



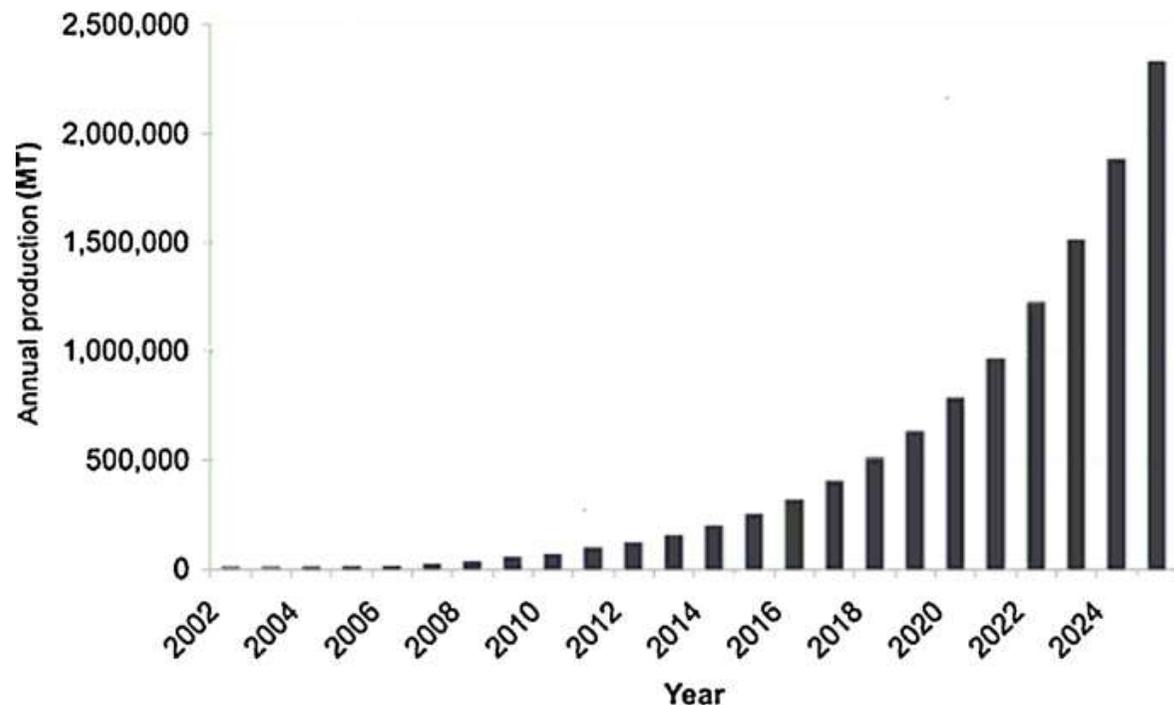
Silica nanoparticles cause pleural and pericardial effusion, pulmonary fibrosis and granuloma in rats as seen in exposed workers

**Beijing Chaoyang Hospital
Capital Medical University, China
Yuguo Song MD, Ph.D**

Nanosafety



- **The potential hazards of nanomaterials to the environment and human health were widely concerned.**



Nanosafety

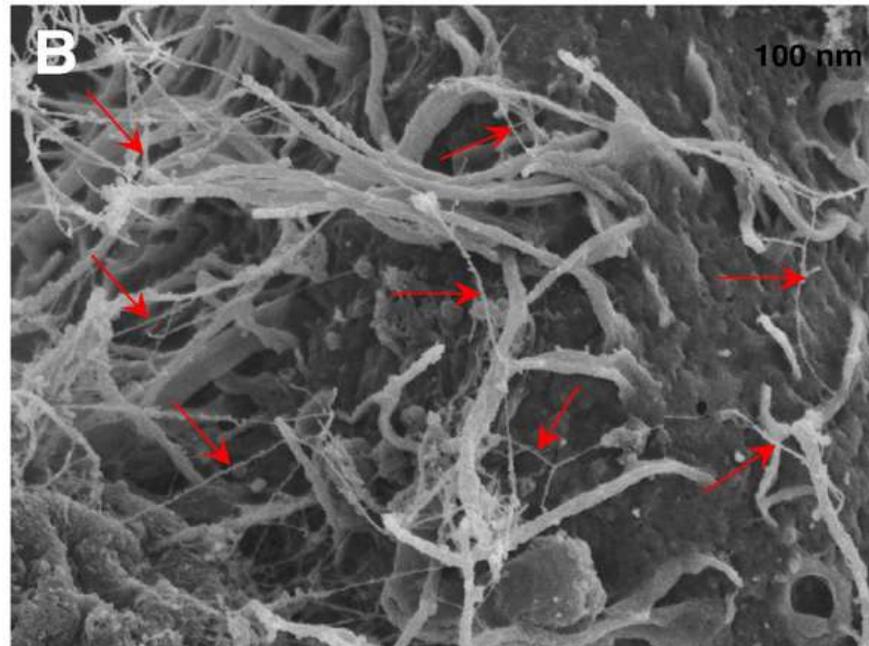
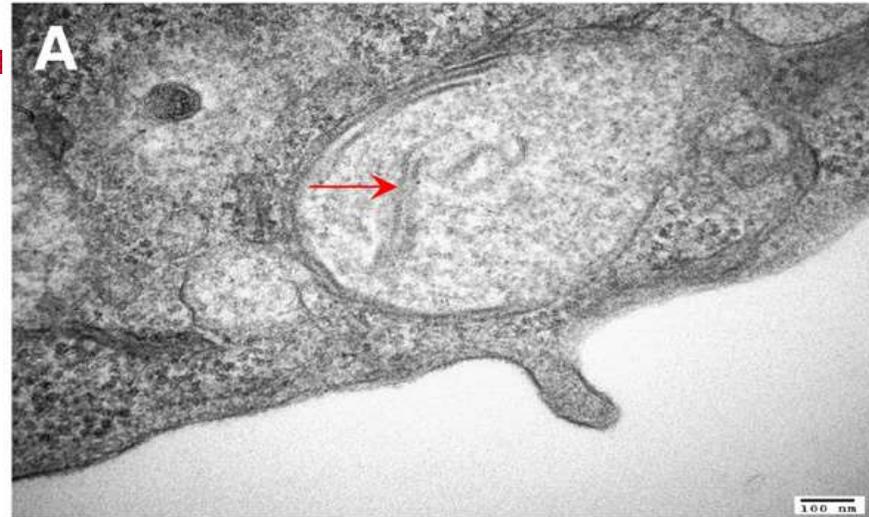


- **Wide-used nanomaterials, heavy exposure**
- **Nanoproducts, nanowaste enter environment.**
- **Not aware of the risk of the nanoproducts that workers are exposed to**

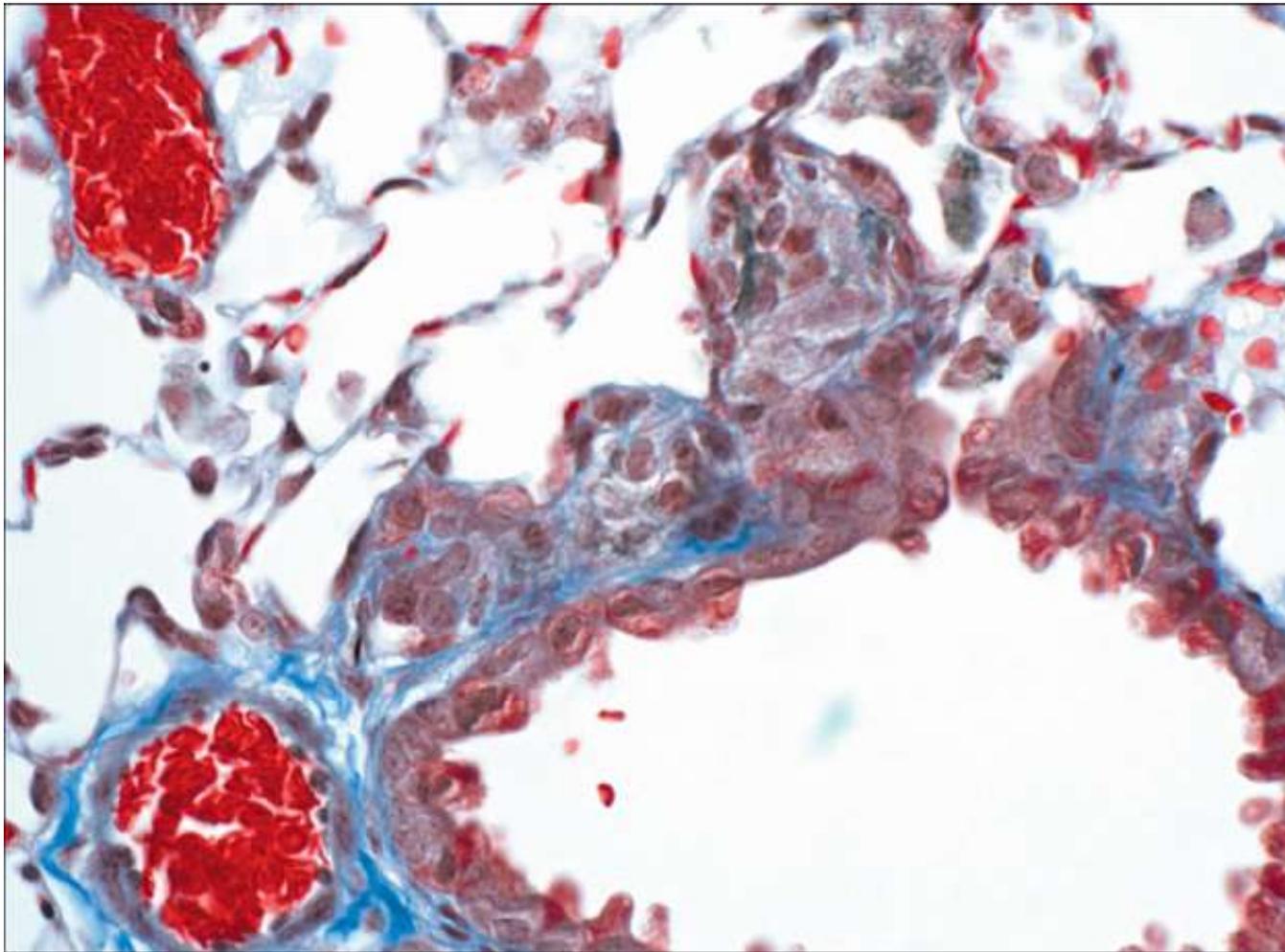
Nanotoxicities are found in animal experiments.



**SWCNT
in a macrophage**



Granuloma formation post SWCNT



Theoretical possibility of human toxicity



It is theoretically possible that nanomaterials may produce some novel toxicity and cause unusual diseases in humans as observed in some animal experiments.



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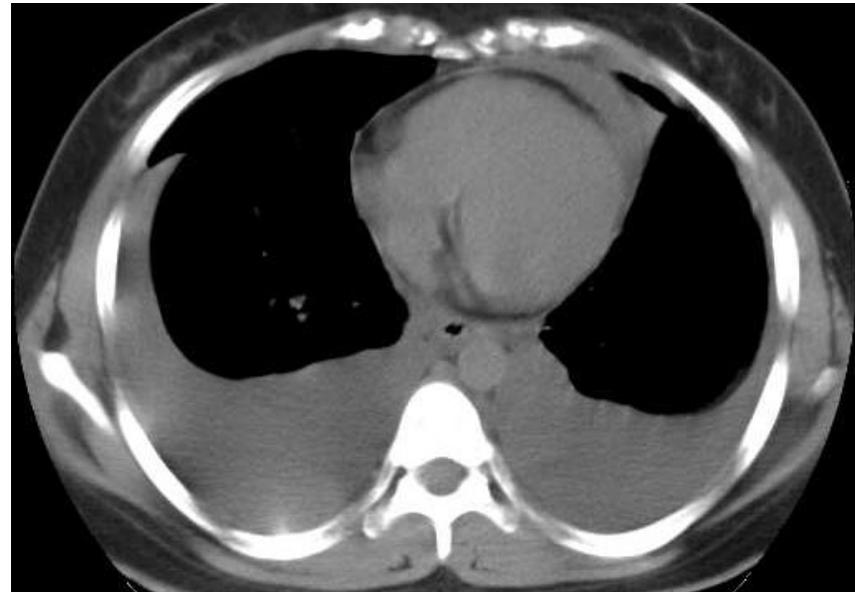
A group of patients with unusual disease



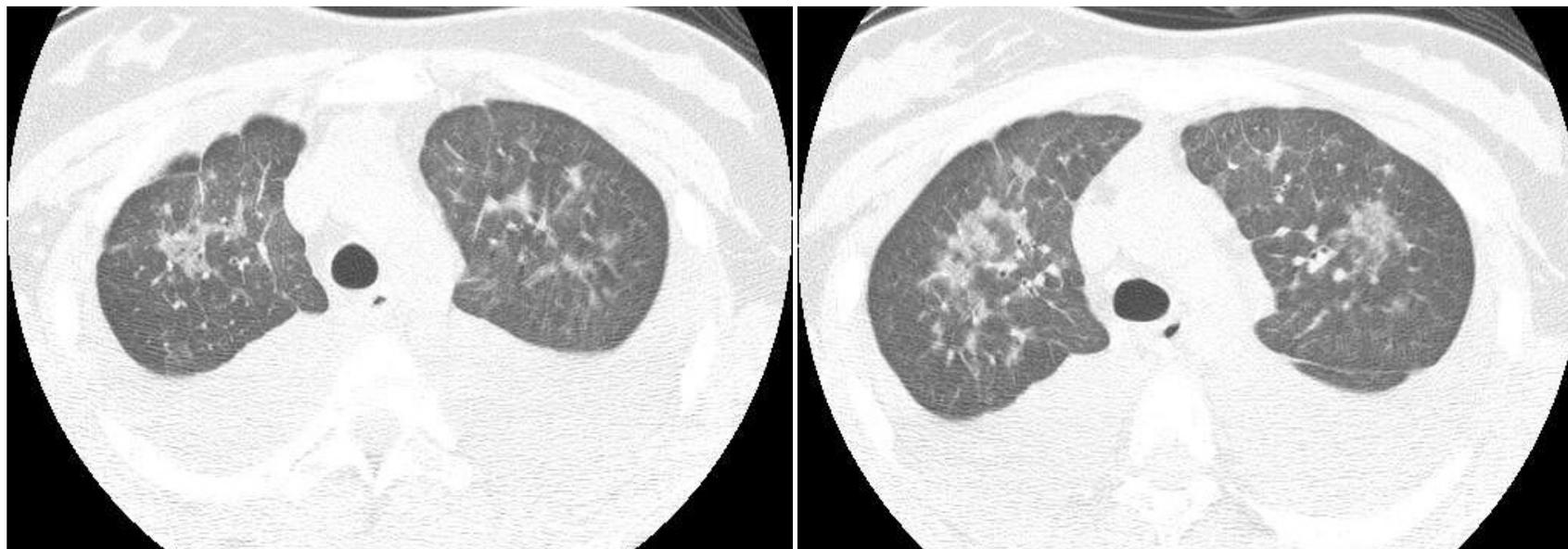
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case	sex	age	Exposure (M)	Plural effusion	Pericardial effusion	coatings
1	F	29	13	++	+	Polyacrylate
2	F	47	11	++	--	PA
3	F	18	13	++	+	PA
4	F	29	12	++	+	PA
5	F	19	10	++	+	PA
6	F	35	10	++	+	PA
7	F	28	5	+	--	PA

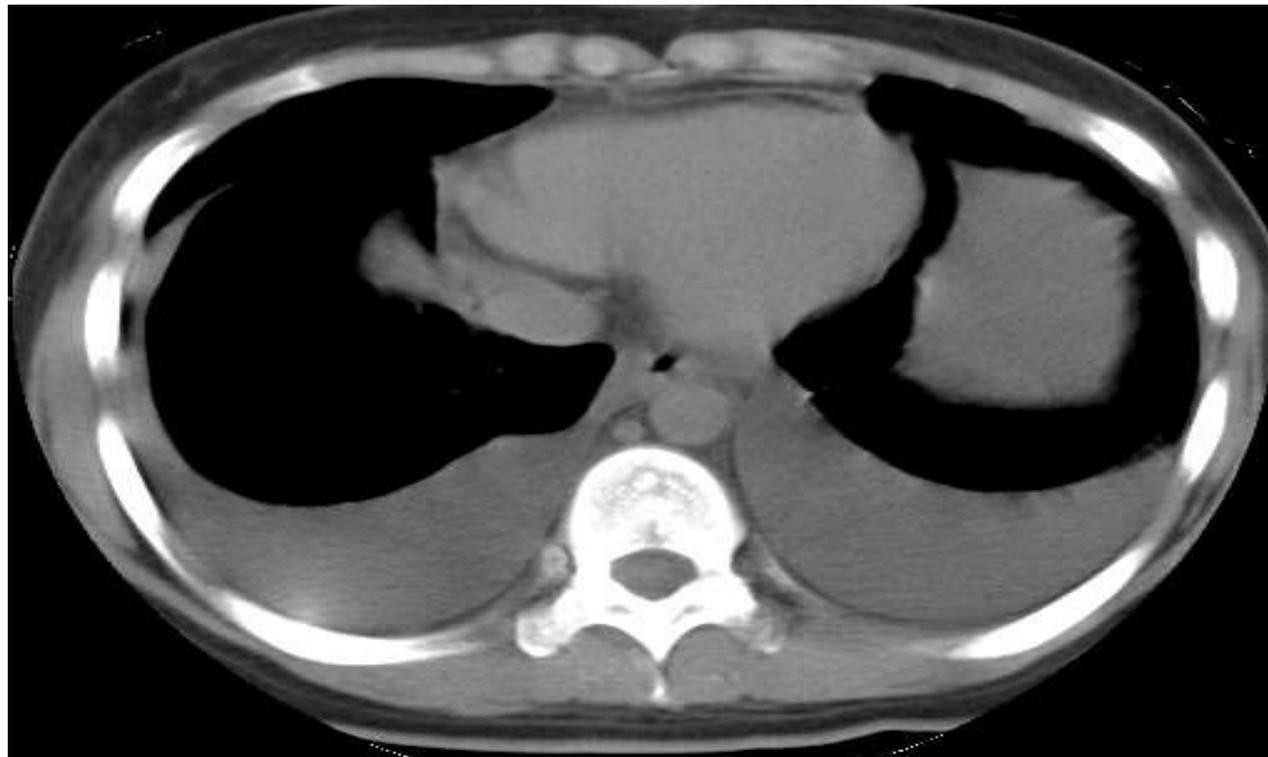
Case 1



Case 2



Case2



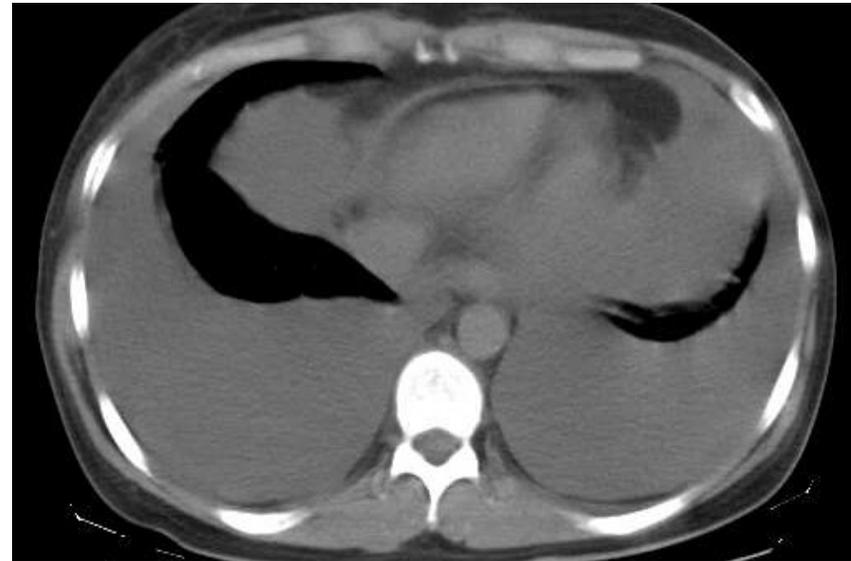
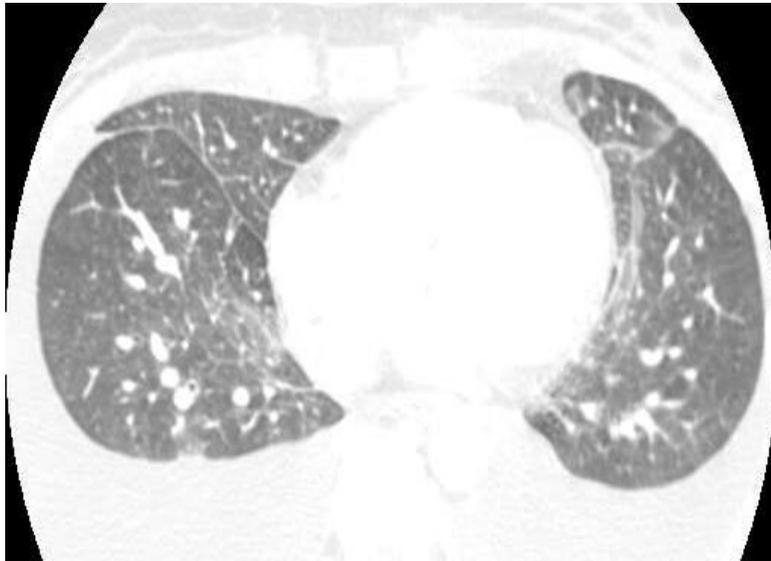
Case 3



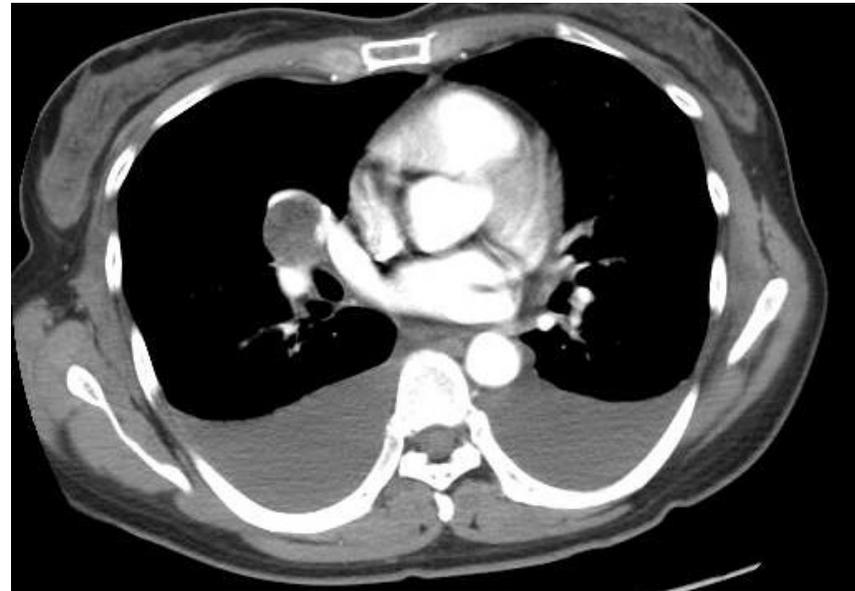
Case 3



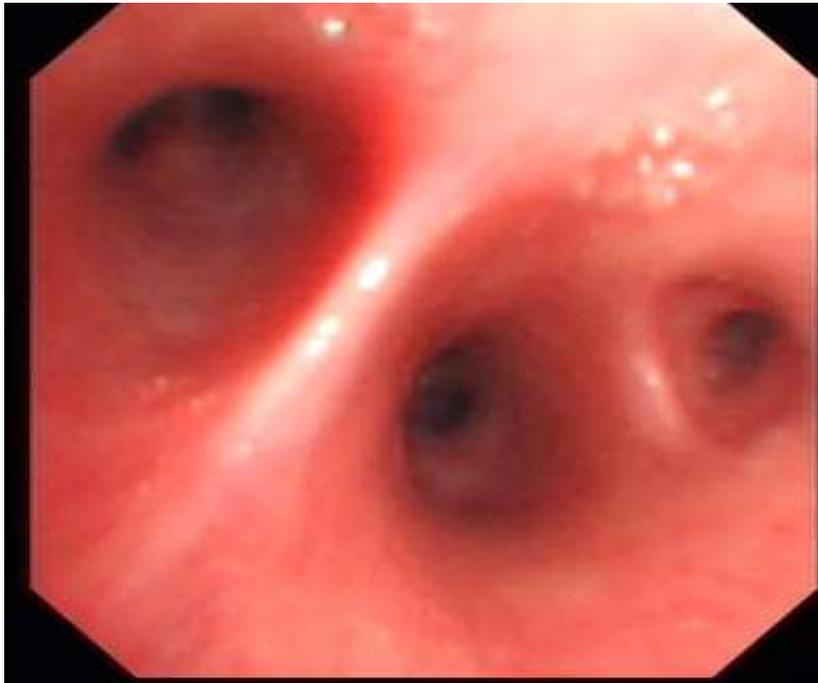
Case 4



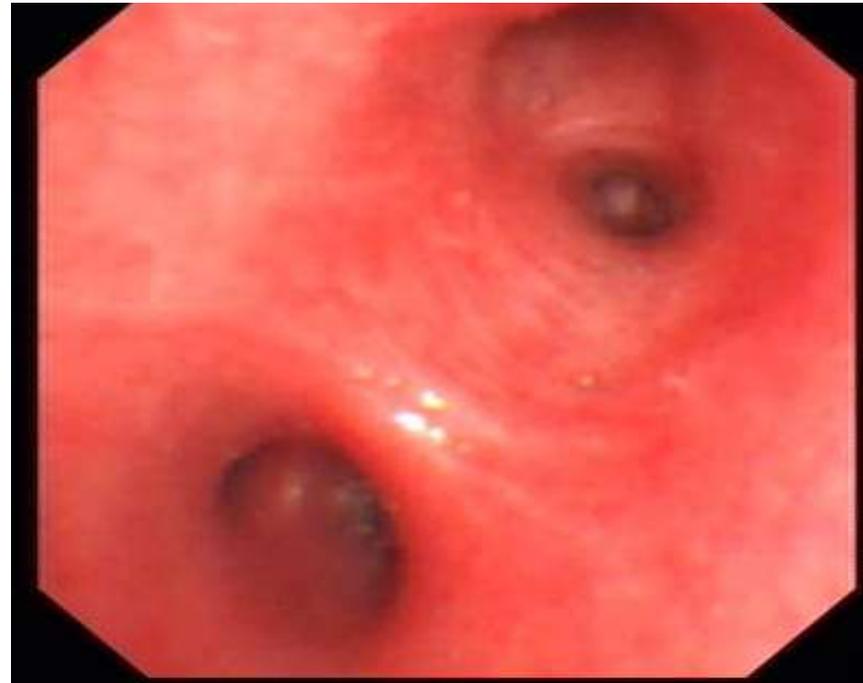
Case 5



Bronchofibroscope



lobus centralis dexte、 inferior lobe



bronchus principalis dexter

Clinical findings



- **Symptoms: shortness of breath.**
- **Signs:**
 - **pleural and pericardial effusion.**
 - **pulmonary inflammation**
(no effect to antibiotics and glucocorticoids)
 - **Pulmonary fibrosis**
Rapid and Progressive



19-yr-female(C5)

- ❑ **Pleural effusion and pericardial effusion**
- ❑ **Pulmonary fibrosis**
- ❑ **Rapid and Progressive**

The group of patients



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- All patients with pleural effusion
- Five out of seven with pericardial effusion.
- Fenestration of the pericardium was performed in the 19-yr-old female, and 170 mL of light-coloured fluid was drained.

Pathology

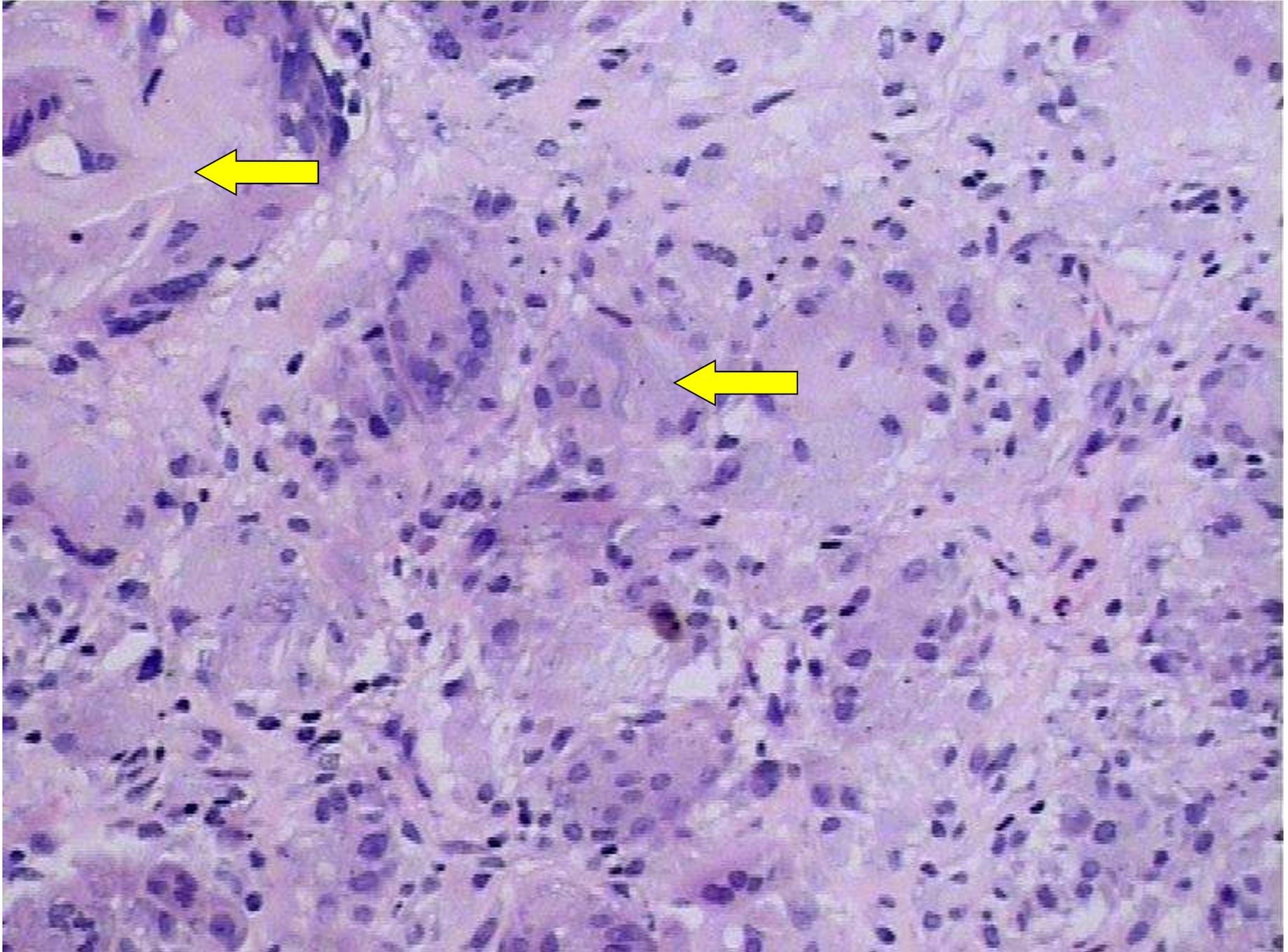


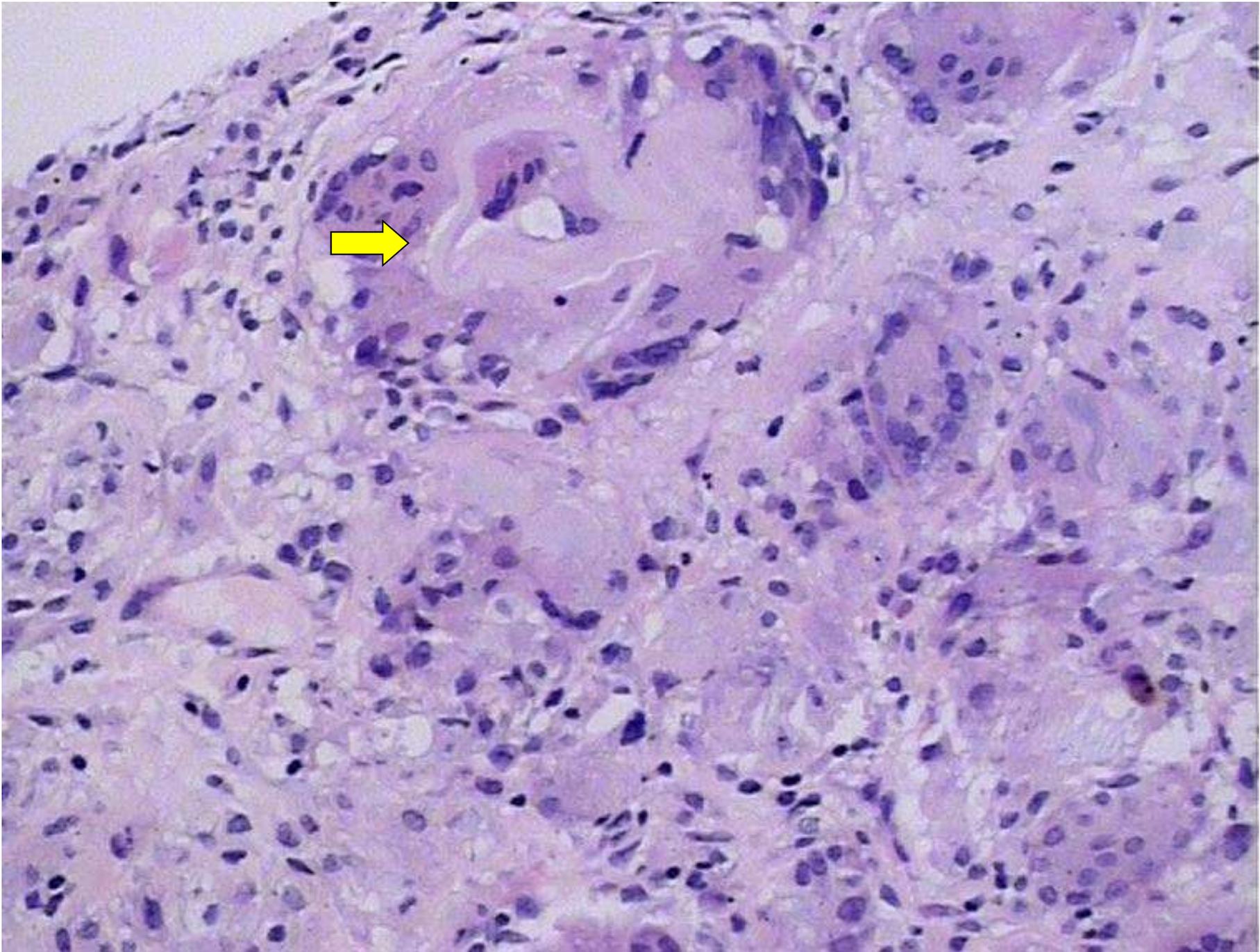
- ❑ **Non-specific inflammation**
- ❑ **Macrophage infiltration**

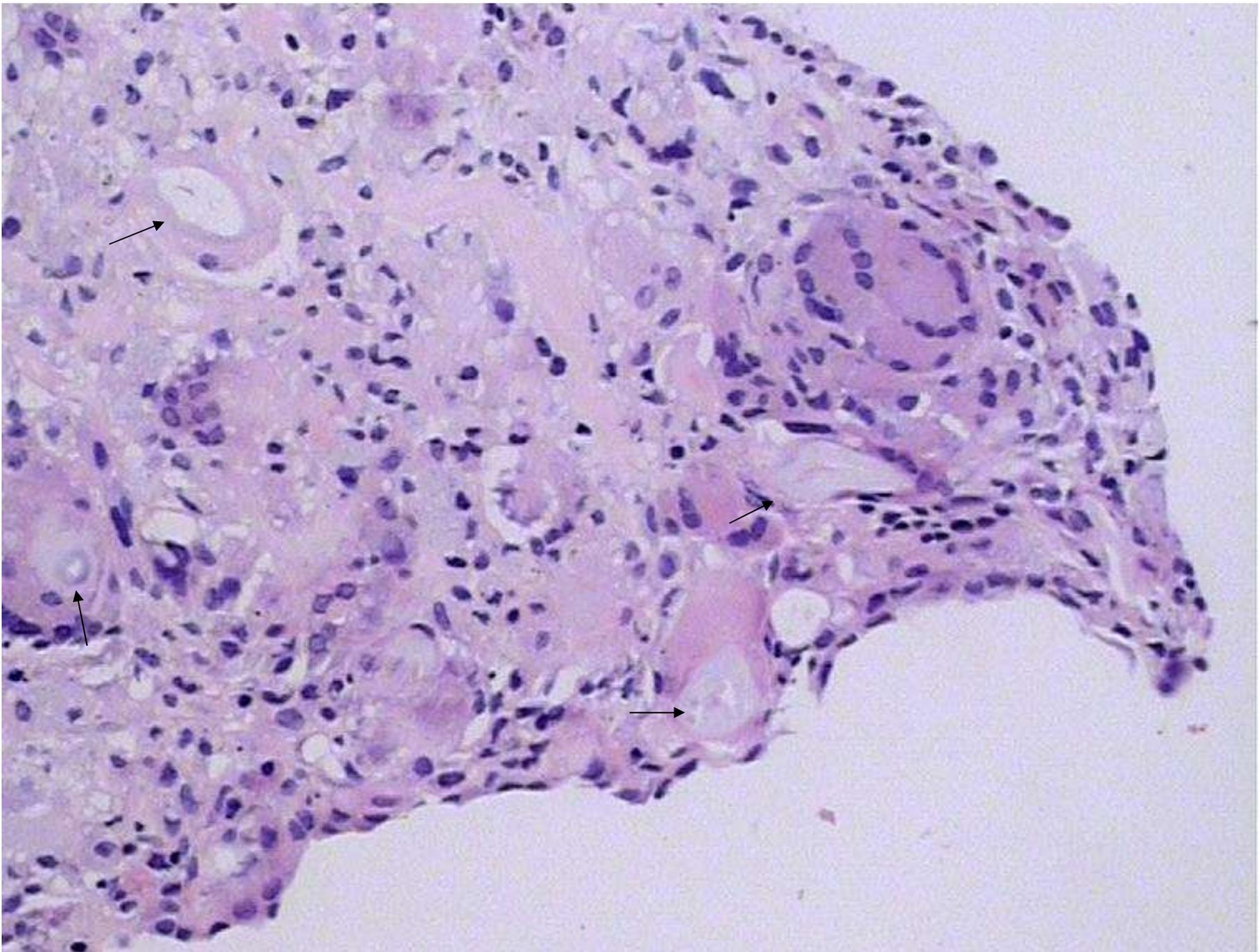


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Pleural membrane(C3)



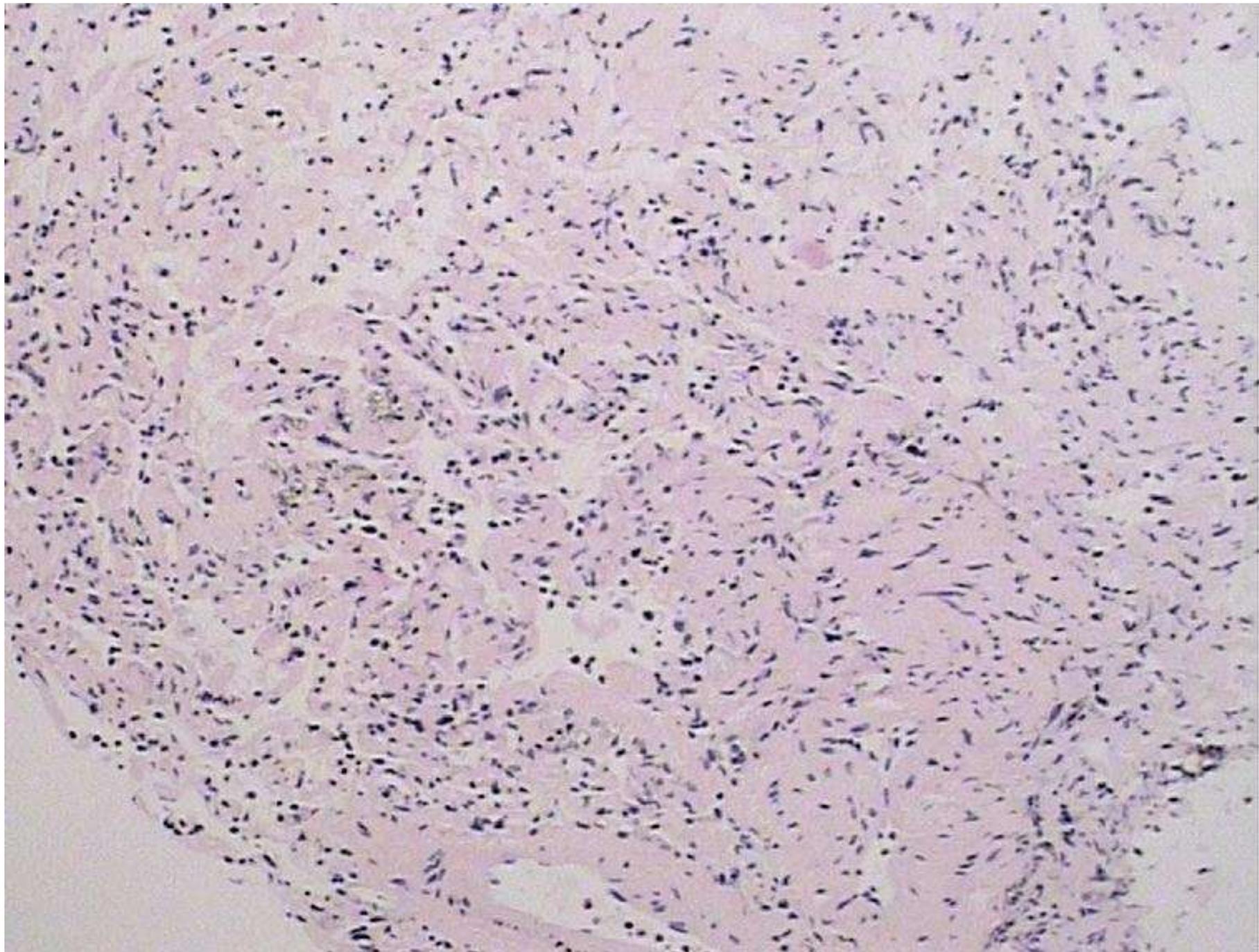


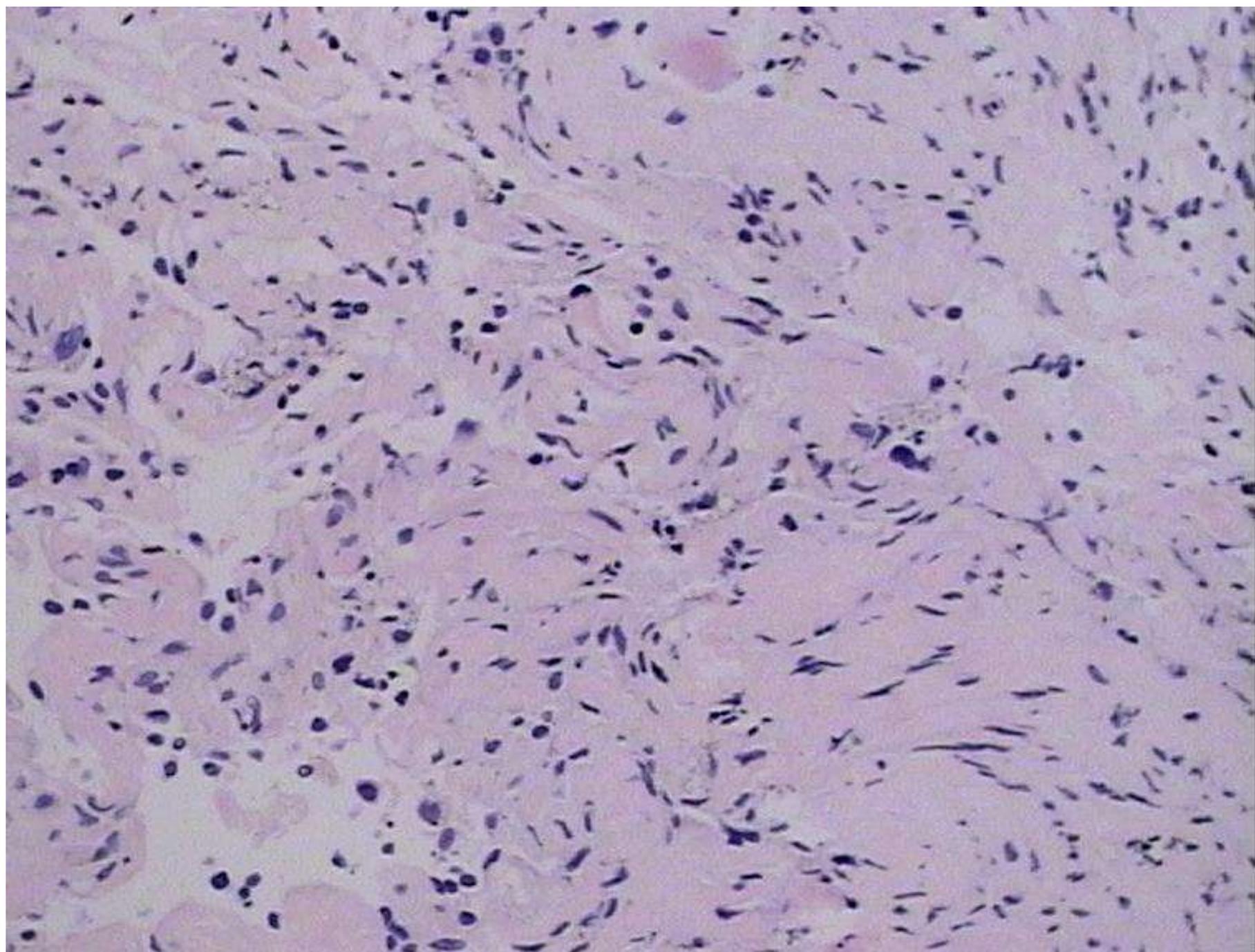




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Pulmonary Biopsy(C1)

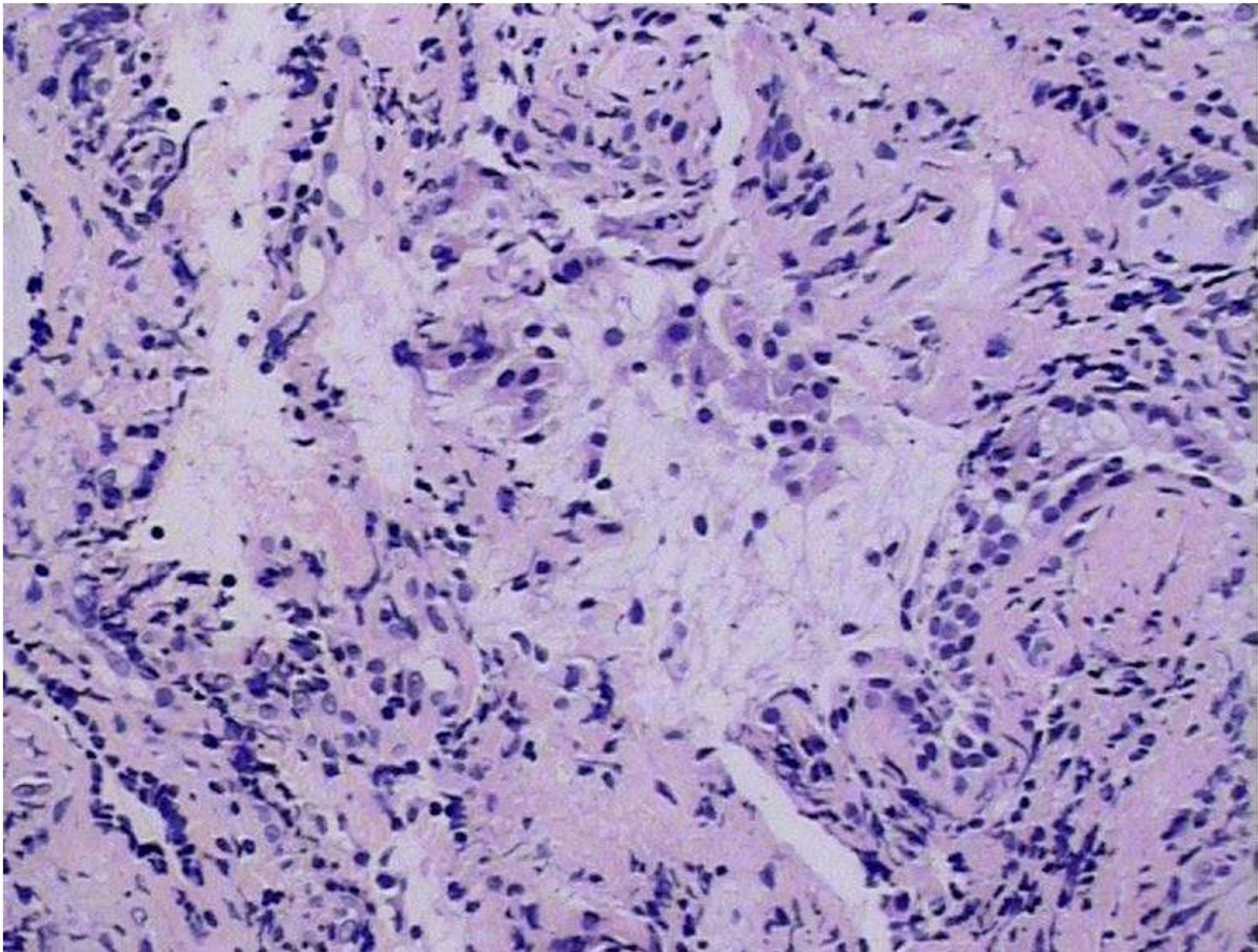


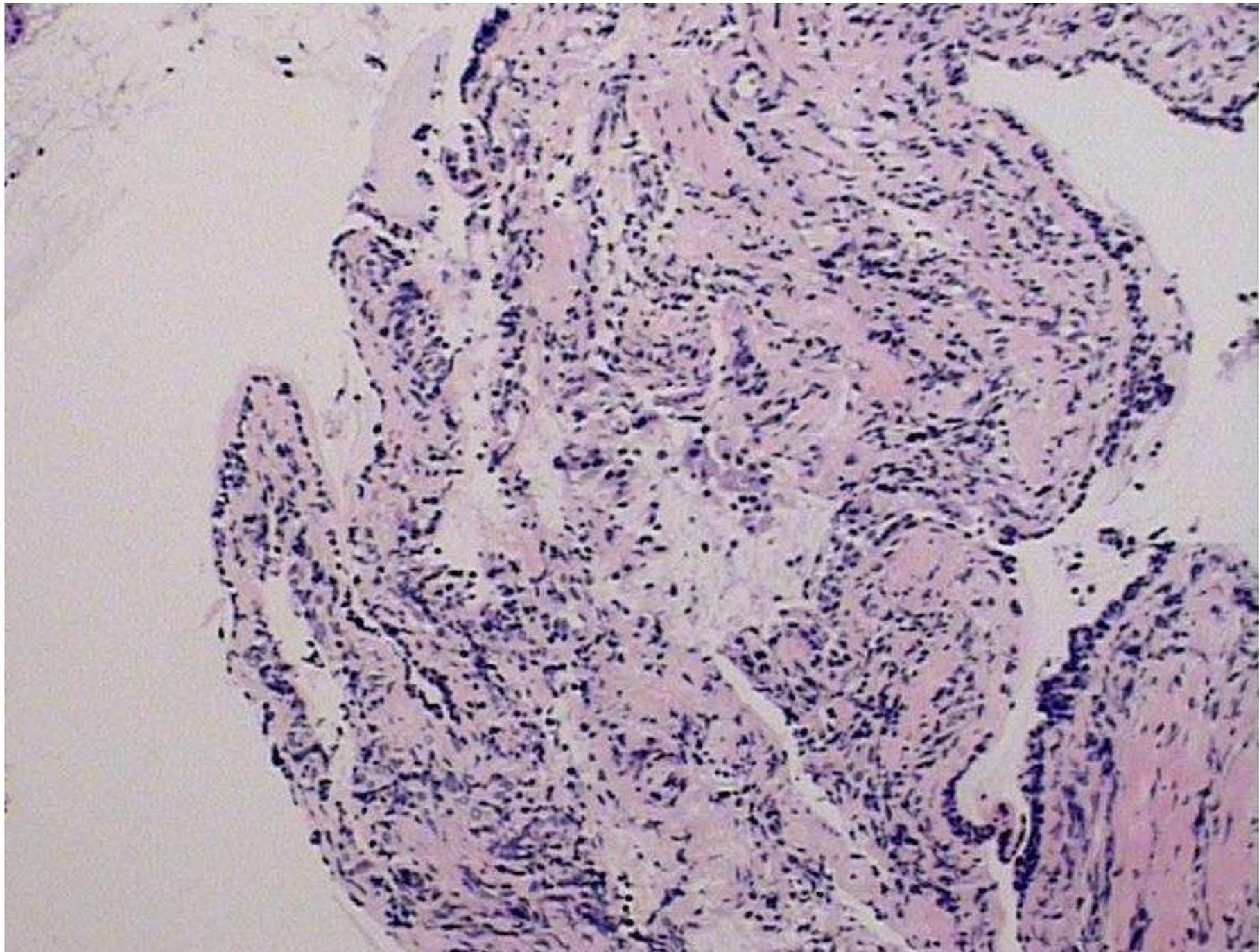




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Transbronchial lung biopsy (C1)

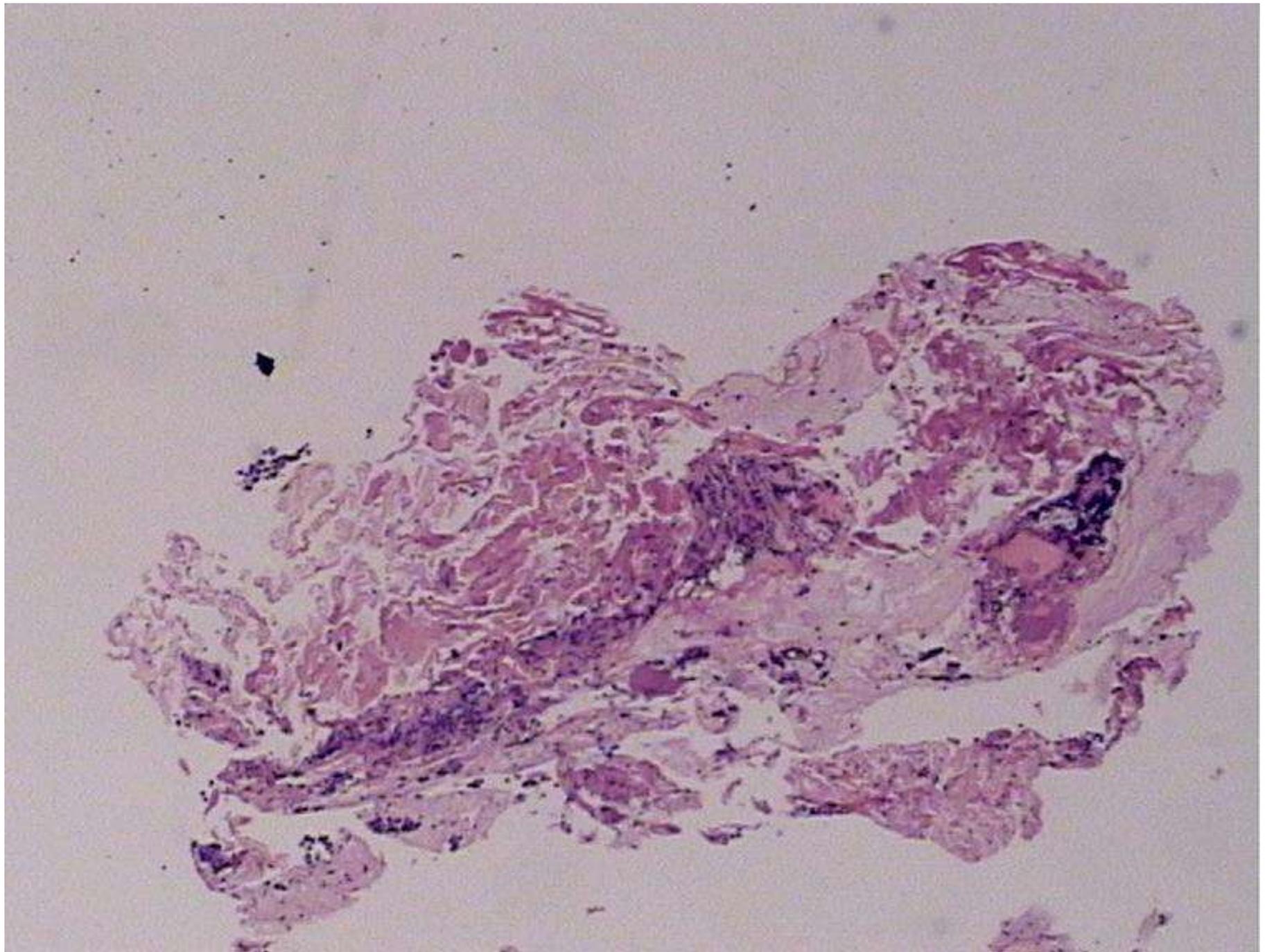


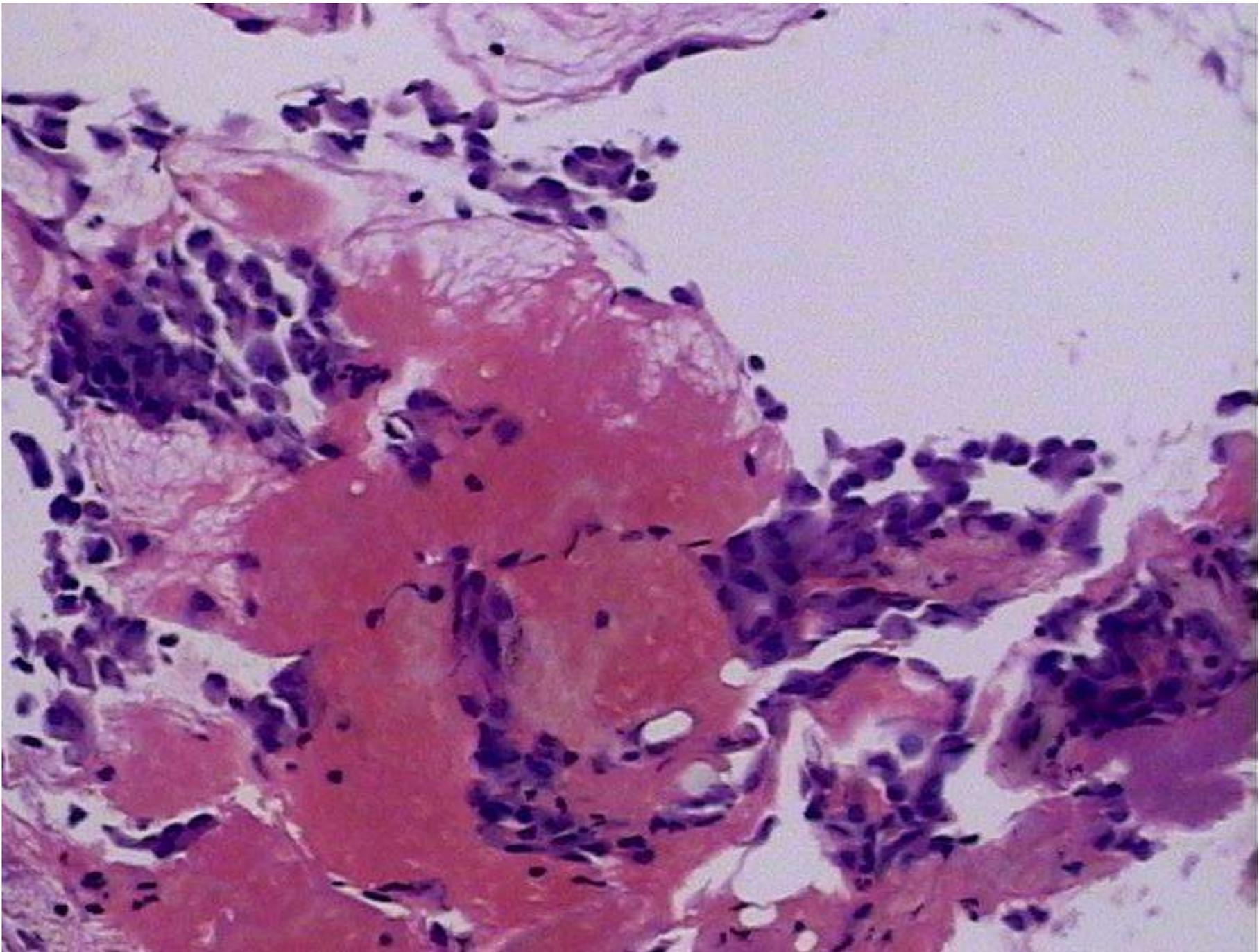




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Pleural mebrane(C2)



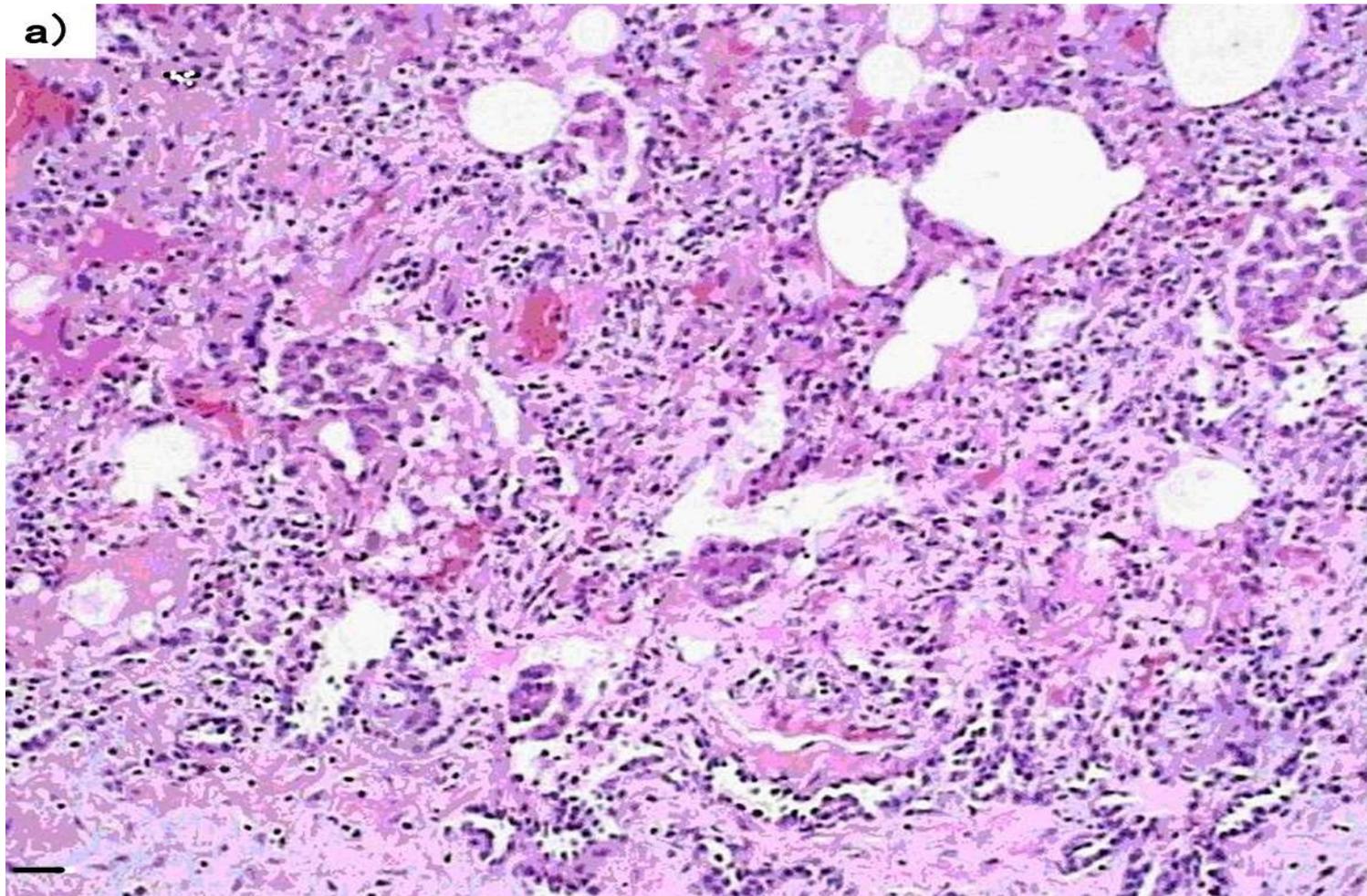




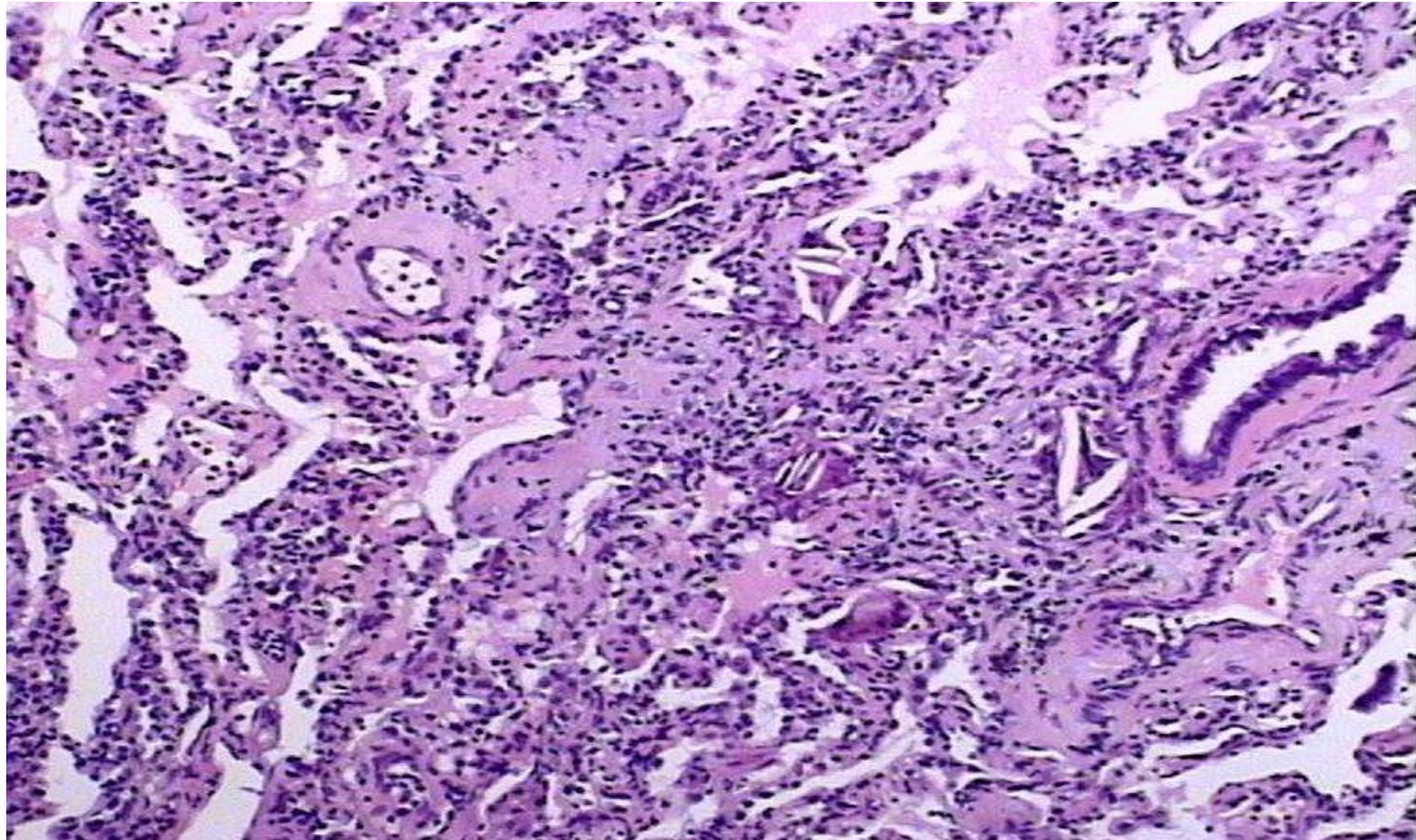
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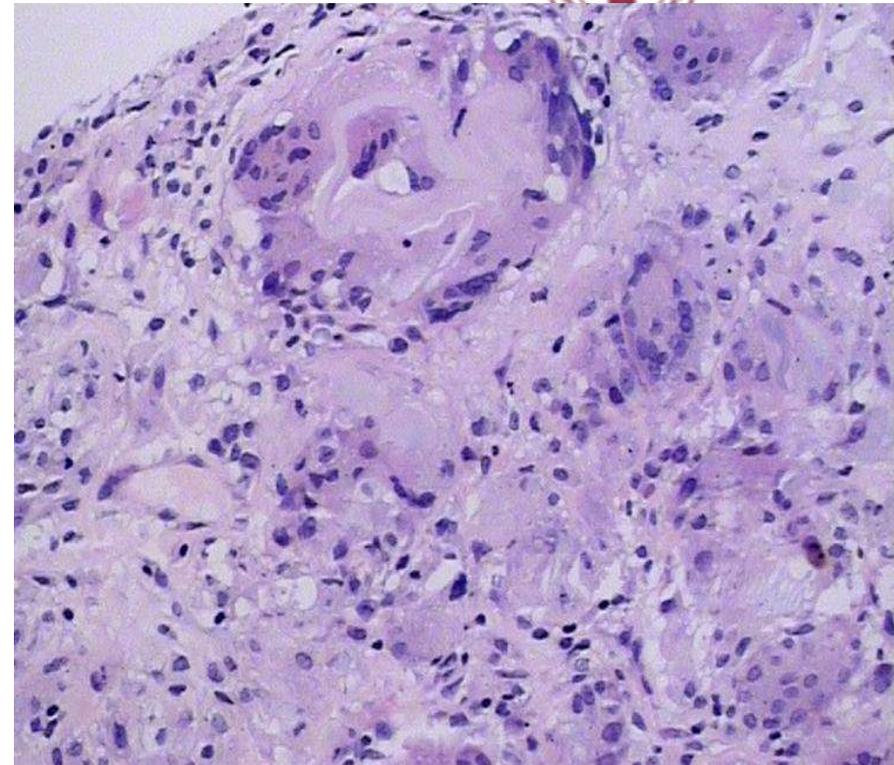
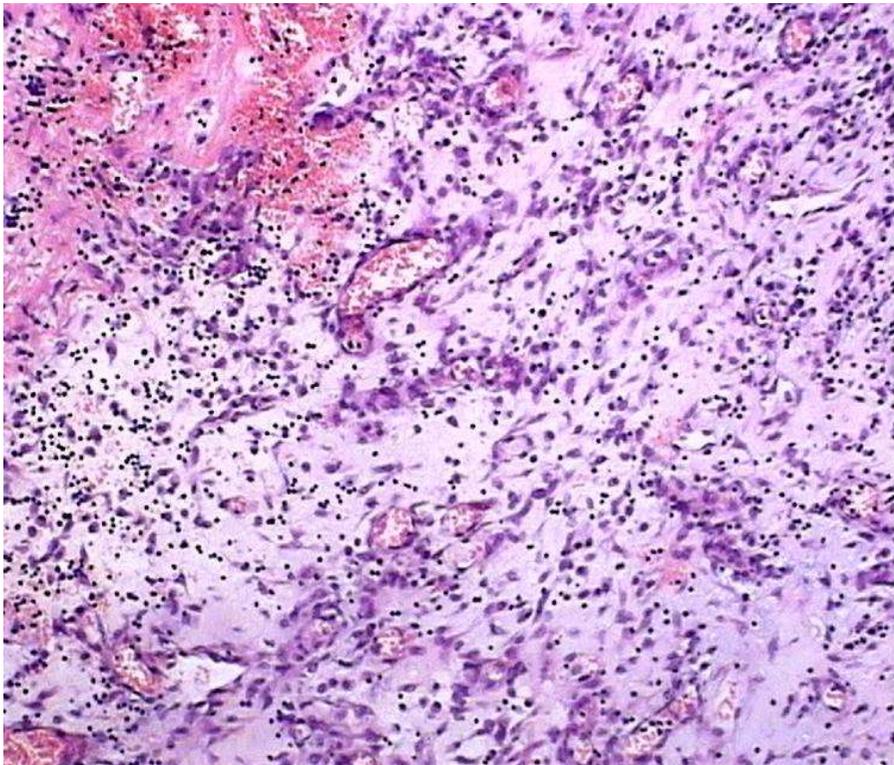
Lung(C5)

In the beginning



18 months later





- ❑ Inflammation with inflammatory cells
- ❑ Pulmonary fibrosis
- ❑ Granuloma formation of pleural membrane

Common characteristics



- Young females**
- In previous good health**
- In the same workplace**
- Same symptoms, signs**
- Similar laboratory results**
- Bad result: 2 died, 5 disabled**

Clinical findings



- ❑ **Symptoms: shortness of breath.**
- ❑ **Signs:**
 - **pleural and pericardial effusion.**
 - **pulmonary inflammation**
(no effect to antibiotics and glucocorticoids)
 - **Pulmonary fibrosis**
Rapid and Progressive

Analysis of the used coating



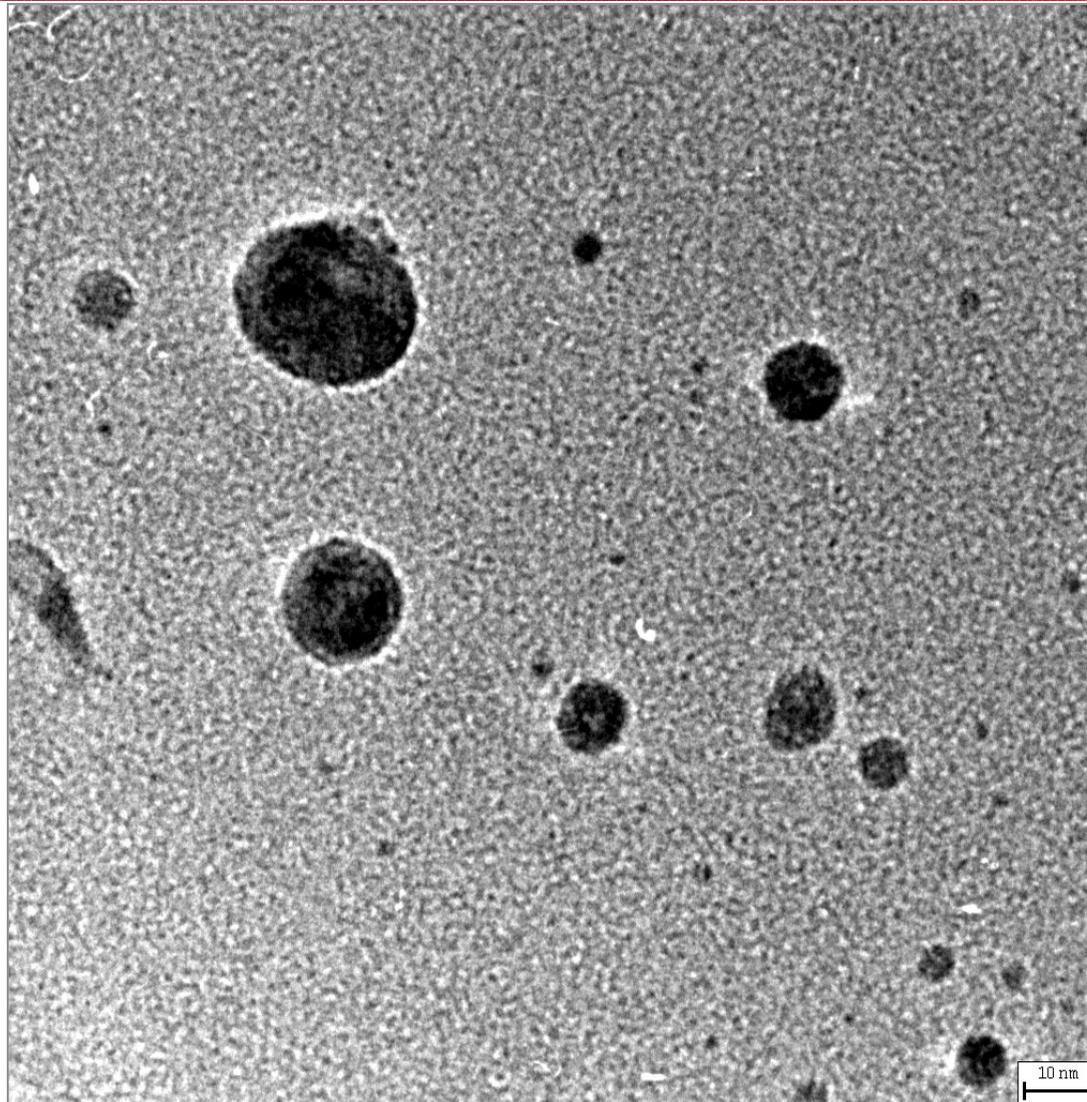
- **Polyacrylate**
- **GC-MS: butanoic acid, butyl ester, N-butyl ether, acetic acid, toluene, di-tert-butyl peroxide, 1-butanol, acetic acid ethenyl ester, isopropyl alcohol, and ethylene dioxide**



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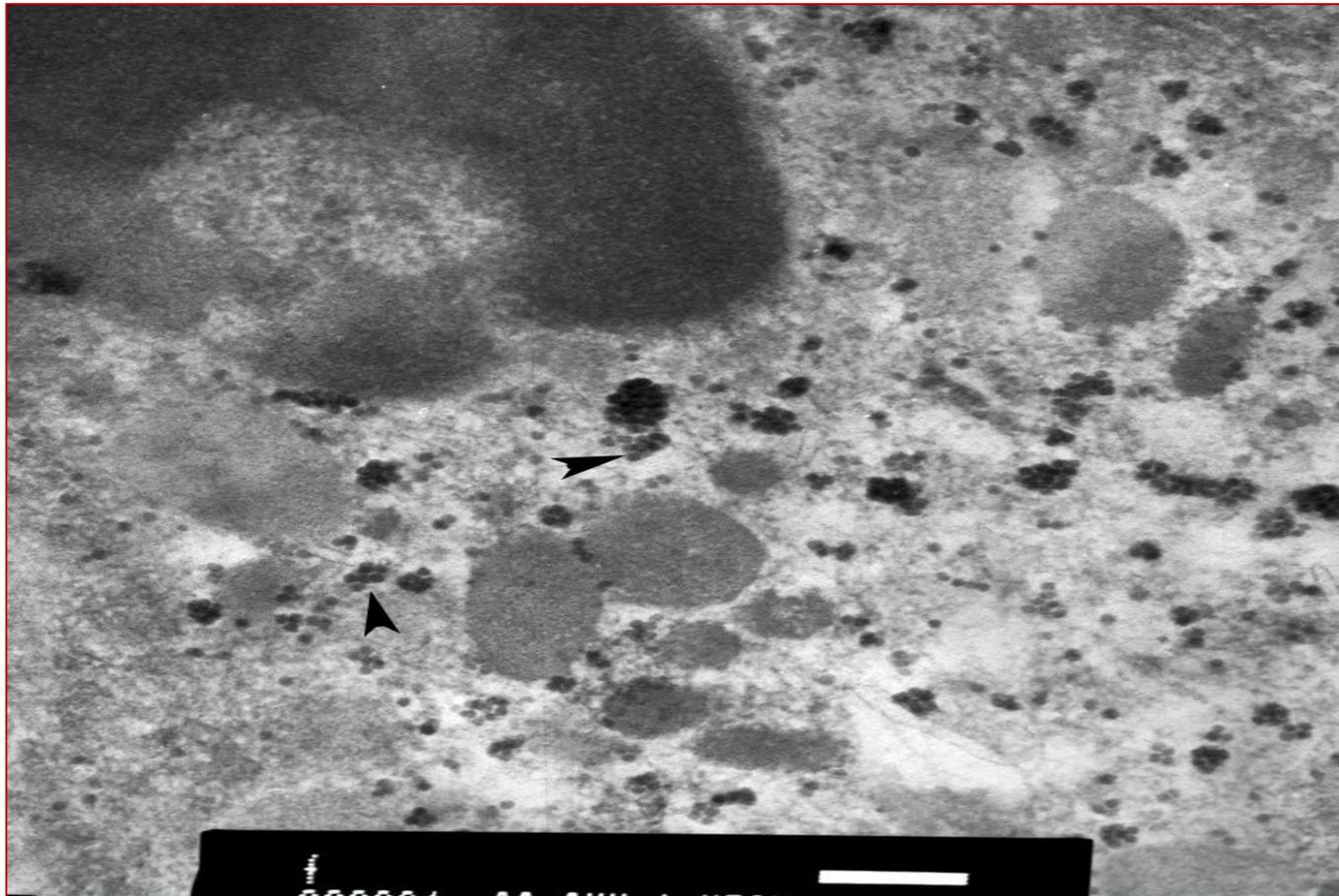


Nanoparticles in pleural effusion





Nanoparticles in a macrophage



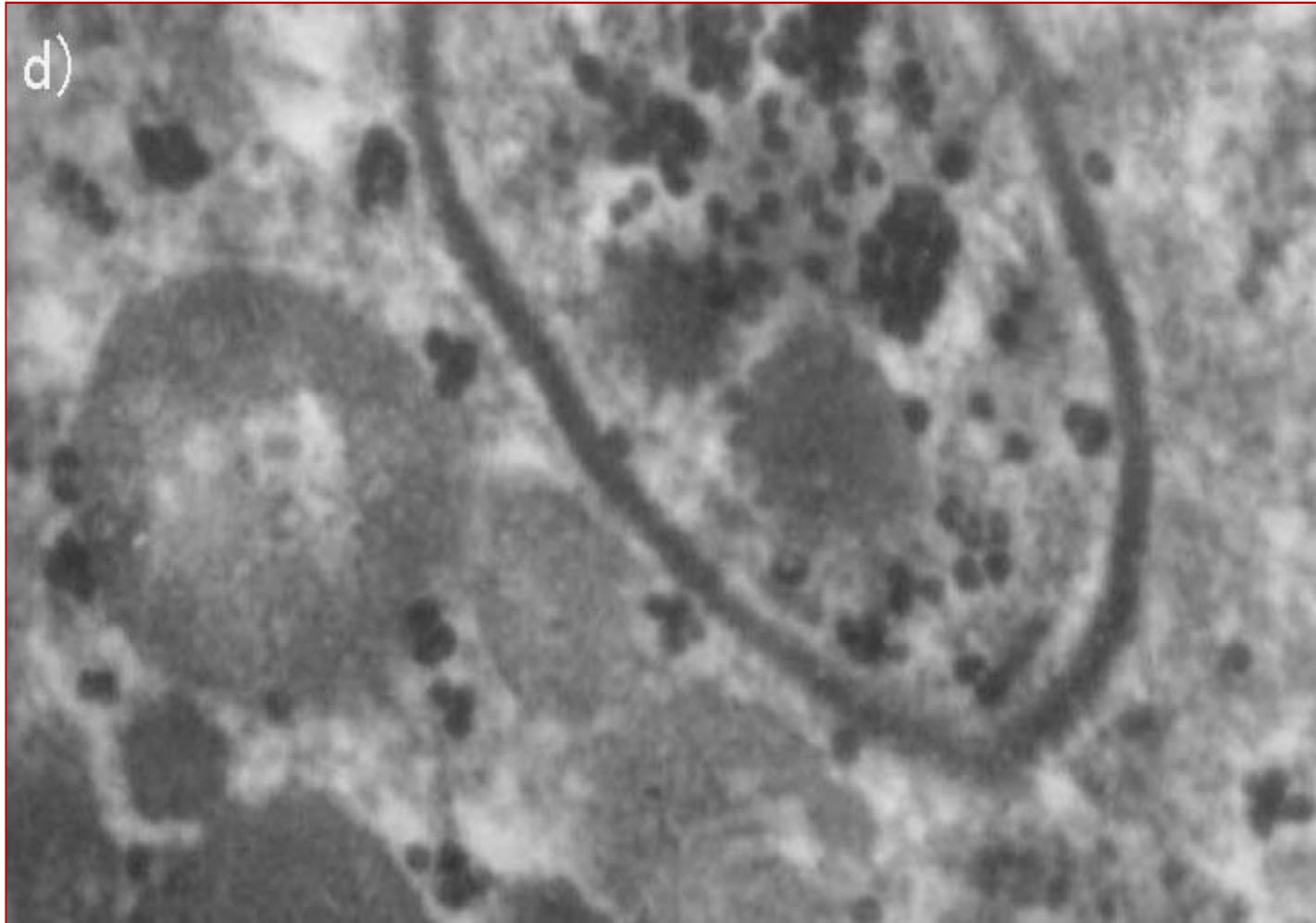


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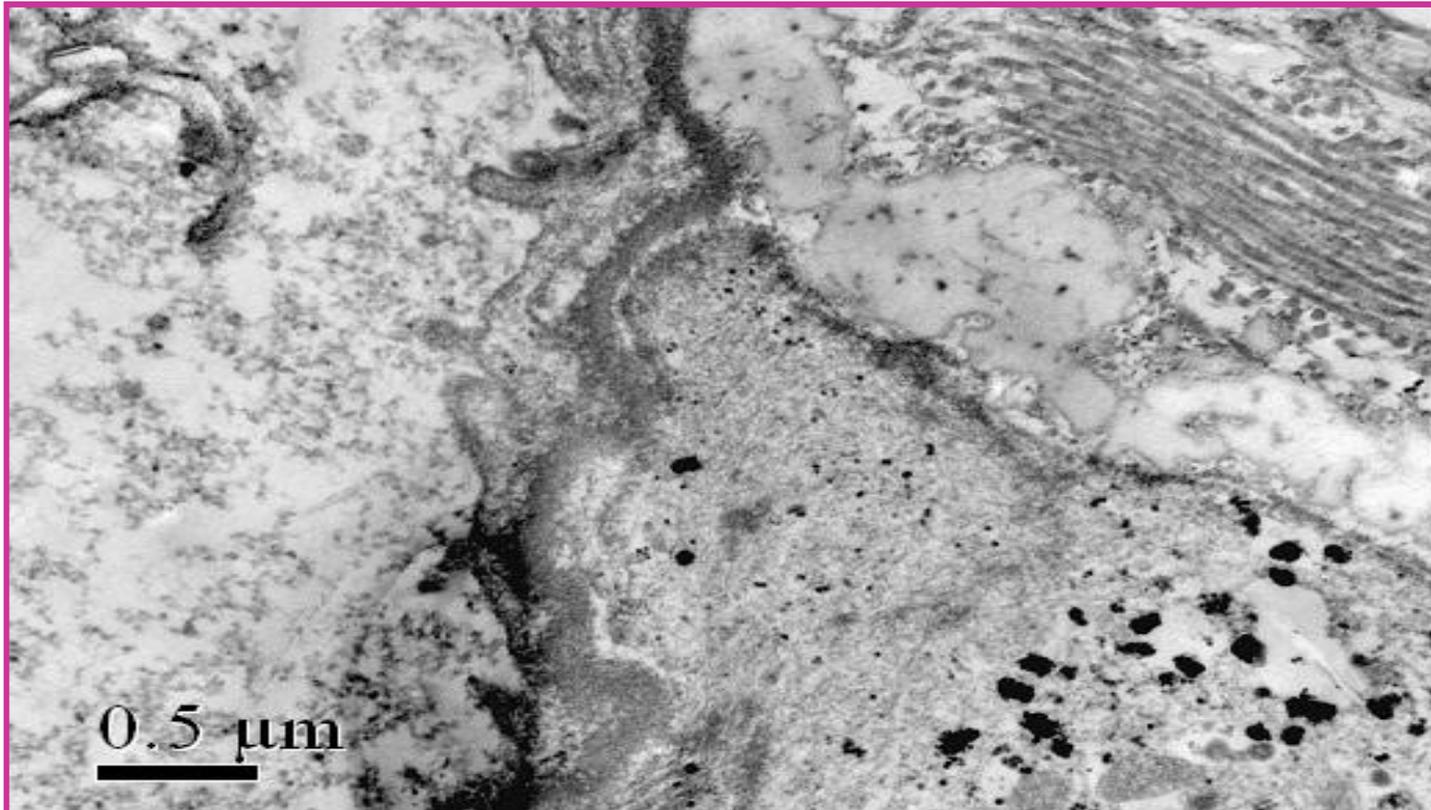


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Nanoparticles in micro-vessels



Specimen :

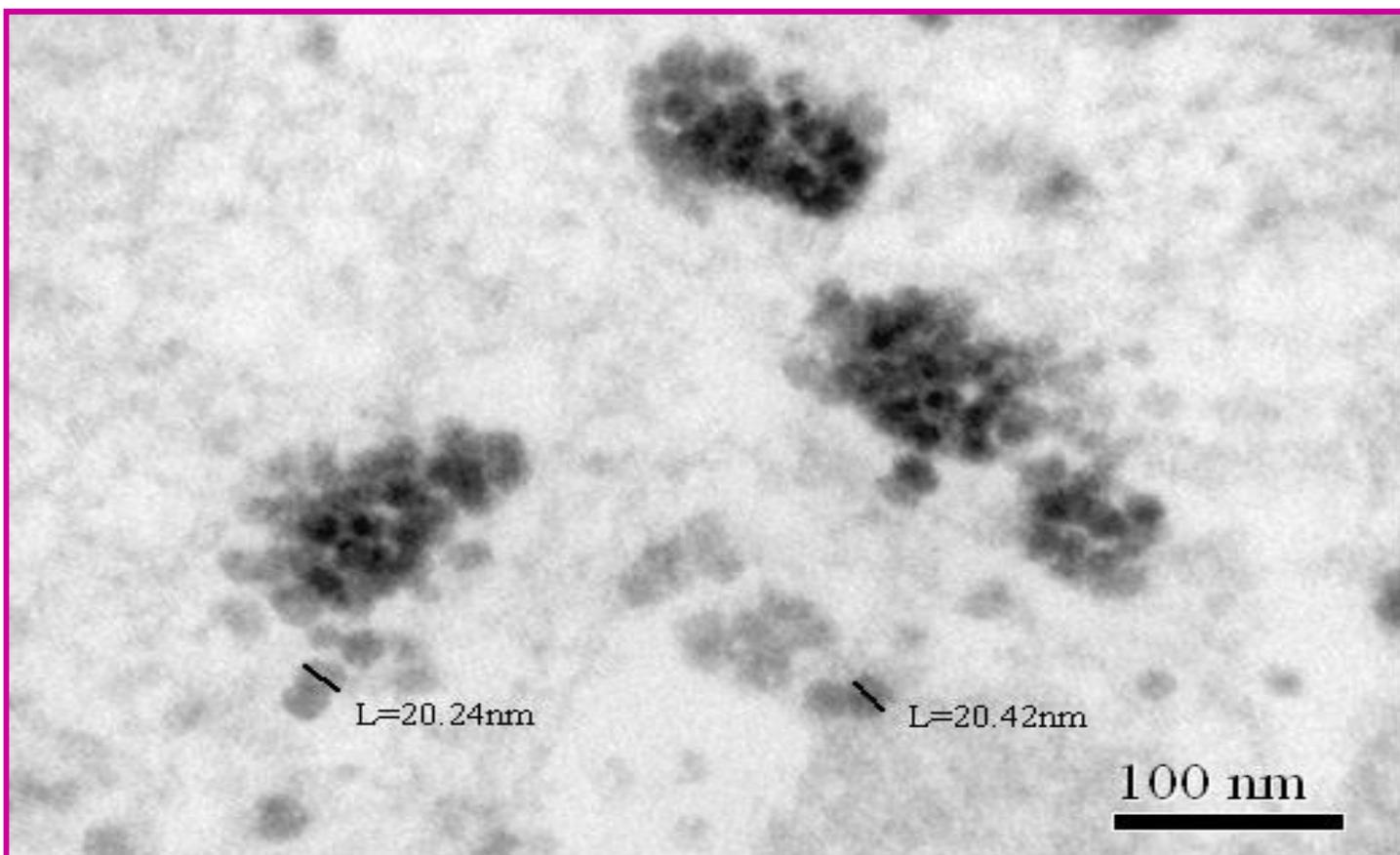
Image Name : Lung-0008

Indicated Magnification : X40000

Acquisition Date : 11/19/2009



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Specimen :

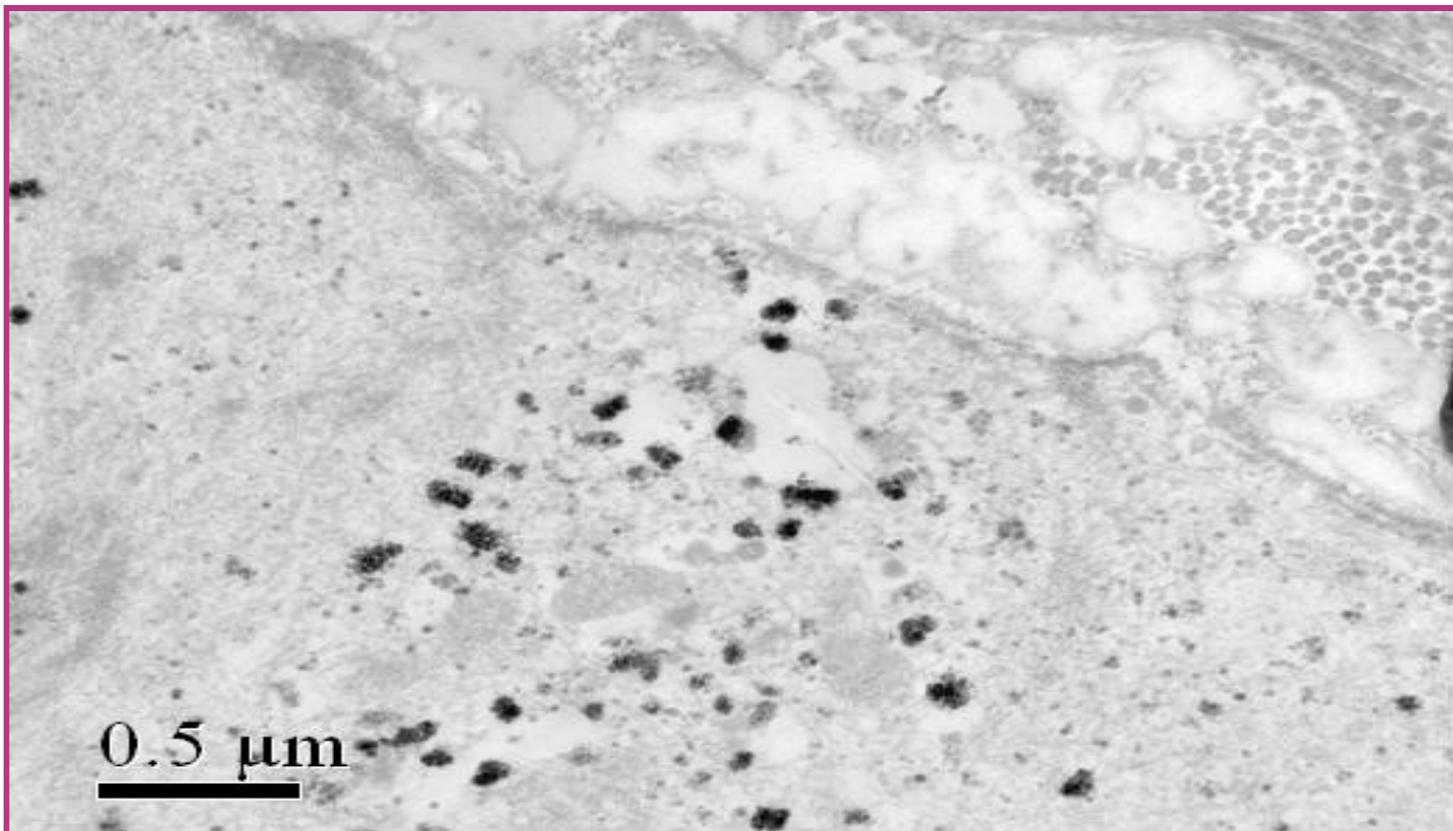
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Indicated Magnification : X300000

Acquisition Date : 11/19/2009



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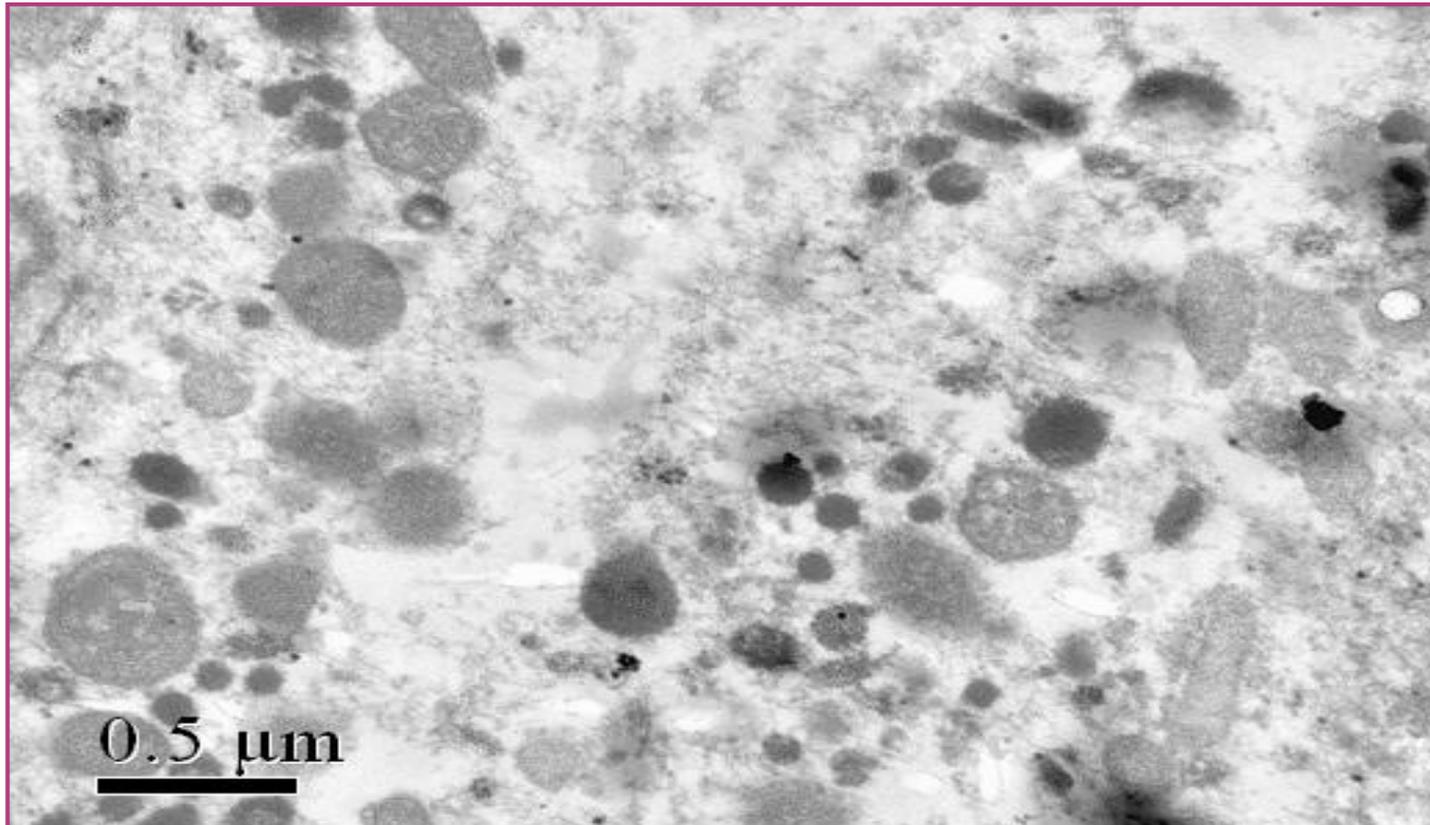
Specimen :

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Indicated Magnification : X50000

Acquisition Date : 11/19/2009

nanoparticles in a vascular endothelial cell



Specimen :

Image Name : Lung-0002

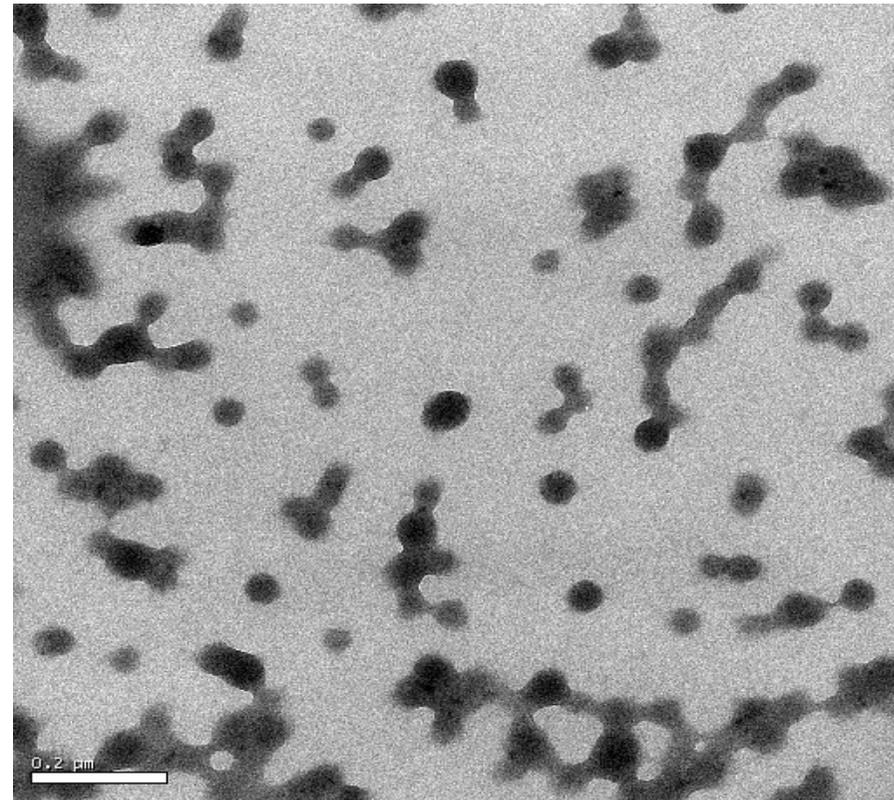
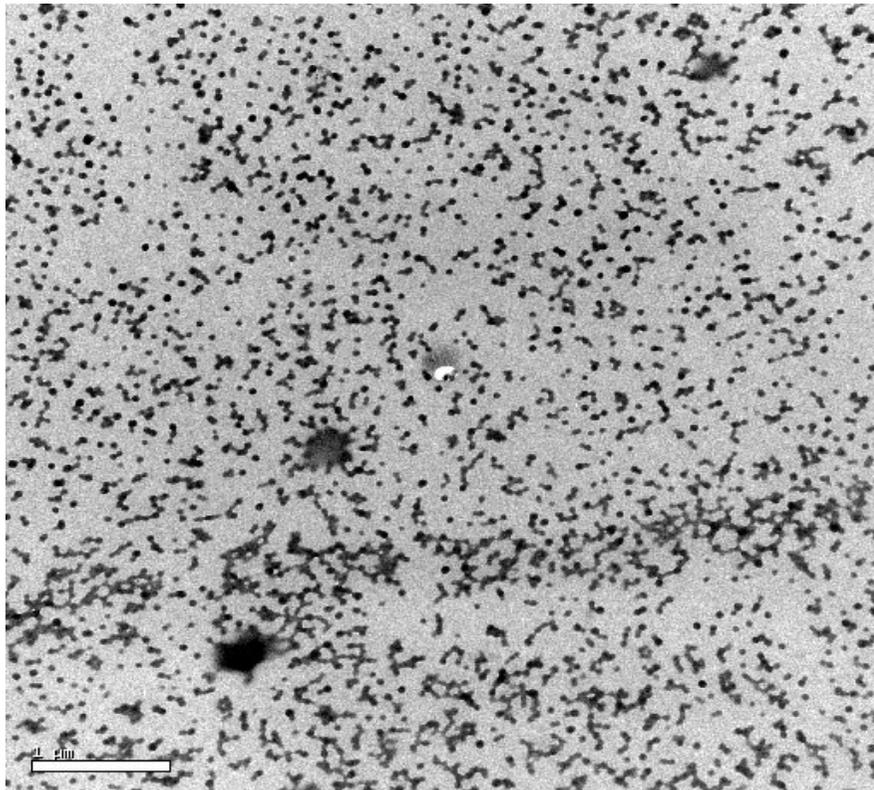
Indicated Magnification : X50000

Acquisition Date : 11/19/2009

Bronchial Alveolar Lavage Fluid (BALF)



Nanoparticles in raw material





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Speculations and questions



- What are these nanoparticles and why exist?
- Are these nanoparticles related to the workers' death and their multi-organ damage?
- May these nanoparticles pose the damage directly or as a carrier?

Eur Respir J 2009; 34: 559–567
DOI: 10.1183/09031936.00178308
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Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma

Y. Song*, X. Li[†] and X. Du*

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

The present study aimed to examine the relationship between a group of workers' presenting with mysterious symptomatic findings and their nanoparticle exposure.

Seven young female workers (aged 18–47 yrs), exposed to nanoparticles for 5–13 months, all with shortness of breath and pleural effusions were admitted to hospital. Immunological tests, examinations of bacteriology, virology and tumour markers, bronchoscopy, internal thoracoscopy and video-assisted thoracic surgery were performed. Surveys of the workplace, clinical observations and examinations of the patients were conducted.

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

KEYWORDS: Foreign-body granuloma, human, hypoxaemia, pleural effusion, polyacrylate nanoparticle, pulmonary fibrosis

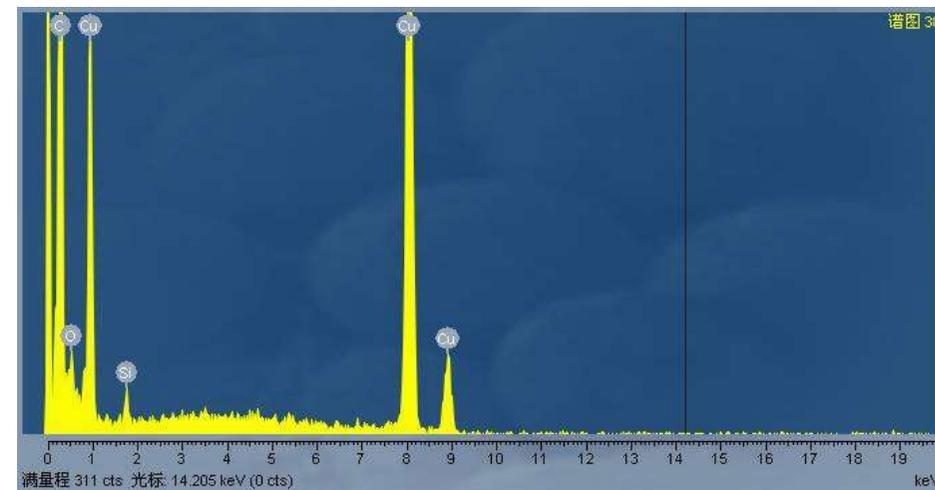
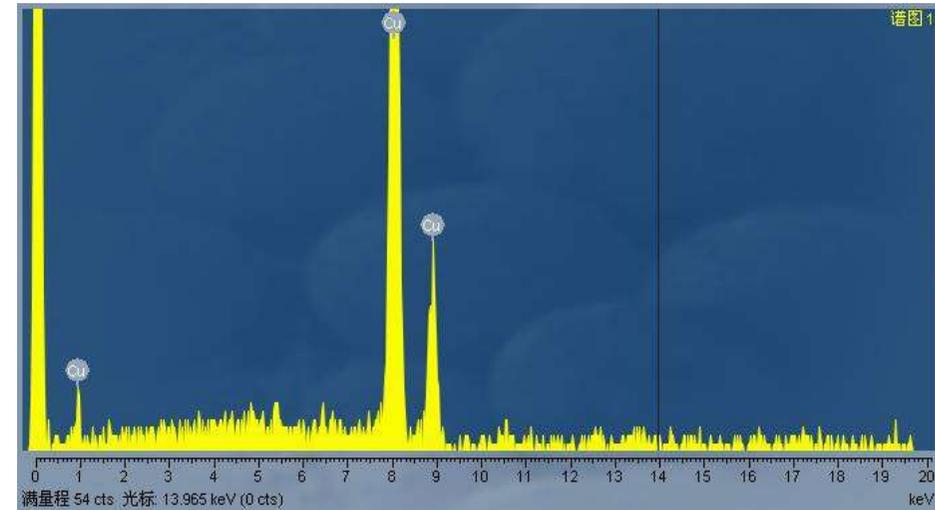
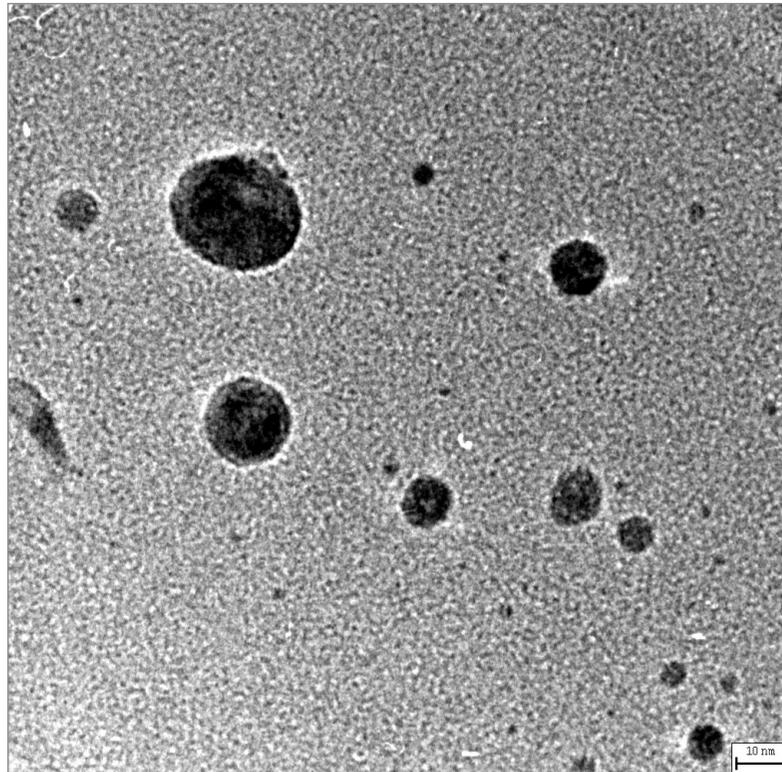


AFFILIATIONS
Depts of *Occupational Medicine and Clinical Toxicology, and
†Pathology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China.

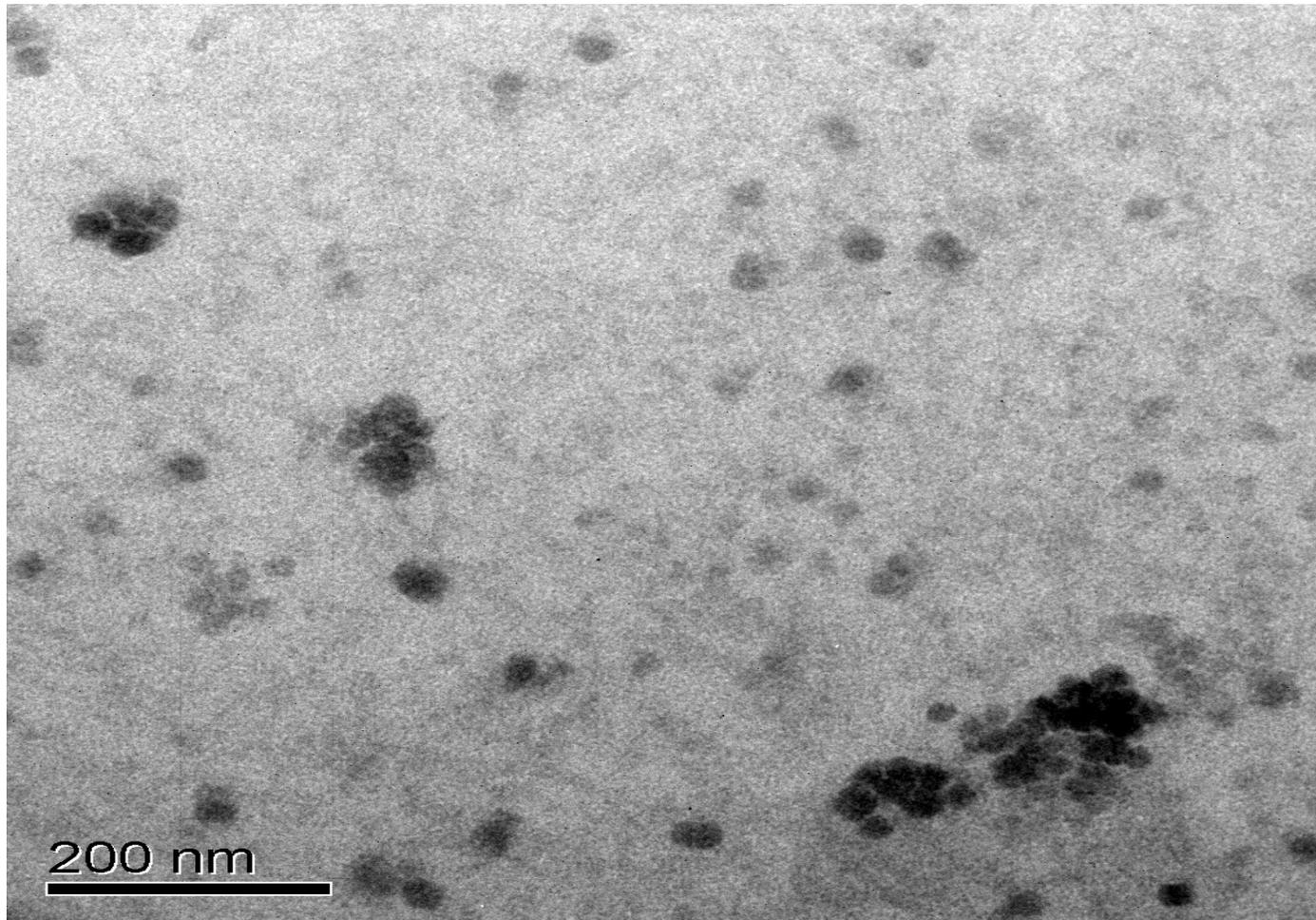
CORRESPONDENCE
Y. Song
Dept of Occupational Medicine and Clinical Toxicology
Beijing Chaoyang Hospital
No. 8
Bajiazhuang Road
Chaoyang District
Beijing
China (100020)
E-mail: songrain123@hotmail.com

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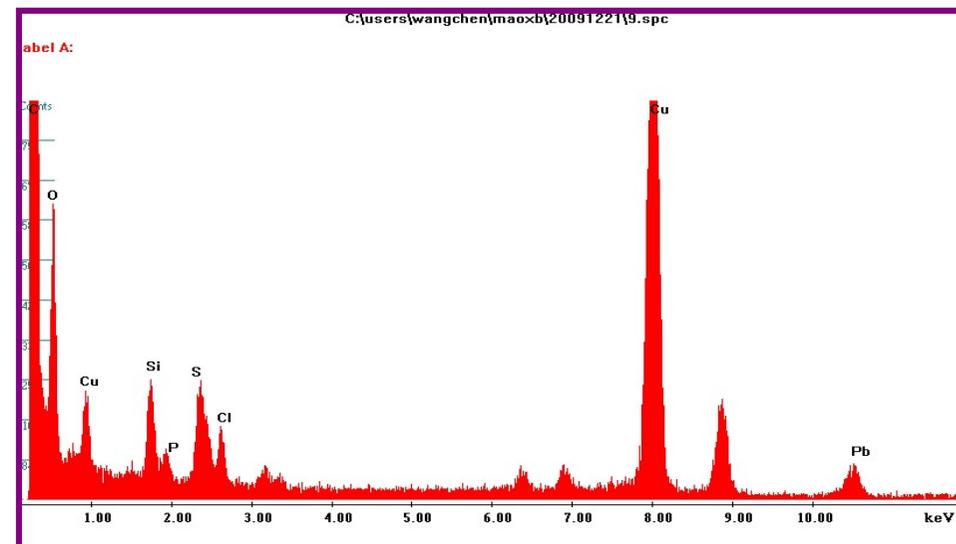
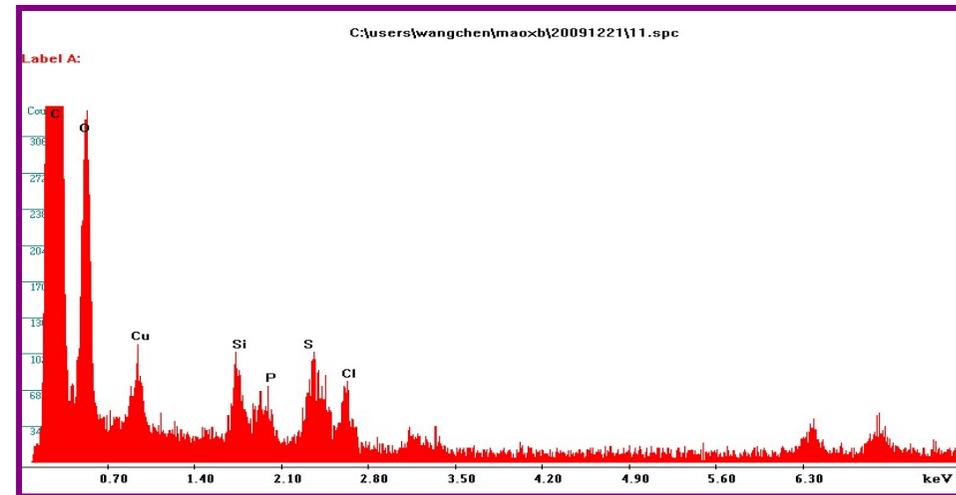
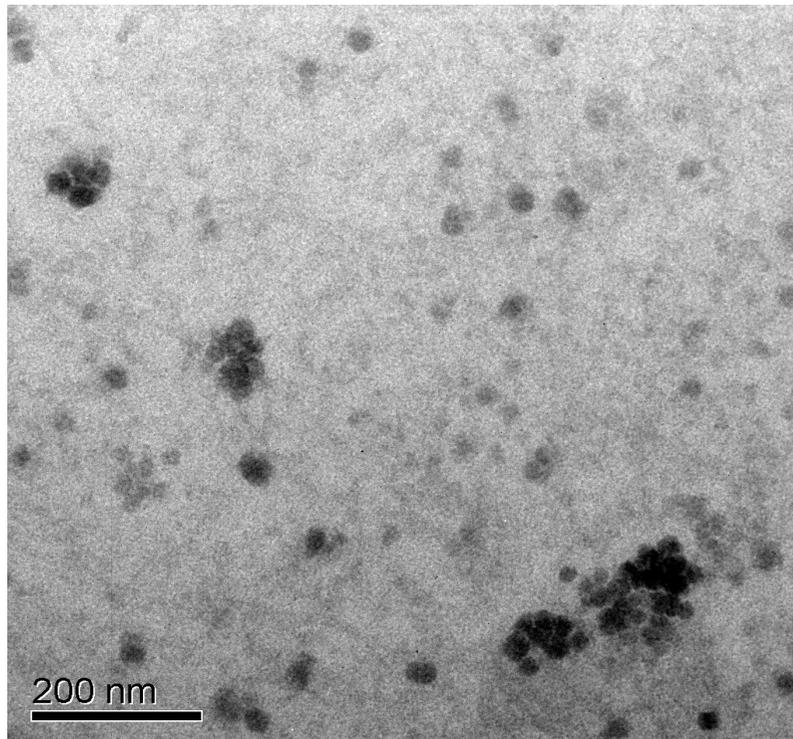
EDX analysis of nanoparticles in pleural effusion



Cytoplasam



Isolation and EDX analysis of nanoparticles in a macrophage





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<i>Elem</i>	<i>Weight %</i>	<i>Atomic %</i>
<i>C K</i>	84.20	94.30
<i>O K</i>	03.10	02.60
<i>Si K</i>	00.70	00.40
<i>P K</i>	00.10	00.00
<i>S K</i>	00.70	00.30
<i>Cl K</i>	00.40	00.20
<i>Cu K</i>	10.80	02.30



Results and speculations

- **Nanosilica entered patients blood vessels, pulmonary tissues, and their subcellular organelles, and potentially exerted toxicity.**
- **Nanosilica may have posed workers multi-organ damage and death .**

Toxicologic Pathology, 39: 841-849, 2011
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Nanomaterials in Humans: Identification, Characteristics, and Potential Damage

YUGUO SONG¹, XUE LI², LIYING WANG³, YON ROJANASAKUL⁴, VINCENT CASTRANOVA³, HUILING LI¹, AND JING MA¹

¹*Department of Occupational Medicine and Clinical Toxicology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China*

²*Department of Pathology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China*

³*Department of Pathology and Physiology, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA*

⁴*Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, West Virginia, USA*

ABSTRACT

Nanomaterials are increasingly being used for commercial purposes. However, concerns about the potential risks of exposure to humans have been raised. We previously reported unusual pulmonary disease and death in a group of patients with occupational exposure to spray paint. However, the nanoparticle and chemical composition of the exposure was not fully described. The present study aimed to isolate and identify the nanoparticles observed in the patients' biopsies and report the potential deleterious effects to human lungs using electron microscopy. Using electron microscopy and energy dispersive x-ray analysis, silica nanoparticles were identified and characterized mainly in macrophages, pulmonary microvessels, vascular endothelial cells, microlymphatic vessels, pleural effusions, and a few in alveolar epithelial cells and pulmonary interstitial tissue (with no microscale particles present). Notably, damage to alveolar epithelial cells, macrophages, vascular endothelial cells, and the blood-gas barrier was observed. Given the well-documented toxicity of microscale silica, it is possible that these silica nanoparticles may have contributed in part to the illness reported in these workers. Such a possibility supports the adoption of controls and prevention strategies to minimize inhalation of nanoparticles by workers, and it highlights the urgent need and the importance of the nanosafety study in humans.

Keywords: human; pleural effusion; pulmonary fibrosis; silica nanoparticle.

Questions ?



□ **Micro-SiO₂** → **Silicosis**

□ **Nano-SiO₂** → ? ?

□ **Toxicity of nanosilica in animals**

More questions ?



- Would nanosilica cause pleural and pericardial effusion and the unusual toxicity as seen in the reported workers?**
- Did the workers' clinical damage result from the single toxicity of nanosilica or combined role of nanosilica together with polyacrylate?**

Animals



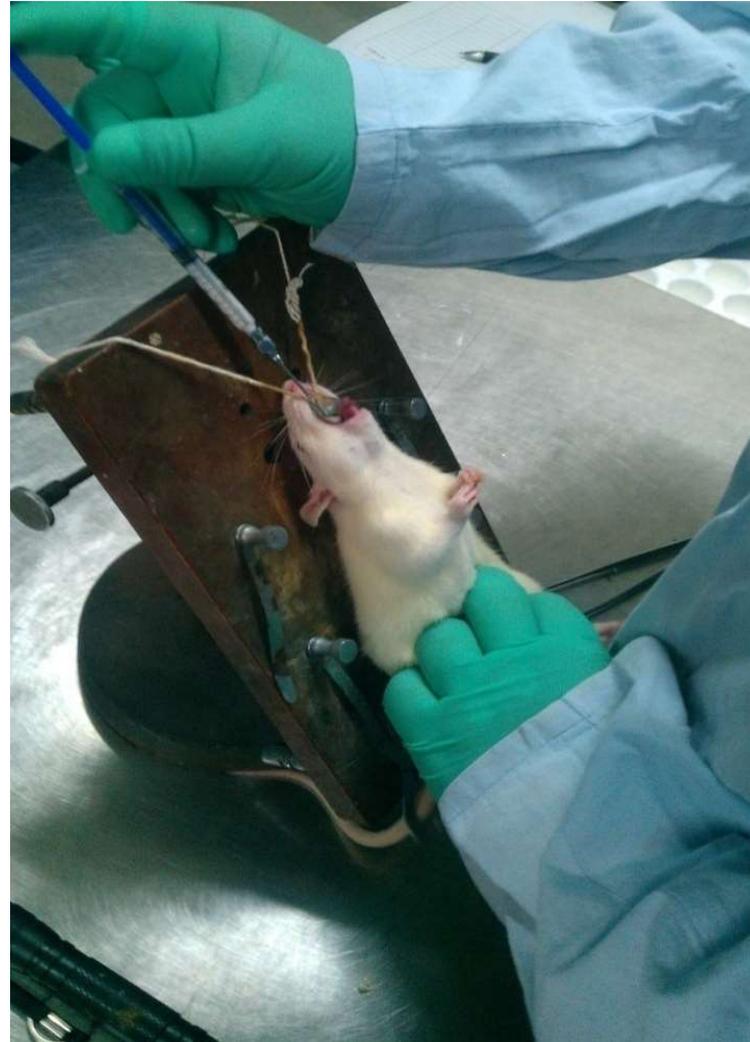
- ❑ **90 adult male SPF Wistar rats**
- ❑ **4–6 weeks old , weights 180 - 220 g.**
- ❑ **Five groups, 18 rats in each group**
- ❑ **Polyacrylate/nanosilica (PA/NSi) were made by order from Fudan University.**

Grouping



- **Control group with NS**
- **Control group with PA**
- **3.125mg/kg PA/NPSi**
- **6.25 mg/kg PA/NPSi**
- **12.5 mg/kg PA/NPSi**

Intratracheal instillation



Experimental design



□ Part 1: 1-week study:

75 rats terminated at 24h, 3d, and 7d post intratracheal instillation

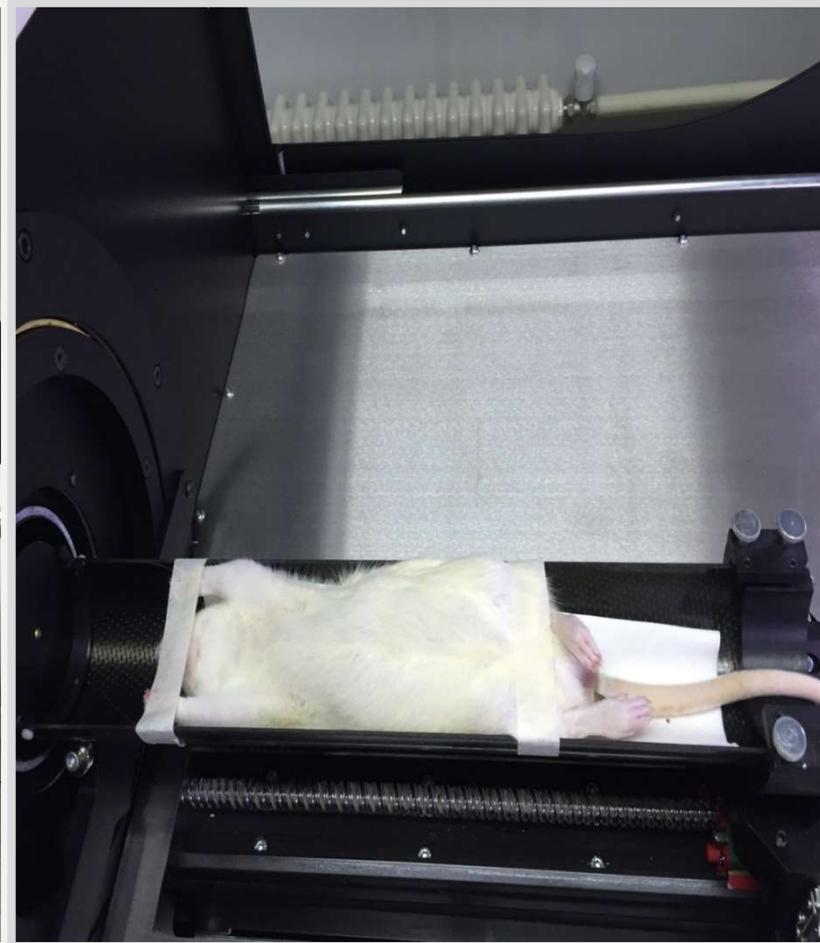
□ Part 2: 2-week study

15 rats (3/group) for repeated ultrasound and CT examinations.

Ultrasound examination for rats



CT examination for rats





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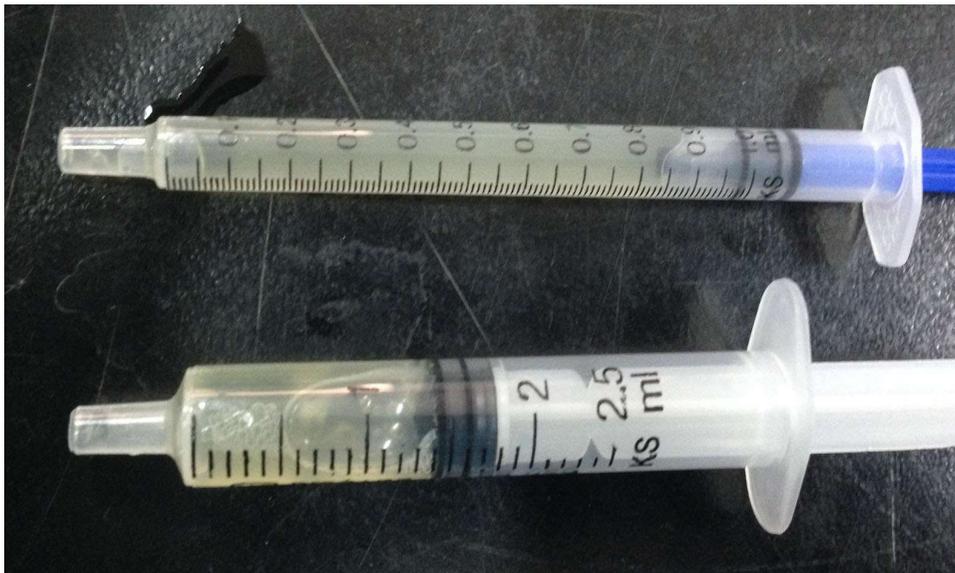
Results



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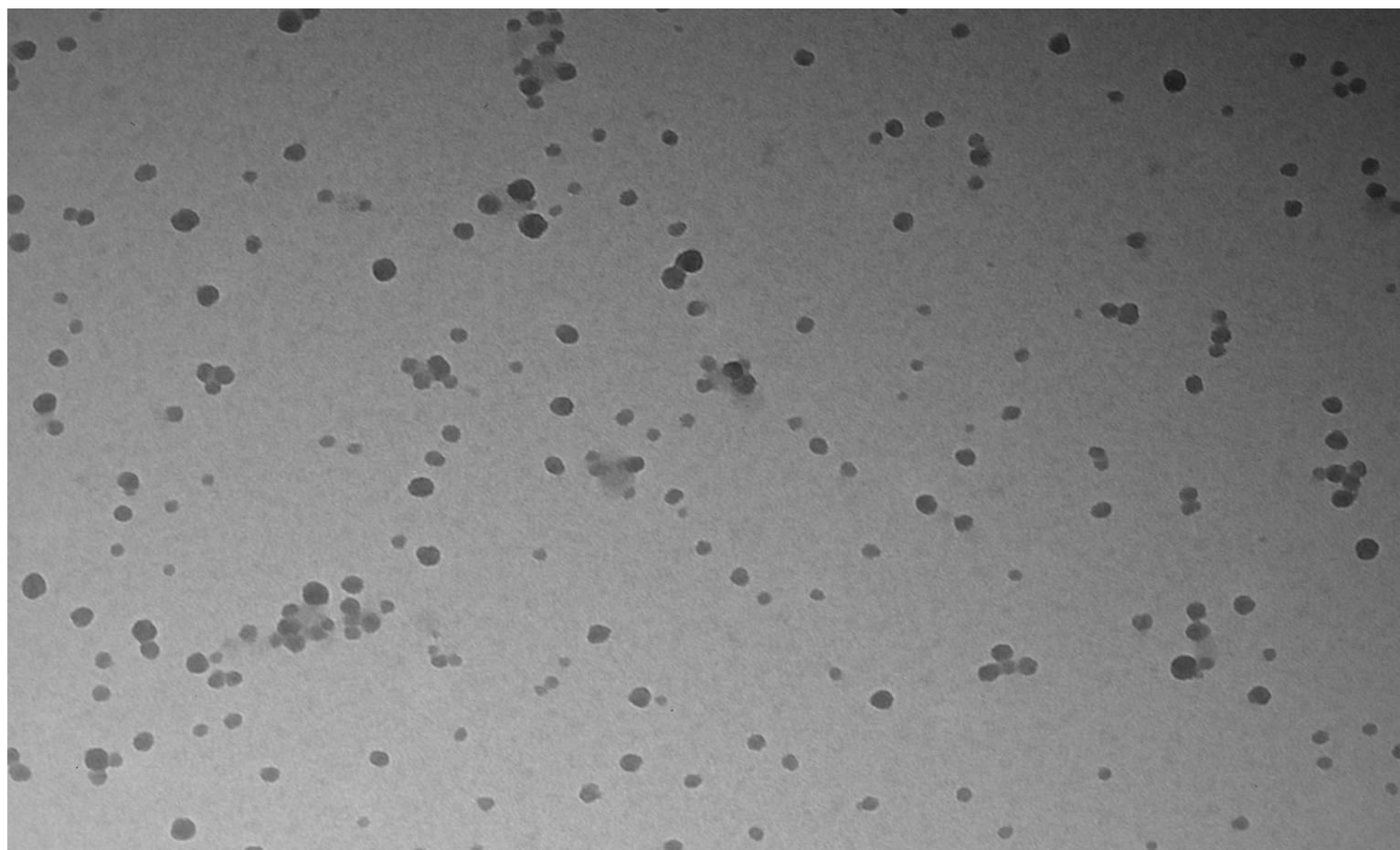


**Pleural fluid
upon dissection**



**1-1.8ml in each
pleural cavity**

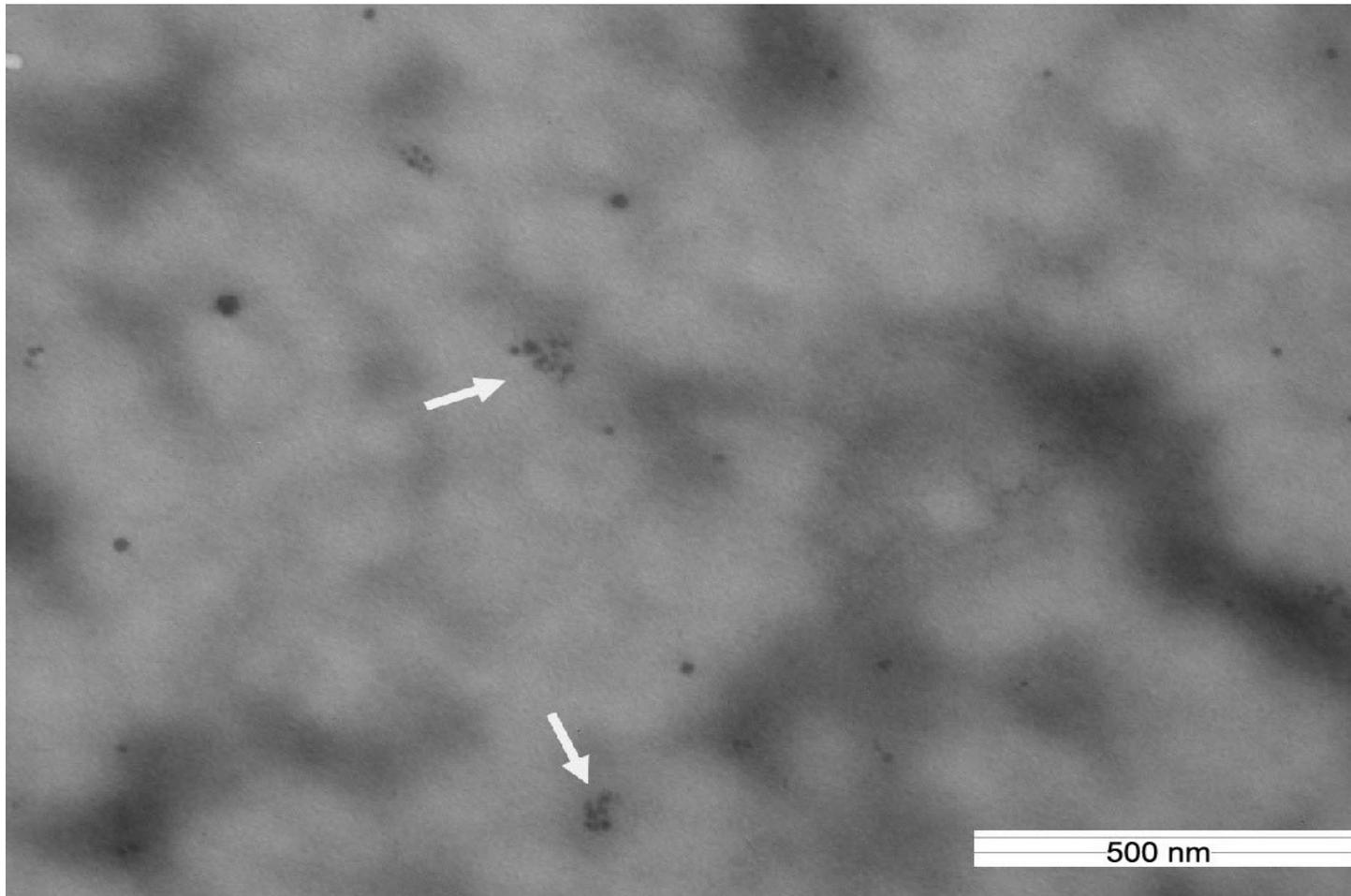
Silica nanoparticles in PA/NSi



	显微镜	加速电压	放大倍率	采集日期
JEM-1400	80 kV	40000 x	14/10/20,	14:43

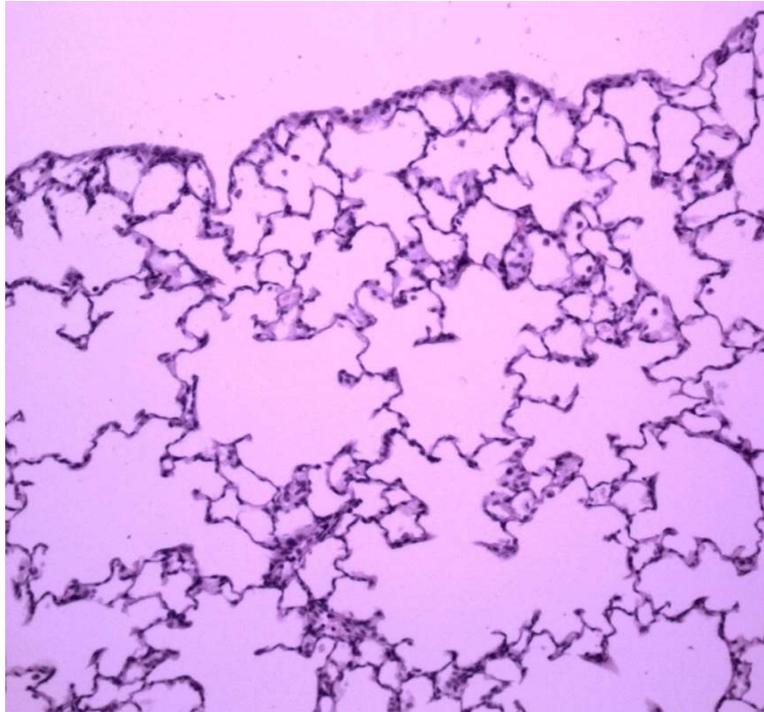
—200 nm—

Nanoparticles in a rat's pleural fluid

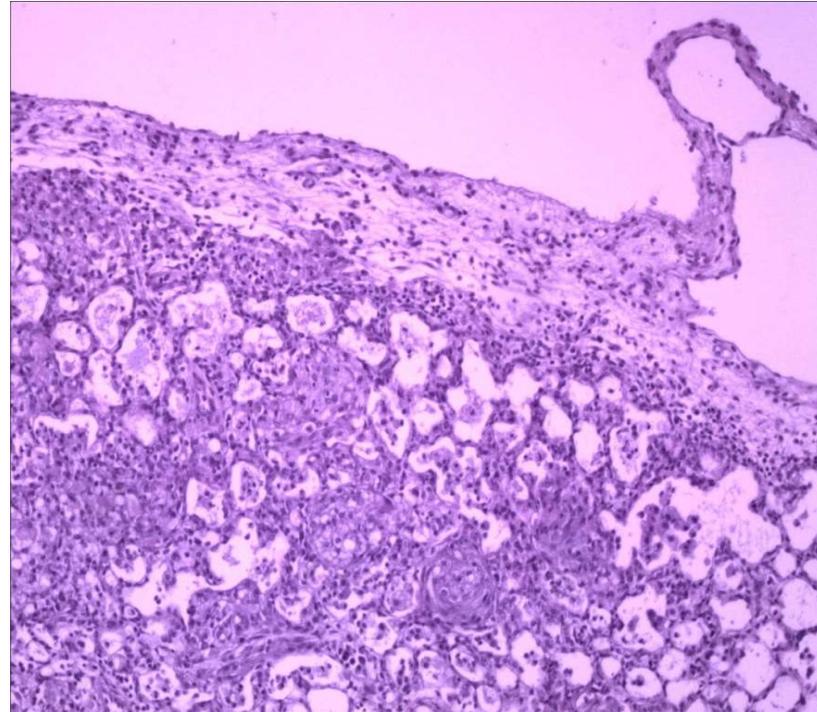




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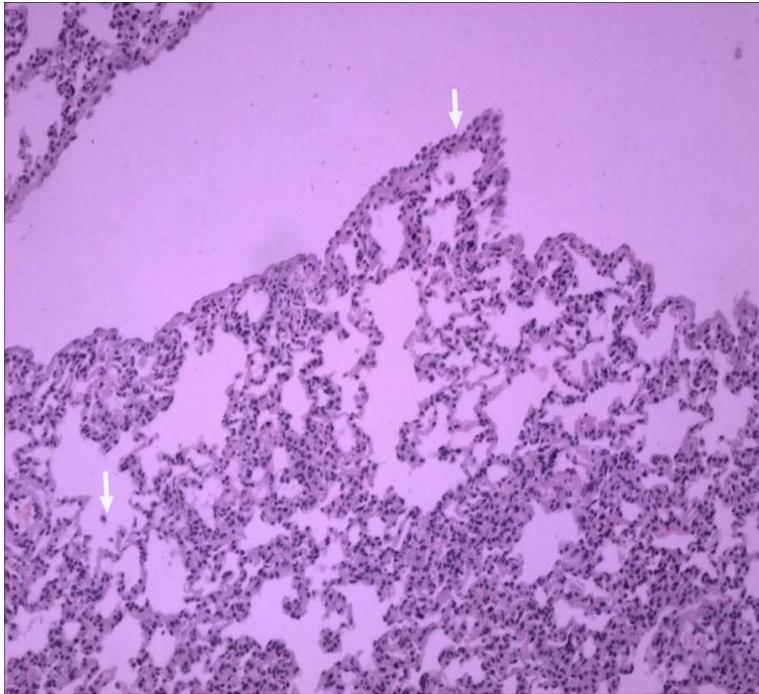
Control (NS)



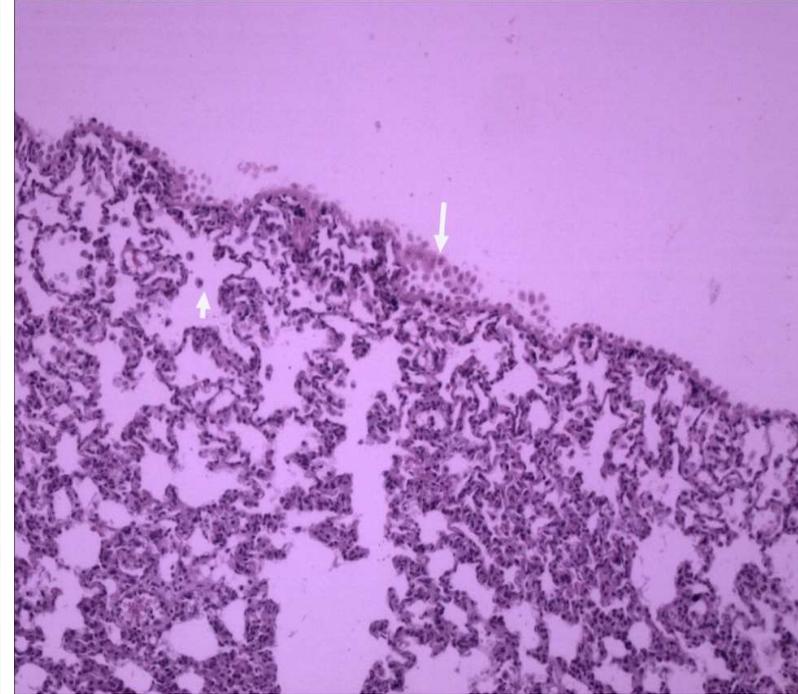
PA/NSi (12.5mg/kg)



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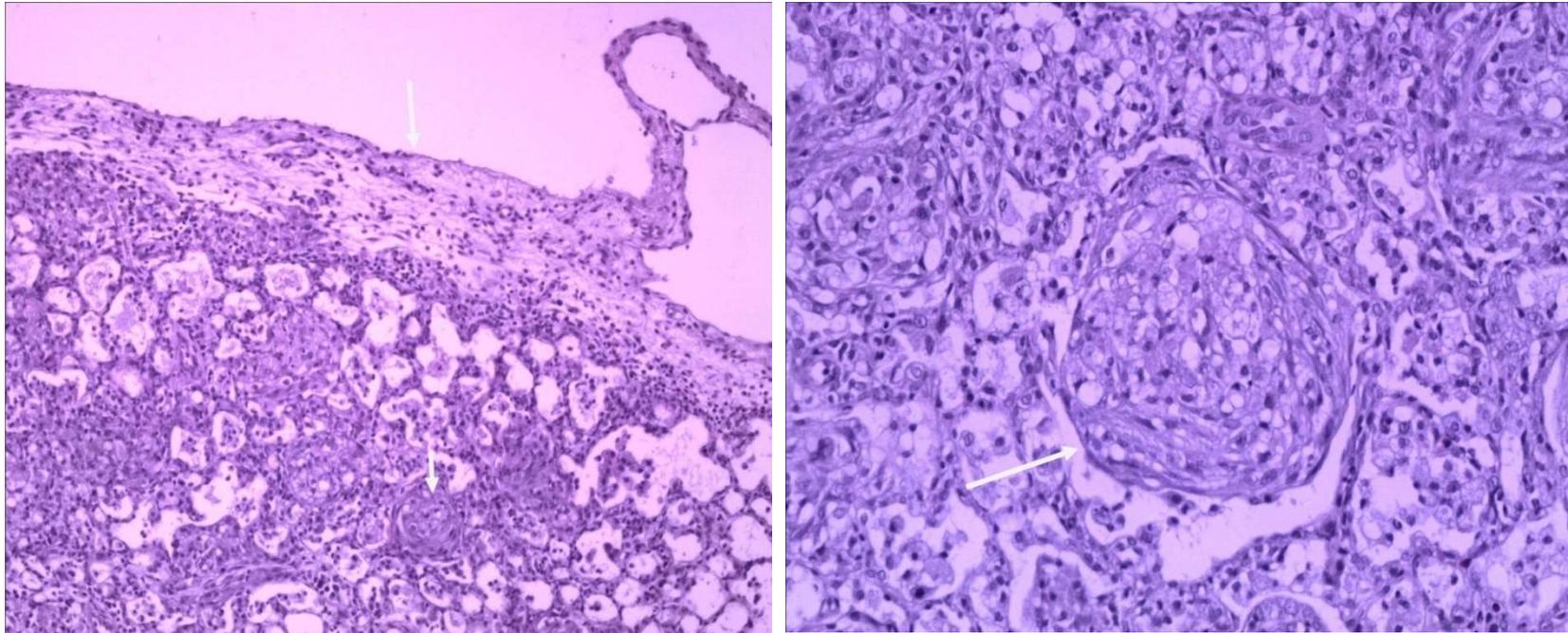
PA/NSi (3.12mg/kg)



PA/NSi (6.25mg/kg)

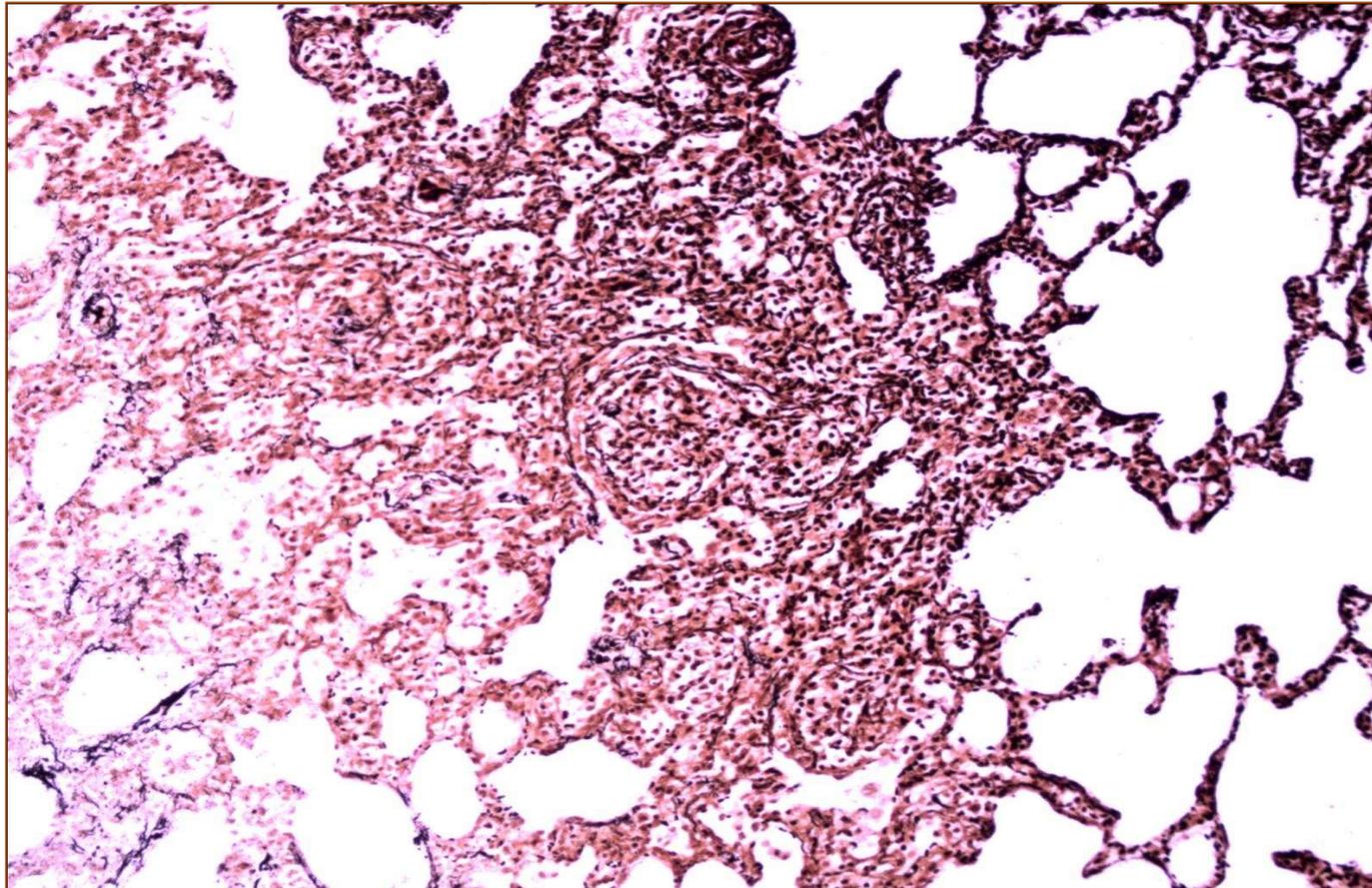


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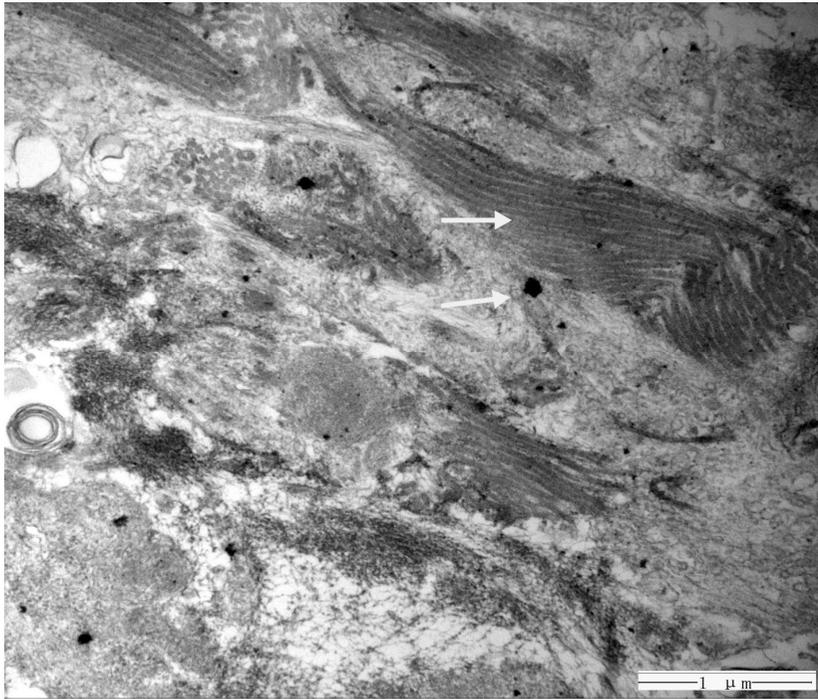


PA/NSi (12.5mg/kg)

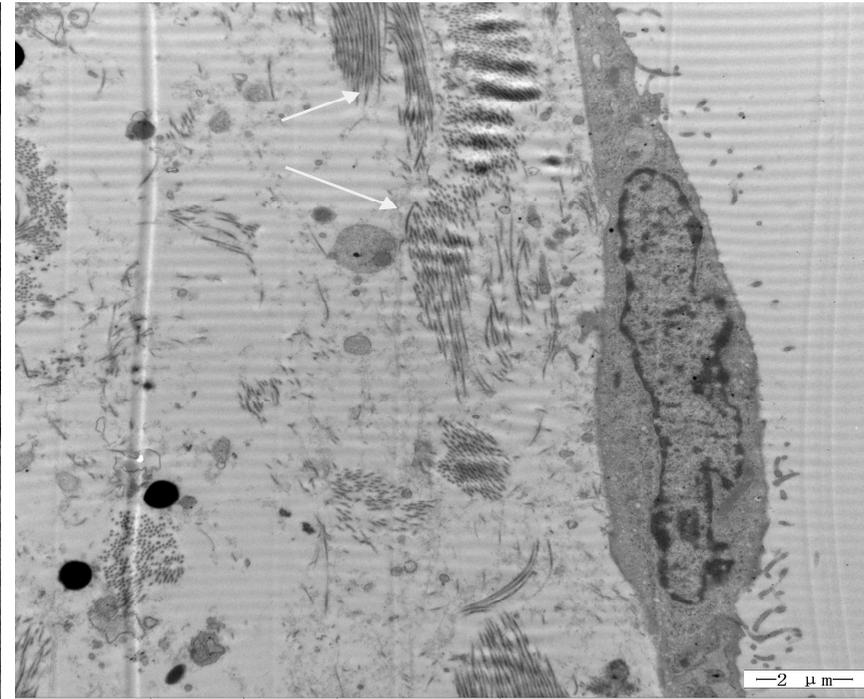
Reticular fiber staining



PA/NSi (12.5mg/kg) on d7

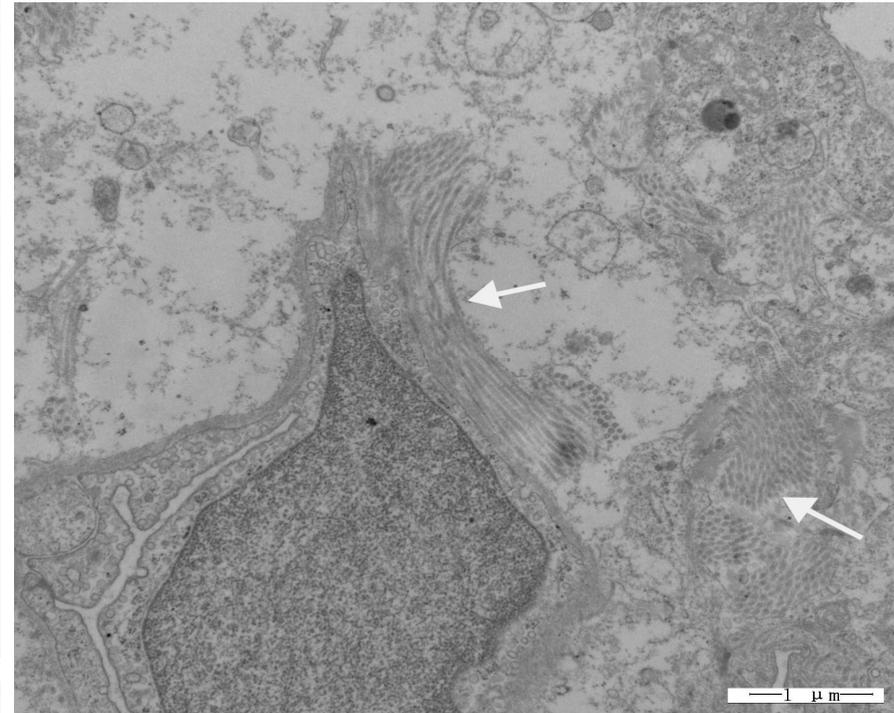
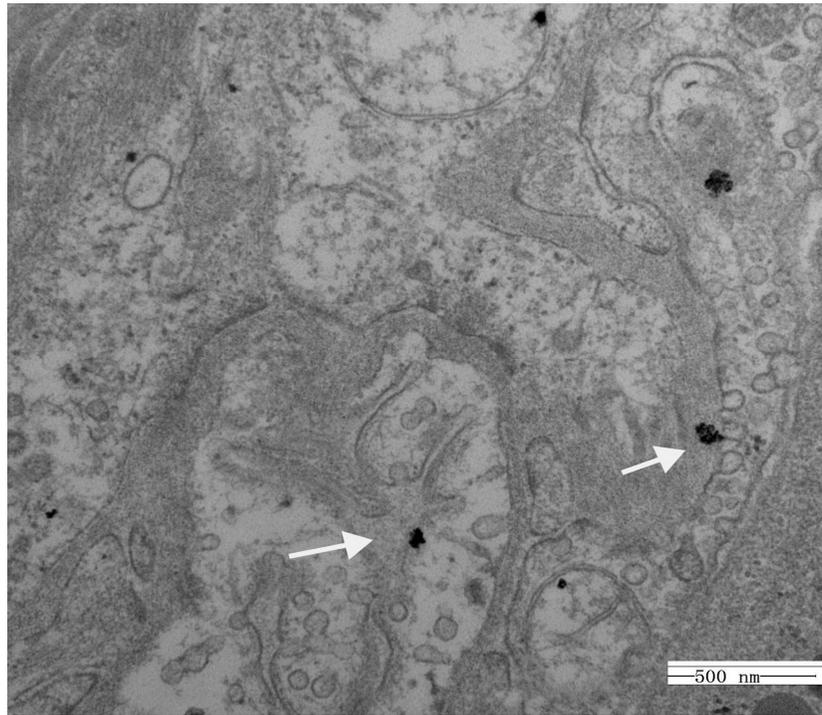


Pulmonary fibers



Pleural membrane

PA/NSi (3.125mg/kg) on d14



Ultrasound examination



Control

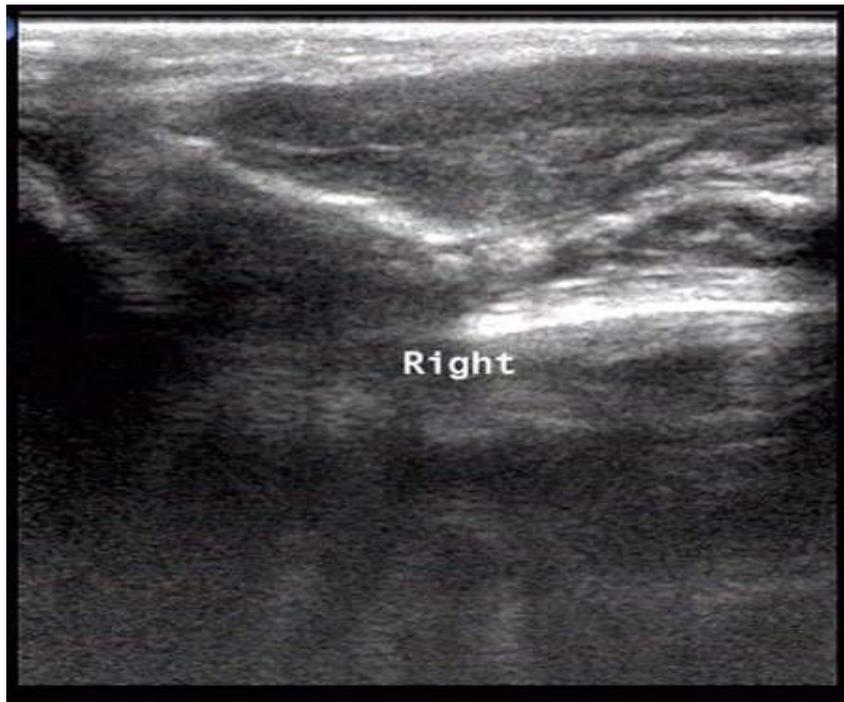


PA/NSi

Pericardial effusion



Pleural effusion



Control

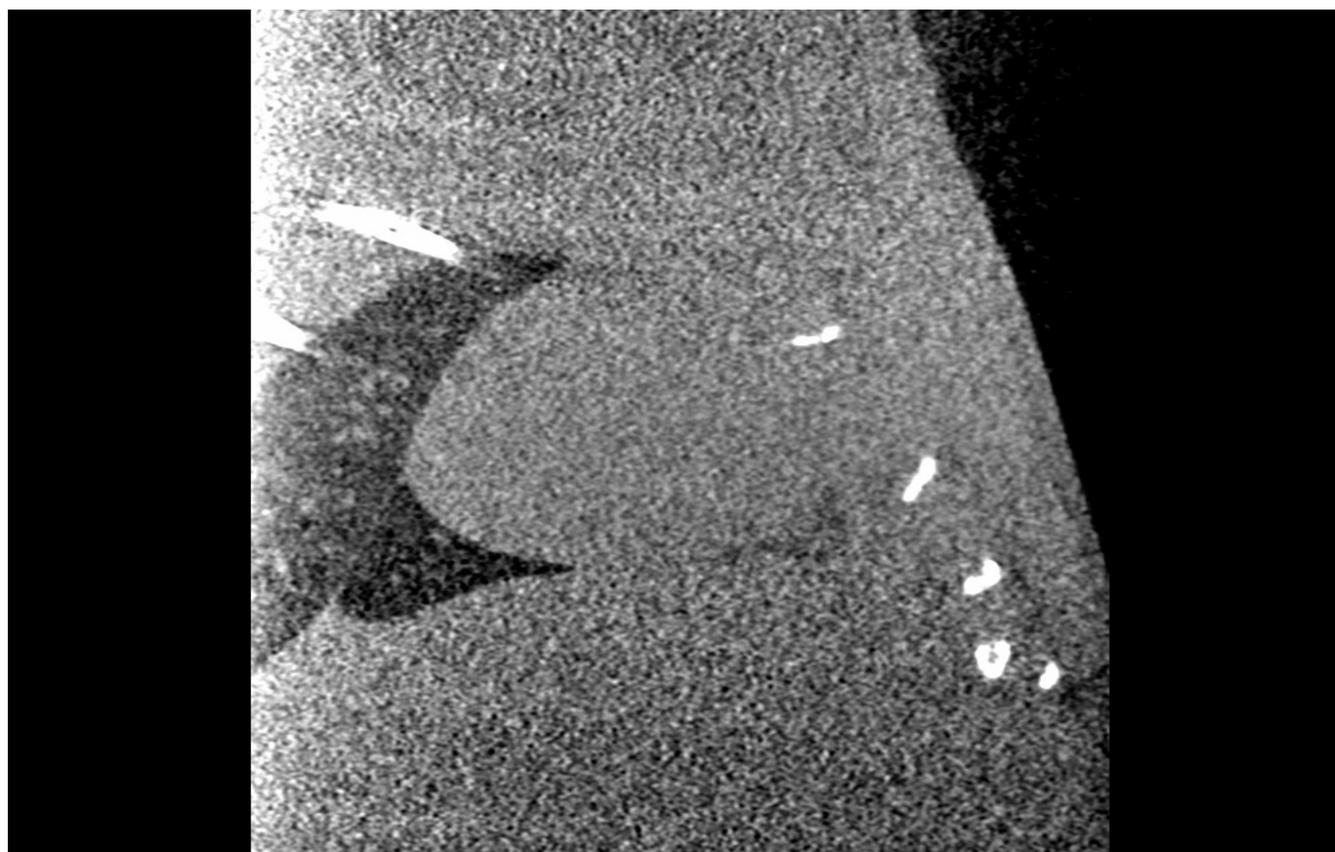


PA/NSi

Chest CT



Interlobular effusion



Results



- ❑ **PA/NSi causes pleural and pericardial effusion as seen in exposed workers**
- ❑ **Effusion occurred on days 3-5, peaking on days 7-10, then decreased and disappeared on day 14.**
- ❑ **No water was found in control groups of NS and PA.**



PA/NSi causes similar toxicity to those observed in exposed workers

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ORIGINAL RESEARCH

Polyacrylate/nanosilica causes pleural and pericardial effusion, and pulmonary fibrosis and granuloma in rats similar to those observed in exposed workers

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Xiaoli Zhu¹
Wen Cao²
Bing Chang³
Linyuan Zhang³
Peihuan Qiao³
Xue Li⁴
Lifang Si⁵
Yingmei Niu¹
Yuguo Song¹

¹Department of Occupational Medicine and Clinical Toxicology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, People's Republic of China; ²Department of Ultrasound, Beijing Chaoyang Hospital, Capital Medical University, Beijing, People's Republic of China; ³Department of Toxicology, National Institute for Occupational Health and Poison Control, China CDC, Beijing, People's Republic of China; ⁴Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, People's Republic of China; ⁵Department of Radiology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, People's Republic of China

Abstract: Nanomaterials offer great benefit as well as potential damage to humans. Workers exposed to polyacrylate coatings have pleural effusion, pericardial effusion, and pulmonary fibrosis and granuloma, which are thought to be related to the high exposure to nanomaterials in the coatings. The study aimed to determine whether polyacrylate/silica nanoparticles cause similar toxicity in rats, as observed in exposed workers. Ninety male Wistar rats were randomly divided into five groups with 18 rats in each group. The groups included the saline control group, another control group of polyacrylate only, and low-, intermediate-, and high-dose groups of polyacrylate/nanosilica with concentrations of 3.125, 6.25, and 12.5 mg/kg. Seventy-five rats for the 1-week study were terminated for scheduled necropsy at 24 hours, 3 days, and 7 days postintratracheal instillation. The remaining 15 rats (three males/group) had repeated ultrasound and chest computed tomography examinations in a 2-week study to observe the pleural and pericardial effusion and pulmonary toxicity. We found that polyacrylate/nanosilica resulted in pleural and pericardial effusions, where nanosilica was isolated and detected. Effusion occurred on day 3 and day 5 post-administration of nanocomposites in the 6.25 and 12.5 mg/kg groups, it gradually rose to a maximum on days 7–10 and then slowly decreased and disappeared on day 14. With an increase in polyacrylate/nanosilica concentrations, pleural effusion increased, as shown by ultrasonographic qualitative observations. Pulmonary fibrosis and granuloma were also observed in the high-dose polyacrylate/nanosilica group. Our study shows that polyacrylate/nanosilica results in specific toxicity presenting as pleural and pericardial effusion, as well as pulmonary fibrosis and granuloma, which are almost identical to results in reported patients. These results indicate the urgent need and importance of nanosafety and awareness of toxicity of polyacrylate/nanosilica.

Keywords: polyacrylate/nanosilica, pleural effusion, pericardial effusion, pulmonary fibrosis, granuloma



Conclusions and Indications

- **PA/NSi causes pleural and pericardial effusion, and pulmonary fibrosis and granuloma in rats as seen in exposed workers.**
- **The unusual disease of the reported workers are closely related to the exposure of PA/NSi.**
- **This highlights the urgent need and importance of nanosafety.**



Conclusions and Indications

- **This also highlights :**
- **Some nanomaterials like nanosilica may cause unusual toxicity upon heavy exposure compared to their micrometer-sized and bulk counterparts, which should be taken seriously in the development of nanoscience and nanotechnology.**

Still more questions



- ❑ Mechanisms of nanosilica causing pleural and pericardial effusion**
- ❑ Prevention of the nanotoxicity in nanoexposed workers**
- ❑ How to detect and evaluate the damage of nanotoxicity in humans?**



朝阳医院
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**Nanomaterials : “double-edged sword”,
more study about its safety and control need!**

**We are looking forward
to your cooperation!**

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Thank you