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Exposure to Nanoparticles is Related to Pleural Effusion, Pulmonary Fibrosis and Granuloma

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Abstract

Nano materials generate great benefits as well as new potential risks. Animal studies and *in vitro* experiments show that nano particles can result in lung damage and other toxicity, but no reports on the clinical toxicity in humans due to nano particles have yet been made.

The study aims to examine the relationship between a group of workers' mysterious symptomatic findings and their nano particle exposure.

Seven young female workers (18-47years), exposed to nano particles for 5-13 months, all with shortness of breath and pleural effusions, were admitted to the hospital. Immunologic tests, examinations of bacteriology, virology and tumor markers, bronchoscope, internal thoracoscopy, and video-assisted thoracic surgery were performed. Survey of the workplace, clinical observations and examinations on patients were conducted.

Polyacrylate, consisting of nano particles, was confirmed in the workplace. Pathological examinations of patients' lung tissue displayed non-specific pulmonary inflammation, pulmonary fibrosis and foreign-body granulomas of pleura. By transmission electron microscopy, nano particles were observed to lodge in the cytoplasm and caryoplasm of pulmonary epithelial and mesothelial cells, but also locate in the chest fluid. These cases arouse concern that long-term exposure to some nano particles without protective measures may be related to serious damage to human lungs.

Key words: foreign-body granuloma; human; hypoxemia; pleural effusion; polyacrylate nano particle; pulmonary fibrosis

Introduction

With the explosion of nano materials, the benefits afforded by them are expected to have significant impacts on almost all industries and all areas of society. New materials based on nanotechnology are already reaching the market in a wide variety of consumer products. It is estimated that the nanotechnology economy will exceed $1 trillion by 2012 (1). However, the vastness and novelty of nano materials leaves open the possibility that they may generate new risks to workers, consumers, and the environment. Several previous studies caution against the possible significant adverse effects of nano materials (2-5).

Animal studies and *in vitro* experiments show that nano particles could result in lung damage and other toxicity. Papageorgiou et al. (6) showed that cobalt-chromium alloy nanoparticles caused more free radicals and induced more DNA damage, more aneuploidy, and more cytotoxicity than micron-sized particles in human fibroblasts in tissue culture. In mice study (7), it was demonstrated that pharyngeal aspiration of single-walled carbon nanotubes (SWCNT) elicited pulmonary effects: acute inflammation with early onset and progressive fibrosis, granulomas, and functional respiratory deficiencies. Study on carbon nanoparticle (CNP) (8), exposure in both *in vitro* and *in vivo* systems, presents evidence that nanoparticles, accumulated in the plasma membranes of cells, increased alveolar macrophage function with antigen-presentation, significantly exacerbated airway hyper-responsiveness, and caused an influx of macrophages into the lungs.

It is possible, theoretically, that long-term exposure to nano particles may cause serious damage to humans as well as animals. However, no reports on the clinical toxicity in humans due to long term exposure to nano particles have been made until now. We report on a group of patients with mysterious findings; pleural effusions, pulmonary

fibrosis, foreign-body granulomas and hypoxemia, which may be the result of toxicity from long - term exposure to nano particles.

Methods

Patients. From January 2007– April 2008, seven young female patients, working in the same department in a print plant, were admitted to our hospital. They suffered from the same symptoms: shortness of breath, and the same clinical findings of pleural effusion and pericardial effusion. These women previously underwent treatment in local hospitals including multiple thoracentesis attempts with recurrence of effusion, antibiotics, anti-tuberculosis drugs, and methylprednisolone or prednisolone. All the patients were previously in good health, and denied any history of smoking or prior occupational exposure to hazard materials, they used to do some housework or farmwork as peasants.

Clinical examinations. All were approved by our hospital's ethics commission, informed consent was obtained before all the examinations and procedures. Routine tests of blood, urine, pleural fluid, and arterial blood gas analysis were performed. Bacteriologic examinations, including cultures of blood, urine, sputum and fluid in chest cavity, were performed. The antibodies of *Mycoplasma pneumoniae* (IgG, IgM), *Chlamydia pneumoniae* (IgM), *Legionella* (IgG, IgM) and tubercle bacillus antibody (TB - Ab) were detected by enzyme linked immunosorbent assay (ELISA) methods. Virologic examinations were conducted including antibodies of cytomegalo virus, epstein-barr virus, human syncytial virus, cache valley virus, rhinovirus, adenovirus, hepatitis virus (A, B, C, E) and human immunodeficiency virus. Positive antibody results were subsequently tested using polymerase chain reaction (PCR) magnification. Immunologic tests, including cell immunity (CD3, CD4, CD8), humoral immunity (IgA, G, D, M, E and C3, C4) and

autoimmune autoantibodies (antinuclear antibody, anti-ribonucleic acid antibody, anti-nucleosome antibody, anti-skin-sensitizing antibodies A, B, anti-SCL-70 antibody, anti histone antibody, rheumatoid factor, C-reacting protein, and antistreptolysin-O test), were conducted. Imaging included X-rays of chest, ultrasound of the thoracic cavity, heart, liver, kidney and spleen, and either CT scanning of chest, or CT pulmonary angiogram. Functional examinations included liver function assays, renal function tests and pulmonary function tests. Patients underwent a variety of procedures according to their symptoms including drainage of pleural effusions, bronchoscopic examinations with bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBLB), internal thoracoscopic examinations with lung biopsy, and video-assisted thoracic surgery（VATS）with wedge resection. Pathologic examinations included light microscopy, and transmission electron microscopy (TEM) of the pleura, pleural fluid and lung tissue.

Survey of the workplace. Epidemiologists from the Chinese Center of Disease Control (CDC), Beijing CDC, local CDC and doctors from our hospital carefully investigated the factory where the patients worked. The paste materials used by the patients and dust particles that accumulated in the local ventilation were analyzed by gas chromatography / mass spectrometry (GC-MS).

Treatment. All patients underwent thoracentesis for both etiologic investigation of the pleural effusions and symptomatic relief. Three patients were performed closed drainage of pleural effusions followed by thoracoscopy, two of seven received video-assisted thoracic surgery (VATS). Antibiotics and supportive therapy, such as oxygen, were used due to persistent inflammation in the lungs demonstrated by chest X-rays and chest CT.

Results

Survey of the workplace. A survey of the patients' workplace was conducted. It measures about 70 square meters (m^2), has one door, no windows, and one machine used to air spray materials, heat and dry boards. This machine has three atomizing spray nozzles, and one gas exhauster (a ventilation unit) that broke 5 months before the occurrence of the disease. The paste material used is an ivory white soft coating mixture of polyacrylic ester.

Eight workers (seven female, one male) were divided into two equal groups each working 8-12 hours shifts. The workers took the above coating material (room temperature), by spoon, to the open-bottom pan of the machine, which automatically air-sprayed the coating material at the pressure of 100-120Kpa onto polystyrene (PS) boards (organic glass), which can then be used in the printing and decorating industry. The PS board was heated and dried at 75-100 degrees Celcius, and the smoke produced in the process was cleared by the gas exhauster. Six kilograms of coating material were typically used each day. The PS board sizes varied from 0.5 - 1m^2 and approximately 5000m^2 were handled each workday. The workers had several tasks in the process including loading the soft coating material in the machine, as well as clipping, heating, and handling the PS board. Each worker participated in all parts of this process.

Accumulated dust particles were found at the intake of the gas exhauster. During the five months preceding illness the door of the workspace was kept closed due to cold outdoor temperatures. The workers were all peasants near the factory, and had no knowledge of industrial hygiene and possible toxicity from the materials they worked with. The only personal protective equipment (PPE) used on an occasional basis was cotton gauze masks. According to the patients, there were often some flocculi produced during air

spraying, which caused itching on their faces and arms. It is estimated that the airflow or turnover rates of indoor air would be very slow, or quiescent due to the lack of windows and the closed door.

Of the 8 workers, the seven women worked there for 5-13 months and all had the described symptoms. The lone male worker was employed there for 3 months and was asymptomatic. The patients' family and the workers in other departments of the same plant did not have similar symptom complaints.

Analysis of used paste and dust particles. The used paste was analyzed using GC-MS. The compound was stated to be polyacrylic ester by the paste producer. It contained the following components: butanoic acid, butyl ester, n-butyl ether, acetic acid, toluene, di-tert-butyl peroxide, 1-butanol, acetic acid ethenyl ester, isopropyl alcohol and ethylene dioxide. Electron microscopy of both the paste and the dust particles found nano particles approximately 30nm in diameter.

The general characteristics of the patients (Table1). All the patients were young women, aged 18-47 years, average 29 years old. Two were single and five were married. They had complaints of dyspnea with exertion and signs consistent with pleural fluid and hypoxemia. Four out of seven patients were found to have hypoxemia ($PO_2 < 10.7$kPa) [normal range (NR): 10.7-13.3kPa], but no carbon dioxide retention. Additionally, all patients suffered from rash with intense itching on their faces, hands and forearms. All patients were found to have pleural fluid by ultrasound, and five patients had pericardial effusions in depth of about 4-8mm. There were not any morphologic abnormalities about the heart, liver, kidney and gynecologic organs.

Results of routine blood, urine, and functional tests of liver, kidney and lung (Table2). All the patients had routine tests of blood and urine. The results of the urine tests were normal. All the patients were noted to have white blood cell counts and red blood cell counts that were in the normal range. Five patients had monocytosis with an elevated absolute monocyte count and neutropenia with a decreased absolute neutrophil count. Additionally, five patients had elevated erythrocyte sedimentation rate (ESR) and one patient had thrombocytopenia (35-51)$\times 10^9$/L (NR:100-300 $\times 10^9$/L).

Six out of seven patients were found, after seven months observation, to have hypoproteinemia (albumin: 25.5-28.9g/L, NR: 32.0-55.0g/L), protein electrophoresis of serum showed decreased albumin (50.2-55.6%, NR: 60.0-71.0%), globulins (α_1 2.9%, NR:1.4-2.9%; α_2 15.4%, NR:7.0-11.0%; β 14.1%, NR:8.0-13%; and γ 12%, NR:9.0-16.0%) were approximately in the normal range. Functional tests of kidney were regularly conducted and within normal limits, but several patients were found with elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Table2). Spirometry showed that all the patients suffered from small airway injury and restrictive ventilation dysfunction, which showed that 3/7 patients with severe lung damage: Maximal vital capacity (VC_{MAX}) (actual value/ predicted value): 24.8-35.4% (NR: >=80%), forced vital capacity (FVC): 24.6-35.1% (NR: >=80%), and forced expiratory volume in one second (FEV_1): 24.8-36.5% (NR: >=80%); 1/7 patients with moderate lung damage VC_{MAX} 63.6%, FVC 61.3%, and FEV_1 63.4%; and 3/7 patients with mild lung damage VC_{MAX} 71.6-92.6%, FVC 75.6- 91.9%, and FEV_1 60 -70.6%.

Immunologic tests and etiologic examinations. As for immunologic tests for cell immunity, humoral immunity and auto immunity, no significant clinical abnormities were

found. As for bacterial investigation, the results of tests on acid-fast bacilli, *Mycoplasma pneumoniae,* and *Chlamydia pneumoniae* were all negative except 2/7 patients with positive results of *Mycoplasma pneumoniae* IgG(+), IgM(+) and *Chlamydia pneumoniae* IgM(+). However, there were no clinical correlations with the above test results. As for virologic examinations and the subsequently tests using PCR magnification on positive antibody results of virus, no clinical correlations were observed between the illness and the test results. Tumor markers, including alpha fetoprotein, carcinoembryonic-like antigen, neurone specific enolase and transferrin, were conducted and all the results were negative both in serum and pleural fluid.

Imaging studies. Repeated examinations of chest X-rays and CT scanning showed all the patients to have pleural effusions and 5 patients with pericardial fluid, 4/7 patients had diffuse ground-glass opacities, 6/7 had pulmonary interstitial nodules, and 3/7 had lymph node adenopathies. No patients had pleural thickening, pleural nodules or pleural plaques. All the seven patients had pulmonary interstitial inflammation and pulmonary fibrosis, and by seven months observation, rapid progressive pulmonary interstitial fibrosis was observed in two patients (case 5 and case 1), also pleural calcification was observed in one patient (case 1), but in the other patients, pulmonary interstitial fibrosis developed very slowly.

Thoracentesis and tests of pleural fluid. The results of the pleural fluid analysis were similar for all the patients showing an amber exudate, which may be produced by physical or chemical irritation. The total cells were $(330\text{-}485)\times10^6$/L; white blood cells were $(10\text{-}470)\times10^6$/L, and subsets of the cells were monocytes (55-85%) and polykaryocytes (15-45%), total protein was (40.1- 49.3)g/L, specific gravity was greater

than 1.018. Rivalta reaction, which is used as a puncture fluid test for differentiation of exudate and transudate, was negative. The concentrations of chloridion in all patients' pleural effusions (108.0-111.8mmol/L, NR: 118.0-129.0mmol/L) were under the lower limit of normal, and concentrations of glucose in all patients' pleural effusions were elevated (5.01- 6.59mmol/L, NR: 2.50-4.44mmol/L). No malignant cells were found in the pleural fluid, mesothelial cells and lymphocytes were common.

Bronchoscope inspection. All patients had bronchoscopy, and four of them had swelling and congestion of the tunica mucosa bronchiorum.

BALF. Examination of the BALF showed decreased macrophages (29-79 %, NR: >84%), increased lymphocytes (15-54%, NR: <13%), elevated neutrophil leukocytes (6-18 %, NR: <3%), and eosinophils in the normal range (0.5 %,NR: <0.5%) in five of the patients. The other two patients were within the normal ranges for BALF cell counts. T cells in the BALF were normal: CD3: 69-73% (NR: 63-83%)□CD4: 40% (NR: 40-70%)□CD8□35% (NR: 20-40%).

TBLB. The results of all patients were consistent showing aggregations of phagocytes and inflammatory cells, proteinaceous effusions in the alveolar space, swollen and widened alveolar septums with scattered neutrophil leucocytes, and pulmonary fibrosis (Figure 1. a). The pathyologic examination of tunica mucosa bronchiorum was also observed the effusion of inflammatory cells (Figure 1. b).

Thoracoscopy. Three out of the seven patients had thoracoscopy. Two patients had mild congestion of the partial pleura, but overall it was smooth and glossy with no neoplasm or exelcosis. The other patient had a normal thoracoscopy. Biopsies of the pleura

and fluid in the chest were also analyzed. Pathologic study of pleura showed foreign-body granulomas (Figure 1. c), hemorrhage, as well as fibrinous and inflammatory cells.

VATS. Two patients underwent VATS, one was the 18-year-old girl (case 3), four months after symptom onset. Wedge resection, lung biopsy and drainage of pleural effusions were performed. Approximately 2000ml of fluid was drained from the chest cavity. Exploration of the chest cavity showed no pleural adhesions, capillary hemorrhage or air leak. On the lung surface, there were scattered small tubercles, which were biopsied from the superior, middle, and inferior lobes. Pleural biopsy was also obtained.

There were non-specific findings by light microscope such as fibrous thickening in the pleural membrane, pulmonary alveoli with emphysema–like alterations, widened alveolar septums, depositions of collagen fibers, engorged capillaries filled with lymphocytes, and infiltrations of phagocytes in alveolar spaces.

The other patient who underwent VATS was the 19-year-old girl (case 5) eighteen months after symptom onset. This was performed emergently due to respiratory failure, secondary to encapsulated pleural effusion formation, pulmonary fibrosis, and severe pericardial effusion development. Moreover, she was in very serious condition due to continuous closed drainage of the thoracic cavity and subsequent loss of protein. Intraoperatively, multi-encapsulated pleural effusions and pleural fibrous lamina formations were observed. The pleural membrane was noted to have severe adhesions and thickening, especially in the visceral and diaphragmatic pleura, which was measured 1cm in thickness and was hard like a helmet. Fenestration of the pericardium was performed, and 170ml of light-colored fluid was drained from the pericardial cavity.

Pathologic examination (Figure 1. d and e) showed fibrous thickening and swelling of the pleural membrane, fibroblast proliferation, cellulosic exudate, lymphocyte aggregation and nodulus lymphaticus formation. The alveolar septum was widened with blood vessel dilatation and congestion. There were type II alveolar epithelial cell proliferations, and pulmonary alveoli were partly emphysematous with scattered multinucleated giant cells.

Examinations of lung pathology and chest fluid by TEM. By TEM, round nano particles about 30nm in diameter were found to scatter in the fluid after a drop was stained by uranyl acetate, air-dried and observed (Figure 2. a). The nano paticles were wrapped up in a fibrous structure, which may have originated from the cytolysis in chest fluid. After the upper liquid of the chest fluid was centrifuged at 1000 rotations/minute, treated as above and observed, the nano particles looked like sperm or comets because the fibrous structure was not completely separated. In the mesothelial cells of the pleural fluid, similar nano particles were found to scatter in the cytoplasm, and lodge in nuclei and other cytoplasmic organoids.

In the lung tissue, nano particles were observed to lodge in the cytoplasm and caryoplasm of the pulmonary epithelial cells (Figure 2. b), with the chromatin condensation and marginalization, like a crescent, showing characteristic cell morphology of cells undergoing apoptosis (Figure 2. c and d). Additionally, around intravascular red blood cells in the pulmonary interstitial tissue, there was a massive aggregation of nano particles**.** No poikilocytosis was noted. In the BALF, similar nano particles were found.

Outcome of the patients. Two patients suffered from iatrogenic pneumothorax, one secondary to thoracoscopy examination and its lung biopsy, another due to VATS and the lung biopsy. Both recovered quickly after treatment. The rashes and itching on the faces

and arms were treated symptomatically. Follow up at 20 months revealed persistent shortness of breath, continued pleural effusions, and slowly progressive pulmonary fibrosis.

The pleural effusions of two patients (case 5 and case 1) developed so quickly that continuous closed drainage of the thoracic cavity was necessary (300-800ml pleural fluid was usually drained everyday). VATS was emergently performed on the 19-year-old woman (case 5) eighteen months after symptom onset. The procedure included fenestration of the pericardium, decortication of pleural fibrous lamina, wedge resection, lung biopsies, and drainage of pleural effusion. Thereafter, she suffered from severe tension pneumothorax 24 hours post-VATS, and acute pericardial tamponade on the tenth day post-surgery, which were all successfully treated. But at last, she died of respiratory failure 16 days post-surgery. The 29-year-old woman (case 1), who was similar with the 19-year-old girl (case 5) both in clinical findings and the development of the illness, died of respiratory failure in local hospital in the twenty-first month after symptom onset.

Discussion

From the report, the patients have several common characteristics. First, they were all young women who were exposed to nano particles consisting of polyacrylic ester without any protective measures for 5-13 months. Also, they presented in the same time frame with the same symptoms: shortness of breath, and the same clinical findings of pleural and pericardial effusions consisting of an amber exudate, which may be produced secondary to physical or chemical irritation. These effusions recurred repeatedly after thoracentesis, and other treatments were rendered ineffective. Additionally, the pathological examinations displayed non-specific pulmonary inflammation, inflammatory infiltration, pulmonary

fibrosis, and foreign-body granulomas of the pleura. These findings were consistent with damage produced by nano particles in animal experiments (7,9). Importantly, nano particles about 30nm in diameter were found in the used paste and dust particles of the workplace as well as in the BALF, chest fluid, and lung biopsies of the patients. By TEM, nano particles were found to lodge in the cytoplasm and caryoplasm of the pulmonary epithelial cells and mesothelial cells of the chest fluid. Also, the chromatin of the pulmonary epithelial cell condensed and marginalized like a crescent, showing characteristic cell morphology of cells undergoing apoptosis. In all of these cases, clinical observations and examinations excluded infections, malignant tumors, immune related disorders and other diseases.

We could infer that the patients may have suffered from damage related to nano particles (30nm in diameter) by comparing these cases with the toxicity of nano materials observed in the animal experiments. These animal studies showed non-specific pulmonary inflammation, granuloma formation of lungs, rapidly progressive fibrosis and lung function deficiency following nano materials aspiration (7-9).

As obseved in animal and *in vitro* experiments, nano materials are found to cause disorders of blood element tests and hepatic function tests of serum ALT and AST(10-11). In the group of patients, five patients had monocytosis with an elevated absolute monocyte count and neutropenia with a decreased absolute neutrophil count; additionally, five patients had elevated ESR and one patient had thrombocytopenia; moreover, several patients were found with elevated levels of ALT and AST; all that was observed above suggests the hematological toxicity of the polyacrylate nano particles. However, the hypoalbuminemia found in some patients is likely due to prolonged drainage of pleural

fluid rather than a specific effect of nano particle exposure, as the levels of serum album in patients were all in normal in the beginning, and furthermore no hypoalbuminemia related to nano particles is reported in animal experiments.

Polyacrylate, widely used as an adhesive in the building, print, and decoration fields, has often been regarded as low toxicity. To make the material stronger and more abrasion-resistant, nano particles, after being modified to turn their surface from hydrophilic to organophilic, can be directly added in organic resin to make organic-inorganic hybrids. These inorganics include silicon nanoparticles (12) , thin zinc oxide (13), titanium dioxide (TiO_2) (14), nanoscale silver cluster (15), and other engineered nano materials. A series of silicon-containing polyacrylate nanoparticles have also been successfully synthesized and widely used (12, 16). However, it is the nano materials containing nano-size particles that appear to produce the toxicities seen in the exposed workers.

Therefore, we have more evidence to show that the nano particles contained in the polyacrylate emulsion had possibly caused the disease. There is an indication from this report that shows the possible dangerous nature of nano particles. Nano particles can penetrate the membrane of pulmonary epithelial cells and lodge in the cytoplasm and caryoplasm, as well as aggregate around the membrane of red blood cells and exert toxicity. Patients may develop clinically serious conditions associated with damaged respiratory function including a progressive pulmonary fibrosis that is resistant to several methods of treatment.

Animal studies show that some nano particles can travel throughout the body, deposit in target organs, penetrate cell membranes, lodge in mitochondria, and trigger injurious

responses. Inflammatory and granulomatous responses are seen in the lungs following exposure to carbon nanotubers (7,17,18). A rapid progressive fibrosis in mice exhibited diffuse interstitial fibrosis and alveolar wall thickening likely associated with dispersed nano particles (7). The major routes for nano particles to enter the body may be through inhalation, ingestion, dermal, and injection (4). In this group of patients, we could infer that exposure to nano particles occurred at least by inhalation and dermal.

Inhalation. The respiratory system is one of the most important portals of entry and organ target for nano particles, which can avoid normal phagocytic defenses in the respiratory system and travel throughout the pulmonary alveoli. Once inhaled and deposited, nano particles appear to translocate to extra pulmonary sites and reach other target organs by different transfer routes and induce toxicological effects (4,19). The generation of smoke at the workplace when the PS board is heated and dried may indicate the formation of a condensation aerosol (containing nano particles) from volatilized material. Particularly, as the freshly-generated fumes are highly reactive and damaging (20), the nano particles entered the body while the spraying, heating and drying of the boards were being performed may exert great toxicities on the exposure workers . In addition, this could help to explain the nano particles deposition in the respiratory tract and in BALF, formation of lung fibrosis, occurrence of multi-serous membrane fluid due to the irritation of nano particles to cell membrane, existence of foreign body granulomas in pleura, inflammatory infiltration and hilar lymphadenopathy.

Dermal. Studies of nanoparticle absorption through the skin are inconclusive and disputable, some demonstrate little penetration into the epidermis while others show deep absorption (21-23). Alvarez-Roman et al show that although polystyrene nano particles (20

to 200 nm) accumulate in the follicle orifices, the particles do not penetrate into the skin or the follicle (24). However, Some studies suggest that skin is surprisingly permeable to nano materials with diverse physicochemical properties, and may serve as a portal of entry for localized or possibly systemic exposure of humans to engineered nano materials (25,26). Nano particles can penetrate intact skin at an occupationally relevant dose within the span of an average-length workday. Recent study on TiO_2 nano particles at workplaces finds that TiO_2 nano particles production workers have significant risk on cytotoxicity response at relatively high airborne concentrations at size range 10–30nm(27). Furthermore, protein tyrosine nitration is a potential hazard of nano TiO_2 on skin, the toxicity of nano TiO_2 to the skin disease should be paid more attention in the production and utilization process (28). In this group of patients, skin may be an available portal of entry into the body by occupational dermal exposure, this could help to explain the rash and itching on the exposed skin of the above patients-likely the dermal toxicity of the polyacrylate nano particles.

The kind of the nano particles and the mechanisms of the injury in patients have not been firmly established. However, animal studies and *in vitro* experiments may provide a clue that the nano particles themselves (6-8), not as carriers, cause the injury directly. Although, the nano particles may also act as carriers facilitating translocation and persistence in the pleural and pericardial spaces. Furthermore, after being analyzed by GC–MS, the compounds in the materials used in the workplace, such as butanoic acid, acetic acid, toluene and ethylene dioxide, are low in toxicity, and are unlikely to cause disease of the severity seen in these patients. So, the patients' illness appears to be a “nano material-related disease”.

There are several limitations about our report. First, this description of patients is limited by the absence of environmental monitoring data of the workplace. Because no one suspected that the illness of the patients would be related to the occupational nano particles exposure in a very long time after the occurrence of the accident, the environmental monitoring of the workplace was not performed in time and the accurate concentrations of the polyacrylate nanoparticles that the workers were likely exposed to was still not known. However, the detailed description of their working, the duration of daily exposure, the dosage of the material used everyday, the space of their workplace and the serious results of long term exposure give us some important information that the concentrations of the polyacrylate nano particles that the workers were exposed to may be very high. Second, the composition of the nano paticles is still unknown though a lot of efforts have been made including our contacting the manufacturer concerning the content of nano materials in the product. Third, there are still many questions to be answered: what are the nano particles (chemistry and composition) found in patients? Would only specific nano particles, or nano particles in general, cause the illness in patients? If it is the nano particles that caused the patients' disease, what does it mean for other workers who use nano particles in their workplace? Was this an isolated incident or could this be occurring on a larger scale? What are the effects of long term exposure to nano particles in animal studies? However, all data given in the report have suggested that the polyacrylate nano particles that the patients were exposed to are linked to the patients' illnesses. As this may be the first report on the clinical toxicity in humans due to long term exposure to nano particles, and so many questions need to be answered, more studies on the possible mechanisms, diagnosis, treatment and prevention of the “nano material-related disease” are needed.

In conclusion, these cases arouse concern that long term exposure to some nano particles without protective measures may be related to serious damage to human lungs. It is impossible to remove nano particles that have penetrated the cell and lodged in the cytoplasm and caryoplasm of pulmonary epithelial cells, or that have aggregated around the red blood cell membrane. Effective protective methods appear to be extremely important in terms of protecting exposed workers from illness caused by nano particles.

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Table1. The general characteristics of the patients

Case	Age (Yr) /Sex	Duration of exposure (M)	PO2 (kPa)	Pleural effusion	Pericardial effusion	Bronchoscope	Thoracoscopy	VATS	Outcome
1	29/F	13	9.6	great	small	(+)	(+)	Not Done	Die
2	47/F	11	10.5	great	None	(+)	(+)	Not Done	Stable
3	18/F	13	9.6	great	small	(+)	(+)	(+)	Stable
4	29/F	12	11.6	great	small	(+)	Not Done	Not Done	Stable
5	19/F	10	10.7	great	small	(+)	Not Done	(+)	Die
6	35/F	10	12.9	great	small	(+)	Not Done	Not Done	Stable
7	28/F	5	12.9	small	None	(+)	Not Done	Not Done	Stable

Abbreviations: F, female; Great, great amounts of; M, months; Small, small amounts of, video-assisted thoracic surgery; Yr, year; “+”, performed.

Table2. The changes of white blood cell counts, the differential counts and the related laboratory items in initial stage

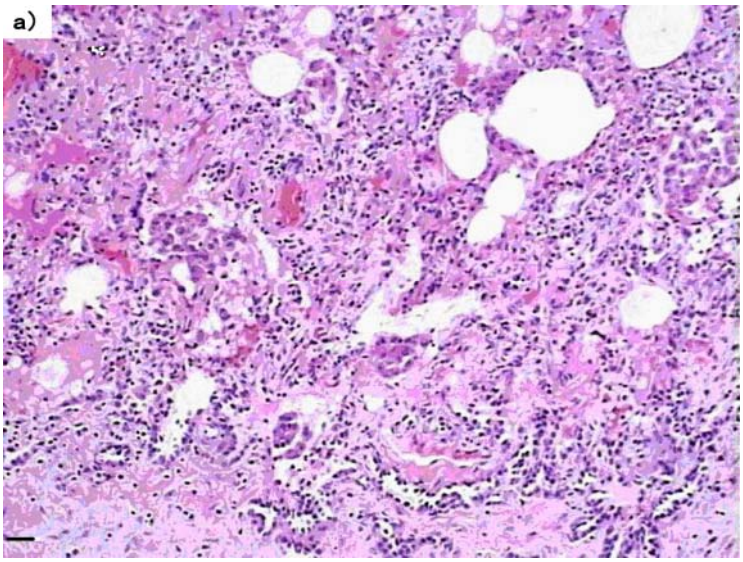
Case	WBC (x109/L)	N (%)	NC (x10^9/L)	M (%)	MC (x10^9/L)	PLT(x10^9/L)	ESR(mm/h)	CRP (mg/L)	ALT(U/L)	AST (U/L)
	NR:4.0-10.0	NR:50.0-70.0	NR:2.0-7.0	NR:3-8	NR: 0.12-0.80	NR:100-300	NR:0-20	NR: <10	NR:10-40	NR:10-42
1	5.83	34.1	2.89	24.5	1.49	210	26	<8	25	21
2	4.15	37.8	1.74	16.5	0.82	150	6	56	30	20
3	6.39	35.5	2.24	16.3	1.44	254	28	<8	17	63
4	4.86	32.6	1.58	9.6	0.45	51	53	<8	73	70
5	6.98	24.5	1.71	13.3	0.96	201	30	<8	42	59
6	5.66	63.5	3.6	7.2	0.41	206	12	<8	20	18
7	5.32	63.6	3.39	6.8	0.36	100	22	<8	15	30

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C- reactive protein; ESR, erythrocyte sedimentation rate; M, monocyte; MC, monocyte counts; N, neutrophilic granulocyte; NC, neutrophilic counts; NR, normal range; PLT, platelets counts; WBC, whiter blood cell counts.

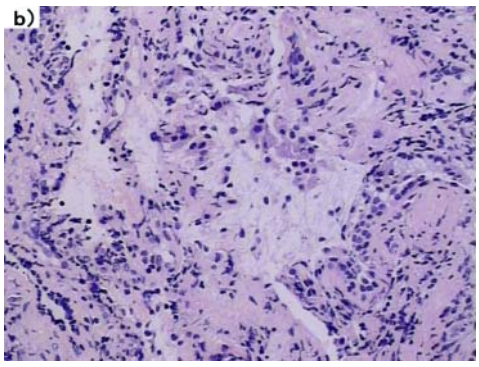
Figure Legends

Figure 1.

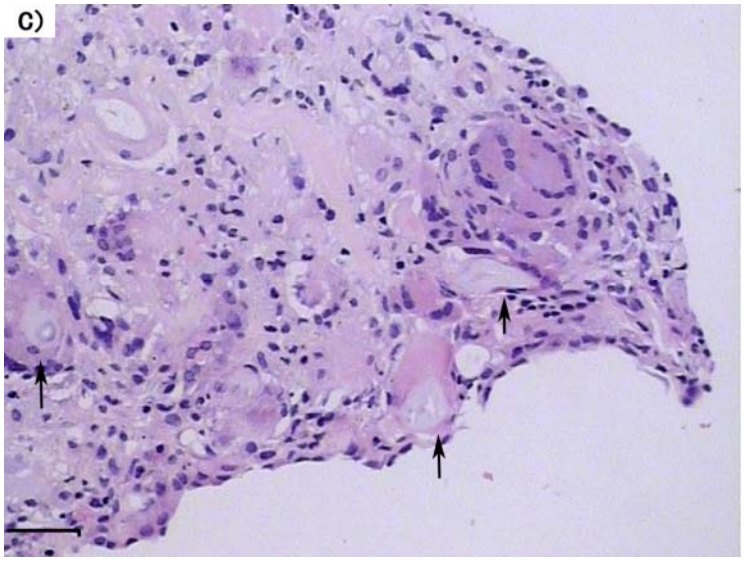
a) Aggregation of phagocytes in the alveolar space, swollen and widened alveolar septums, and pulmonary fibrosis were observed (H&E stain). Scale bar= 20 μm.



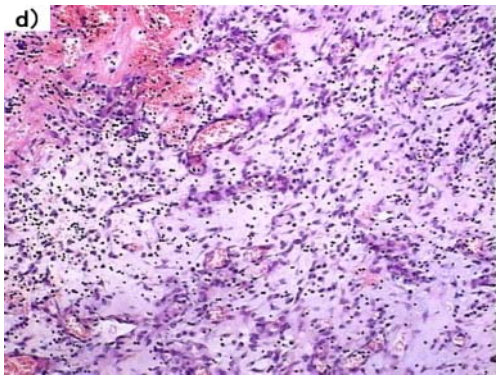
b) Effusion of inflammatory cells was observed in tunica mucosa bronchiorum (H&E stain ×200) .



c) Pathologic examination of the pleura showed foreign-body granulomas with fibrinous and inflammatory cell effusions (H&E stain). Scale bar= 25 μm.



d) Eighteen months later, pathologic examination of the pleural membrane showed fibrous thickening and swelling, fibroblast proliferation, cellulosic exudate and lymphocyte aggregation (H&E stain ×100).



e) Eighteen months later, pulmonary pathologic examination showed the alveolar septum was widened with blood vessel dilatation and congestion. Pulmonary alveoli were partly emphysematous with scattered multinucleated giant cells (H&E stain ×200).

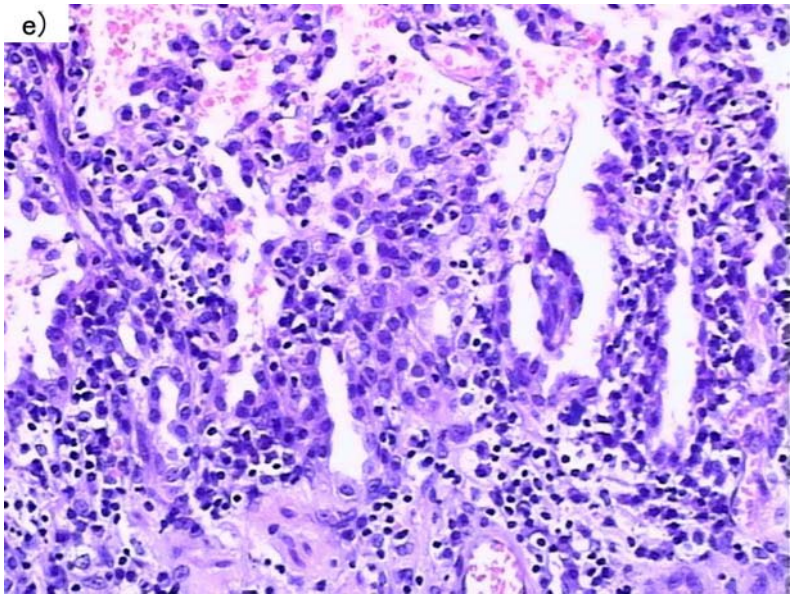
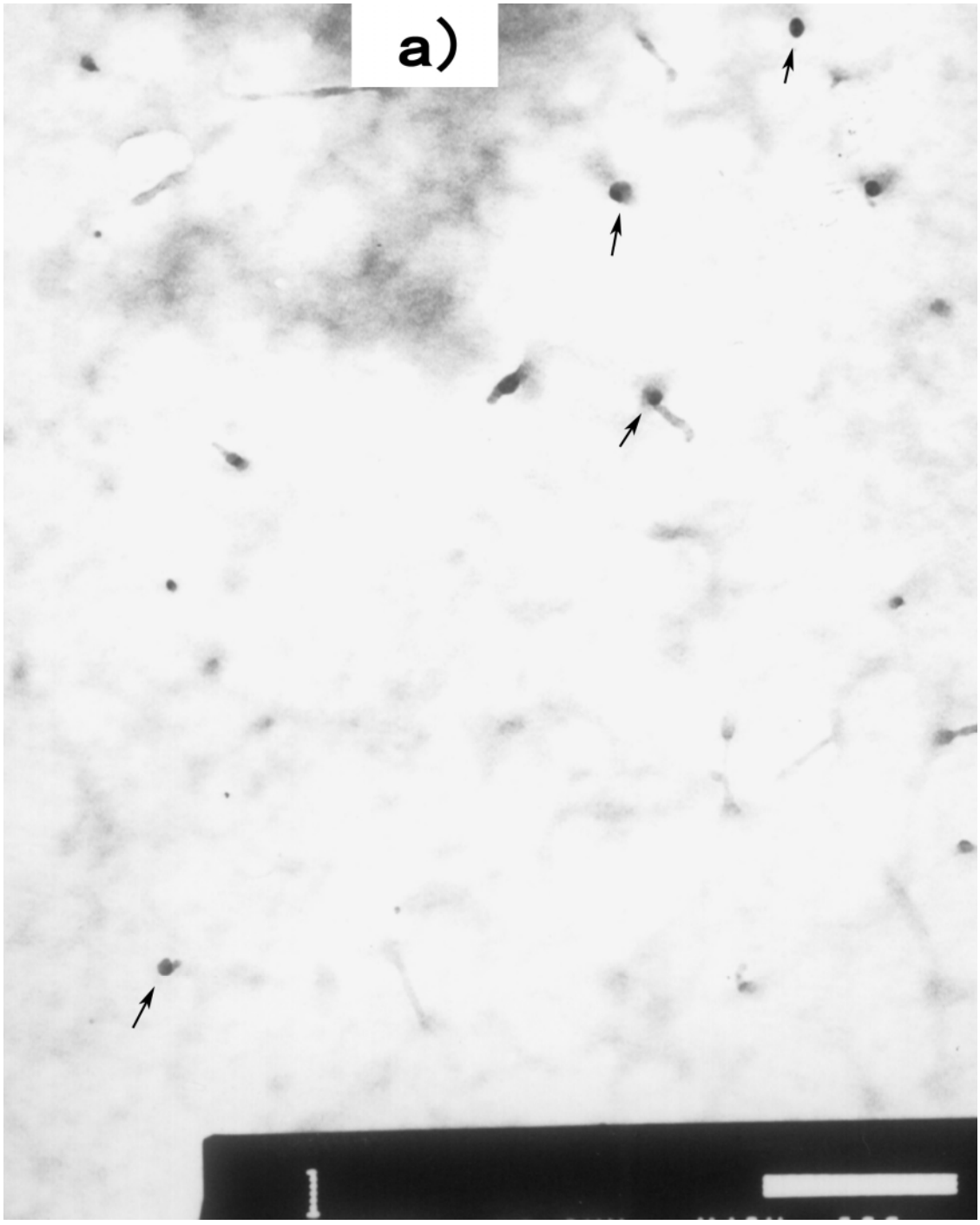
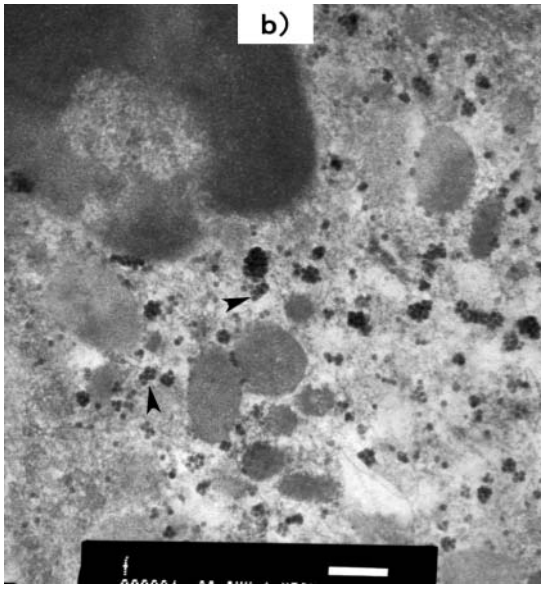


Figure 2.

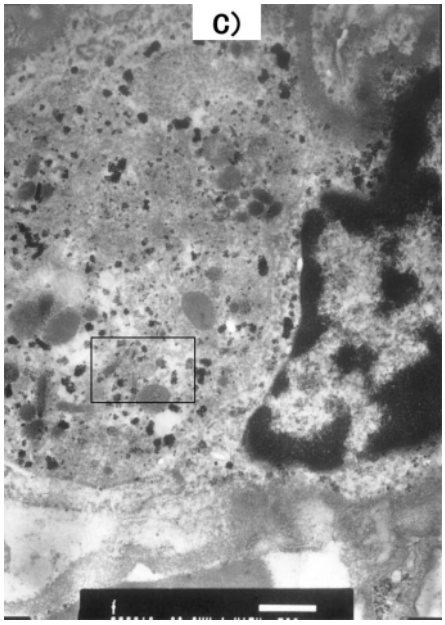
a) Image of nano particles in chest fluid: round nano particles about 30nm in diameter were scattered in the fluid, being wrapped up by fibrous structure, which may have originated from cytolysis in pleural fluid. Scale bar= 200 nm.



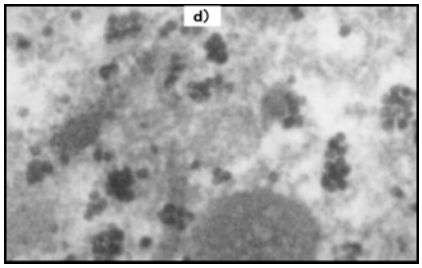
b) Clusters of nano particles (30nm in diameter) were observed to lodge in the caryoplasm of a pulmonary epithelial cell. Scale bar= 200 nm.



c) With nano particles lodging in the cytoplasm and caryoplasm of the pulmonary epithelial cell, the chromatin had condensed and marginalized like a crescent, showing characteristic cell morphology of cells undergoing apoptosis. Scale bar= 500 nm.



d) Clusters of nano particles were obviously found when the selected part of Figure 2.c was zoomed in.


d)