Chemokine (C-C motif) ligand 3 detection in the serum of persons exposed to asbestos: A patient-based study

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Exposure to asbestos results in serious risk of developing lung and mesothelial diseases. Currently, there are no biomarkers that can be used to diagnose asbestos exposure. The purpose of the present study was to determine whether the levels or detection rate of chemokine (C-C motif) ligand 3 (CCL3) in the serum are elevated in persons exposed to asbestos. The primary study group consisted of 76 healthy subjects not exposed to asbestos and 172 healthy subjects possibly exposed to asbestos. The secondary study group consisted of 535 subjects possibly exposed to asbestos and diagnosed with pleural plaque (412), benign hydrothorax (10), asbestosis (86), lung cancer (17), and malignant mesothelioma (10). All study subjects who were possibly exposed to asbestos had a certificate of asbestos exposure issued by the Japanese Ministry of Health, Labour and Welfare. For the primary study group, levels of serum CCL3 did not differ between the two groups. However, the detection rate of CCL3 in the serum of healthy subjects possibly exposed to asbestos (30.2%) was significantly higher (P < 0.001) than for the control group (6.6%). The pleural plaque, benign hydrothorax, asbestosis, and lung cancer groups had serum CCL3 levels and detection rates similar to that of healthy subjects possibly exposed to asbestos. The CCL3 chemokine was detected in the serum of 9 of the 10 patients diagnosed with malignant mesothelioma. Three of the patients with malignant mesothelioma had exceptionally high CCL3 levels. Malignant mesothelioma cells from four biopsy cases and an autopsy case were positive for CCL3, possibly identifying the source of the CCL3 in the three malignant mesothelioma patients with exceptionally high serum CCL3 levels. In conclusion, a significantly higher percentage of healthy persons possibly exposed to asbestos had detectable levels of serum CCL3 compared to healthy unexposed control subjects.
important goal. Testing for asbestos exposure is particularly relevant for persons who work or previously worked in asbestos factories, residents who lived near asbestos factories, workers processing rubble resulting from destruction of asbestos-containing homes and buildings, and firefighters and other rescue workers.

Numerous studies searching for biomarkers of asbestos exposure and malignant mesothelioma, with the majority concentrating on malignant mesothelioma, have been carried out, and a number of markers have been proposed. Most of these studies, however, suffer from small patient numbers, and consequently, the diagnostic value of most proposed markers requires further evaluation. Osteopontin (OPN) and soluble mesothelin-related proteins (SMRP), as defined in Cristaudo et al. 2011, have generally been regarded as the most promising biomarkers. Application of OPN, however, is limited: OPN is not able to discriminate between asbestos-exposed subjects without malignant mesothelioma and unexposed subjects, and OPN is not specific to mesothelioma. Initially, SMRP was also found to be limited to detection of malignant mesothelioma, however, a later study reported that SMRP might also serve as a marker of asbestos exposure. These conflicting results remain to be resolved. Another promising biomarker is fibulin-3; however, it is not a distinguishing marker between asbestos-exposed subjects without malignant mesothelioma from unexposed subjects.

Therefore, establishment of biomarkers that detect asbestos exposure, and subsequently identify persons at risk of developing asbestos-associated diseases, including malignant mesothelioma, remains an important goal.

In rats treated with nanoscale titanium dioxide by intrapulmonary instillation, macrophages interact with TiO2 aggregates in the lung and produce chemokine (C-C motif) ligand 3 (CCL3), also known as macrophage inflammatory protein 1-α, resulting in increased levels of CCL3 in the blood. Based on this finding, we undertook the current patient-based study to determine whether the serum levels or the detection rate of CCL3 are elevated in asbestos-exposed subjects.

In this study, we determined the serum CCL3 levels in healthy asymptomatic subjects possibly exposed to asbestos and in healthy unexposed subjects. We also determined the serum CCL3 levels in patients possibly exposed to asbestos and diagnosed with pleural plaque, benign hydrothorax, asbestosis, lung cancer, and malignant mesothelioma. Our primary finding was that a significantly higher percentage of healthy asymptomatic persons possibly exposed to asbestos had detectable levels of serum CCL3 compared to healthy unexposed control subjects.

Materials and Methods

Ethics statement. This study was approved by the Ethics Review Committee of the respective participating institutes and hospitals: Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan), Asahi Rosai Hospital (Owaraisashi, Japan), Saiseikai Chuwa Hospital (Sakura, Japan), and Nagoyashi (Nagoya City) Koseiin Medical Welfare Center (Nagoya, Japan), and conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo 2004). Participants provided written informed consent before inclusion in the study, after which serum samples were obtained, coded, and stored in aliquots at −80°C until use.

Subjects. Serum of unexposed subjects. Control sera were collected from the teaching and research staff at the Nagoya City University Medical School and healthy inmate residents/patients at Nagoyashi Koseiin Medical Welfare Center Hospital (Koseiin Hospital) (n = 76; mean age, 50.9 ± 17.7 years). These subjects had no history of work or tenancy at asbestos-related workplaces or residences. They were free from lung and pleural lesions on periodical (once or twice a year) institutional health examinations including physical, chest x-ray, blood biochemical, and electrocardiogram examinations.

Serum of exposed subjects. The sera of subjects possibly exposed to asbestos (n = 707; mean age, 69.1 ± 8.2 years) were collected from patients who visited or were hospitalized in the Japan Labour Health and Welfare Organization Asahi Rosai (work-related accident) Hospital, the Saiseikai Chuwa Hospital, or the Nagoya City University Hospital from 2008 to 2012. All of the enrolled subjects potentially exposed to asbestos had certified documents issued by the Japanese Ministry of Health, Labour and Welfare for the compensation of medical care. The exposed subjects were grouped as follows: no detectable lesions (n = 172), pleural plaque (including 12 cases of pneumoconiosis complication) (n = 412), benign hydrothorax (n = 10), asbestosis lung (asbestosis) (n = 86), lung cancer (n = 17), and malignant mesothelioma (n = 10). The diagnosis for all lung and mesothelial disease cases was made by chest x-ray and/or computed tomography examinations. The diagnosis of malignant mesothelioma was confirmed by immunohistochemical examination coupled with histopathological examination of biopsy specimens. Pathological examination of malignant mesothelioma included an immunohistochemical antibody panel: positive markers were calretinin, mesothelin, WT1 (Wilms tumor 1), D2-40 (mAb directed against M2A antigen), and CK5/6 (cytokeratin 5/6). For malignant mesothelioma diagnosis, staining with at least two positive markers must be positive and carcinoembryonic antigen must be negative. In addition, thyroid transcription factor 1 and Ber-EP4 staining should be negative (see also ref. 50). All the malignant mesothelioma cases were epithelial-type tumors. For all subjects, job history and the site of residence were recorded. Residents near asbestos factories without any history of asbestos-related occupation were certified as asbestos-exposed and included in the asbestos-exposed groups. For smokers, previous or current smoking status was recorded and expressed as smoking index (Brinkman index: daily number of cigarettes × years of smoking).

Enzyme-linked immunosorbent assay. Human serum CCL3 was measured using the Quantikine Human CCL3/MIP-1α Immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions, except that the samples added to the ELISA plates were incubated at room temperature for 3 h instead of 2 h. Levels of CCL3 below the detection limit (7.8 pg/mL) were arbitrarily regarded as 0. The association of CCL3 levels with the subject’s work place, work duties, length of exposure, lapse of time after the last exposure, and smoking habit was analyzed.

Immunohistochemistry of malignant mesothelioma. Four biopsy cases and one autopsy case with malignant mesothelioma were available and examined by immunohistochemistry for the presence of CCL3, C-ERC/mesothelin (mesothelin), and CD68, a macrophage marker. (The autopsy case and three of the four biopsy cases were available for analysis of serum CCL3.) Slides of malignant mesothelioma were deparaffinized and heated in 10 mM sodium citrate, 0.05% Tween 20 (pH 6.0) for 10 min for antigen retrieval. The slides were blocked with Blocking One (03953-95; Nacalai Tesque, Kyoto, Japan) and incubated with rabbit anti-human CCL3 polyclonal antibodies (LS-B1056: Lifespan Biosciences, Seattle, WA, USA)
diluted 1:100 at 4°C overnight and then washed and incubated with Alexa Fluor 488 labeled anti-rabbit secondary antibodies diluted 1:500 (Invitrogen Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. The slides were then washed with Blocking One for 30 min and incubated with rabbit anti-human mesothelin mAbs (ab93620; Abcom, Tokyo, Japan) diluted 1:100 at 4°C overnight, and then washed and incubated with Alexa Fluor 546 labeled anti-rabbit secondary antibodies (Invitrogen Molecular Probes) diluted 1:500 for 1 h at room temperature.

**Statistics.** The Kruskal–Wallis test was used to analyze the levels of CCL3 in the serum. Spearman’s rank correlation coefficient was used to analyze the associations of CCL3 level and background factors: age, gender, cigarette consumption (scored by the Brinkman index), the length of exposure time to asbestos, and the lapse of time after the last potential exposure to asbestos. The Steel–Dwass method was used to compare CCL3 levels among the asbestos-exposed subgroups: no lesion, pleural plaque, benign hydrothorax, asbestosis lung cancer, and malignant mesothelioma. The effects of background factors on detection of serum CCL3 was analyzed using multivariable logistic regression, and CCL3 detection was analyzed using multivariable logistic regression adjusted by background factors. P-values < 0.05 were considered to indicate statistical significance. Statistical analyses were carried out using JMP version 9.0 (SAS Institute, Cary, NC, USA).

**Results**

**Study population.** The primary study population was composed of 76 healthy subjects not exposed to asbestos and 172 healthy, asymptomatic (i.e., no detectable lung or pleural lesions) patients possibly exposed to asbestos. The general characteristics of the primary study group are summarized in Table 1a.

The secondary study population was composed of 535 subjects possibly exposed to asbestos and diagnosed with pleural plaque (412), asbestosis (86), benign hydrothorax (10), lung cancer (17), and malignant mesothelioma (10). The general characteristics of the secondary study group are summarized in Table 1b.

All study participants, with the exception of the 76 healthy subjects not exposed to asbestos, had certificates of asbestos exposure issued by the Japanese Ministry of Health, Labour and Welfare. However, confirmation of the presence of asbestos fibers in the lung or pleural tissues of healthy, asymptomatic persons is not possible. Therefore, in the primary study group the study participants with certificates of asbestos exposure must be assumed to be possibly exposed to asbestos, resulting in this study group being composed of an above average number of persons exposed to asbestos rather than being composed entirely of asbestos-exposed persons. Consequently, these study subjects are referred to as healthy, asymptomatic subjects possibly exposed to asbestos in this report.

**Serum CCL3 levels: Primary study group.** The serum CCL3 levels in the unexposed group and the healthy, asymptomatic subjects possibly exposed to asbestos are shown in Figure 1. For the study participants with detectable serum CCL3, there was no difference in CCL3 levels between the healthy control subjects and the healthy, asymptomatic subjects possibly exposed to asbestos. The study data can be downloaded from Table S1.

**Serum CCL3 levels and background factors: Primary study group.** Age and cigarette consumption (scored by the Brinkman index) showed a significant association with serum CCL3 levels (Table 2). Gender, the length of exposure time to asbestos, and the lapse of time after the last potential exposure to asbestos did not show a significant association with serum CCL3 levels.

**Detection of serum CCL3: Primary study group.** Subjects with CCL3 levels higher than 7.8 pg/mL, the detection limit of the ELISA assay, were defined as positive for serum CCL3. The detection rate of CCL3 in the serum of the primary study group is shown in Table 3. The detection rate of serum CCL3 in healthy, asymptomatic subjects possibly exposed to asbestos (52/172; 30.2%) was significantly higher (see Table 4) than in the unexposed control group (5/76; 6.6%).

**Detection of serum CCL3 and background factors: Primary study group.** Age, gender, smoking habit (never, previous, or current smoker), cigarette consumption (scored by the Brinkman index), the length of exposure time to asbestos, and the lapse of time after the last exposure to asbestos did not show a significant association with detection of CCL3 in the serum.

**Levels of CCL3: Secondary study group.** The serum CCL3 levels in the secondary study population are shown, alongside the levels in the primary study population, in Figure 2. For the study participants with detectable serum CCL3 in the pleural plaque, asbestosis, benign hydrothorax, and lung cancer groups, CCL3 levels were not different between groups or from the healthy, asymptomatic subjects possibly exposed to asbestos. In contrast, detectable serum CCL3 levels in the 10 patients constituting the mesothelioma group were significantly higher compared to the other groups. Notably, the higher levels of serum CCL3 in the mesothelioma group was entirely due to the levels in three patients with extraordinarily high – 611, 1007, and 2012 pg/mL – serum CCL3 levels. The study data can be downloaded from Table S1.

**Detection of serum CCL3: Secondary study group.** The detection rate of CCL3 in the serum of the study subjects with pleural plaque (139/412; 33.7%), asbestosis (34/86; 39.5%), benign hydrothorax (3/10; 30.0%), and lung cancer (5/17; 29.4%) was similar to that of the healthy, asymptomatic subjects possibly exposed to asbestos (Table 5): there were no
significant differences in the detection rate of serum CCL3 between any of these groups. In contrast, the detection rate of CCL3 in the serum of the 10 patients constituting the mesothelioma group (9\text{/}10) was significantly higher than in the other groups (Table 5).

Immunohistochemical localization of CCL3 in malignant mesotheliomas. All biopsy specimens (4) and the autopsy specimen (1) showed clear expression of CCL3 in the tumor cells. In Figure 3, panel A is a malignant mesothelioma with glandular formation, and panel B is a malignant mesothelioma with solid proliferation. The tumor cells co-express CCL3 and mesothelin with CCL3 localizing primarily to the cytoplasm and mesothelin localizing more to the plasma membrane. These specimens were negative for the macrophage marker CD68 (data not shown). The levels of serum CCL3 of these two cases were 40.2 (panel A) and 2012.4 (panel B) pg\text{/}mL.

Discussion
Asbestos has a long history of use worldwide, and annual global production of asbestos remains at over 2 million tons.\textsuperscript{3} The extensive use of asbestos has resulted in widespread risk of developing asbestos-associated diseases due to deposition of asbestos in the lung and pleural tissue, which can persist for the remainder of the exposed person’s lifetime, causing foreign body inflammation in the lung and pleura. The ability to iden-

Table 2. Associations between chemokine (C-C motif) ligand 3 levels with background factors

| Background factor | Rho  | P-value |
|------------------|------|---------|
| Age              | +0.196 | 0.002  |
| Cigarette consumption | +0.171 | 0.026  |
| Gender (M = 0; F = 1) | −0.052 | 0.417  |
| Length of exposure time | +0.070 | 0.431  |
| Lapse of time since last exposure | −0.080 | 0.359  |

Table 3. Detection of serum chemokine (C-C motif) ligand 3 in the primary study group, consisting of healthy subjects exposed or not exposed to asbestos

| Lesion category | Total number of subjects | Number of positive subjects | Detection rate, % | 95% confidence interval |
|----------------|--------------------------|----------------------------|------------------|------------------------|
| Unexposed      |                          |                            |                  |                        |
| No lesions     | 76                       | 5                          | 6.6              | 0.3–14.5               |
| Asbestos exposed |                        |                            |                  |                        |
| No lesions     | 172                      | 52                         | 30.2             | 23.9–37.5              |

Table 4. Odds ratio for asbestos exposure. The detection rate of serum CCL3 in healthy, asymptomatic subjects possibly exposed to asbestos was significantly higher than in the unexposed control group

| Odds ratio | 95% confidence interval | P-value |
|------------|-------------------------|---------|
| No lesion group/control | 6.15 | 2.56–18.3 | <0.001  |
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Fig. 2. Serum chemokine (C-C motif) ligand 3 (CCL3) levels in the secondary study group. Levels of CCL3 in study participants possibly exposed to asbestos and diagnosed with pleural plaque, benign hydrothorax, asbestosis, lung cancer, and mesothelioma are shown. For ease of comparison, the primary study group is shown alongside the secondary study group. The upper region of the graph is a log plot and is not continuous with the lower region of the graph. The serum CCL3 levels of the three patients plotted in the upper region are shown. Excluding the subjects without detectable CCL3 and the three subjects with exceptionally high levels of CCL3, there are no differences in CCL3 levels between any of the groups.

Table 5. Detection of serum chemokine (C-C motif) ligand 3 in the secondary study group, composed of subjects possibly exposed to asbestos and diagnosed with lung disease

| Lesion category | Total number of subjects | Number of positive subjects | Detection rate, % | 95% confidence interval |
|-----------------|--------------------------|-----------------------------|------------------|------------------------|
| Exposed to asbestos |                        |                             |                  |                        |
| Pleural plaque† | 412                      | 143                         | 34.7             | 30.3–39.4              |
| Asbestosis      | 86                       | 34                          | 39.5             | 29.9–50.1              |
| Benign hydrothorax | 10                    | 3                           | 30.0             | 10.8–60.3              |
| Lung cancer     | 17                       | 5                           | 29.4             | 13.3–53.1              |
| Malignant mesothelioma | 10                | 9                           | 90.0             | 59.6–98.2              |

†Includes 12 cases of pleural plaque with pneumoconiosis (mainly silicosis).
with asbestos fibers even after the cessation of exposure to airborne asbestos.

Notably, the detection rate of CCL3 in the serum of the asbestos-exposed pleural plaque and asbestosis groups was not significantly different from the asbestos-exposed no lesion group. As the percentage of subjects diagnosed with pleural plaque and asbestosis that have asbestos fibers in their lung or pleural tissues is likely to be much higher than for the healthy, asymptomatic subjects possibly exposed to asbestos that constitute the asbestos-exposed no lesion group, this suggests that the detection rate of CCL3 in the serum of asbestos-exposed persons with certain asbestos-associated diseases is lower than for asymptomatic asbestos-exposed persons. One possible explanation for this seeming anomaly is that development of fibrotic tissue associated with inhaled asbestos fibers can prevent interaction of macrophages with the fibers.

Our study included 10 patients diagnosed with malignant mesothelioma. While this small number of malignant mesothelioma patients precludes assessment of CCL3 as a marker of malignant mesothelioma, two important observations were made. First, biopsy and autopsy specimens of malignant mesothelioma showed CCL3 expression by the tumor cells (see Fig. 3). Second, 9 of the 10 patients with malignant mesothelioma had detectable serum CCL3, and serum CCL3 levels in three of the malignant mesothelioma patients were dramatically higher than in the other study subjects. These observations suggest that expression of CCL3 by malignant mesothelioma tumor cells may be a source of the CCL3 in the serum of these patients and may result in extremely high levels of CCL3 in the serum of some malignant mesothelioma patients. We are currently accessing more malignant mesothelioma cases and appropriate controls to investigate the possibility that CCL3 may be a biomarker for malignant mesothelioma. The use of CCL3 in the pleural fluid and biopsy specimens for diagnosis of mesothelioma is also being examined.

In conclusion, inhalation of asbestos elicits a high risk of developing lung and mesothelial diseases, including fatal malignant mesothelioma. Careful follow-up of patients exposed to asbestos is a key issue in controlling the development of asbestos-associated diseases. Accordingly, identification of healthy, asymptomatic persons exposed to asbestos is an important goal. The CCL3 chemokine is detectable in the serum of a significant percentage of asymptomatic persons exposed to asbestos. Persistent detection over time of CCL3 in the serum of a healthy individual possibly exposed to asbestos...
References
1 Marsili D, Comba P. Asbestos case and its current implications for global health. Am Jt Super Sana 2013; 49: 249–51.
2 Robinson BW, Lake RA. Advances in malignant mesothelioma. N Engl J Med 2005; 353: 1591–603.
3 Ramazzini C. Asbestos is still with us: repeat call for a universal ban. Am J Ind Med 2011; 54: 168–73.
4 Robinson BW, Creaney J, Lake R et al. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 2003; 362: 1612–6.
5 Maeda M, Hino O. Molecular tumor markers for asbestos-related mesothelioma: serum diagnostic markers. Pathol Int 2006; 56: 649–54.
6 Hino O, Maeda M. Diagnostic tumor marker of asbestos-related mesothelioma. Environ Health Prev Med 2008; 13: 71–4.
7 Iwahori K, Osaki T, Serada S et al. Megakaryocyte potentiating factor as a tumor marker of malignant pleural mesothelioma: evaluation in comparison with mesothelin. Lung Cancer 2008; 62: 45–54.
8 Scherpereel A, Grigoriu B, Conti M et al. Soluble mesothelin-related peptides in the diagnosis of malignant pleural pleural mesothelioma. Am J Respir Crit Care Med 2006; 173: 1155–60.
9 Shionni K, Hagiwara Y, Sonoue K et al. Specific and sensitive new enzyme-linked immunosorbent assay for N-ERC/mesothelin increases its potential as a useful serum tumor marker for mesothelioma. Clin Cancer Res 2008; 14: 1431–7.
10 Rodriguez Portal JA, Rodriguez Becerra E, Rodriguez Rodriguez D et al. Serum levels of soluble mesothelin-related peptides in malignant and nonmalignant asbestos-related pleural disease: relation with past asbestos exposure. Cancer Epidemiol Biomarkers Prev 2009; 18: 646–50.
11 Pass HI, Lott D, Lonardo F et al. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 2005; 353: 1564–73.
12 Amati M, Tomasetti M, Mariotti L, Tarquini LM, Valentinio M, Santarelli L. Assessment of biomarkers in asbestos-exposed workers as indicators of cancer risk. Mutat Res 2008; 655: 52–8.
13 Amati M, Tomasetti M, Scartozzi M et al. Profiling tumor-associated markers for early detection of malignant mesothelioma: an epidemiologic study. Cancer Epidemiol Biomarkers Prev 2008; 17: 163–70.
14 Yasumitsu A, Tabata C, Tabata R et al. Clinical significance of vascular endothelial growth factor in malignant pleural mesothelioma. J Thorac Oncol 2010; 5: 479–83.
15 Hirayama N, Tabata C, Tabata R et al. Pleural effusion VEGF levels as a prognostic factor of malignant pleural mesothelioma. Respir Med 2011; 105: 137–42.
16 Raiko I, Sander I, Weber DG et al. Development of an enzyme-linked immunosorbent assay for the detection of human calretinin in plasma and serum of mesothelioma patients. BMC Cancer 2010; 10: 242.
17 Yasuda M, Hanagiri T, Shigematsu Y et al. Identification of a tumour associated antigen in lung cancer patients with asbestos exposure. Anticancer Res 2010; 30: 2631–9.
18 Maeda R, Tabata C, Tabata R, Eguchi R, Fujimori Y, Nakano T. Is serum thioredoxin-1 a useful clinical marker for malignant pleural mesothelioma? Antioxid Redox Signal 2011; 15: 685–9.
19 Tabata C, Terada T, Tabata R et al. Serum thioredoxin-1 as a diagnostic marker for malignant peritoneal mesothelioma. J Clin Gastroenterol 2013; 47: 7–11.
20 Watzka SB, Porsch F, Pass HI et al. Detection of integrin-linked kinase in the serum of patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2011; 142: 384–9.
21 Leivo-Korpela S, Lehtimaki L, Nieminen R et al. Adipokine adipin is associated with the degree of lung fibrosis in asbestos-exposed workers. Respir Med 2012; 106: 1435–40.
22 Pass HI, Levin SM, Harbut MR et al. Fibrin-3 as a blood and effusion biomarker for pleural mesothelioma. N Engl J Med 2012; 367: 1417–27.
23 Rostila A, Puustinen A, Toljamo T et al. Peroxiredoxins and tropomyosins as plasma biomarkers for lung cancer and asbestos exposure. Lung Cancer 2012; 77: 450–9.
24 Santarelli L, Strafella E, Staffolani S et al. Association of MiR-126 with soluble mesothelin-related peptides, a marker for malignant mesothelioma. PLoS One 2011; 6: e18232.
25 Fuji M, Fujimoto N, Hiraki A et al. Aberrant DNA methylation profile in pleural fluid for differential diagnosis of malignant pleural mesothelioma. Cancer Sci 2012; 103: 510–4.
26 Lehtomin H, Oksa P, Lehtimaki L et al. Increased alveolar nitric oxide concentration and high levels of leukotriene B(4) and 8-iso prostane in exhaled breath condensate in patients with asbestosis. Thorax 2007; 62: 602–7.
27 Pelcova D, Fenclova Z, Kacer P, Kuzma M, Navratil T, Lebedova J. Increased 8-iso prostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. Ind Health 2008; 46: 484–9.
28 Chow S, Campbell C, Sandrini A, Thomas PS, Johnson AR, Yates DH. Exhaled breath condensate biomarkers in asbestos-related lung disorders. Respir Med 2009; 103: 1091–7.
29 Syslova K, Kacer P, Kuzma M et al. Rapid and easy method for monitoring oxidative stress markers in body fluids of patients with asbestos or silica-induced lung diseases. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877: 2477–86.
30 de Gennaro G, Dragonieri S, Longobardi F et al. Chemical characterization of exhaled breath to differentiate between patients with malignant pleural mesothelioma from subjects with similar professional asbestos exposure. Anal Bioanal Chem 2010; 398: 3043–50.
31 Lehtimaki L, Oksa P, Jarvenpaa R et al. Pulmonary inflammation in asbestos-exposed subjects with borderline parenchymal changes on HRCT. Respir Med 2010; 104: 1042–9.
32 Dragonieri S, van der Schee MP, Massaro T et al. An electronic nose distinguishes exhaled breath of patients with Malignant Pleural Mesothelioma from controls. Lung Cancer 2012; 75: 326–31.
33 Cristaudo A, Bonotti A, Simonini S, Bruno R, Foddis R. Soluble markers for diagnosis of malignant pleural mesothelioma. Biomark Med 2011; 5: 261–73.
34 Creaney J, Yeoman D, Demelker Y et al. Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. J Thorac Oncol 2008; 3: 851–7.
35 Grigoriu BD, Chahine B, Zerimech F et al. Serum mesothelin has a higher diagnostic utility than hyaluronic acid in malignant mesothelioma. Clin Biochem 2009; 42: 1046–50.
36 Grigoriu BD, Grigoriu C, Chahine B, Gey T, Scherpereel A. Clinical utility of diagnostic markers for malignant pleural mesothelioma. Monaldi Arch Chest Dis 2009; 71: 31–8.
37 Gube M, Taeger D, Weigl DG et al. Performance of biomarkers SMRP, CA125, and CYFRA 21-1 as potential tumor markers for malignant mesothelioma and lung cancer in a cohort of workers formerly exposed to asbestos. Arch Toxicol 2011; 85: 185–92.
38 Hollevest K, Nackauts K, Thimpont J et al. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am J Respir Crit Care Med 2010; 181: 620–5.
39 Luo L, Shi HZ, Liang QL, Jiang J, Qin SM, Deng JM. Diagnostic value of soluble mesothelin-related peptides for malignant mesothelioma: a meta-analysis. Respir Med 2010; 104: 149–56.
40 Pass HI, Wali A, Tang N et al. Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann Thorac Surg 2008; 85: 265–72; discussion 72.
41 Ray M, Kindler HL. Malignant pleural mesothelioma: an update on biomarkers and treatment. Chest 2009; 136: 888–96.

Disclosure Statement
Hiroyuki Tsuda has submitted a patent application for CCL3: #PCT/JP2012/056321. None of the other authors has a conflict of interest.
Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Study participant data.