, DAD	+	-	-
13	VATS	lt. S1+2/S6	GIP	+	-	-
14	VATS	lt. S4/S9	GIP, UIP/NSIP?	+	-	+
15	VATS	rt. S6	GIP	+	+	-
16	VATS/autopsy	lt. S1+2/whole	upper lobe fibrosis	+	-	+
17	TBB/Lobectomy	-/RLL	UIP	+	-	-
18	VATS	lt. S1+2/S9	UIP	+	+	-
19	VATS	rt. S3/S10	UIP, centrilobular fibrosis	+	-	+

Abbreviation; TBB, trans-bronchial biopsy; VATS, video-assisted thoracic surgery; GIP, giant cell interstitial pneumonia; NA, not available; RLL, right lower lobectomy; DAD, diffuse alveolar damage; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia

Comparison of clinical features

We then classified the patients with hard metal lung disease into two groups according to their pathological findings. We grouped GIP and centrilobular inflammation/fibrosis together, because the latter pattern was considered to be a variant

of GIP due to the similar distribution of lesions. One patient was pathologically diagnosed as upper lobe fibrosis. It has such characteristic findings of subpleural, zonal, rather well defined fibrosis with small cysts and honeycomb lesions similar to that of UIP pattern that we grouped UIP pattern and upper lobe fibrosis together and named them the fibrosis group. We then compared clinical features between the GIP group and the fibrosis group. The GIP group was younger, had shorter exposure duration, lower serum KL-6, and higher lymphocyte percentage in BAL fluid compared with the fibrosis group (Table 5).

Table 5. Comparison of clinical features between GIP group and fibrosis group

	GIP group (n=14)	Fibrosis group (n=5)	p-value
Age (yrs)	43.1 ± 10.8	58.6 ± 5.41	0.007
Gender (M/F)	7/7	5/0	0.106
Exposure duration (months)	73.0 ± 48.8	285.6 ± 140.3	0.007
Pneumothorax (+/-)	6/8	2/3	1.000
KL-6 (U/ml)	398.7 ± 189.4	710.8 ± 297.7	0.023
SP-D (ng/ml)	260.3 ± 257.5	161.0 ± 54.75	0.903
PaO_2 (Torr)	84.3 ± 14.3	84.4 ± 11.2	0.922
$PaCO_2$ (Torr)	42.8 ± 2.75	56.0 ± 34.6	0.657
%VC (%)	64.4 ± 27.1	65.5 ± 24.1	0.734
FEV_1% (%)	85.4 ± 12.9	86.1 ± 2.62	0.910
%DLco (%)	50.8 ± 16.7	57.2 ± 18.8	0.371
Bronchoalveolar lavage			
Total cell count ($\times 10^5$/ml)	3.52 ± 2.41	2.26 ± 0.96	0.395
Lymphocytes (%)	31.5 ± 23.0	8.40 ± 9.08	0.015
CD4/8 ratio	.76 ± 0.51	3.22 ± 4.85	0.298

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

DISCUSSION

Pathological features of GIP are interstitial pneumonia with centrilobular fibrosis with multinucleated giant cells in the airspaces.[15] Sometimes centrilobular inflammation/fibrosis is only noted with few giant cells. EPMA-WDS analysis of lung tissue of hard metal lung disease demonstrated that tungsten was distributed in a relatively high concentration almost throughout the centrilobular fibrosis and in giant cells.[7] Comparison of distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements were associated with centrilobular inflammation/fibrosis by $CD163^+$ macrophages in cooperation with $CD8^+$ lymphocytes. Thus, centrilobular inflammation/fibrosis without giant cells should also be a variant of hard metal lung disease. GIP was also found in Belgian diamond polishers exposed not to hard metal dust, but to cobalt-containing dust, which confirmed that cobalt plays a dominant role in hard metal lung disease.[16] Cobalt is a well-known skin sensitizer, causing allergic contact dermatitis, and it can also cause occupational asthma.[17] Four patients were positive for patch testing for cobalt. Although such patch testing has been claimed to carry some risk of aggravation of disease in the situation with beryllium, cobalt is included in the routine metal allergy test panel and caused no worsening of hard metal lung disease. Hard metal lung disease cases show features of hypersensitivity pneumonitis (HP) with small interstitial granulomas, although well formed granulomas as in chronic beryllium disease are very rarely seen in the disease or HP. These data suggest that allergic inflammation may be different between hard metal lung disease/HP and berylliosis.

Respiratory symptoms of hard metal lung diseases sometimes improve on holidays and exacerbate during workdays, which resemble those of HP. Histopathology findings in HP may also include centrilobular fibrosis in association with isolated giant cells.[18] However, they do not show cannibalism as those in hard metal lung disease. BAL is the most sensitive tool to detect HP: a marked lymphocytosis with decreased CD4/8 ratio is characteristic of BAL findings.[19] BAL findings of patients with hard metal lung disease show increased total cell counts with increased lymphocytes and decreased CD4/CD8 ratio.[4, 20-22] Reduced CD4/8 ratio is consistent with the findings of immunohistochemistry in the previous study.[7] In this study, we found that lymphocyte percentage in BAL fluid was increased with rather low CD4/8 ratio in the GIP group, but they were not recognized in fibrosis group.

UIP pattern is the pathological abnormality associated with various restrictive lung diseases, including idiopathic pulmonary fibrosis (IPF). Interstitial inflammation and fibrosis in UIP pattern does not usually involve centrilobular area and peribronchioles. Three cases who were pathologically diagnosed as UIP pattern also had centrilobular micronodular opacities in HRCT findings. One patient was pathologically diagnosed as UIP pattern and centrilobular fibrosis. Element analysis of the deposition in lung tissues from patients with IPF/UIP usually demonstrates following elements; Si, Al, Fe, and Ti with various degrees (unpublished data). While we found tungsten accumulated in periarteriolar area and subpleural fibrosis in lung specimens of UIP pattern in this study. However, tungsten in periarteriolar area was hardly associated with any fibrosis or inflammatory cells. These results suggest that individual immune

susceptibility/response to inhaled hard metal elements may decide pathological patterns of UIP, GIP, or their mixture in varying degrees. Patients develop hard metal lung disease usually after mean exposure duration of more than 10 years. Although most studies have found no relation between disease occurrence and length of occupational exposure, individuals with increased susceptibility may develop hard metal lung disease after relatively short and low levels of exposure. The GIP group was younger and had shorter exposure duration suggesting that those who had UIP pattern were individuals with decreased susceptibility. Upper lobe fibrosis was pathologically diagnosed in one patient. Although it is significantly different from UIP pattern, tungsten in the fibrosis was not associated with inflammation around the element, either. With regard to the relationship between hard metal elements and surrounding inflammation, upper lobe fibrosis looks similar to UIP pattern in the other cases.

Liebow first described GIP as a form of idiopathic interstitial pneumonia.[23] It is now recognized that GIP is pathognomonic for hard metal lung disease.[24] Since tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals, the presence of tungsten and/or cobalt in BAL fluid or lung specimens leads to a definite diagnosis of hard metal lung disease. According to the results of elemental analyses in this study, five cases with UIP pattern or upper lobe fibrosis should be diagnosed as hard metal lung disease. The pathological findings of UIP pattern demonstrated no physical connection between centrilobular fibrosis and the UIP area, dense fibrosis with fibroblastic foci. Since centrilobular fibrosis is usually irreversible, if GIP evolved to UIP, sequels of centrilobular fibrosis would be somewhat

linked to peripheral UIP lesion. EPMA-WDS analyses of lung specimens of UIP pattern revealed that tungsten and tantalum in periarteriolar area were not accompanied by centrilobular inflammation/fibrosis as seen in typical GIP. In addition, clinical features of the fibrosis group were different from those of the GIP group. We identified tungsten in subpleural fibrosis with dense acellular collagen from UIP pattern and in the fibrotic region from apical cap-like fibrosis. Fibrotic reactions of these patients could have caused accumulation of hard metal particles as the scars contract and cut off lymphatic drainage. Those who are not sensitive to hard metal elements, particularly cobalt, might simply have idiopathic UIP or upper lobe fibrosis by accident as everyone with interstitial lung disease and a history of asbestos exposure does not have asbestosis.[25] However, microscopic findings of the lung specimen of UIP pattern included mild centrilobular inflammation and multinucleated giant cells with cannibalism, which could never been seen in idiopathic UIP/IPF. If we find tungsten or cobalt in the biopsies of UIP/fibrosis from the subjects who worked in the hard-metal industry, we cannot help but make a diagnosis of hard-metal lung disease. Given present information, we only conclude that the UIP/fibrosis may be induced by hard metal elements, or just a coincidence. Longitudinal data of the relative frequencies of incidence of the two diseases, hard metal lung disease and IPF, allow us to infer the likelihood of someone with hard metal exposure developing idiopathic UIP/IPF.

Hard metal lung disease is caused by exposure to cobalt and tungsten carbide. Toxicity stems from reactive oxygen species generation in a mechanism involving both elements in mutual contact.[26] Inhaled cobalt and tungsten carbides may cause lung

toxicity even in those who are less sensitive to those elements, which can result in lung fibrosis with GIP features. Qualitative elemental analysis of fibrosing lesion in GIP also demonstrated the presence of miscellaneous elements: Al, Si, Ti, Cr, and Fe, in addition to tungsten, cobalt, and/or Ta.[7] Several sources of evidence suggest that environmental agents may have an etiologic role in IPF. A meta-analysis of six case-control studies demonstrated that six exposures including cigarette smoking, agriculture/farming, livestock, wood dust, metal dust, and stone/sand were significantly associated with IPF.[27] Metal dust must contain various metal elements. In an EPMA analysis field of the lung biopsy specimen from upper lobe fibrosis, we found tungsten scattered throughout the fibrosis as well as aluminum, silicon, and titanium.[14] Miscellaneous metal dust inhaled in addition to tungsten and cobalt may cause UIP pattern in less sensitive individuals.

Acknowledgement

The authors thank the following doctors for the supply of cases: Dr. Y. Endo from Nagaoka Chuo General Hospital, Dr. M. Amano and Dr. S. Aoki from Showa General Hospital, Dr. T. Ishiguro from Gifu Municipal Hospital, Dr. M. Sakai from Saga Social Insurance Hospital, Dr. M. Tajiri from Kurume University, Dr T. Ishida from Niigata Prefectural Central Hospital, Dr. K. Koreeda from Minami Kyusyu National Hospital, Dr. K. Okuno from Kasai City Hospital, Dr. Y. Shimaoka from Nagaoka Red Cross Hospital, Dr. K. Kashiwada from Nippon Medical School, Dr. T. Sawada and Dr. A. Shiihara from Kanagawa Cardiovascular and Respiratory Center, Dr. K. Tachibana from

National Hospital Organization Kinki-chuo Chest Medical Center, Dr. T. Azuma from Shinshu University, Dr. K. Hara and Dr. T. Ishihara from NTT east corporation Kanto Medical Center, Dr. Y. Waseda from Kanazawa University, Dr. H. Ishii from Oita University, Dr. H. Matsuoka from Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dr. A Hara from Nagasaki University, Dr. O. Hisata from Tohoku University, and Dr. H. Tokuda from Social Insurance Chuo General Hospital. The authors also would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens.

References

1. Nemery B. Metal toxicity and the respiratory tract. *Eur Respir J*. 1990;**3**:202-19.

2. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environmental health perspectives*. 2000;**108 Suppl 4**:685-96.

3. Takada T, Moriyama H. Hard Metal Lung Disease. In: Huang Y-CT, Ghio AJ, Maier LA, editors. A Clinical Guide to Occupational and Environmental Lung Diseases Respiratory Medicine. New York: Springer; 2012. p. 217-30.

4. Davison AG, Haslam PL, Corrin B et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;**38**:119-28.

5. Cugell DW. The hard metal diseases. *Clin Chest Med*. 1992;**13**:269-79.

6. Anttila S, Sutinen S, Paananen M et al. Hard metal lung disease: a clinical, histological, ultrastructural and X-ray microanalytical study. *Eur J Respir Dis*. 1986;**69**:83-94.

7. Moriyama H, Kobayashi M, Takada T et al. Two-dimensional analysis of elements and mononuclear cells in hard metal lung disease. *Am J Respir Crit Care Med*. 2007;**176**:70-7.

8. Ohori NP, Sciurba FC, Owens GR et al. Giant-cell interstitial pneumonia and hard-metal pneumoconiosis. A clinicopathologic study of four cases and review of the literature. *Am J Surg Pathol*. 1989;**13**:581-7.

9. Travis WD, Matsui K, Moss J et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with

usual interstitial pneumonia and desquamative interstitial pneumonia. *Am J Surg Pathol*. 2000;**24**:19-33.

10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;**157**:1301-15.

11. Moriyama H, Yamamoto T, Takatsuka H et al. Expression of macrophage colony-stimulating factor and its receptor in hepatic granulomas of Kupffer-cell-depleted mice. *Am J Pathol*. 1997;**150**:2047-60.

12. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000;**161**:646-64.

13. Shiota S, Shimizu K, Suzuki M et al. [Seven cases of marked pulmonary fibrosis in the upper lobe]. *Nihon Kokyuki Gakkai Zasshi*. 1999;**37**:87-96.

14. Kaneko Y, Kikuchi N, Ishii Y et al. Upper lobe-dominant pulmonary fibrosis showing deposits of hard metal component in the fibrotic lesions. *Intern Med*. 2010;**49**:2143-5.

15. Naqvi AH, Hunt A, Burnett BR et al. Pathologic spectrum and lung dust burden in giant cell interstitial pneumonia (hard metal disease/cobalt pneumonitis): review of 100 cases. *Arch Environ Occup Health*. 2008;**63**:51-70.

16. Demedts M, Gheysens B, Nagels J et al. Cobalt lung in diamond polishers. *The American review of respiratory disease*. 1984;**130**:130-5.

17. Nemery B, Verbeken EK, Demedts M. Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung). *Semin Respir Crit Care Med*. 2001;**22**:435-48.

18. Churg A, Muller NL, Flint J et al. Chronic hypersensitivity pneumonitis. *Am J Surg Pathol*. 2006;**30**:201-8.

19. D'Ippolito R, Chetta A, Foresi A et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med*. 2004;**98**:977-83.

20. Okuno K, Kobayashi K, Kotani Y et al. A case of hard metal lung disease resembling a hypersensitive pneumonia in radiological images. *Intern Med*. 2010;**49**:1185-9.

21. Kakugawa T, Mukae H, Nagata T et al. Giant cell interstitial pneumonia in a 15-year-old boy. *Intern Med*. 2002;**41**:1007-12.

22. Forni A. Bronchoalveolar lavage in the diagnosis of hard metal disease. *Sci Total Environ*. 1994;**150**:69-76.

23. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. *Prog Respir Res*. 1975:1-33.

24. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc*. 1991;**5**:95-104; discussion 5-8.

25. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. *The American review of respiratory disease*. 1991;**144**:689-96.

26. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environmental health perspectives*. 1997;**105 Suppl 5**:1013-20.

27. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease?

Proc Am Thorac Soc. 2006;**3**:293-8.

FIGURE LEGENDS

Figure 1

High-resolution computed tomography of the chest illustrating differences in the radiographic appearance of the lungs in giant cell interstitial pneumonia (GIP) and in usual interstitial pneumonia (UIP) pattern. (A, B) In GIP of case 9, centriolobular micronodular opacities pathologically correspond to centrilobular fibrosis and giant cell accumulation within the alveolar space. (C, D) In UIP pattern of case 10, reticular opacities and traction bronchiectasis are present with centriolobular micronodular opacities.

Figure 2

Representative images of light microscopic findings and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of S6 specimen from case 9 pathologically diagnosed as giant cell interstitial pneumonia. (A, B, and C) The black square area in centrilobular fibrosis is stepwise magnified to show multinucleated giant cells with cannibalism. (A, D) The green square area in subpleural zone is elementally analyzed by EPMA-WDS to show (E) many orange spots corresponding to tungsten. A qualitative colored image of tungsten distribution is superimposed onto a lung tissue image of amino nitrogen colored green. Note that tungsten is widely distributed in centrilobular fibrosis as well as surrounding alveolar walls. Original magnification, (A) panoramic view, (B) x 4, (C) x 60, and (D) x 8.

Figure 3

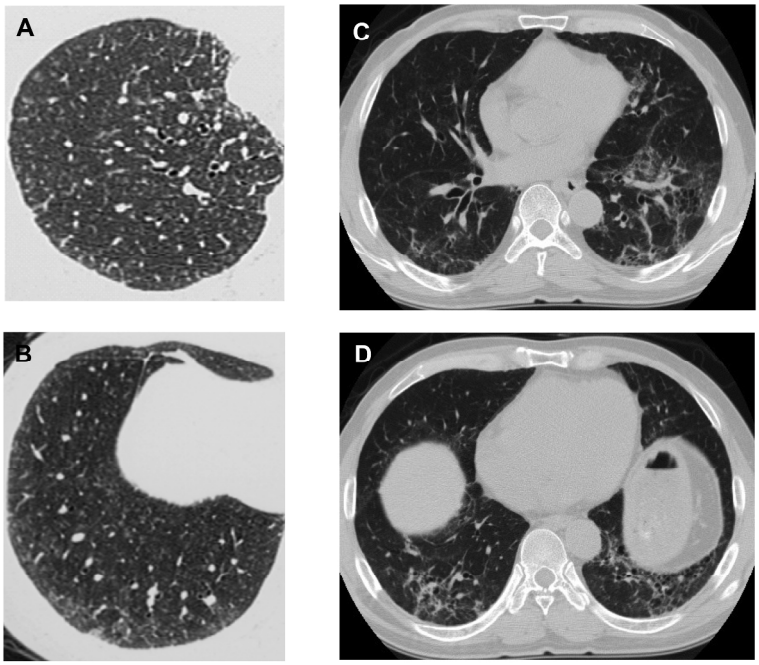
Representative images of light microscopic findings of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern. (A, B) A low magnification view of left S1+2 specimen demonstrates a combination of patchy interstitial fibrosis with alternating areas of normal lung and architectural alteration due to chronic scarring or honeycomb change. Note that there are several small bronchioles with mild centrilobular inflammation (blue arrows). (B, C) Multinucleated giant cells with cannibalism are also shown in a stepwise-magnified black square area located in subpleural fibrosis. (D, E, F) Left S10 specimen from the same patient also shows characteristic fibroblastic foci (black arrows) in the background of dense acellular collagen in a stepwise-magnified square area located in subpleural fibrosis. Original magnification, (A, D) panoramic view, (B) x 2, (C) x 40, (E) x 4 and (F) x 20.

Figure 4

Representative images of light micrographs and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern (A). (B, C) An arteriole and its surrounding interstitium (orange square) are elementally analyzed by EPMA-WDS to demonstrate that (D) tungsten and (E) tantalum are distributed in periarteriolar area with little fibrosis. Elemental analysis by EPMA-WDS of subpleural fibrosis with dense acellular collagen (green square in B, F,

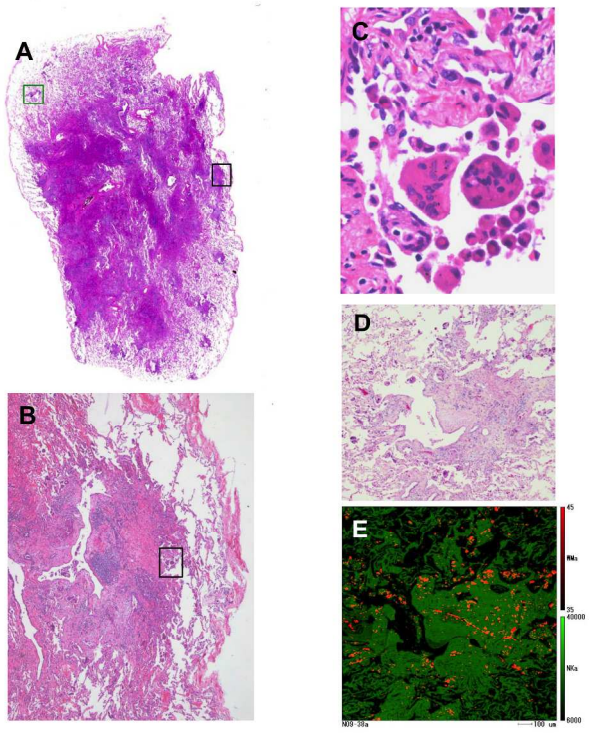
I) also shows (G, J) tungsten and (H, K) tantalum almost randomly distributed in magnified images (yellow squares in G and H are magnified to show (J) tungsten and (K) tantalum). We did not further analyze the centrilobular pattern or the cannibalistic giant cells shown in Fig 3. Note that the distribution of tungsten is not completely the same as that of tantalum. Original magnification, (A) panoramic view and (B) x 4. Scale bars for the magnification and scan areas for (E), (H), and (K) correspond to 100μm (0.768 x 0.768 mm), 200μm (1.536 x 1.536 mm), and 25μm (0.1792 x 0.1792 mm), respectively.

Fig 1



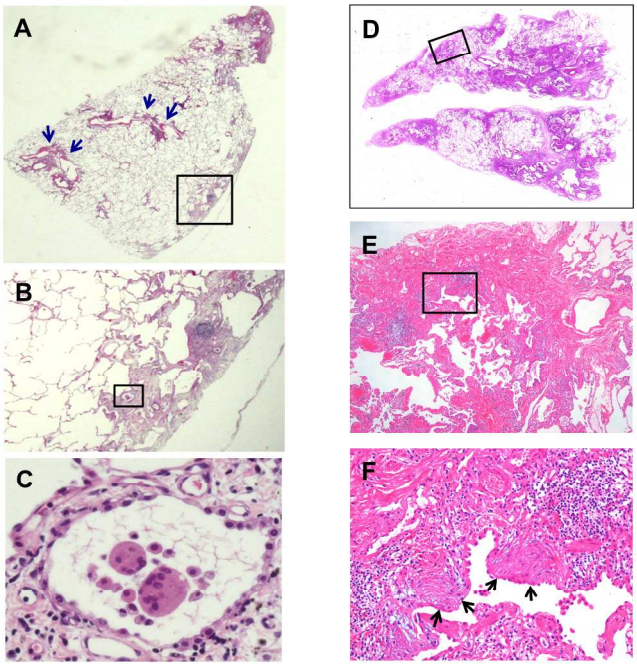
253x190mm (300 x 300 DPI)

Fig 2



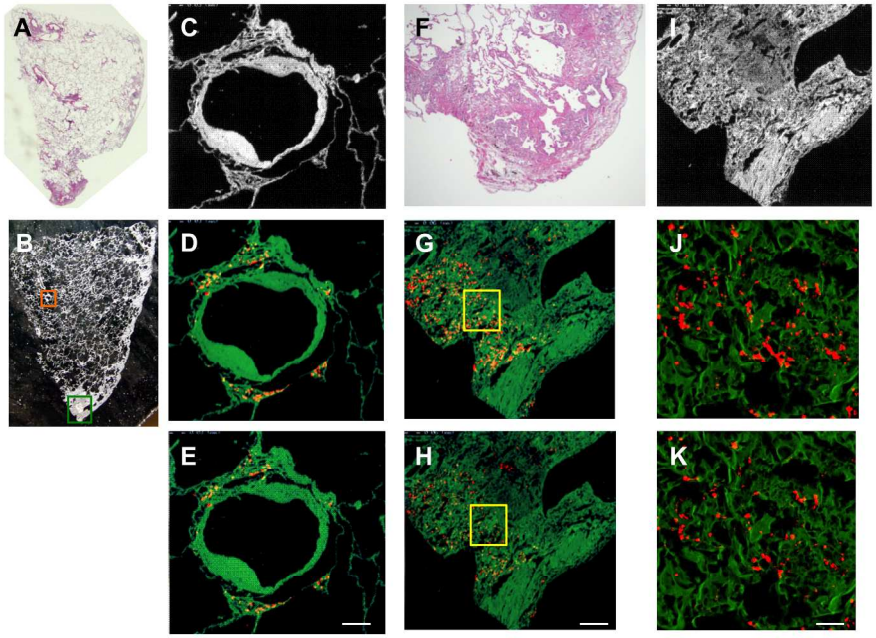
253x190mm (300 x 300 DPI)

Fig 3



253x190mm (300 x 300 DPI)

Fig 4



253x190mm (300 x 300 DPI)

An Observational Study of Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

[1]Junichi Tanaka, MD, [1]Hiroshi Moriyama, MD, [1]Masaki Terada, MD, [1]Toshinori Takada, MD, [2]Eiichi Suzuki, MD, [1]Ichiei Narita, MD, [3]Yoshinori Kawabata, MD, [3]Tetsuo Yamaguchi, MD, [3]Akira Hebisawa, MD, [3]Fumikazu Sakai, MD, and [3]Hiroaki Arakawa, MD,

[1]Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan, [3]Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Tokyo, Japan

Corresponding author: Toshinori Takada, M.D., PhD

Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University

1-757 Asahimachi-dori, Chuo-ku, Niigata, 951-8510, Japan

Tel; +81-25-227-2200, Fax; +81-25-227-0775, Email; ttakada@med.niigata-u.ac.jp

Keywords: hard metal, pulmonary fibrosis, electron probe microanalysis

Word count: 2,910

Statements

a. contributorship,

JT and HM, elemental analysis; ES, IN, and TY, interpretation of the results; MT, ES, YK, AH, pathological study; JT and TT, manuscript preparation; and FS and HA, radiological examination.

b. funding,

This research received no specific funding.

c. ethics,

We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

d. data sharing,

There are no data shared in the study.

ABSTRACT

Background: Hard metal lung disease has pathological patterns including giant cell interstitial pneumonia (GIP) and usual interstitial pneumonia (UIP). Although UIP pattern is considered the prominent feature in advanced disease, it is unknown whether GIP finally progresses to UIP pattern.

Objective: To clarify clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Methods: We obtained the clinical records, chest CT, and lung tissue from nineteen cases diagnosed as hard metal lung disease. Lung tissue was elementally analyzed by electron probe microanalyser. We classified the patients into two groups according to the pathological findings and statistically compared clinical data.

Results: Fourteen cases were pathologically diagnosed as GIP or centrilobular inflammation/fibrosing. The other five cases were UIP pattern or upper lobe fibrosis. Elemental analyses of lung specimens of GIP showed tungsten throughout the centrilobular fibrotic areas. In UIP pattern, tungsten was detected in periarteriolar area and subpleural fibrosis in no association with centrilobular fibrosis or inflammatory cell infiltration. The GIP group was younger (43.1 vs 58.6 yrs) with shorter exposure duration (73 vs 285 months) ($p<0.01$), lower serum KL-6 (398 vs 710 U/ml), and higher lymphocyte percentage in bronchoalveolar lavage fluid (31.5 vs 3.22 %) ($p<0.05$) than the fibrosis group.

Conclusions UIP pattern or upper lobe fibrosis is remarkably different from GIP in distribution of hard metal elements, associated interstitial inflammation and fibrosis,

and clinical features. In hard metal lung disease, UIP pattern or upper lobe fibrosis may not be an advanced form of GIP.

Strengths and limitations of this study

1, Nineteen cases of hard metal lung disease, a rare occupational lung disease, were collected and their clinical features were documented.

2, Lung tissue from all the patients was elementally analyzed by a patented technique, an improved element analysis using electron probe microanalyzers with wavelength dispersive spectrometer.

3, Since the relative frequencies of incidence of hard metal lung disease and IPF, the probability that someone with hard metal exposure will develop idiopathic UIP/IPF cannot be inferred.

INTRODUCTION

Hard metal is a synthetic compound that combines tungsten carbide with cobalt. Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, or interstitial lung disease which is recognized as hard metal lung disease.[1-3] In many cases with hard metal lung disease, multinucleated giant cells with centrilobular fibrosis are prominent resulting in a pattern of giant cell interstitial pneumonia (GIP).[4-6] We demonstrated that hard metal accumulated in the centrilobular area may trigger the inflammation in cooperation with $CD163^+$ monocyte-macrophages and $CD8^+$ lymphocytes using electron probe microanalyzers with wavelength dispersive spectrometer (EPMA-WDS).[7] In addition to classical GIP, hard metal lung disease has a variety of pathological patterns, desquamative interstitial pneumonia, obliterative bronchiolitis, and usual interstitial pneumonia (UIP) pattern.[4, 8] The lesions of classical GIP are usually centered on the centrilobular areas. On the other hand, the key histologic features of UIP are predominantly distributed at the periphery of the acinus or lobule.[9, 10] Hard metal lung disease has pathological patterns of both GIP and UIP, and the UIP pattern is thought to be the prominent feature in advanced cases of the disease.[8] The key question is whether UIP pattern is an advanced form of GIP or not. In order to elucidate relationship between GIP and lung fibrosis with detection of hard metal elements, we collected cases with tungsten in lung tissue and reviewed their clinical records. We then elementally reexamined lung specimens by EPMA-WDS. We finally classified the patients into two groups according to the histological findings and

statistically compared their clinical features. Pathological and elemental analyses in the study suggest that UIP pattern or upper lobe fibrosis may be different from an end-stage form of GIP.

METHODS

Patient population

We collected patients by announcing inquiry for cases of hard metal lung disease to the major medical institutes and hospitals all over Japan for the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases. We obtained information of patient profile such as age, gender, duration of hard metal exposure, history of pneumothorax, history of allergy, symptoms, physical findings, serum levels of Krebs von den Lungen-6 (KL-6) and SP-D, arterial blood gas data, pulmonary function tests, bronchoalveolar lavage (BAL) cell profiles and treatment and prognosis in order to make a data base. We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

HRCT scan findings

All patients with hard metal lung disease except one had undergone high-resolution computed tomography (HRCT) scanning. Two radiologists (observers) who were blinded to clinical, laboratory, or pulmonary function test results evaluated CT scan

findings. The observers judged each CT scan for the presence or absence of three main features of centrilobular nodules, ground glass opacity, and pneumothorax. They also noted other remarkable findings; traction bronchiectasis, reticular pattern, subpleural linear opacity, consolidation, bulla, centrilobular emphysema, atelectasis, and bronchial wall thickening and entered these results into a data sheet independently. After evaluation, disagreement on the results between the observers for some HRCT scans was resolved by discussion and consensus.

Sample preparation and pathological study

Each tissue sample was serially cut into 3 μm-thickness sections and subjected to pathological study and EPMA-WDS analysis. For pathological study, formalin-fixed 3 μm serial sections were stained with hematoxylin-eosine and Elastica van Gieson method. Two pathologists (observers), who were blinded to clinical, laboratory, or pulmonary function test results, evaluated pathological findings. After evaluation, disagreement on the pathological diagnoses between the observers for some specimens was resolved by discussion and consensus.

Electron probe microanalysis

Examination of tissue sections with EMPA-WDS was performed according to procedures previously described.[11] X-ray data were

obtained with an EPMA-WDS (EPMA 8705, EPMA-1610, Shimadzu Ltd, Kyoto, Japan). In order to have representative element maps, we at first microscopically

scanned tissue specimens and looked for lesions of centrilobular fibrosis with low magnification because hard metal related elements, tungsten/cobalt were always found around centrilobular areas according to our experiences. For EMPA analysis, we at first screened areas of about 1.5 mm x 1.5 mm at largest covering centrilobular lesions or fibrosing lesion of interstitial lung diseases observed by pathological study to make rough element maps. Then we focused into areas from 5x5 to 10x10 μm at smallest to draw fine maps for elements. Each pixel in the focused areas in the tissue was scanned by three wavelength dispersive crystals; RAP, PET, and LiF for screening elements of Al, K, RAP; Si, K, PET; Ti, K, LiF; Cr, K, LiF; Fe, K, LiF; Co, K, LiF; Ta, M, PET; W, M, PET, and Zn, L, RAP. Since generated X-ray signals from each pixel were the smallest part of a distribution map, we simultaneously obtained element maps with qualitative analyses of pixels in the focused area. The distribution of amino nitrogen corresponding to the pathological image was also mapped for each sample.

Statistical analysis

Comparisons of categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by Mann-Whitney's U-test. A p Value <0.05 was considered significant.

RESULTS

Characteristics of subject

When we held the Tokyo ILD Meeting, 22 cases were collected and suspected to be

hard metal lung diseases due to occupational history and pathological findings, but 3 cases were excluded because tungsten or cobalt were not detected in the lung tissue. Nineteen patients were finally diagnosed as hard metal lung disease because of presence of tungsten in lung specimens detected by EPMA-WDS. In 4 of 19 patients, the presence of tungsten, cobalt, or tantalum was not known in the first place and proved by element analysis at the meeting.

Occupational history and clinical features are summarized in Table 1 and 2. Demographic findings in 6 of these patients have been reported previously (case 2, 5, 7, 8, 10, and 16 corresponding to case 1, 3, 5, 6, 14, and 16 in 2007 report, respectively).[7] All the subjects had an occupational history of hard metal industry for 1 to 36 years. One patient (case 15) was doing deskwork in an insufficiently ventilated room of a hard metal grinding company. Five patients had occupational history of hard metal industry but were not exposed at the diagnosis of hard metal lung disease. The delay between cessation of exposure and biopsy in the patients were 5 years, 4 months, 2 months, and 6 months for case 1, 2, 8, and 14, respectively. Case 10 had worked as a metal grinder for 6 years and then as a chimney cleaner at a copper mine for 32 years. He visited a hospital complaining of dry cough after 32-year work as a chimney cleaner and was finally diagnosed as hard metal lung diseases 4 years later by surgical biopsy. Five patients (case 2, 5, 7, 8, and 15) had an allergic history and were patch tested for Co, Ni, Cr, Hg, Au, Zn, Mn, Ag, Pd, Pt, Sn, Cu, Fe, Al, In, Ir, Ti. 4 of 5 patients who had undergone patch testing (case 2, 5, 7, and 15) were found to be positive for cobalt. Pulmonary function tests revealed restrictive lung defect

characterized by reduced vital capacity and lung diffusing capacity. BAL findings showed increased total cell counts, increased lymphocytes and eosinophils, with normal CD4/CD8 ratio. Bizarre multinucleated giant cells were noted in 3 patients.

Table 1. Demographic features of subjects

Case	Age	Sex	Smoking history	Occupational history (hard metal exposure)	Exposure (y/m) start/duration	Bx year	Exposure at Dx
1	39	M	non	Hard metal shaping/drilling	2000/12	2006	No
2	53	M	ex	Hard metal shaping/drilling	2002/30	2002	No
3	21	M	non	Metal grinding	2005/32	2008	Yes
4	42	M	ex	Hard metal shaping/drilling	2005/36	2009	Yes
5	48	M	non	Metal grinding	2000/48	2004	NA
6	45	M	non	Hard metal shaping/drilling	1982/60	1987	Yes
7	32	F	non	Metal grinding	1988/60	1993	Yes
8	32	F	non	Metal grinding	1997/72	2003	No
9	44	F	non	Hard metal shaping/drilling	1990/72	1996	Yes
10	62	M	non	Metal grinding	1963/72	2003	No
11	40	F	non	Hard metal shaping/drilling	1997/96	2005	NA
12	48	M	non	Metal grinding	1981/120	1992	NA
13	49	F	non	Hard metal shaping/drilling	1999/120	2009	Yes
14	65	F	non	Metal grinding	1988/144	2000	No
15	50	F	non	Desk worker in hard metal factory	1985/168	1996	Yes
16	53	M	non	Quality control of hard metals	1974/264	2001	NA
17	60	M	ex	Hard metal shaping/drilling	1972/276	1995	Yes
18	53	M	non	Hard metal shaping/drilling	1971/372	2005	Yes
19	65	M	non	Hard metal shaping/drilling	1963/444	2008	Yes

Abbreviation; Bx, biopsy; Dx, diagnosis; NA, not available.

Table 2. Clinical characteristics of Patients with Hard metal lung disease

		Value
Mean age at diagnosis (yrs)		46.4 ± 14.1 (21 - 65)
Gender	M/F	12/7
Smoking history	Cur/Ex/Never	0/3/16
Chief complaints	dry cough	13/19
	breath shortness	8/19
Pneumothorax	Yes	8/19
Allergic history	Yes	5/19
Patch test to cobalt	positive	4/5
Mean exposure duration (yrs)		10.7 ± 10.3 (1 - 36)
Physical findings	rales on auscultation	11/19
	fine crackles	8/19
	finger clubbing	4/18
	edema of leg	1/16
Laboratory tests	KL-6	502.7 ± 267.5 U/ml
	SP-D	216.1 ± 192.4 ng/ml
Pulmonary function tests		
	%VC	64.8 ± 25.3 %
	FEV_1%	85.6 ± 10.7 %
	%DLco	53.4 ± 17.0 %
Bronchoalveolar lavage		
	Total cell count	3.13 ± 2.11 $\times 10^5$ /ml
	Lymphocytes	24.3 ± 22.3 %
	Neutrophils	3.07 ± 2.86 %
	Eosinophils	3.01 ± 5.03 %
	CD4/8 ratio	1.65 ± 2.96

The mean numbers ± standard deviations and ranges in parentheses are shown.

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

Radiological findings

HRCT of all patients except one with hard metal lung disease were available for review of radiological findings. Conventional CT findings of case 12 were added to the table (Table 3). Centrilobular nodules (Fig 1 A, B) and ground glass opacity were identified in chest CT of 16 patients. In some patients, reticular opacities, traction bronchiectasis, and subpleural curvilinear opacities were also present (Fig 1 C, D). Although centrilobular micronodular opacities were noted in those patients, they were not predominant.

Table 3. Radiologic findings of patients with hard metal lung disease

	CT features				
Case	CL nodules	GGO	PTx	other findings	radiological diagnosis
1	+	-	-	bronchial wall thickening	bronchitis (DPB like)
2	+	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
3	+	+	+		subacute HP
4	+	-	+	subpleural curvilinear opacities	subacute HP
5	+	+	-		subacute HP
6	-	+	-	reticular opacities, consolidation	Interstitial pneumonia NOS
7	+	+	+		subacute HP
8	+	+	-	traction bronchiectasis	subacute HP
9	+	+	-		subacute HP
10	+	+	-	reticular opacities traction bronchiectasis	UIP
11	+	-	+		subacute HP
12	+	+	+	subpleural curvilinear opacities	chronic HP
13	+	+	-		subacute HP
14	+	+	-	traction bronchiectasis, apical cap	chronic HP
15	+	+	+	traction bronchiectasis	subacute HP
16	-	+	+	subpleural/peribronchovascular consolidation, atelectasis, bulla	upper lobe predominant IP or chronic IP NOS
17	+	+	-	bulla, centrilobular emphysema	UIP
18	-	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
19	+	+	-	reticular opacities	chronic HP

Abbreviation; CL, centrilobular; GGO, ground-glass opacities; PTx, pneumothorax; DPB, diffuse panbronchiolitis; IP, interstitial pneumonia; NOS, not otherwise specified; NSIP, non-specific interstitial pneumonia; UIP, usual interstitial pneumonia; HP, hypersensitivity pneumonitis

Pathological findings and elemental analysis

Pathological findings and detected elements in lung tissue of 19 cases were summarized in Table 4. Four major histological features noted in this study were as follows: GIP

characterized with centrilobular fibrosis (Fig 2 A, B) and characteristic giant cells showing cannibalism (Fig 2 C), centrilobular inflammation/fibrosis similar to GIP but without giant cells, UIP pattern characterized with patchy distribution and temporal heterogeneity, and dense fibrosis with fibroblastic foci (Fig 3 A, B, D, E, F) [12], upper lobe fibrosis characterized with apical scar/cap type fibrosis mainly in the upper lobe.[13] In the case of upper lobe fibrosis, biopsy specimen contained apical cap-like subpleural dense fibrosis which was composed of airspace fibrosis (intraluminar organization) with collapse and increased elastic framework. In autopsy taken 4 years later, we recognized remarkable subpleural elastosis with a few of cannibalistic giant cells.

Elemental analyses of lung specimens of GIP and centrilobular inflammation/fibrosis demonstrated that tungsten was mapped almost throughout the centrilobular fibrotic areas (Fig 2 D, E). Analyses of lung specimens of UIP pattern by EPMA-WDS revealed that tungsten and tantalum were distributed in periarteriolar area (Fig 4, D, E) and in subpleural fibrosis with dense acellular collagen (Fig 4 G, H, J, K). However, these elements were not accompanied by centrilobular inflammation/fibrosis (Fig 4, A, B). Lung histopathology in one case showed apical cap-like fibrosis with tungsten deposits detected in the fibrotic region but without GIP.[14] In total, elemental analysis by EPMA-WDS detected tungsten but no cobalt or tantalum in 10 patients, tungsten and cobalt in 5 patients, and tungsten and tantalum in 4 patients (Table 4).

Table 4. Pathological findings and elemental analysis of patients with hard metal lung disease

	sampling			elements detected		
Case	method	site(s)	pathological findings	W	Co	Ta
1	VATS	rt. S5/S8	centrilobular inflammation/fibrosis	+	-	-
2	VATS	lt. S2/S9	GIP	+	-	-
3	TBB/VATS	rt. apex	GIP	+	-	-
4	VATS	rt. S9	centrilobular inflammation/fibrosis	+	-	-
5	VATS	rt. S4/S9	GIP	+	-	-
6	Autopsy	NA	GIP, DAD	+	-	-
7	VATS	rt. S8	centrilobular inflammation/fibrosis	+	+	-
8	VATS	rt. S4/S6	GIP	+	-	+
9	VATS	rt. S2/S6	GIP	+	+	-
10	VATS	lt. S1+2/S10	UIP, GIP	+	-	+
11	VATS	lt. S1+2/S9	GIP	+	+	-
12	Autopsy	NA	GIP, DAD	+	-	-
13	VATS	lt. S1+2/S6	GIP	+	-	-
14	VATS	lt. S4/S9	GIP, UIP/NSIP?	+	-	+
15	VATS	rt. S6	GIP	+	+	-
16	VATS/autopsy	lt. S1+2/whole	upper lobe fibrosis	+	-	+
17	TBB/Lobectomy	-/RLL	UIP	+	-	-
18	VATS	lt. S1+2/S9	UIP	+	+	-
19	VATS	rt. S3/S10	UIP, centrilobular fibrosis	+	-	+

Abbreviation; TBB, trans-bronchial biopsy; VATS, video-assisted thoracic surgery; GIP, giant cell interstitial pneumonia; NA, not available; RLL, right lower lobectomy; DAD, diffuse alveolar damage; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia

Comparison of clinical features

We then classified the patients with hard metal lung disease into two groups according to their pathological findings. We grouped GIP and centrilobular inflammation/fibrosis together, because the latter pattern was considered to be a variant

of GIP due to the similar distribution of lesions. One patient was pathologically diagnosed as upper lobe fibrosis. It has such characteristic findings of subpleural, zonal, rather well defined fibrosis with small cysts and honeycomb lesions similar to that of UIP pattern that we grouped UIP pattern and upper lobe fibrosis together and named them the fibrosis group. We then compared clinical features between the GIP group and the fibrosis group. The GIP group was younger, had shorter exposure duration, lower serum KL-6, and higher lymphocyte percentage in BAL fluid compared with the fibrosis group (Table 5).

Table 5. Comparison of clinical features between GIP group and fibrosis group

	GIP group (n=14)	Fibrosis group (n=5)	p-value
Age (yrs)	43.1 ± 10.8	58.6 ± 5.41	0.007
Gender (M/F)	7/7	5/0	0.106
Exposure duration (months)	73.0 ± 48.8	285.6 ± 140.3	0.007
Pneumothorax (+/-)	6/8	2/3	1.000
KL-6 (U/ml)	398.7 ± 189.4	710.8 ± 297.7	0.023
SP-D (ng/ml)	260.3 ± 257.5	161.0 ± 54.75	0.903
PaO_2 (Torr)	84.3 ± 14.3	84.4 ± 11.2	0.922
$PaCO_2$ (Torr)	42.8 ± 2.75	56.0 ± 34.6	0.657
%VC (%)	64.4 ± 27.1	65.5 ± 24.1	0.734
FEV_1% (%)	85.4 ± 12.9	86.1 ± 2.62	0.910
%DLco (%)	50.8 ± 16.7	57.2 ± 18.8	0.371
Bronchoalveolar lavage			
Total cell count ($\times 10^5$/ml)	3.52 ± 2.41	2.26 ± 0.96	0.395
Lymphocytes (%)	31.5 ± 23.0	8.40 ± 9.08	0.015
CD4/8 ratio	.76 ± 0.51	3.22 ± 4.85	0.298

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

DISCUSSION

Pathological features of GIP are interstitial pneumonia with centrilobular fibrosis with multinucleated giant cells in the airspaces.[15] Sometimes centrilobular inflammation/fibrosis is only noted with few giant cells. EPMA-WDS analysis of lung tissue of hard metal lung disease demonstrated that tungsten was distributed in a relatively high concentration almost throughout the centrilobular fibrosis and in giant cells.[7] Comparison of distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements were associated with centrilobular inflammation/fibrosis by $CD163^{+}$ macrophages in cooperation with $CD8^{+}$ lymphocytes. Thus, centrilobular inflammation/fibrosis without giant cells should also be a variant of hard metal lung disease. GIP was also found in Belgian diamond polishers exposed not to hard metal dust, but to cobalt-containing dust, which confirmed that cobalt plays a dominant role in hard metal lung disease.[16] Cobalt is a well-known skin sensitizer, causing allergic contact dermatitis, and it can also cause occupational asthma.[17] Four patients were positive for patch testing for cobalt. Although such patch testing has been claimed to carry some risk of aggravation of disease in the situation with beryllium, cobalt is included in the routine metal allergy test panel and caused no worsening of hard metal lung disease. Hard metal lung disease cases show features of hypersensitivity pneumonitis (HP) with small interstitial granulomas, although well formed granulomas as in chronic beryllium disease are very rarely seen in the disease or HP. These data suggest that allergic inflammation may be different between hard metal lung disease/HP and berylliosis.

Respiratory symptoms of hard metal lung diseases sometimes improve on holidays and exacerbate during workdays, which resemble those of HP. Histopathology findings in HP may also include centrilobular fibrosis in association with isolated giant cells.[18] However, they do not show cannibalism as those in hard metal lung disease. BAL is the most sensitive tool to detect HP: a marked lymphocytosis with decreased CD4/8 ratio is characteristic of BAL findings.[19] BAL findings of patients with hard metal lung disease show increased total cell counts with increased lymphocytes and decreased CD4/CD8 ratio.[4, 20-22] Reduced CD4/8 ratio is consistent with the findings of immunohistochemistry in the previous study.[7] In this study, we found that lymphocyte percentage in BAL fluid was increased with rather low CD4/8 ratio in the GIP group, but they were not recognized in fibrosis group.

UIP pattern is the pathological abnormality associated with various restrictive lung diseases, including idiopathic pulmonary fibrosis (IPF). Interstitial inflammation and fibrosis in UIP pattern does not usually involve centrilobular area and peribronchioles. Three cases who were pathologically diagnosed as UIP pattern also had centrilobular micronodular opacities in HRCT findings. One patient was pathologically diagnosed as UIP pattern and centrilobular fibrosis. Element analysis of the deposition in lung tissues from patients with IPF/UIP usually demonstrates following elements; Si, Al, Fe, and Ti with various degrees (unpublished data). While we found tungsten accumulated in periarteriolar area and subpleural fibrosis in lung specimens of UIP pattern in this study. However, tungsten in periarteriolar area was hardly associated with any fibrosis or inflammatory cells. These results suggest that individual immune

susceptibility/response to inhaled hard metal elements may decide pathological patterns of UIP, GIP, or their mixture in varying degrees. Patients develop hard metal lung disease usually after mean exposure duration of more than 10 years. Although most studies have found no relation between disease occurrence and length of occupational exposure, individuals with increased susceptibility may develop hard metal lung disease after relatively short and low levels of exposure. The GIP group was younger and had shorter exposure duration suggesting that those who had UIP pattern were individuals with decreased susceptibility. Upper lobe fibrosis was pathologically diagnosed in one patient. Although it is significantly different from UIP pattern, tungsten in the fibrosis was not associated with inflammation around the element, either. With regard to the relationship between hard metal elements and surrounding inflammation, upper lobe fibrosis looks similar to UIP pattern in the other cases.

Liebow first described GIP as a form of idiopathic interstitial pneumonia.[23] It is now recognized that GIP is pathognomonic for hard metal lung disease.[24] Since tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals, the presence of tungsten and/or cobalt in BAL fluid or lung specimens leads to a definite diagnosis of hard metal lung disease. According to the results of elemental analyses in this study, five cases with UIP pattern or upper lobe fibrosis should be diagnosed as hard metal lung disease. The pathological findings of UIP pattern demonstrated no physical connection between centrilobular fibrosis and the UIP area, dense fibrosis with fibroblastic foci. Since centrilobular fibrosis is usually irreversible, if GIP evolved to UIP, sequels of centrilobular fibrosis would be somewhat

linked to peripheral UIP lesion. EPMA-WDS analyses of lung specimens of UIP pattern revealed that tungsten and tantalum in periarteriolar area were not accompanied by centrilobular inflammation/fibrosis as seen in typical GIP. In addition, clinical features of the fibrosis group were different from those of the GIP group. We identified tungsten in subpleural fibrosis with dense acellular collagen from UIP pattern and in the fibrotic region from apical cap-like fibrosis. Fibrotic reactions of these patients could have caused accumulation of hard metal particles as the scars contract and cut off lymphatic drainage. Those who are not sensitive to hard metal elements, particularly cobalt, might simply have idiopathic UIP or upper lobe fibrosis by accident as everyone with interstitial lung disease and a history of asbestos exposure does not have asbestosis.[25] However, microscopic findings of the lung specimen of UIP pattern included mild centrilobular inflammation and multinucleated giant cells with cannibalism, which could never been seen in idiopathic UIP/IPF. If we find tungsten or cobalt in the biopsies of UIP/fibrosis from the subjects who worked in the hard-metal industry, we cannot help but make a diagnosis of hard-metal lung disease. Given present information, we only conclude that the UIP/fibrosis may be induced by hard metal elements, or just a coincidence. Longitudinal data of the relative frequencies of incidence of the two diseases, hard metal lung disease and IPF, allow us to infer the likelihood of someone with hard metal exposure developing idiopathic UIP/IPF.

Hard metal lung disease is caused by exposure to cobalt and tungsten carbide. Toxicity stems from reactive oxygen species generation in a mechanism involving both elements in mutual contact.[26] Inhaled cobalt and tungsten carbides may cause lung

toxicity even in those who are less sensitive to those elements, which can result in lung fibrosis with GIP features. Qualitative elemental analysis of fibrosing lesion in GIP also demonstrated the presence of miscellaneous elements: Al, Si, Ti, Cr, and Fe, in addition to tungsten, cobalt, and/or Ta.[7] Several sources of evidence suggest that environmental agents may have an etiologic role in IPF. A meta-analysis of six case-control studies demonstrated that six exposures including cigarette smoking, agriculture/farming, livestock, wood dust, metal dust, and stone/sand were significantly associated with IPF.[27] Metal dust must contain various metal elements. In an EPMA analysis field of the lung biopsy specimen from upper lobe fibrosis, we found tungsten scattered throughout the fibrosis as well as aluminum, silicon, and titanium.[14] Miscellaneous metal dust inhaled in addition to tungsten and cobalt may cause UIP pattern in less sensitive individuals.

Acknowledgement

The authors thank the following doctors for the supply of cases: Dr. Y. Endo from Nagaoka Chuo General Hospital, Dr. M. Amano and Dr. S. Aoki from Showa General Hospital, Dr. T. Ishiguro from Gifu Municipal Hospital, Dr. M. Sakai from Saga Social Insurance Hospital, Dr. M. Tajiri from Kurume University, Dr T. Ishida from Niigata Prefectural Central Hospital, Dr. K. Koreeda from Minami Kyusyu National Hospital, Dr. K. Okuno from Kasai City Hospital, Dr. Y. Shimaoka from Nagaoka Red Cross Hospital, Dr. K. Kashiwada from Nippon Medical School, Dr. T. Sawada and Dr. A. Shiihara from Kanagawa Cardiovascular and Respiratory Center, Dr. K. Tachibana from

National Hospital Organization Kinki-chuo Chest Medical Center, Dr. T. Azuma from Shinshu University, Dr. K. Hara and Dr. T. Ishihara from NTT east corporation Kanto Medical Center, Dr. Y. Waseda from Kanazawa University, Dr. H. Ishii from Oita University, Dr. H. Matsuoka from Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dr. A Hara from Nagasaki University, Dr. O. Hisata from Tohoku University, and Dr. H. Tokuda from Social Insurance Chuo General Hospital. The authors also would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens.

References

1. Nemery B. Metal toxicity and the respiratory tract. *Eur Respir J*. 1990;**3**:202-19.

2. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environmental health perspectives*. 2000;**108 Suppl 4**:685-96.

3. Takada T, Moriyama H. Hard Metal Lung Disease. In: Huang Y-CT, Ghio AJ, Maier LA, editors. A Clinical Guide to Occupational and Environmental Lung Diseases Respiratory Medicine. New York: Springer; 2012. p. 217-30.

4. Davison AG, Haslam PL, Corrin B et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;**38**:119-28.

5. Cugell DW. The hard metal diseases. *Clin Chest Med*. 1992;**13**:269-79.

6. Anttila S, Sutinen S, Paananen M et al. Hard metal lung disease: a clinical, histological, ultrastructural and X-ray microanalytical study. *Eur J Respir Dis*. 1986;**69**:83-94.

7. Moriyama H, Kobayashi M, Takada T et al. Two-dimensional analysis of elements and mononuclear cells in hard metal lung disease. *Am J Respir Crit Care Med*. 2007;**176**:70-7.

8. Ohori NP, Sciurba FC, Owens GR et al. Giant-cell interstitial pneumonia and hard-metal pneumoconiosis. A clinicopathologic study of four cases and review of the literature. *Am J Surg Pathol*. 1989;**13**:581-7.

9. Travis WD, Matsui K, Moss J et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with

usual interstitial pneumonia and desquamative interstitial pneumonia. *Am J Surg Pathol*. 2000;**24**:19-33.

10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;**157**:1301-15.

11. Moriyama H, Yamamoto T, Takatsuka H et al. Expression of macrophage colony-stimulating factor and its receptor in hepatic granulomas of Kupffer-cell-depleted mice. *Am J Pathol*. 1997;**150**:2047-60.

12. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000;**161**:646-64.

13. Shiota S, Shimizu K, Suzuki M et al. [Seven cases of marked pulmonary fibrosis in the upper lobe]. *Nihon Kokyuki Gakkai Zasshi*. 1999;**37**:87-96.

14. Kaneko Y, Kikuchi N, Ishii Y et al. Upper lobe-dominant pulmonary fibrosis showing deposits of hard metal component in the fibrotic lesions. *Intern Med*. 2010;**49**:2143-5.

15. Naqvi AH, Hunt A, Burnett BR et al. Pathologic spectrum and lung dust burden in giant cell interstitial pneumonia (hard metal disease/cobalt pneumonitis): review of 100 cases. *Arch Environ Occup Health*. 2008;**63**:51-70.

16. Demedts M, Gheysens B, Nagels J et al. Cobalt lung in diamond polishers. *The American review of respiratory disease*. 1984;**130**:130-5.

17. Nemery B, Verbeken EK, Demedts M. Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung). *Semin Respir Crit Care Med*. 2001;**22**:435-48.

18. Churg A, Muller NL, Flint J et al. Chronic hypersensitivity pneumonitis. *Am J Surg Pathol*. 2006;**30**:201-8.

19. D'Ippolito R, Chetta A, Foresi A et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med*. 2004;**98**:977-83.

20. Okuno K, Kobayashi K, Kotani Y et al. A case of hard metal lung disease resembling a hypersensitive pneumonia in radiological images. *Intern Med*. 2010;**49**:1185-9.

21. Kakugawa T, Mukae H, Nagata T et al. Giant cell interstitial pneumonia in a 15-year-old boy. *Intern Med*. 2002;**41**:1007-12.

22. Forni A. Bronchoalveolar lavage in the diagnosis of hard metal disease. *Sci Total Environ*. 1994;**150**:69-76.

23. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. *Prog Respir Res*. 1975:1-33.

24. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc*. 1991;**5**:95-104; discussion 5-8.

25. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. *The American review of respiratory disease*. 1991;**144**:689-96.

26. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environmental health perspectives*. 1997;**105 Suppl 5**:1013-20.

27. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease?

Proc Am Thorac Soc. 2006;**3**:293-8.

FIGURE LEGENDS

Figure 1

High-resolution computed tomography of the chest illustrating differences in the radiographic appearance of the lungs in giant cell interstitial pneumonia (GIP) and in usual interstitial pneumonia (UIP) pattern. (A, B) In GIP of case 9, centriolobular micronodular opacities pathologically correspond to centrilobular fibrosis and giant cell accumulation within the alveolar space. (C, D) In UIP pattern of case 10, reticular opacities and traction bronchiectasis are present with centriolobular micronodular opacities.

Figure 2

Representative images of light microscopic findings and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of S6 specimen from case 9 pathologically diagnosed as giant cell interstitial pneumonia. (A, B, and C) The black square area in centrilobular fibrosis is stepwise magnified to show multinucleated giant cells with cannibalism. (A, D) The green square area in subpleural zone is elementally analyzed by EPMA-WDS to show (E) many orange spots corresponding to tungsten. A qualitative colored image of tungsten distribution is superimposed onto a lung tissue image of amino nitrogen colored green. Note that tungsten is widely distributed in centrilobular fibrosis as well as surrounding alveolar walls. Original magnification, (A) panoramic view, (B) x 4, (C) x 60, and (D) x 8.

Figure 3

Representative images of light microscopic findings of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern. (A, B) A low magnification view of left S1+2 specimen demonstrates a combination of patchy interstitial fibrosis with alternating areas of normal lung and architectural alteration due to chronic scarring or honeycomb change. Note that there are several small bronchioles with mild centrilobular inflammation (blue arrows). (B, C) Multinucleated giant cells with cannibalism are also shown in a stepwise-magnified black square area located in subpleural fibrosis. (D, E, F) Left S10 specimen from the same patient also shows characteristic fibroblastic foci (black arrows) in the background of dense acellular collagen in a stepwise-magnified square area located in subpleural fibrosis. Original magnification, (A, D) panoramic view, (B) x 2, (C) x 40, (E) x 4 and (F) x 20.

Figure 4

Representative images of light micrographs and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern (A). (B, C) An arteriole and its surrounding interstitium (orange square) are elementally analyzed by EPMA-WDS to demonstrate that (D) tungsten and (E) tantalum are distributed in periarteriolar area with little fibrosis. Elemental analysis by EPMA-WDS of subpleural fibrosis with dense acellular collagen (green square in B, F,

I) also shows (G, J) tungsten and (H, K) tantalum almost randomly distributed in magnified images (yellow squares in G and H are magnified to show (J) tungsten and (K) tantalum). We did not further analyze the centrilobular pattern or the cannibalistic giant cells shown in Fig 3. Note that the distribution of tungsten is not completely the same as that of tantalum. Original magnification, (A) panoramic view and (B) x 4. Scale bars for the magnification and scan areas for (E), (H), and (K) correspond to 100μm (0.768 x 0.768 mm), 200μm (1.536 x 1.536 mm), and 25μm (0.1792 x 0.1792 mm), respectively.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract **p. 1, 3-4**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale p. 5	2	Explain the scientific background and rationale for the investigation being reported
Objectives, p. 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design, p.6	4	Present key elements of study design early in the paper
Setting, p.6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants, p.6	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables, p.6	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement, p.6-8	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias, p.6	9	Describe any efforts to address potential sources of bias
Study size, p. 8, 9	10	Explain how the study size was arrived at
Quantitative variables, p. 18	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods, p. 8	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants, p. 8, 9	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data, p. 10	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data, p. 12	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results, p. 13, 14	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses, p. 18	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results, p. 15, 16	18	Summarise key results with reference to study objectives
Limitations, p. 18	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation, p.17, 18	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability, p 18	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding NA	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

An Observational Study of Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004407.R2
Article Type:	Research
Date Submitted by the Author:	08-Mar-2014
Complete List of Authors:	Tanaka, Junichi; Niigata University, Division of Respiratory Medicine Moriyama, Hiroshi; Niigata University, Division of Respiratory Medicine Terada, Masaki; Niigata University, Division of Respiratory Medicine Takada, Toshinori; Niigata University, Division of Respiratory Medicine Suzuki, Eiichi; Niigata University Medical and Dental Hospital, Department of General Medicine Narita, Ichiei; Niigata University, Division of Respiratory Medicine Kawabata, Yoshinori; Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Yamaguchi, Tetsuo; Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Hebisawa, Akira; Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Sakai, Fumikazu; Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Arakawa, Hiroaki; Tokyo Research Group for Diffuse Parenchymal Lung Diseases,
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Occupational and environmental medicine
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, Thoracic medicine < INTERNAL MEDICINE, Interstitial lung disease < THORACIC MEDICINE

An Observational Study of Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

[1]Junichi Tanaka, MD, [1]Hiroshi Moriyama, MD, [1]Masaki Terada, MD, [1, 2]Toshinori Takada, MD, [3]Eiichi Suzuki, MD, [1]Ichiei Narita, MD, [4]Yoshinori Kawabata, MD, [4]Tetsuo Yamaguchi, MD, [4]Akira Hebisawa, MD, [4]Fumikazu Sakai, MD, and [4]Hiroaki Arakawa, MD,

[1]Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital [3]Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan, [4]Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Tokyo, Japan

Corresponding author: Toshinori Takada, M.D., PhD

Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University

1-757 Asahimachi-dori, Chuo-ku, Niigata, 951-8510, Japan

Tel; +81-25-227-2200, Fax; +81-25-227-0775, Email; ttakada@med.niigata-u.ac.jp

Keywords: hard metal, pulmonary fibrosis, electron probe microanalysis

Word count: 2,921

ABSTRACT

Objectives: Hard metal lung disease has various pathological patterns including giant cell interstitial pneumonia (GIP) and usual interstitial pneumonia (UIP). Although UIP pattern is considered the prominent feature in advanced disease, it is unknown whether GIP finally progresses to UIP pattern. The aim of our study is to clarify clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Setting: A cross-sectional study for patients of 17 institutes participating in the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases, 2009.

Participants: Nineteen patients with 7 females diagnosed as hard metal lung disease by the presence of tungsten in lung specimens.

Primary and secondary outcome measures: Clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Results: Fourteen cases were pathologically diagnosed as GIP or centrilobular inflammation/fibrosing. The other five cases were UIP pattern or upper lobe fibrosis. Elemental analyses of lung specimens of GIP showed tungsten throughout the centrilobular fibrotic areas. In UIP pattern, tungsten was detected in periarteriolar area and subpleural fibrosis in no association with centrilobular fibrosis or inflammatory cell infiltration. The GIP group was younger (43.1 vs 58.6 yrs) with shorter exposure duration (73 vs 285 months) ($p<0.01$), lower serum KL-6 (398 vs 710 U/ml), and higher lymphocyte percentage in bronchoalveolar lavage fluid (31.5 vs 3.22 %) ($p<0.05$) than

the fibrosis group.

Conclusions: UIP pattern or upper lobe fibrosis is remarkably different from GIP in distribution of hard metal elements, associated interstitial inflammation and fibrosis, and clinical features. In hard metal lung disease, UIP pattern or upper lobe fibrosis may not be an advanced form of GIP.

Strengths and limitations of this study

1, Nineteen cases of hard metal lung disease, a rare occupational lung disease, were collected and their clinical features were documented.

2, Lung tissue from all the patients was elementally analyzed by a patented technique, an improved element analysis using electron probe microanalyzers with wavelength dispersive spectrometer.

3, Since the incidences of hard metal lung disease and IPF in potentially exposed populations and in the general population are unknown, the probability that someone with hard metal exposure will develop "idiopathic" UIP/IPF is also unknown.

INTRODUCTION

Hard metal is a synthetic compound that combines tungsten carbide with cobalt. Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, or interstitial lung disease which is recognized as hard metal lung disease.[1-3] In many cases with hard metal lung disease, multinucleated giant cells with centrilobular fibrosis are prominent resulting in a pattern of giant cell interstitial pneumonia (GIP).[4-6] We demonstrated that hard metal accumulated in the centrilobular area may trigger the inflammation in cooperation with $CD163^+$ monocyte-macrophages and $CD8^+$ lymphocytes using electron probe microanalyzers with wavelength dispersive spectrometer (EPMA-WDS).[7] In addition to classical GIP, hard metal lung disease has a variety of pathological patterns, desquamative interstitial pneumonia, obliterative bronchiolitis, and usual interstitial pneumonia (UIP) pattern.[4, 8] The lesions of classical GIP are usually centered on the centrilobular areas. On the other hand, the key histologic features of UIP are predominantly distributed at the periphery of the acinus or lobule.[9, 10] Hard metal lung disease has pathological patterns of both GIP and UIP, and the UIP pattern is thought to be the prominent feature in advanced cases of the disease.[8] The key question is whether UIP pattern is an advanced form of GIP or not. In order to elucidate relationship between GIP and lung fibrosis with detection of hard metal elements, we collected cases with tungsten in lung tissue and reviewed their clinical records. We then elementally reexamined lung specimens by EPMA-WDS. We finally classified the patients into two groups according to the histological findings and

statistically compared their clinical features. Pathological and elemental analyses in the study suggest that UIP pattern or upper lobe fibrosis may be different from an end-stage form of GIP.

METHODS

Patient population

We collected patients by announcing inquiry for cases of hard metal lung disease to the major medical institutes and hospitals all over Japan for the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases, 2009. We obtained information of patient profile such as age, gender, duration of hard metal exposure, history of pneumothorax, history of allergy, symptoms, physical findings, serum levels of Krebs von den Lungen-6 (KL-6) and SP-D, arterial blood gas data, pulmonary function tests, bronchoalveolar lavage (BAL) cell profiles and treatment and prognosis in order to make a data base. We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

HRCT scan findings

All patients with hard metal lung disease except one had undergone high-resolution computed tomography (HRCT) scanning. Two radiologists (observers) who were blinded to clinical, laboratory, or pulmonary function test results evaluated CT scan

findings. The observers judged each CT scan for the presence or absence of three main features of centrilobular nodules, ground glass opacity, and pneumothorax. They also noted other remarkable findings; traction bronchiectasis, reticular pattern, subpleural linear opacity, consolidation, bulla, centrilobular emphysema, atelectasis, and bronchial wall thickening and entered these results into a data sheet independently. After evaluation, disagreement on the results between the observers for some HRCT scans was resolved by discussion and consensus.

Sample preparation and pathological study

Each tissue sample was serially cut into 3 μm-thickness sections and subjected to pathological study and EPMA-WDS analysis. For pathological study, formalin-fixed 3 μm serial sections were stained with hematoxylin-eosine and Elastica van Gieson method. Two pathologists (observers), who were blinded to clinical, laboratory, or pulmonary function test results, evaluated pathological findings. After evaluation, disagreement on the pathological diagnoses between the observers for some specimens was resolved by discussion and consensus.

Electron probe microanalysis

Examination of tissue sections with EMPA-WDS was performed according to procedures previously described.[11] X-ray data were obtained with an EPMA-WDS (EPMA 8705, EPMA-1610, Shimadzu Ltd, Kyoto, Japan). In order to have representative element maps, we at first microscopically scanned tissue specimens and

looked for lesions of centrilobular fibrosis with low magnification because hard metal related elements, tungsten/cobalt were always found around centrilobular areas according to our experiences. For EMPA analysis, we at first screened areas of about 1.5 mm x 1.5 mm at largest covering centrilobular lesions or fibrosing lesion of interstitial lung diseases observed by pathological study to make rough element maps. Then we focused into areas from 5x5 to 10x10 μm at smallest to draw fine maps for elements. Each pixel in the focused areas in the tissue was scanned by three wavelength dispersive crystals; RAP, PET, and LiF for screening elements of Al, K, RAP; Si, K, PET; Ti, K, LiF; Cr, K, LiF; Fe, K, LiF; Co, K, LiF; Ta, M, PET; W, M, PET, and Zn, L, RAP. Since generated X-ray signals from each pixel were the smallest part of a distribution map, we simultaneously obtained element maps with qualitative analyses of pixels in the focused area. The distribution of amino nitrogen corresponding to the pathological image was also mapped for each sample.

Statistical analysis

Comparisons of categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by Mann-Whitney's U-test. A p Value <0.05 was considered significant.

RESULTS

Characteristics of subject

When we held the Tokyo ILD Meeting, 22 cases were collected and suspected to be

hard metal lung diseases due to occupational history and pathological findings, but 3 cases were excluded because tungsten or cobalt were not detected in the lung tissue. Nineteen patients were finally diagnosed as hard metal lung disease because of presence of tungsten in lung specimens detected by EPMA-WDS. In 4 of 19 patients, the presence of tungsten, cobalt, or tantalum was not known in the first place and proved by element analysis at the meeting.

Occupational history and clinical features are summarized in Table 1 and 2. Demographic findings in 6 of these patients have been reported previously (case 2, 5, 7, 8, 10, and 16 corresponding to case 1, 3, 5, 6, 14, and 16 in 2007 report, respectively).[7] All the subjects had an occupational history of hard metal industry for 1 to 36 years. One patient (case 15) was doing deskwork in an insufficiently ventilated room of a hard metal grinding company. Five patients had occupational history of hard metal industry but were not exposed at the diagnosis of hard metal lung disease. The delay between cessation of exposure and biopsy in the patients were 5 years, 4 months, 2 months, and 6 months for case 1, 2, 8, and 14, respectively. Case 10 had worked as a metal grinder for 6 years and then as a chimney cleaner at a copper mine for 32 years. He visited a hospital complaining of dry cough after 32-year work as a chimney cleaner and was finally diagnosed as hard metal lung diseases 4 years later by surgical biopsy. Five patients (case 2, 5, 7, 8, and 15) had an allergic history and were patch tested for Co, Ni, Cr, Hg, Au, Zn, Mn, Ag, Pd, Pt, Sn, Cu, Fe, Al, In, Ir, Ti. 4 of 5 patients who had undergone patch testing (case 2, 5, 7, and 15) were found to be positive for cobalt. Pulmonary function tests revealed restrictive lung defect

characterized by reduced vital capacity and lung diffusing capacity. BAL findings showed increased total cell counts, increased lymphocytes and eosinophils, with normal CD4/CD8 ratio. Bizarre multinucleated giant cells were noted in 3 patients.

Table 1. Demographic features of subjects

Case	Age	Sex	Smoking history	Occupational history (hard metal exposure)	Exposure (y/m) start/duration	Bx year	Exposure at Dx
1	39	M	non	Hard metal shaping/drilling	2000/12	2006	No
2	53	M	ex	Hard metal shaping/drilling	2002/30	2002	No
3	21	M	non	Metal grinding	2005/32	2008	Yes
4	42	M	ex	Hard metal shaping/drilling	2005/36	2009	Yes
5	48	M	non	Metal grinding	2000/48	2004	NA
6	45	M	non	Hard metal shaping/drilling	1982/60	1987	Yes
7	32	F	non	Metal grinding	1988/60	1993	Yes
8	32	F	non	Metal grinding	1997/72	2003	No
9	44	F	non	Hard metal shaping/drilling	1990/72	1996	Yes
10	62	M	non	Metal grinding	1963/72	2003	No
11	40	F	non	Hard metal shaping/drilling	1997/96	2005	NA
12	48	M	non	Metal grinding	1981/120	1992	NA
13	49	F	non	Hard metal shaping/drilling	1999/120	2009	Yes
14	65	F	non	Metal grinding	1988/144	2000	No
15	50	F	non	Desk worker in hard metal factory	1985/168	1996	Yes
16	53	M	non	Quality control of hard metals	1974/264	2001	NA
17	60	M	ex	Hard metal shaping/drilling	1972/276	1995	Yes
18	53	M	non	Hard metal shaping/drilling	1971/372	2005	Yes
19	65	M	non	Hard metal shaping/drilling	1963/444	2008	Yes

Abbreviation; Bx, biopsy; Dx, diagnosis; NA, not available.

Table 2. Clinical characteristics of Patients with Hard metal lung disease

		Value
Mean age at diagnosis (yrs)		46.4 ± 14.1 (21 - 65)
Gender	M/F	12/7
Smoking history	Cur/Ex/Never	0/3/16
Chief complaints	dry cough	13/19
	breath shortness	8/19
Pneumothorax	Yes	8/19
Allergic history	Yes	5/19
Patch test to cobalt	positive	4/5
Mean exposure duration (yrs)		10.7 ± 10.3 (1 - 36)
Physical findings	rales on auscultation	11/19
	fine crackles	8/19
	finger clubbing	4/18
	edema of leg	1/16
Laboratory tests	KL-6	502.7 ± 267.5 U/ml
	SP-D	216.1 ± 192.4 ng/ml
Pulmonary function tests		
	VC, % predicted	64.8 ± 25.3 %
	FEV_1	1.71 ± 0.70 L
	FEV_1/FVC	85.6 ± 10.7 %
	DLco, % predicted	53.4 ± 17.0 %
Bronchoalveolar lavage		
	Total cell count	3.13 ± 2.11 $\times 10^5$ /ml
	Lymphocytes	24.3 ± 22.3 %
	Neutrophils	3.07 ± 2.86 %
	Eosinophils	3.01 ± 5.03 %
	CD4/8 ratio	1.65 ± 2.96

The mean numbers ± standard deviations and ranges in parentheses are shown.

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

Radiological findings

HRCT of all patients except one with hard metal lung disease were available for review of radiological findings. Conventional CT findings of case 12 were added to the table (Table 3). Centrilobular nodules (Fig 1 A, B) and ground glass opacity were identified in chest CT of 16 patients. In some patients, reticular opacities, traction bronchiectasis, and subpleural curvilinear opacities were also present (Fig 1 C, D). Although centrilobular micronodular opacities were noted in those patients, they were not predominant.

Table 3. Radiologic findings of patients with hard metal lung disease

	CT features				
Case	CL nodules	GGO	PTx	other findings	radiological diagnosis
1	+	-	-	bronchial wall thickening	bronchitis (DPB like)
2	+	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
3	+	+	+		subacute HP
4	+	-	+	subpleural curvilinear opacities	subacute HP
5	+	+	-		subacute HP
6	-	+	-	reticular opacities, consolidation	Interstitial pneumonia NOS
7	+	+	+		subacute HP
8	+	+	-	traction bronchiectasis	subacute HP
9	+	+	-		subacute HP
10	+	+	-	reticular opacities traction bronchiectasis	UIP
11	+	-	+		subacute HP
12	+	+	+	subpleural curvilinear opacities	chronic HP
13	+	+	-		subacute HP
14	+	+	-	traction bronchiectasis, apical cap	chronic HP
15	+	+	+	traction bronchiectasis	subacute HP
16	-	+	+	subpleural/peribronchovascular consolidation, atelectasis, bulla	upper lobe predominant IP or chronic IP NOS
17	+	+	-	bulla, centrilobular emphysema	UIP
18	-	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
19	+	+	-	reticular opacities	chronic HP

Abbreviation; CL, centrilobular; GGO, ground-glass opacities; PTx, pneumothorax; DPB, diffuse panbronchiolitis; IP, interstitial pneumonia; NOS, not otherwise specified; NSIP, non-specific interstitial pneumonia; UIP, usual interstitial pneumonia; HP, hypersensitivity pneumonitis

Pathological findings and elemental analysis

Pathological findings and detected elements in lung tissue of 19 cases were summarized

in Table 4. Four major histological features noted in this study were as follows: GIP characterized with centrilobular fibrosis (Fig 2 A, B) and characteristic giant cells showing cannibalism (Fig 2 C), centrilobular inflammation/fibrosis similar to GIP but without giant cells, UIP pattern characterized with patchy distribution and temporal heterogeneity, and dense fibrosis with fibroblastic foci (Fig 3 A, B, D, E, F) [12], upper lobe fibrosis characterized with apical scar/cap type fibrosis mainly in the upper lobe.[13] In the case of upper lobe fibrosis, biopsy specimen contained apical cap-like subpleural dense fibrosis which was composed of airspace fibrosis (intraluminar organization) with collapse and increased elastic framework. In autopsy taken 4 years later, we recognized remarkable subpleural elastosis with a few of cannibalistic giant cells.

Elemental analyses of lung specimens of GIP and centrilobular inflammation/fibrosis demonstrated that tungsten was mapped almost throughout the centrilobular fibrotic areas (Fig 2 D, E). Analyses of lung specimens of UIP pattern by EPMA-WDS revealed that tungsten and tantalum were distributed in periarteriolar area (Fig 4, D, E) and in subpleural fibrosis with dense acellular collagen (Fig 4 G, H, J, K). However, these elements were not accompanied by centrilobular inflammation/fibrosis (Fig 4, A, B). Lung histopathology in one case showed apical cap-like fibrosis with tungsten deposits detected in the fibrotic region but without GIP.[14] In total, elemental analysis by EPMA-WDS detected tungsten but no cobalt or tantalum in 10 patients, tungsten and cobalt in 5 patients, and tungsten and tantalum in 4 patients (Table 4).

Table 4. Pathological findings and elemental analysis of patients with hard metal lung disease

	sampling			elements detected		
Case	method	site(s)	pathological findings	W	Co	Ta
1	VATS	rt. S5/S8	centrilobular inflammation/fibrosis	+	-	-
2	VATS	lt. S2/S9	GIP	+	-	-
3	TBB/VATS	rt. apex	GIP	+	-	-
4	VATS	rt. S9	centrilobular inflammation/fibrosis	+	-	-
5	VATS	rt. S4/S9	GIP	+	-	-
6	Autopsy	NA	GIP, DAD	+	-	-
7	VATS	rt. S8	centrilobular inflammation/fibrosis	+	+	-
8	VATS	rt. S4/S6	GIP	+	-	+
9	VATS	rt. S2/S6	GIP	+	+	-
10	VATS	lt. S1+2/S10	UIP, GIP	+	-	+
11	VATS	lt. S1+2/S9	GIP	+	+	-
12	Autopsy	NA	GIP, DAD	+	-	-
13	VATS	lt. S1+2/S6	GIP	+	-	-
14	VATS	lt. S4/S9	GIP, UIP/NSIP?	+	-	+
15	VATS	rt. S6	GIP	+	+	-
16	VATS/autopsy	lt. S1+2/whole	upper lobe fibrosis	+	-	+
17	TBB/Lobectomy	-/RLL	UIP	+	-	-
18	VATS	lt. S1+2/S9	UIP	+	+	-
19	VATS	rt. S3/S10	UIP, centrilobular fibrosis	+	-	+

Abbreviation; TBB, trans-bronchial biopsy; VATS, video-assisted thoracic surgery; GIP, giant cell interstitial pneumonia; NA, not available; RLL, right lower lobectomy; DAD, diffuse alveolar damage; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia

Comparison of clinical features

We then classified the patients with hard metal lung disease into two groups according to their pathological findings. We grouped GIP and centrilobular inflammation/fibrosis together, because the latter pattern was considered to be a variant

of GIP due to the similar distribution of lesions. One patient was pathologically diagnosed as upper lobe fibrosis. It has such characteristic findings of subpleural, zonal, rather well defined fibrosis with small cysts and honeycomb lesions similar to that of UIP pattern that we grouped UIP pattern and upper lobe fibrosis together and named them the fibrosis group. We then compared clinical features between the GIP group and the fibrosis group. The GIP group was younger, had shorter exposure duration, lower serum KL-6, and higher lymphocyte percentage in BAL fluid compared with the fibrosis group (Table 5).

Table 5. Comparison of clinical features between GIP group and fibrosis group

	GIP group (n=14)	Fibrosis group (n=5)	p-value
Age (yrs)	43.1 ± 10.8	58.6 ± 5.41	0.007
Gender (M/F)	7/7	5/0	0.106
Exposure duration (months)	73.0 ± 48.8	285.6 ± 140.3	0.007
Pneumothorax (+/-)	6/8	2/3	1.000
KL-6 (U/ml)	398.7 ± 189.4	710.8 ± 297.7	0.023
SP-D (ng/ml)	260.3 ± 257.5	161.0 ± 54.75	0.903
PaO_2 (Torr)	84.3 ± 14.3	84.4 ± 11.2	0.922
$PaCO_2$ (Torr)	42.8 ± 2.75	56.0 ± 34.6	0.657
VC, % predicted (%)	64.4 ± 27.1	65.5 ± 24.1	0.734
FEV_1 (L)	1.63 ± 0.23	1.88 ± 0.32	0.537
FEV_1/FVC (%)	85.4 ± 12.9	86.1 ± 2.62	0.910
DLco, % predicted (%)	50.8 ± 16.7	57.2 ± 18.8	0.371
Bronchoalveolar lavage			
Total cell count ($\times 10^5$/ml)	3.52 ± 2.41	2.26 ± 0.96	0.395
Lymphocytes (%)	31.5 ± 23.0	8.40 ± 9.08	0.015
CD4/8 ratio	.76 ± 0.51	3.22 ± 4.85	0.298

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

DISCUSSION

Pathological features of GIP are interstitial pneumonia with centrilobular fibrosis with multinucleated giant cells in the airspaces.[15] Sometimes centrilobular inflammation/fibrosis is only noted with few giant cells. EPMA-WDS analysis of lung tissue of hard metal lung disease demonstrated that tungsten was distributed in a relatively high concentration almost throughout the centrilobular fibrosis and in giant cells.[7] Comparison of distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements were associated with centrilobular inflammation/fibrosis by $CD163^+$ macrophages in cooperation with $CD8^+$ lymphocytes. Thus, centrilobular inflammation/fibrosis without giant cells should also be a variant of hard metal lung disease. GIP was also found in Belgian diamond polishers exposed not to hard metal dust, but to cobalt-containing dust, which confirmed that cobalt plays a dominant role in hard metal lung disease.[16] Cobalt is a well-known skin sensitizer, causing allergic contact dermatitis, and it can also cause occupational asthma.[17] Four patients were positive for patch testing for cobalt. Although such patch testing has been claimed to carry some risk of aggravation of disease in the situation with beryllium, cobalt is included in the routine metal allergy test panel and caused no worsening of hard metal lung disease. Hard metal lung disease cases show features of hypersensitivity pneumonitis (HP) with small interstitial granulomas, although well formed granulomas as in chronic beryllium disease are very rarely seen in the disease or HP. These data

suggest that allergic inflammation may be different between hard metal lung disease/HP and berylliosis.

Respiratory symptoms of hard metal lung diseases sometimes improve on holidays and exacerbate during workdays, which resemble those of HP. Histopathology findings in HP may also include centrilobular fibrosis in association with isolated giant cells.[18] However, they do not show cannibalism as those in hard metal lung disease. BAL is the most sensitive tool to detect HP: a marked lymphocytosis with decreased CD4/8 ratio is characteristic of BAL findings.[19] BAL findings of patients with hard metal lung disease show increased total cell counts with increased lymphocytes and decreased CD4/CD8 ratio.[4, 20-22] Reduced CD4/8 ratio is consistent with the findings of immunohistochemistry in the previous study.[7] In this study, we found that lymphocyte percentage in BAL fluid was increased with rather low CD4/8 ratio in the GIP group, but they were not recognized in fibrosis group.

UIP pattern is the pathological abnormality associated with various restrictive lung diseases, including idiopathic pulmonary fibrosis (IPF). Interstitial inflammation and fibrosis in UIP pattern does not usually involve centrilobular area and peribronchioles. Three cases who were pathologically diagnosed as UIP pattern also had centrilobular micronodular opacities in HRCT findings. One patient was pathologically diagnosed as UIP pattern and centrilobular fibrosis. Element analysis of the deposition in lung tissues from patients with IPF/UIP usually demonstrates following elements; Si, Al, Fe, and Ti with various degrees (unpublished data). While we found tungsten accumulated in periarteriolar area and subpleural fibrosis in lung specimens of UIP pattern in this

study. However, tungsten in periarteriolar area was hardly associated with any fibrosis or inflammatory cells. These results suggest that individual immune susceptibility/response to inhaled hard metal elements may decide pathological patterns of UIP, GIP, or their mixture in varying degrees. Patients develop hard metal lung disease usually after mean exposure duration of more than 10 years. Although most studies have found no relation between disease occurrence and length of occupational exposure, individuals with increased susceptibility may develop hard metal lung disease after relatively short and low levels of exposure. The GIP group was younger and had shorter exposure duration suggesting that those who had UIP pattern were individuals with decreased susceptibility. Upper lobe fibrosis was pathologically diagnosed in one patient. Although it is significantly different from UIP pattern, tungsten in the fibrosis was not associated with inflammation around the element, either. With regard to the relationship between hard metal elements and surrounding inflammation, upper lobe fibrosis looks similar to UIP pattern in the other cases.

Liebow first described GIP as a form of idiopathic interstitial pneumonia.[23] It is now recognized that GIP is pathognomonic for hard metal lung disease.[24] Since tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals, the presence of tungsten and/or cobalt in BAL fluid or lung specimens leads to a definite diagnosis of hard metal lung disease. According to the results of elemental analyses in this study, five cases with UIP pattern or upper lobe fibrosis should be diagnosed as hard metal lung disease. The pathological findings of UIP pattern demonstrated no physical connection between centrilobular fibrosis and the

UIP area, dense fibrosis with fibroblastic foci. Since centrilobular fibrosis is usually irreversible, if GIP evolved to UIP, sequels of centrilobular fibrosis would be somewhat linked to peripheral UIP lesion. EPMA-WDS analyses of lung specimens of UIP pattern revealed that tungsten and tantalum in periarteriolar area were not accompanied by centrilobular inflammation/fibrosis as seen in typical GIP. In addition, clinical features of the fibrosis group were different from those of the GIP group. We identified tungsten in subpleural fibrosis with dense acellular collagen from UIP pattern and in the fibrotic region from apical cap-like fibrosis. Fibrotic reactions of these patients could have caused accumulation of hard metal particles as the scars contract and cut off lymphatic drainage. Those who are not sensitive to hard metal elements, particularly cobalt, might simply have idiopathic UIP or upper lobe fibrosis by accident as everyone with interstitial lung disease and a history of asbestos exposure does not have asbestosis.[25] However, microscopic findings of the lung specimen of UIP pattern included mild centrilobular inflammation and multinucleated giant cells with cannibalism, which could never been seen in idiopathic UIP/IPF. If we find tungsten or cobalt in the biopsies of UIP/fibrosis from the subjects who worked in the hard-metal industry, we cannot help but make a diagnosis of hard-metal lung disease. Given present information, we only conclude that the UIP/fibrosis may be induced by hard metal elements, or just a coincidence. Since the incidences of hard metal lung disease and IPF in potentially exposed populations and in the general population are unknown, the probability that someone with hard metal exposure will develop "idiopathic" UIP/IPF is also unknown.

Hard metal lung disease is caused by exposure to cobalt and tungsten carbide. Toxicity stems from reactive oxygen species generation in a mechanism involving both elements in mutual contact.[26] Inhaled cobalt and tungsten carbides may cause lung toxicity even in those who are less sensitive to those elements, which can result in lung fibrosis with GIP features. Qualitative elemental analysis of fibrosing lesion in GIP also demonstrated the presence of miscellaneous elements: Al, Si, Ti, Cr, and Fe, in addition to tungsten, cobalt, and/or Ta.[7] Several sources of evidence suggest that environmental agents may have an etiologic role in IPF. A meta-analysis of six case-control studies demonstrated that six exposures including cigarette smoking, agriculture/farming, livestock, wood dust, metal dust, and stone/sand were significantly associated with IPF.[27] Metal dust must contain various metal elements. In an EPMA analysis field of the lung biopsy specimen from upper lobe fibrosis, we found tungsten scattered throughout the fibrosis as well as aluminum, silicon, and titanium.[14] Miscellaneous metal dust inhaled in addition to tungsten and cobalt may cause UIP pattern in less sensitive individuals.

Acknowledgement

The authors thank the following doctors for the supply of cases: Dr. Y. Endo from Nagaoka Chuo General Hospital, Dr. M. Amano and Dr. S. Aoki from Showa General Hospital, Dr. T. Ishiguro from Gifu Municipal Hospital, Dr. M. Sakai from Saga Social Insurance Hospital, Dr. M. Tajiri from Kurume University, Dr T. Ishida from Niigata Prefectural Central Hospital, Dr. K. Koreeda from Minami Kyusyu National Hospital,

Dr. K. Okuno from Kasai City Hospital, Dr. Y. Shimaoka from Nagaoka Red Cross Hospital, Dr. K. Kashiwada from Nippon Medical School, Dr. T. Sawada and Dr. A. Shiihara from Kanagawa Cardiovascular and Respiratory Center, Dr. K. Tachibana from National Hospital Organization Kinki-chuo Chest Medical Center, Dr. T. Azuma from Shinshu University, Dr. K. Hara and Dr. T. Ishihara from NTT east corporation Kanto Medical Center, Dr. Y. Waseda from Kanazawa University, Dr. H. Ishii from Oita University, Dr. H. Matsuoka from Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dr. A Hara from Nagasaki University, Dr. O. Hisata from Tohoku University, and Dr. H. Tokuda from Social Insurance Chuo General Hospital. The authors also would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens.

Statements

a. contributorship,

JT and HM, elemental analysis; ES, IN, and TY, interpretation of the results; MT, ES, YK, AH, pathological study; JT and TT, manuscript preparation; and FS and HA, radiological examination.

b. funding,

This research received no specific funding.

c. ethics,

We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

d. data sharing,

There are no data shared in the study.

e. competing interests

None

References

1. Nemery B. Metal toxicity and the respiratory tract. *Eur Respir J*. 1990;**3**:202-19.

2. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environmental health perspectives*. 2000;**108 Suppl 4**:685-96.

3. Takada T, Moriyama H. Hard Metal Lung Disease. In: Huang Y-CT, Ghio AJ, Maier LA, editors. A Clinical Guide to Occupational and Environmental Lung Diseases Respiratory Medicine. New York: Springer; 2012. p. 217-30.

4. Davison AG, Haslam PL, Corrin B et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;**38**:119-28.

5. Cugell DW. The hard metal diseases. *Clin Chest Med*. 1992;**13**:269-79.

6. Anttila S, Sutinen S, Paananen M et al. Hard metal lung disease: a clinical, histological, ultrastructural and X-ray microanalytical study. *Eur J Respir Dis*. 1986;**69**:83-94.

7. Moriyama H, Kobayashi M, Takada T et al. Two-dimensional analysis of elements and mononuclear cells in hard metal lung disease. *Am J Respir Crit Care Med*. 2007;**176**:70-7.

8. Ohori NP, Sciurba FC, Owens GR et al. Giant-cell interstitial pneumonia and hard-metal pneumoconiosis. A clinicopathologic study of four cases and review of the literature. *Am J Surg Pathol*. 1989;**13**:581-7.

9. Travis WD, Matsui K, Moss J et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with

usual interstitial pneumonia and desquamative interstitial pneumonia. *Am J Surg Pathol*. 2000;**24**:19-33.

10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;**157**:1301-15.

11. Moriyama H, Yamamoto T, Takatsuka H et al. Expression of macrophage colony-stimulating factor and its receptor in hepatic granulomas of Kupffer-cell-depleted mice. *Am J Pathol*. 1997;**150**:2047-60.

12. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000;**161**:646-64.

13. Shiota S, Shimizu K, Suzuki M et al. [Seven cases of marked pulmonary fibrosis in the upper lobe]. *Nihon Kokyuki Gakkai Zasshi*. 1999;**37**:87-96.

14. Kaneko Y, Kikuchi N, Ishii Y et al. Upper lobe-dominant pulmonary fibrosis showing deposits of hard metal component in the fibrotic lesions. *Intern Med*. 2010;**49**:2143-5.

15. Naqvi AH, Hunt A, Burnett BR et al. Pathologic spectrum and lung dust burden in giant cell interstitial pneumonia (hard metal disease/cobalt pneumonitis): review of 100 cases. *Arch Environ Occup Health*. 2008;**63**:51-70.

16. Demedts M, Gheysens B, Nagels J et al. Cobalt lung in diamond polishers. *The American review of respiratory disease*. 1984;**130**:130-5.

17. Nemery B, Verbeken EK, Demedts M. Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung). *Semin Respir Crit Care Med*. 2001;**22**:435-48.

18. Churg A, Muller NL, Flint J et al. Chronic hypersensitivity pneumonitis. *Am J Surg Pathol*. 2006;**30**:201-8.

19. D'Ippolito R, Chetta A, Foresi A et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med*. 2004;**98**:977-83.

20. Okuno K, Kobayashi K, Kotani Y et al. A case of hard metal lung disease resembling a hypersensitive pneumonia in radiological images. *Intern Med*. 2010;**49**:1185-9.

21. Kakugawa T, Mukae H, Nagata T et al. Giant cell interstitial pneumonia in a 15-year-old boy. *Intern Med*. 2002;**41**:1007-12.

22. Forni A. Bronchoalveolar lavage in the diagnosis of hard metal disease. *Sci Total Environ*. 1994;**150**:69-76.

23. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. *Prog Respir Res*. 1975:1-33.

24. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc*. 1991;**5**:95-104; discussion 5-8.

25. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. *The American review of respiratory disease*. 1991;**144**:689-96.

26. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environmental health perspectives*. 1997;**105 Suppl 5**:1013-20.

27. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease?

Proc Am Thorac Soc. 2006;**3**:293-8.

FIGURE LEGENDS

Figure 1

High-resolution computed tomography of the chest illustrating differences in the radiographic appearance of the lungs in giant cell interstitial pneumonia (GIP) and in usual interstitial pneumonia (UIP) pattern. (A, B) In GIP of case 9, centriolobular micronodular opacities pathologically correspond to centrilobular fibrosis and giant cell accumulation within the alveolar space. (C, D) In UIP pattern of case 10, reticular opacities and traction bronchiectasis are present with centriolobular micronodular opacities.

Figure 2

Representative images of light microscopic findings and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of S6 specimen from case 9 pathologically diagnosed as giant cell interstitial pneumonia. (A, B, and C) The black square area in centrilobular fibrosis is stepwise magnified to show multinucleated giant cells with cannibalism. (A, D) The green square area in subpleural zone is elementally analyzed by EPMA-WDS to show (E) many orange spots corresponding to tungsten. A qualitative colored image of tungsten distribution is superimposed onto a lung tissue image of amino nitrogen colored green. Note that tungsten is widely distributed in centrilobular fibrosis as well as surrounding alveolar walls. Original magnification, (A) panoramic view, (B) x 4, (C) x 60, and (D) x 8.

Figure 3

Representative images of light microscopic findings of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern. (A, B) A low magnification view of left S1+2 specimen demonstrates a combination of patchy interstitial fibrosis with alternating areas of normal lung and architectural alteration due to chronic scarring or honeycomb change. Note that there are several small bronchioles with mild centrilobular inflammation (blue arrows). (B, C) Multinucleated giant cells with cannibalism are also shown in a stepwise-magnified black square area located in subpleural fibrosis. (D, E, F) Left S10 specimen from the same patient also shows characteristic fibroblastic foci (black arrows) in the background of dense acellular collagen in a stepwise-magnified square area located in subpleural fibrosis. Original magnification, (A, D) panoramic view, (B) x 2, (C) x 40, (E) x 4 and (F) x 20.

Figure 4

Representative images of light micrographs and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern (A). (B, C) An arteriole and its surrounding interstitium (orange square) are elementally analyzed by EPMA-WDS to demonstrate that (D) tungsten and (E) tantalum are distributed in periarteriolar area with little fibrosis. Elemental analysis by EPMA-WDS of subpleural fibrosis with dense acellular collagen (green square in B, F,

I) also shows (G, J) tungsten and (H, K) tantalum almost randomly distributed in magnified images (yellow squares in G and H are magnified to show (J) tungsten and (K) tantalum). We did not further analyze the centrilobular pattern or the cannibalistic giant cells shown in Fig 3. Note that the distribution of tungsten is not completely the same as that of tantalum. Original magnification, (A) panoramic view and (B) x 4. Scale bars for the magnification and scan areas for (E), (H), and (K) correspond to 100μm (0.768 x 0.768 mm), 200μm (1.536 x 1.536 mm), and 25μm (0.1792 x 0.1792 mm), respectively.

An Observational Study of Giant Cell Interstitial Pneumonia and Lung Fibrosis in Hard Metal Lung Disease

[1]Junichi Tanaka, MD, [1]Hiroshi Moriyama, MD, [1]Masaki Terada, MD, [1, 2]Toshinori Takada, MD, [3]Eiichi Suzuki, MD, [1]Ichiei Narita, MD, [4]Yoshinori Kawabata, MD, [4]Tetsuo Yamaguchi, MD, [4]Akira Hebisawa, MD, [4]Fumikazu Sakai, MD, and [4]Hiroaki Arakawa, MD,

[1]Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital [3]Department of General Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan, [4]Tokyo Research Group for Diffuse Parenchymal Lung Diseases, Tokyo, Japan

Corresponding author: Toshinori Takada, M.D., PhD

Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University

1-757 Asahimachi-dori, Chuo-ku, Niigata, 951-8510, Japan

Tel; +81-25-227-2200, Fax; +81-25-227-0775, Email; ttakada@med.niigata-u.ac.jp

Keywords: hard metal, pulmonary fibrosis, electron probe microanalysis

Word count: 2,921

Statements

a. contributorship,

JT and HM, elemental analysis; ES, IN, and TY, interpretation of the results; MT, ES, YK, AH, pathological study; JT and TT, manuscript preparation; and FS and HA, radiological examination.

b. funding,

This research received no specific funding.

c. ethics,

We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

d. data sharing,

There are no data shared in the study.

ABSTRACT

Objectives: Hard metal lung disease has various pathological patterns including giant cell interstitial pneumonia (GIP) and usual interstitial pneumonia (UIP). Although UIP pattern is considered the prominent feature in advanced disease, it is unknown whether GIP finally progresses to UIP pattern. The aim of our study is to clarify clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Setting: A cross-sectional study for patients of 17 institutes participating in the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases, 2009.

Participants: Nineteen patients with 7 females diagnosed as hard metal lung disease by the presence of tungsten in lung specimens.

Primary and secondary outcome measures: Clinical, pathological, and elemental differences between GIP and UIP pattern in hard metal lung disease.

Results: Fourteen cases were pathologically diagnosed as GIP or centrilobular inflammation/fibrosing. The other five cases were UIP pattern or upper lobe fibrosis. Elemental analyses of lung specimens of GIP showed tungsten throughout the centrilobular fibrotic areas. In UIP pattern, tungsten was detected in periarteriolar area and subpleural fibrosis in no association with centrilobular fibrosis or inflammatory cell infiltration. The GIP group was younger (43.1 vs 58.6 yrs) with shorter exposure duration (73 vs 285 months) ($p<0.01$), lower serum KL-6 (398 vs 710 U/ml), and higher lymphocyte percentage in bronchoalveolar lavage fluid (31.5 vs 3.22 %) ($p<0.05$) than

the fibrosis group.

Conclusions: UIP pattern or upper lobe fibrosis is remarkably different from GIP in distribution of hard metal elements, associated interstitial inflammation and fibrosis, and clinical features. In hard metal lung disease, UIP pattern or upper lobe fibrosis may not be an advanced form of GIP.

Strengths and limitations of this study

1, Nineteen cases of hard metal lung disease, a rare occupational lung disease, were collected and their clinical features were documented.

2, Lung tissue from all the patients was elementally analyzed by a patented technique, an improved element analysis using electron probe microanalyzers with wavelength dispersive spectrometer.

3, Since the incidences of hard metal lung disease and IPF in potentially exposed populations and in the general population are unknown, the probability that someone with hard metal exposure will develop "idiopathic" UIP/IPF is also unknown.

INTRODUCTION

Hard metal is a synthetic compound that combines tungsten carbide with cobalt. Patients exposed to hard metal may develop occupational asthma, a syndrome resembling hypersensitivity pneumonitis, or interstitial lung disease which is recognized as hard metal lung disease.[1-3] In many cases with hard metal lung disease, multinucleated giant cells with centrilobular fibrosis are prominent resulting in a pattern of giant cell interstitial pneumonia (GIP).[4-6] We demonstrated that hard metal accumulated in the centrilobular area may trigger the inflammation in cooperation with $CD163^+$ monocyte-macrophages and $CD8^+$ lymphocytes using electron probe microanalyzers with wavelength dispersive spectrometer (EPMA-WDS).[7] In addition to classical GIP, hard metal lung disease has a variety of pathological patterns, desquamative interstitial pneumonia, obliterative bronchiolitis, and usual interstitial pneumonia (UIP) pattern.[4, 8] The lesions of classical GIP are usually centered on the centrilobular areas. On the other hand, the key histologic features of UIP are predominantly distributed at the periphery of the acinus or lobule.[9, 10] Hard metal lung disease has pathological patterns of both GIP and UIP, and the UIP pattern is thought to be the prominent feature in advanced cases of the disease.[8] The key question is whether UIP pattern is an advanced form of GIP or not. In order to elucidate relationship between GIP and lung fibrosis with detection of hard metal elements, we collected cases with tungsten in lung tissue and reviewed their clinical records. We then elementally reexamined lung specimens by EPMA-WDS. We finally classified the patients into two groups according to the histological findings and

statistically compared their clinical features. Pathological and elemental analyses in the study suggest that UIP pattern or upper lobe fibrosis may be different from an end-stage form of GIP.

METHODS

Patient population

We collected patients by announcing inquiry for cases of hard metal lung disease to the major medical institutes and hospitals all over Japan for the 10th annual meeting of the Tokyo Research Group for Diffuse Parenchymal Lung Diseases, 2009. We obtained information of patient profile such as age, gender, duration of hard metal exposure, history of pneumothorax, history of allergy, symptoms, physical findings, serum levels of Krebs von den Lungen-6 (KL-6) and SP-D, arterial blood gas data, pulmonary function tests, bronchoalveolar lavage (BAL) cell profiles and treatment and prognosis in order to make a data base. We acquired consent from all treating physicians for each identified case according to the Guidelines for Epidemiological Studies from The Ministry of Health, Labor and Welfare. The Committee of Ethics, Niigata University, approved the EPMA-WDS study protocol (#396).

HRCT scan findings

All patients with hard metal lung disease except one had undergone high-resolution computed tomography (HRCT) scanning. Two radiologists (observers) who were blinded to clinical, laboratory, or pulmonary function test results evaluated CT scan

findings. The observers judged each CT scan for the presence or absence of three main features of centrilobular nodules, ground glass opacity, and pneumothorax. They also noted other remarkable findings; traction bronchiectasis, reticular pattern, subpleural linear opacity, consolidation, bulla, centrilobular emphysema, atelectasis, and bronchial wall thickening and entered these results into a data sheet independently. After evaluation, disagreement on the results between the observers for some HRCT scans was resolved by discussion and consensus.

Sample preparation and pathological study

Each tissue sample was serially cut into 3 μm-thickness sections and subjected to pathological study and EPMA-WDS analysis. For pathological study, formalin-fixed 3 μm serial sections were stained with hematoxylin-eosine and Elastica van Gieson method. Two pathologists (observers), who were blinded to clinical, laboratory, or pulmonary function test results, evaluated pathological findings. After evaluation, disagreement on the pathological diagnoses between the observers for some specimens was resolved by discussion and consensus.

Electron probe microanalysis

Examination of tissue sections with EMPA-WDS was performed according to procedures previously described.[11] X-ray data were obtained with an EPMA-WDS (EPMA 8705, EPMA-1610, Shimadzu Ltd, Kyoto, Japan). In order to have representative element maps, we at first microscopically scanned tissue specimens and

looked for lesions of centrilobular fibrosis with low magnification because hard metal related elements, tungsten/cobalt were always found around centrilobular areas according to our experiences. For EMPA analysis, we at first screened areas of about 1.5 mm x 1.5 mm at largest covering centrilobular lesions or fibrosing lesion of interstitial lung diseases observed by pathological study to make rough element maps. Then we focused into areas from 5x5 to 10x10 μm at smallest to draw fine maps for elements. Each pixel in the focused areas in the tissue was scanned by three wavelength dispersive crystals; RAP, PET, and LiF for screening elements of Al, K, RAP; Si, K, PET; Ti, K, LiF; Cr, K, LiF; Fe, K, LiF; Co, K, LiF; Ta, M, PET; W, M, PET, and Zn, L, RAP. Since generated X-ray signals from each pixel were the smallest part of a distribution map, we simultaneously obtained element maps with qualitative analyses of pixels in the focused area. The distribution of amino nitrogen corresponding to the pathological image was also mapped for each sample.

Statistical analysis

Comparisons of categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by Mann-Whitney's U-test. A p Value <0.05 was considered significant.

RESULTS

Characteristics of subject

When we held the Tokyo ILD Meeting, 22 cases were collected and suspected to be

hard metal lung diseases due to occupational history and pathological findings, but 3 cases were excluded because tungsten or cobalt were not detected in the lung tissue. Nineteen patients were finally diagnosed as hard metal lung disease because of presence of tungsten in lung specimens detected by EPMA-WDS. In 4 of 19 patients, the presence of tungsten, cobalt, or tantalum was not known in the first place and proved by element analysis at the meeting.

Occupational history and clinical features are summarized in Table 1 and 2. Demographic findings in 6 of these patients have been reported previously (case 2, 5, 7, 8, 10, and 16 corresponding to case 1, 3, 5, 6, 14, and 16 in 2007 report, respectively).[7] All the subjects had an occupational history of hard metal industry for 1 to 36 years. One patient (case 15) was doing deskwork in an insufficiently ventilated room of a hard metal grinding company. Five patients had occupational history of hard metal industry but were not exposed at the diagnosis of hard metal lung disease. The delay between cessation of exposure and biopsy in the patients were 5 years, 4 months, 2 months, and 6 months for case 1, 2, 8, and 14, respectively. Case 10 had worked as a metal grinder for 6 years and then as a chimney cleaner at a copper mine for 32 years. He visited a hospital complaining of dry cough after 32-year work as a chimney cleaner and was finally diagnosed as hard metal lung diseases 4 years later by surgical biopsy. Five patients (case 2, 5, 7, 8, and 15) had an allergic history and were patch tested for Co, Ni, Cr, Hg, Au, Zn, Mn, Ag, Pd, Pt, Sn, Cu, Fe, Al, In, Ir, Ti. 4 of 5 patients who had undergone patch testing (case 2, 5, 7, and 15) were found to be positive for cobalt. Pulmonary function tests revealed restrictive lung defect

characterized by reduced vital capacity and lung diffusing capacity. BAL findings showed increased total cell counts, increased lymphocytes and eosinophils, with normal CD4/CD8 ratio. Bizarre multinucleated giant cells were noted in 3 patients.

Table 1. Demographic features of subjects

Case	Age	Sex	Smoking history	Occupational history (hard metal exposure)	Exposure (y/m) start/duration	Bx year	Exposure at Dx
1	39	M	non	Hard metal shaping/drilling	2000/12	2006	No
2	53	M	ex	Hard metal shaping/drilling	2002/30	2002	No
3	21	M	non	Metal grinding	2005/32	2008	Yes
4	42	M	ex	Hard metal shaping/drilling	2005/36	2009	Yes
5	48	M	non	Metal grinding	2000/48	2004	NA
6	45	M	non	Hard metal shaping/drilling	1982/60	1987	Yes
7	32	F	non	Metal grinding	1988/60	1993	Yes
8	32	F	non	Metal grinding	1997/72	2003	No
9	44	F	non	Hard metal shaping/drilling	1990/72	1996	Yes
10	62	M	non	Metal grinding	1963/72	2003	No
11	40	F	non	Hard metal shaping/drilling	1997/96	2005	NA
12	48	M	non	Metal grinding	1981/120	1992	NA
13	49	F	non	Hard metal shaping/drilling	1999/120	2009	Yes
14	65	F	non	Metal grinding	1988/144	2000	No
15	50	F	non	Desk worker in hard metal factory	1985/168	1996	Yes
16	53	M	non	Quality control of hard metals	1974/264	2001	NA
17	60	M	ex	Hard metal shaping/drilling	1972/276	1995	Yes
18	53	M	non	Hard metal shaping/drilling	1971/372	2005	Yes
19	65	M	non	Hard metal shaping/drilling	1963/444	2008	Yes

Abbreviation; Bx, biopsy; Dx, diagnosis; NA, not available.

Table 2. Clinical characteristics of Patients with Hard metal lung disease

		Value
Mean age at diagnosis (yrs)		46.4 ± 14.1 (21 - 65)
Gender	M/F	12/7
Smoking history	Cur/Ex/Never	0/3/16
Chief complaints	dry cough	13/19
	breath shortness	8/19
Pneumothorax	Yes	8/19
Allergic history	Yes	5/19
Patch test to cobalt	positive	4/5
Mean exposure duration (yrs)		10.7 ± 10.3 (1 - 36)
Physical findings	rales on auscultation	11/19
	fine crackles	8/19
	finger clubbing	4/18
	edema of leg	1/16
Laboratory tests	KL-6	502.7 ± 267.5 U/ml
	SP-D	216.1 ± 192.4 ng/ml
Pulmonary function tests		
	VC, % predicted	64.8 ± 25.3 %
	FEV_1	1.71 ± 0.70 L
	FEV_1/FVC	85.6 ± 10.7 %
	DLco, % predicted	53.4 ± 17.0 %
Bronchoalveolar lavage		
	Total cell count	3.13 ± 2.11 $\times 10^5$ /ml
	Lymphocytes	24.3 ± 22.3 %
	Neutrophils	3.07 ± 2.86 %
	Eosinophils	3.01 ± 5.03 %
	CD4/8 ratio	1.65 ± 2.96

The mean numbers ± standard deviations and ranges in parentheses are shown.

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

Radiological findings

HRCT of all patients except one with hard metal lung disease were available for review of radiological findings. Conventional CT findings of case 12 were added to the table (Table 3). Centrilobular nodules (Fig 1 A, B) and ground glass opacity were identified in chest CT of 16 patients. In some patients, reticular opacities, traction bronchiectasis, and subpleural curvilinear opacities were also present (Fig 1 C, D). Although centrilobular micronodular opacities were noted in those patients, they were not predominant.

Table 3. Radiologic findings of patients with hard metal lung disease

	CT features				
Case	CL nodules	GGO	PTx	other findings	radiological diagnosis
1	+	-	-	bronchial wall thickening	bronchitis (DPB like)
2	+	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
3	+	+	+		subacute HP
4	+	-	+	subpleural curvilinear opacities	subacute HP
5	+	+	-		subacute HP
6	-	+	-	reticular opacities, consolidation	Interstitial pneumonia NOS
7	+	+	+		subacute HP
8	+	+	-	traction bronchiectasis	subacute HP
9	+	+	-		subacute HP
10	+	+	-	reticular opacities traction bronchiectasis	UIP
11	+	-	+		subacute HP
12	+	+	+	subpleural curvilinear opacities	chronic HP
13	+	+	-		subacute HP
14	+	+	-	traction bronchiectasis, apical cap	chronic HP
15	+	+	+	traction bronchiectasis	subacute HP
16	-	+	+	subpleural/peribronchovascular consolidation, atelectasis, bulla	upper lobe predominant IP or chronic IP NOS
17	+	+	-	bulla, centrilobular emphysema	UIP
18	-	+	-	reticular opacities	chronic IP, NOS (NSIP or UIP)
19	+	+	-	reticular opacities	chronic HP

Abbreviation; CL, centrilobular; GGO, ground-glass opacities; PTx, pneumothorax; DPB, diffuse panbronchiolitis; IP, interstitial pneumonia; NOS, not otherwise specified; NSIP, non-specific interstitial pneumonia; UIP, usual interstitial pneumonia; HP, hypersensitivity pneumonitis

Pathological findings and elemental analysis

Pathological findings and detected elements in lung tissue of 19 cases were summarized

in Table 4. Four major histological features noted in this study were as follows: GIP characterized with centrilobular fibrosis (Fig 2 A, B) and characteristic giant cells showing cannibalism (Fig 2 C), centrilobular inflammation/fibrosis similar to GIP but without giant cells, UIP pattern characterized with patchy distribution and temporal heterogeneity, and dense fibrosis with fibroblastic foci (Fig 3 A, B, D, E, F) [12], upper lobe fibrosis characterized with apical scar/cap type fibrosis mainly in the upper lobe.[13] In the case of upper lobe fibrosis, biopsy specimen contained apical cap-like subpleural dense fibrosis which was composed of airspace fibrosis (intraluminar organization) with collapse and increased elastic framework. In autopsy taken 4 years later, we recognized remarkable subpleural elastosis with a few of cannibalistic giant cells.

Elemental analyses of lung specimens of GIP and centrilobular inflammation/fibrosis demonstrated that tungsten was mapped almost throughout the centrilobular fibrotic areas (Fig 2 D, E). Analyses of lung specimens of UIP pattern by EPMA-WDS revealed that tungsten and tantalum were distributed in periarteriolar area (Fig 4, D, E) and in subpleural fibrosis with dense acellular collagen (Fig 4 G, H, J, K). However, these elements were not accompanied by centrilobular inflammation/fibrosis (Fig 4, A, B). Lung histopathology in one case showed apical cap-like fibrosis with tungsten deposits detected in the fibrotic region but without GIP.[14] In total, elemental analysis by EPMA-WDS detected tungsten but no cobalt or tantalum in 10 patients, tungsten and cobalt in 5 patients, and tungsten and tantalum in 4 patients (Table 4).

Table 4. Pathological findings and elemental analysis of patients with hard metal lung disease

	sampling			elements detected		
Case	method	site(s)	pathological findings	W	Co	Ta
1	VATS	rt. S5/S8	centrilobular inflammation/fibrosis	+	-	-
2	VATS	lt. S2/S9	GIP	+	-	-
3	TBB/VATS	rt. apex	GIP	+	-	-
4	VATS	rt. S9	centrilobular inflammation/fibrosis	+	-	-
5	VATS	rt. S4/S9	GIP	+	-	-
6	Autopsy	NA	GIP, DAD	+	-	-
7	VATS	rt. S8	centrilobular inflammation/fibrosis	+	+	-
8	VATS	rt. S4/S6	GIP	+	-	+
9	VATS	rt. S2/S6	GIP	+	+	-
10	VATS	lt. S1+2/S10	UIP, GIP	+	-	+
11	VATS	lt. S1+2/S9	GIP	+	+	-
12	Autopsy	NA	GIP, DAD	+	-	-
13	VATS	lt. S1+2/S6	GIP	+	-	-
14	VATS	lt. S4/S9	GIP, UIP/NSIP?	+	-	+
15	VATS	rt. S6	GIP	+	+	-
16	VATS/autopsy	lt. S1+2/whole	upper lobe fibrosis	+	-	+
17	TBB/Lobectomy	-/RLL	UIP	+	-	-
18	VATS	lt. S1+2/S9	UIP	+	+	-
19	VATS	rt. S3/S10	UIP, centrilobular fibrosis	+	-	+

Abbreviation; TBB, trans-bronchial biopsy; VATS, video-assisted thoracic surgery; GIP, giant cell interstitial pneumonia; NA, not available; RLL, right lower lobectomy; DAD, diffuse alveolar damage; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia

Comparison of clinical features

We then classified the patients with hard metal lung disease into two groups according to their pathological findings. We grouped GIP and centrilobular inflammation/fibrosis together, because the latter pattern was considered to be a variant

of GIP due to the similar distribution of lesions. One patient was pathologically diagnosed as upper lobe fibrosis. It has such characteristic findings of subpleural, zonal, rather well defined fibrosis with small cysts and honeycomb lesions similar to that of UIP pattern that we grouped UIP pattern and upper lobe fibrosis together and named them the fibrosis group. We then compared clinical features between the GIP group and the fibrosis group. The GIP group was younger, had shorter exposure duration, lower serum KL-6, and higher lymphocyte percentage in BAL fluid compared with the fibrosis group (Table 5).

Table 5. Comparison of clinical features between GIP group and fibrosis group

	GIP group (n=14)	Fibrosis group (n=5)	p-value
Age (yrs)	43.1 ± 10.8	58.6 ± 5.41	0.007
Gender (M/F)	7/7	5/0	0.106
Exposure duration (months)	73.0 ± 48.8	285.6 ± 140.3	0.007
Pneumothorax (+/-)	6/8	2/3	1.000
KL-6 (U/ml)	398.7 ± 189.4	710.8 ± 297.7	0.023
SP-D (ng/ml)	260.3 ± 257.5	161.0 ± 54.75	0.903
PaO_2 (Torr)	84.3 ± 14.3	84.4 ± 11.2	0.922
$PaCO_2$ (Torr)	42.8 ± 2.75	56.0 ± 34.6	0.657
VC, % predicted (%)	64.4 ± 27.1	65.5 ± 24.1	0.734
FEV_1 (L)	1.63 ± 0.23	1.88 ± 0.32	0.537
FEV_1/FVC (%)	85.4 ± 12.9	86.1 ± 2.62	0.910
DLco, % predicted (%)	50.8 ± 16.7	57.2 ± 18.8	0.371
Bronchoalveolar lavage			
Total cell count ($\times 10^5$/ml)	3.52 ± 2.41	2.26 ± 0.96	0.395
Lymphocytes (%)	31.5 ± 23.0	8.40 ± 9.08	0.015
CD4/8 ratio	.76 ± 0.51	3.22 ± 4.85	0.298

Abbreviation; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; VC, vital capacity; FEV_1, Forced expiratory volume in 1 second; DLco, Carbon monoxide diffusing capacity

DISCUSSION

Pathological features of GIP are interstitial pneumonia with centrilobular fibrosis with multinucleated giant cells in the airspaces.[15] Sometimes centrilobular inflammation/fibrosis is only noted with few giant cells. EPMA-WDS analysis of lung tissue of hard metal lung disease demonstrated that tungsten was distributed in a relatively high concentration almost throughout the centrilobular fibrosis and in giant cells.[7] Comparison of distribution of inflammatory cells and tungsten suggested that inhaled hard metal elements were associated with centrilobular inflammation/fibrosis by $CD163^+$ macrophages in cooperation with $CD8^+$ lymphocytes. Thus, centrilobular inflammation/fibrosis without giant cells should also be a variant of hard metal lung disease. GIP was also found in Belgian diamond polishers exposed not to hard metal dust, but to cobalt-containing dust, which confirmed that cobalt plays a dominant role in hard metal lung disease.[16] Cobalt is a well-known skin sensitizer, causing allergic contact dermatitis, and it can also cause occupational asthma.[17] Four patients were positive for patch testing for cobalt. Although such patch testing has been claimed to carry some risk of aggravation of disease in the situation with beryllium, cobalt is included in the routine metal allergy test panel and caused no worsening of hard metal lung disease. Hard metal lung disease cases show features of hypersensitivity pneumonitis (HP) with small interstitial granulomas, although well formed granulomas as in chronic beryllium disease are very rarely seen in the disease or HP. These data

suggest that allergic inflammation may be different between hard metal lung disease/HP and berylliosis.

Respiratory symptoms of hard metal lung diseases sometimes improve on holidays and exacerbate during workdays, which resemble those of HP. Histopathology findings in HP may also include centrilobular fibrosis in association with isolated giant cells.[18] However, they do not show cannibalism as those in hard metal lung disease. BAL is the most sensitive tool to detect HP: a marked lymphocytosis with decreased CD4/8 ratio is characteristic of BAL findings.[19] BAL findings of patients with hard metal lung disease show increased total cell counts with increased lymphocytes and decreased CD4/CD8 ratio.[4, 20-22] Reduced CD4/8 ratio is consistent with the findings of immunohistochemistry in the previous study.[7] In this study, we found that lymphocyte percentage in BAL fluid was increased with rather low CD4/8 ratio in the GIP group, but they were not recognized in fibrosis group.

UIP pattern is the pathological abnormality associated with various restrictive lung diseases, including idiopathic pulmonary fibrosis (IPF). Interstitial inflammation and fibrosis in UIP pattern does not usually involve centrilobular area and peribronchioles. Three cases who were pathologically diagnosed as UIP pattern also had centrilobular micronodular opacities in HRCT findings. One patient was pathologically diagnosed as UIP pattern and centrilobular fibrosis. Element analysis of the deposition in lung tissues from patients with IPF/UIP usually demonstrates following elements; Si, Al, Fe, and Ti with various degrees (unpublished data). While we found tungsten accumulated in periarteriolar area and subpleural fibrosis in lung specimens of UIP pattern in this

study. However, tungsten in periarteriolar area was hardly associated with any fibrosis or inflammatory cells. These results suggest that individual immune susceptibility/response to inhaled hard metal elements may decide pathological patterns of UIP, GIP, or their mixture in varying degrees. Patients develop hard metal lung disease usually after mean exposure duration of more than 10 years. Although most studies have found no relation between disease occurrence and length of occupational exposure, individuals with increased susceptibility may develop hard metal lung disease after relatively short and low levels of exposure. The GIP group was younger and had shorter exposure duration suggesting that those who had UIP pattern were individuals with decreased susceptibility. Upper lobe fibrosis was pathologically diagnosed in one patient. Although it is significantly different from UIP pattern, tungsten in the fibrosis was not associated with inflammation around the element, either. With regard to the relationship between hard metal elements and surrounding inflammation, upper lobe fibrosis looks similar to UIP pattern in the other cases.

Liebow first described GIP as a form of idiopathic interstitial pneumonia.[23] It is now recognized that GIP is pathognomonic for hard metal lung disease.[24] Since tungsten and cobalt are only observed within the lungs of subjects who have been exposed to hard metals, the presence of tungsten and/or cobalt in BAL fluid or lung specimens leads to a definite diagnosis of hard metal lung disease. According to the results of elemental analyses in this study, five cases with UIP pattern or upper lobe fibrosis should be diagnosed as hard metal lung disease. The pathological findings of UIP pattern demonstrated no physical connection between centrilobular fibrosis and the

UIP area, dense fibrosis with fibroblastic foci. Since centrilobular fibrosis is usually irreversible, if GIP evolved to UIP, sequels of centrilobular fibrosis would be somewhat linked to peripheral UIP lesion. EPMA-WDS analyses of lung specimens of UIP pattern revealed that tungsten and tantalum in periarteriolar area were not accompanied by centrilobular inflammation/fibrosis as seen in typical GIP. In addition, clinical features of the fibrosis group were different from those of the GIP group. We identified tungsten in subpleural fibrosis with dense acellular collagen from UIP pattern and in the fibrotic region from apical cap-like fibrosis. Fibrotic reactions of these patients could have caused accumulation of hard metal particles as the scars contract and cut off lymphatic drainage. Those who are not sensitive to hard metal elements, particularly cobalt, might simply have idiopathic UIP or upper lobe fibrosis by accident as everyone with interstitial lung disease and a history of asbestos exposure does not have asbestosis.[25] However, microscopic findings of the lung specimen of UIP pattern included mild centrilobular inflammation and multinucleated giant cells with cannibalism, which could never been seen in idiopathic UIP/IPF. If we find tungsten or cobalt in the biopsies of UIP/fibrosis from the subjects who worked in the hard-metal industry, we cannot help but make a diagnosis of hard-metal lung disease. Given present information, we only conclude that the UIP/fibrosis may be induced by hard metal elements, or just a coincidence. Since the incidences of hard metal lung disease and IPF in potentially exposed populations and in the general population are unknown, the probability that someone with hard metal exposure will develop "idiopathic" UIP/IPF is also unknown.

Hard metal lung disease is caused by exposure to cobalt and tungsten carbide. Toxicity stems from reactive oxygen species generation in a mechanism involving both elements in mutual contact.[26] Inhaled cobalt and tungsten carbides may cause lung toxicity even in those who are less sensitive to those elements, which can result in lung fibrosis with GIP features. Qualitative elemental analysis of fibrosing lesion in GIP also demonstrated the presence of miscellaneous elements: Al, Si, Ti, Cr, and Fe, in addition to tungsten, cobalt, and/or Ta.[7] Several sources of evidence suggest that environmental agents may have an etiologic role in IPF. A meta-analysis of six case-control studies demonstrated that six exposures including cigarette smoking, agriculture/farming, livestock, wood dust, metal dust, and stone/sand were significantly associated with IPF.[27] Metal dust must contain various metal elements. In an EPMA analysis field of the lung biopsy specimen from upper lobe fibrosis, we found tungsten scattered throughout the fibrosis as well as aluminum, silicon, and titanium.[14] Miscellaneous metal dust inhaled in addition to tungsten and cobalt may cause UIP pattern in less sensitive individuals.

Acknowledgement

The authors thank the following doctors for the supply of cases: Dr. Y. Endo from Nagaoka Chuo General Hospital, Dr. M. Amano and Dr. S. Aoki from Showa General Hospital, Dr. T. Ishiguro from Gifu Municipal Hospital, Dr. M. Sakai from Saga Social Insurance Hospital, Dr. M. Tajiri from Kurume University, Dr T. Ishida from Niigata Prefectural Central Hospital, Dr. K. Koreeda from Minami Kyusyu National Hospital,

Dr. K. Okuno from Kasai City Hospital, Dr. Y. Shimaoka from Nagaoka Red Cross Hospital, Dr. K. Kashiwada from Nippon Medical School, Dr. T. Sawada and Dr. A. Shiihara from Kanagawa Cardiovascular and Respiratory Center, Dr. K. Tachibana from National Hospital Organization Kinki-chuo Chest Medical Center, Dr. T. Azuma from Shinshu University, Dr. K. Hara and Dr. T. Ishihara from NTT east corporation Kanto Medical Center, Dr. Y. Waseda from Kanazawa University, Dr. H. Ishii from Oita University, Dr. H. Matsuoka from Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dr. A Hara from Nagasaki University, Dr. O. Hisata from Tohoku University, and Dr. H. Tokuda from Social Insurance Chuo General Hospital. The authors also would like to acknowledge Dr. Kouichi Watanabe and Mr. Masayoshi Kobayashi of EPMA Laboratory, Center of Instrumental Analysis, Niigata University, who contributed to elemental analysis of lung specimens.

References

1. Nemery B. Metal toxicity and the respiratory tract. *Eur Respir J*. 1990;**3**:202-19.

2. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environmental health perspectives*. 2000;**108 Suppl 4**:685-96.

3. Takada T, Moriyama H. Hard Metal Lung Disease. In: Huang Y-CT, Ghio AJ, Maier LA, editors. A Clinical Guide to Occupational and Environmental Lung Diseases Respiratory Medicine. New York: Springer; 2012. p. 217-30.

4. Davison AG, Haslam PL, Corrin B et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;**38**:119-28.

5. Cugell DW. The hard metal diseases. *Clin Chest Med*. 1992;**13**:269-79.

6. Anttila S, Sutinen S, Paananen M et al. Hard metal lung disease: a clinical, histological, ultrastructural and X-ray microanalytical study. *Eur J Respir Dis*. 1986;**69**:83-94.

7. Moriyama H, Kobayashi M, Takada T et al. Two-dimensional analysis of elements and mononuclear cells in hard metal lung disease. *Am J Respir Crit Care Med*. 2007;**176**:70-7.

8. Ohori NP, Sciurba FC, Owens GR et al. Giant-cell interstitial pneumonia and hard-metal pneumoconiosis. A clinicopathologic study of four cases and review of the literature. *Am J Surg Pathol*. 1989;**13**:581-7.

9. Travis WD, Matsui K, Moss J et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with

usual interstitial pneumonia and desquamative interstitial pneumonia. *Am J Surg Pathol*. 2000;**24**:19-33.

10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;**157**:1301-15.

11. Moriyama H, Yamamoto T, Takatsuka H et al. Expression of macrophage colony-stimulating factor and its receptor in hepatic granulomas of Kupffer-cell-depleted mice. *Am J Pathol*. 1997;**150**:2047-60.

12. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000;**161**:646-64.

13. Shiota S, Shimizu K, Suzuki M et al. [Seven cases of marked pulmonary fibrosis in the upper lobe]. *Nihon Kokyuki Gakkai Zasshi*. 1999;**37**:87-96.

14. Kaneko Y, Kikuchi N, Ishii Y et al. Upper lobe-dominant pulmonary fibrosis showing deposits of hard metal component in the fibrotic lesions. *Intern Med*. 2010;**49**:2143-5.

15. Naqvi AH, Hunt A, Burnett BR et al. Pathologic spectrum and lung dust burden in giant cell interstitial pneumonia (hard metal disease/cobalt pneumonitis): review of 100 cases. *Arch Environ Occup Health*. 2008;**63**:51-70.

16. Demedts M, Gheysens B, Nagels J et al. Cobalt lung in diamond polishers. *The American review of respiratory disease*. 1984;**130**:130-5.

17. Nemery B, Verbeken EK, Demedts M. Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung). *Semin Respir Crit Care Med*. 2001;**22**:435-48.

18. Churg A, Muller NL, Flint J et al. Chronic hypersensitivity pneumonitis. *Am J Surg Pathol*. 2006;**30**:201-8.

19. D'Ippolito R, Chetta A, Foresi A et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med*. 2004;**98**:977-83.

20. Okuno K, Kobayashi K, Kotani Y et al. A case of hard metal lung disease resembling a hypersensitive pneumonia in radiological images. *Intern Med*. 2010;**49**:1185-9.

21. Kakugawa T, Mukae H, Nagata T et al. Giant cell interstitial pneumonia in a 15-year-old boy. *Intern Med*. 2002;**41**:1007-12.

22. Forni A. Bronchoalveolar lavage in the diagnosis of hard metal disease. *Sci Total Environ*. 1994;**150**:69-76.

23. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. *Prog Respir Res*. 1975:1-33.

24. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc*. 1991;**5**:95-104; discussion 5-8.

25. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. *The American review of respiratory disease*. 1991;**144**:689-96.

26. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environmental health perspectives*. 1997;**105 Suppl 5**:1013-20.

27. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease?

Proc Am Thorac Soc. 2006;**3**:293-8.

FIGURE LEGENDS

Figure 1

High-resolution computed tomography of the chest illustrating differences in the radiographic appearance of the lungs in giant cell interstitial pneumonia (GIP) and in usual interstitial pneumonia (UIP) pattern. (A, B) In GIP of case 9, centriolobular micronodular opacities pathologically correspond to centrilobular fibrosis and giant cell accumulation within the alveolar space. (C, D) In UIP pattern of case 10, reticular opacities and traction bronchiectasis are present with centriolobular micronodular opacities.

Figure 2

Representative images of light microscopic findings and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of S6 specimen from case 9 pathologically diagnosed as giant cell interstitial pneumonia. (A, B, and C) The black square area in centrilobular fibrosis is stepwise magnified to show multinucleated giant cells with cannibalism. (A, D) The green square area in subpleural zone is elementally analyzed by EPMA-WDS to show (E) many orange spots corresponding to tungsten. A qualitative colored image of tungsten distribution is superimposed onto a lung tissue image of amino nitrogen colored green. Note that tungsten is widely distributed in centrilobular fibrosis as well as surrounding alveolar walls. Original magnification, (A) panoramic view, (B) x 4, (C) x 60, and (D) x 8.

Figure 3

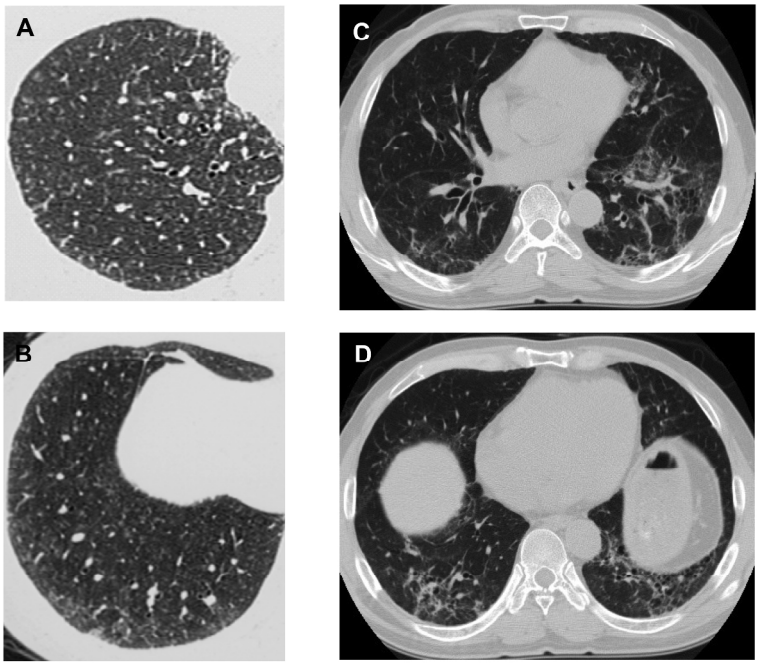
Representative images of light microscopic findings of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern. (A, B) A low magnification view of left S1+2 specimen demonstrates a combination of patchy interstitial fibrosis with alternating areas of normal lung and architectural alteration due to chronic scarring or honeycomb change. Note that there are several small bronchioles with mild centrilobular inflammation (blue arrows). (B, C) Multinucleated giant cells with cannibalism are also shown in a stepwise-magnified black square area located in subpleural fibrosis. (D, E, F) Left S10 specimen from the same patient also shows characteristic fibroblastic foci (black arrows) in the background of dense acellular collagen in a stepwise-magnified square area located in subpleural fibrosis. Original magnification, (A, D) panoramic view, (B) x 2, (C) x 40, (E) x 4 and (F) x 20.

Figure 4

Representative images of light micrographs and electron probe microanalyser with wavelength dispersive spectrometer (EPMA-WDS) of lung specimen from case 10 with hard metal lung disease pathologically diagnosed as usual interstitial pneumonia pattern (A). (B, C) An arteriole and its surrounding interstitium (orange square) are elementally analyzed by EPMA-WDS to demonstrate that (D) tungsten and (E) tantalum are distributed in periarteriolar area with little fibrosis. Elemental analysis by EPMA-WDS of subpleural fibrosis with dense acellular collagen (green square in B, F,

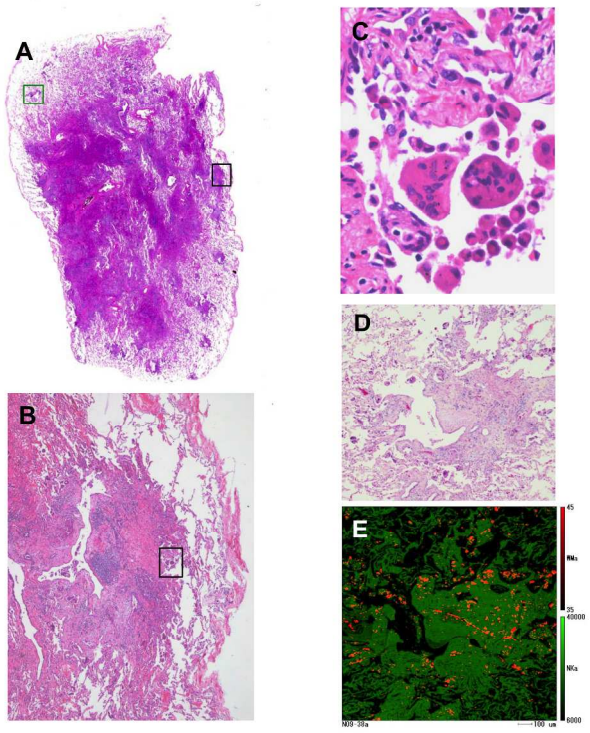
I) also shows (G, J) tungsten and (H, K) tantalum almost randomly distributed in magnified images (yellow squares in G and H are magnified to show (J) tungsten and (K) tantalum). We did not further analyze the centrilobular pattern or the cannibalistic giant cells shown in Fig 3. Note that the distribution of tungsten is not completely the same as that of tantalum. Original magnification, (A) panoramic view and (B) x 4. Scale bars for the magnification and scan areas for (E), (H), and (K) correspond to 100μm (0.768 x 0.768 mm), 200μm (1.536 x 1.536 mm), and 25μm (0.1792 x 0.1792 mm), respectively.

Fig 1



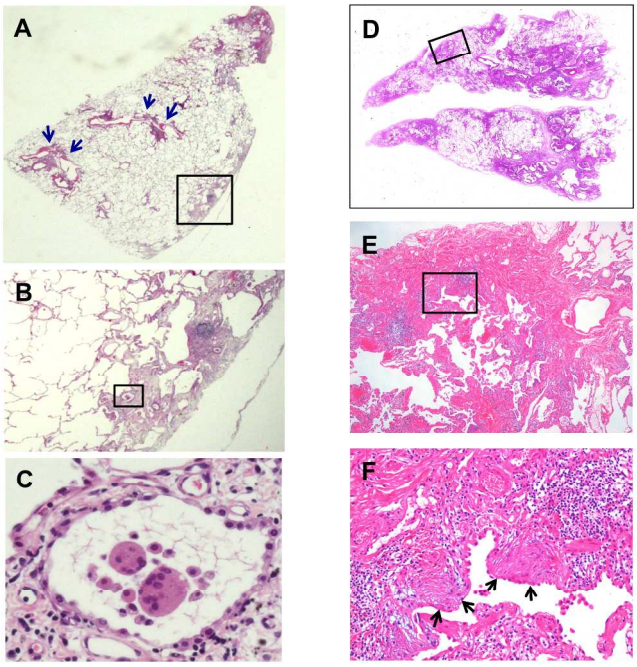
253x190mm (300 x 300 DPI)

Fig 2



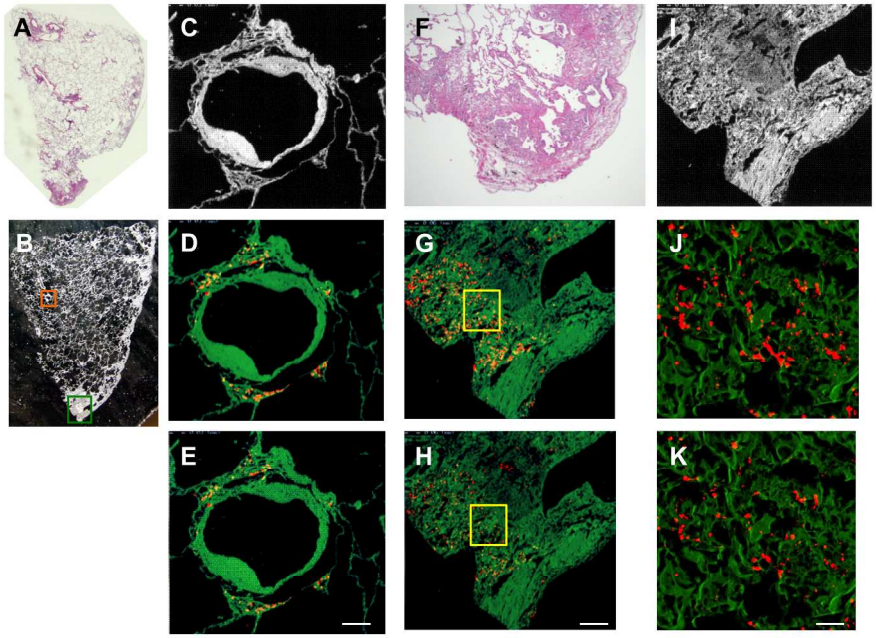
253x190mm (300 x 300 DPI)

Fig 3



253x190mm (300 x 300 DPI)

Fig 4



253x190mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract **p. 1, 3-4**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale p. 5	2	Explain the scientific background and rationale for the investigation being reported
Objectives, p. 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design, p.6	4	Present key elements of study design early in the paper
Setting, p.6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants, p.6	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables, p.6	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement, p.6-8	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias, p.6	9	Describe any efforts to address potential sources of bias
Study size, p. 8, 9	10	Explain how the study size was arrived at
Quantitative variables, p. 18	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods, p. 8	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants, p. 8, 9	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data, p. 10	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data, p. 12	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results, p. 13, 14	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses, p. 18	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results, p. 15, 16	18	Summarise key results with reference to study objectives
Limitations, p. 18	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation, p.17, 18	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability, p 18	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding NA	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.