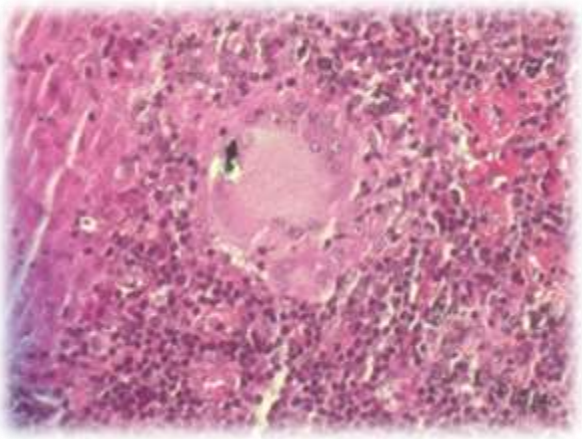
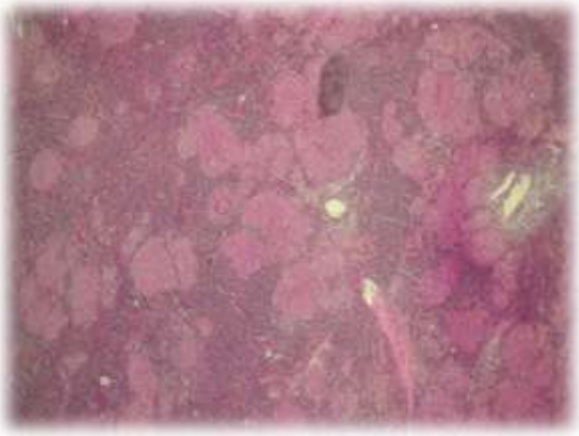
Lyon's Colloquium 5 - 6 - 7 December 2016
Organised by ALCTMP
(Association for Thoracic Cancer and Pulmonary Disease Fight)
Jean Bosco Center Lyon (14 Rue Roger Radisson Lyon 69005)

GRANULOMATOSIS

SARCOIDOSIS, MINERALOGICAL ANALYSIS AND INORGANIC PARTICLES HYPERSENSIBILITY





ALLP

La Région Auvergne-Rhône-Alpes

CENTRE HOSPITALIER Saint Joseph • Saint Luc

Fondation Saint Gabriel

Association Saint Luc

erc European Research Council

MINAPATH Mineral exposome

French Sarcoidosis Group

JEOL

GROUPE CARSO

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Welcome

Dear Participants,

Welcome to the Lyon International Workshop on Granulomatosis, Sarcoidosis and Inorganic Particles.
Welcome to those who have travelled far : United States, China, Israel.
Welcome to our European Colleagues : Sweden, Germany, The Netherlands, Belgium, Italy.
Welcome for those joining us from Paris and Auvergne Rhône-Alpes.

Thank you to Benoit Nemery, Lisy Fireman and Vincent Bonneterre for helping us put together this scientific program.

Thank you to our sponsors : JEOL, ALLP (Association Lyonnaise de Logistique Post Hospitalière), the Auvergne-Rhône-Alpes Region, Laboratoire CARSO, Centre Hospitalier Saint-Joseph . Saint-Luc, Association Saint-Luc, Fond de dotation Saint Gabriel, Minapath Development…

We have gathered quite a mix of disciplines : social sciences, internal medicine, pneumology, pathology, occupational and environmental medicine, physicists, chemists, spéci molecular biologistes and electron microscopists.

There are two good reasons to organize this meeting in Lyon :

In 1929, the International Labour Organization had a preliminary meeting in Lyon ahead of the 1930 Johannesburg Conference. It was the fourth meeting of the permanent International Committee on Occupational Diseases. France declined to attend the Johannesburg Conference and only recognized Silicosis as an Occupational disease in 1945…

Furthermore, the pathologist regarded by many as one of the precursors of pulmonary pathology, Policard, worked in Lyon. He is recognised by a citation in Dean E Schraufnagel 1990 book on Electron Microscopy of the lung (Vol 48 in Lung Biology in Health and Disease directed by National Heart, Lung and Blood Institute). The introduction of this book by Claude Lenfant from Bethesda quotes Policard and then adds « A. Policard is credited by the French to be the master, if not the creator of pulmonary histopathology. In 1929, he published the first edition of Poumon (the lungs). It says that if we want to understand lung pathology we must understand lung structure ».

Policard worked with a famous Lyonnais pulmonologist, Pierre Galy who was a clinician, physiopathologist and pathologist and tought us a lot of what we know in pulmonology.

The Association de Lutte contre les Cancers Thoraciques et les Maladies Pulmonaires (ALCTMP), Association for fight against Lung Cancer and Pulmonary Disease, works in synergy with Minapath Development which bought a Scanning Electron Microscope with the aim of providing mineralogical analysis and try to develop such analysis at a manageable cost.

There will also be some social events. We have cancelled the trip to Perouges for two reasons : December is not a good time to visit this medieval city by night and staying in Lyon may allow us to see some of the Light Festival attractions (which starts on 8th december).

We hope you enjoy your stay.

Dr JF Alex - President ALCTMP
Mme A Auroux - Vice-Presidente ALCTMP
Dr M Vincent - Scientific coordinator

Lyon's Colloquium 5 - 6 - 7 December 2016

ALCTMP (Association pour la Lutte contre les Cancers Thoraciques et les Maladies Pulmonaires)

Organised at Centre Jean Bosco by M Vincent, L Fireman et B Nemery 5, 6, 7 th December 2016

Granulomatosis - Sarcoidosis. Mineralogical analysis and Inorganic Particles

Definitive program

Monday 5 December

11 to 12.30 pm : Welcome at Jean Bosco Center by A Auroux, M Vincent, L Fireman, Ben Nemery and registration desk

12.30 to 2 pm: Lunch

2 pm : Assessing the Mineral Exposome

Chairs : Benoit Nemery (Department of Public Health University of Leuven, Belgium) and F. Thivolet (Department of Cytology and Pathology Pole Est HCL Lyon)

2 - 2.40 pm: Measuring methods for assessing exposure to micron and nanoparticles in workplaces and in environment - M Chouvet (ITGA Saint Etienne France)

2.40 - 3.20 pm: Questionnaires about Mineral Exposome - C. Cavalin (Center for European Studies ERC Grant Silicosis Sciences-Po Paris), M Vincent (Minapath Development and ERC Grant Silicosis).

3.20 - 4 pm: Mineral exposome and sarcoidosis: Sarcoidosis and occupational exposures: lessons from the French National network for occupational disease surveillance and prevention network. V. Bonneterre (Occupational and health department - Grenoble-Alpes Teaching Hospital)

4 - 4.40 pm: Chinese experience Y. Song (Department of Occupational medicine and clinical toxicology. Beijing China)

4.40 - 5.10 pm: General discussion

5.10 - 5.50 pm: Coffee break

6 pm - 7 pm: Plenary Conference by B. Nemery (Department of Public Health University of Leuven Belgium) : Exposure to particles and health : from the workplace to the urban space

_____ 8 pm: Welcome dinner in Restaurant « Le Vivarais » _____

1 Place Gailleton Lyon 69002 - Tél 04 78 37 85 15

Tuesday 6 December

7 - 8.15 am: Breakfast

8.30 - 10 am: Inorganic particle and organic tissue: a chemical approach

Chairs: A. Auroux (Vice-President of the French Chemical Society, IRCELYON CNRS, ALCTMP) – M Kambouchner (CHU Paris)

8.30 - 9 am: Revisiting the paradigm of silica quartz pathogenicity: the role of crystallinity and surface disorder - C Pavan (Department of Chemistry University of Torino)

9 - 9.30 am: In situ analysis of Interstitial Pathologies from TEM to SEM, preliminary results of Minasarc study - AM Trunfio-Sfarghiu (CNRS LAMCOS Insa Lyon), M Catinon (Minapath Development), M Vincent (Minapath Development)

9.30 - 10 am : Tissue Elemental Imaging with LIBS (Laser-Induced Breakdown Spectro-scopy) : recent advances and medical applications - V. Motto-Ros (Light and Matter Institute, Villeurbanne) and B Busser (Grenoble University Hospital France)

10 to 10.30 am: Coffee break

10.30 - 12.30 pm: Granulomatosis, Hypersensitivity : from Beryllium to metal and silica

Chairs : J Muller Quernheim (Medicine Unit University Hospital Freiburg Germany), Fireman L (Laboratory Pulmonary and allergic disease, Tel Aviv University, Israel)

10.30-11.10 am: The use of lymphocytes proliferation test in the assessment of occupational diseases - L.Fireman, Laboratory Pulmonary and allergic disease, Tel Aviv University, Israel

11.10-11.50 am: Netherland Experience - M. Veltkamp, St Antonius Hospital Nieuwegein Netherland

11.50-12.30 pm: An Italian Experience - A Gatti (Laboratory of Biomaterials University of Modena and Reggio Emilia Italy)

12.30 - 2 pm: Lunch

2 - 4.30 pm : Mineral exposome, sarcoidosis, genome and immunology

Chairs : JF Mornex (Department of Pneumology Louis Pradel Hospital, CHU Lyon), M Vincent (ALCTMP and Minapath Development)

2 - 2.40 pm: Sweden experience with genome and sarcoidosis - Grunewald J (Respiratory medicine Unit Karolinska University Hospital Solna Sweden)

2.40 - 3 .20 pm: Genome, sarcoidosis French - A. Calender (Molecular Genetic Laboratory Edouard Herriot Hospital Lyon), Y. Pacheco (Pneumology Department CHU Lyon)

3.20 - 4 pm: Immunology, genome and sarcoidosis - J Muller Quernheim (Medicine Unit University Hospital Freiburg Germany)

4 - 4.30 pm: Discussion

4.30 - 5 pm: Coffee break

5 - 6 pm: Plenary conference: S Sanyal and J Abraham Upstate Medical University Syracuse NY 13210 USA: Thirty year experience of in situ mineralogical analysis

6 - 8.30 pm: **free time and dinner at Chez Paul Restaurant, 11 Rue Major Martin Lyon 69001 - Tel : 04 78 28 35 83**

Wednesday 7 December

7.30 - 8.45 am: Breakfast

9 - 10.30 : Nanoparticle and granuloma and fibrosis

Chairs : Y Song (Department of Occupational medicine and clinical toxicology. Beijing China), Y Pacheco (CHU Lyon)

9 - 9.30 am: Saint Etienne Experience - JM Vergnon (Pneumology Department St Etienne University).

9.30 - 10.10 am: WTC and respiratory diseases and granulomatous diseases - R De La Hoz (Icahn School of Medicine at Mount Sinai New York)

10.10 - 10.40 am: Animal experimental Data - A Bentaher (INSERM Lyon) and Y Pacheco (Pneumology Department CHU Lyon)

10.40 - 11.10 am: Coffee break

11.10 - 12 am: BAL Minasarc study data : Healthy subjects - C Chemarin, Sarcoidosis cases - M Catinon, Inorganic particles and causal relationship in Sarcoidosis from Hill's criteria's – M Vincent, St Joseph-St Luc Hospital, Minapath Development Lyon

12 - 12.30 pm: For a declaration about paraclinic tests needed for granulomatous disease. General discussion.

12.30 pm : Lunch and End of the colloquium.

Curriculum Vitae
of the speakers and contributors

ABRAHAM Jerrold

Dr Jerrold L. Abraham is currently Professor of Pathology and Director of Environmental and Occupational Pathology in the Department of Pathology, College of Medicine, at the State University of New York (SUNY) Upstate Medical University in Syracuse NY.

Dr Abraham has practiced pathology for over 44 years, specializing in occupational and environmental lung disease and in analytical methodology to detect and quantify foreign particulate materials in lungs and other tissues. He served 3 years as a medical officer in the United States Public Health Service in NIOSH at Morgantown. He is board-certified in anatomic pathology. His curriculum vita lists 156 scientific publications and 168 abstracts. His laboratory has created and maintained a database on the lung burden of inorganic particulates in hundreds of individuals with a wide range of exposures, and has published widely on the results of such analyses. One study dealt with an outbreak of accelerated silicosis in sandblasters. He is considered an expert in occupational respiratory disease pathology, has been an invited participant for numerous national and international activities, and in particular is considered a world authority on micro-analytical methodology for characterizing the lung burden of inorganic particulates. Dr. Abraham has worked with NIOSH on panels of experts related to the pathology related to silica and asbestos, and with the American Thoracic Society (ATS) on expert committees related to silica, tremolite, and beryllium. Currently he is working 2 projects with NIOSH on newly recognized hazards from coal mining and metal-working fluids. His interest in particulate material has logically extended to his interest in air pollution and its health effects, resulting in his work as co-investigator and PI on projects supported by the EPA, the American Lung Association and the Department of HUD, with resultant publications.

He was born in Los Angeles, CA, where he received his public school education. He earned his Bachelor's degree in Biology from MIT in Cambridge, MA, and his M.D from UC San Francisco. After internship and residency in pathology in Boston at Children's Hospital and Beth Israel Hospital, he joined the US Public Health Service at the NIOSH lab in Morgantown, WV. That experience was vital to his subsequent career emphasis in Occupational and Environmental Lung Disease. He was on the pathology faculty at Univ. Calif. San Diego 1975-1983, and has been in his current department since 1983. He has been active in the American Thoracic Society, serving on the Tremolite, Silica and Beryllium committees, and was a member of the NIOSH/CAP Task Forces on Pathology Standards for Asbestos Related Disease, 1982, and for Silica and Silicate Related Disease, 1988. He has over 170 publications, some of which are included in the references to the program abstract.

AUROUX Aline

PhD IRCELYON-CNRS, 2 Avenue Einstein, 69626 Villeurbanne, France.
Aline.auroux@ircelyon.univ-lyon1.fr
Tel : 33-472445398 (office) 33-614105858 (mobile)

Professional experience

1973 : PhD in Physical Sciences, University of Lyon, France
CNRS researcher, Director of research, Emeritus
2006 - 2013 Head of the "Clean and Renewable Energies" group of IRCELYON

Main research areas

Catalysis, Catalytic processes, Synthesis of catalysts and nanoparticles, Oxides, Zeolites, Clean and renewable energies, Clean combustion of hydrocarbons, Hydrogen production and storage, Nanoparticles, Catalytic reforming, Fuel cells, Heat measurements , Calorimetry, Acid-base and redox properties of solids , Water and Air depollution, DeNOx, CO_2 capture, Biomass conversion, Biofuels, Interseasonal heat storage. Publications : over 317 in international journals. H-index=46. Citations=8452

17 book chapters, Editor of one book (2013, Springer Ed) and guest-editor of 3 reviews (Thermochim.Acta).

Presentation at congresses : 63 invited conferences, 174 oral communications, 305 posters.

Training of more than 90 PhD, Postdoc and Master students.
Numerous industrial, European (Inco-Copernicus, Eurobioref, Marie Curie) and national contracts (10 ANR projects).
Organization of 3 international congresses (CTEC) and 10 international summer schools in calorimetry and thermal analysis.

Awards : Award "I.G. Murgulescu" of the Romanian Academy of Sciences (2003).
Award CALVET in calorimetry (2007).

Implication in scientific societies

Member of SCF (Société Chimique de France) since 1975 and ACS (American Chemical Society)
Tresorier of Rhône-Alpes section of French Society of chemistry since 2013 and inter-division Energy since 2015
Vice-President of Administration Council of French Society of Chemistry (Paris, since 2015)
Nominated member of Comité National du CNRS (section 14 et CDI 43) de 2004 à 2008.
Nominated member of CSE 31 de l'Université de Montpellier de 2005 à 2009

BENTAHER Abderrazzak, Ph.D.

Research Director at National Institute of Health and Medical Research (INSERM), France

CAREER

- Assistant professor, Pulmonary Division of Medicine Department and Joint Appointment in Molecular Microbiology Department, Washington University, St Louis, MO.
- Director, Avenir Program : Neutrophils and lung Inflammation in the seting of infection, Reims, France.
- Present : Director, Research team «Inflammation and Immunity of the Respiratory Epithelium», EA 7426 (PI-3) Lyon, France.

MEMBERSHIP OF PROFESSIONAL SOCIETIES

- American Thoracic Society (Microbiology, Tuberculosis and Pulmonary Infection Assembly)
- European Respiratory Society (Assembly of Respiratory Infections)

FIELDS OF EXPERTISE AND APPLICATIONS

- Lung infection, innate immunity, inflammation, and tissue injury - Gene targeting - MicroRNAs - Mouse experimental models.
- Translational Research, Therapeutic approaches and Development.
- Acute lung injury: pneumonia, cystic fibrosis.
- Chronic pulmonary obstructive diseases: Cigarette smoke-induced emphysema and bacterial-mediated COPD exacerbations.

RESEARCH FUNDINGS (Past and Present) (Selection)

- National Institute of Health (Lung Pathology), USA.
- French Foundation for Medical Research (FRM), France.
- French National Agency for Research, France.

RESEARCH AWARDS

- Research Award, Barnes-Jewish Hospital Foundation, St Louis, USA.
- American Lung Association Career Investigator Award.
- Inserm Avenir Laureat.
- Lung Science Conference Travel Award, European Respiratory Society.
- Grant Award « Environmental Chemical Threats and Lung Injury: Mechanisms and Countermeasures ».
- Grant for best COPD research project, European Respiratory Society.

RELEVANT PUBLICATIONS (SELECTION)

- Boxio R et al. ; Neutrophil elastase cleaves epithelial cadherin in acutely injured lung epithelium. Respir Res. 2016 Oct ; 17 (1) : 129.
- Aloui R et al. ; Effects of fine particulate matter on bronchial epithelial cells. Rev Mal Respir. 2016, S0761-8425 (16) 30041 - 9.
- Guyot N et al. ; Unopposed cathepsin G, neutrophil elastase, and proteinase 3 cause severe lung damage and emphysema. Am J Pathol 2014 ; 184 (8) : 2197 - 210.
- Gehrig S et al. Lack of neutrophil elastase reduces inflammation, mucus hypersecretion, and emphysema, but not mucus obstruction, in mice with cystic fibrosis-like lung disease. Am J Respir Crit Care Med 2014 ; 189 (9) : 1082 - 92.
- Bergin DA et al. ; Activation of the epidermal growth factor receptor (EGFR) by a novel metalloprotease pathway. J Biol Chem 2008 ; 283 (46) : 31736 - 44.

BONNETERRE Vincent

Institution : CHU Grenoble-Alpes
Mail : VBonneterre@chu-grenoble.fr
Téléphone : 04 7676 5851 / 06 64 29 62 71

Professor of Occupational Medicine in Grenoble-Alpes teaching Hospital, in charge of Grenoble Occupational Diseases clinic, Vincent Bonneterre has, from a clinical point of view, a strong interest for the investigation of occupational diseases (OD) of toxic origin. He is chair of the "Emergence Working Group" of the French National Network for surveillance and prevention of occupational diseases gathering the 31 French OD clinics (acronym : rnv3p for "Réseau National de Surveillance et de Vigilance des Pathologies Professionnelles"). He developed, and validated with the national agency for health safety in food, work and environment (Anses) a 3 step procedure for the early detection of new potential work-related diseases, their investigation and the launching of due actions (including alerts). The early signals might arise from clinical experts of OD clinics, from data mining of rnv3p database, or from bibliographic/documentary watch. Investigations as well as actions are standardized. This has led to cooperations at EU level (Modernet network).

BUSSER Benoit

Institution : Grenoble University Hospital (CHU Grenoble Alpes)
Mail : bbusser@chu-grenoble.fr
Téléphone : 06 32 39 37 65

Benoit Busser is a 35 y Associated Professor in University Grenoble Alpes (France). He is the head of the Cancer & Biotherapy clinical laboratory at the Grenoble University Hospital. He is also researcher in the Institute for Advanced Biosciences (IAB) in Grenoble. He is deeply specialized in solid malignancies and he mainly focuses his interest in lung cancer and melanoma. For the last 3 years, he also worked in close collaboration with physicists from University of Lyon (France) on the biological and medical applications of LIBS multi-elemental imaging (Laser Induced Breakdown Spectroscopy). He is currently doing a 1-year research mobility in Lyon to work as a full-time investigator to demonstrate the versatility of LIBS elemental imaging as a tool for pathologists, biologists or physicians, especially for lung (e.g. Interstitial Lung Diseases - ILD) and skin diseases.

CALENDER Alain

Adresse professionnelle : Service de génétique clinique et moléculaire, Hôpital Edouard Herriot, B7, 5 Place d'Arsonval – 69437 LYON cedex 03 – LYON - FRANCE
Tel : 00 33 (0)4 72 11 73 80
Tel : 00 33 (0)4 72 11 73 81
alain.calender@chu-lyon.fr

Main Areas of Research

- **Familial sarcoidosis** (1st main topic)
- **Multiple Endocrine Neoplasia type 1** (2nd main topic)
- Neuro genetics (3rd main topic) – mental retardation, genetics of autism, Alzheimer
- Cancer predisposition
- Breast Cancer
- Hereditary Hemorrhagic Telangiectasia (HHT)

Academic career

- Since September 2001 : **Chief of the Medical and Molecular Genetics Department** (Hospital Edouard Herriot - Hospices Civils de Lyon) and co-manager of the Genetic Unit on familial breast and colon cancers (Centre Léon Bérard – LYON)
- **Medical practice in oncogenetics and neurogenetics**
- Regular medical practice in emergency intensive care unit (night care service)
- Assistant doctor intensive care and emergency medicine (Hôpital Edouard Herriot – Emergency Unit)
- **Professor of Genetics, Oncology** in University Lyon I (Medical University Lyon EST)
- **Professor of molecular pathology in** '**ENS**' (Ecole Normale Supérieure) – LYON – F
- **Chairman** of '**Pathological and clinical forms of handicap**' – University Lyon II
- Management of master degrees in human genetics (Medical University in Lyon)
- 1993 – 2001 : associated Professor of Genetics in Medical University Lyon EST - Medical practice in Onco genetics and intensive care
- 1991 – 1993 : assistant in an **intensive care unit** (Lyon and Villefranche sur Saône)
- 1991 : Medical degree
- 1981 – 1991 : Medical University and research activities in 1) pathogenic role of HTLV-I and HIV (1981-1985) Pasteur Institute / **Chappel Hill** – **USA - North Carolina University** and 2) basic mechanisms involved in B lymphocytes immortalization by the Epstein Barr Virus (EBV) and its role in Burkitt's lymphoma (1985-1991 International Agency for Research on Cancer in Lyon – France) - 1974 – 1981 – Basic Science university cursus and PhD in 1981 – topic : mechanisms involved in the replication of herpes viruses

Academic degree – **PhD, MD**

1981: **PhD of cellular and molecular biology**
1991: **Medical Doctor thesis**, Lyon1
1999: **Inter University Diploma (DIU) of Genetics** (4 yrs national) formation (DIU)
1999: **Research Habilitation Degree** (**H**abilitation à **D**iriger les **R**echerches – **HDR**)
2001: University diploma (**DU**) in **Molecular Endocrinology**
2007: University diploma (**DMU**) in **intensive care medicine** (interregional formation)
Total number of PMID REF publications (all positions): **140** (12.nov. 2016)

CATINON Mickaël

Mickael Catinon is a researcher at Minapath Development a Business Social Society. He specialises in physico-chemical analysis, notably the mineralogical analysis of micro- and nanoparticles deposited in living and inert matrix. He holds a doctorate in Ecology, Environment and Health from the University of Grenoble1, where he subsequently completed postdoctoral research. His doctoral and postdoctoral work studied inorganic atmospheric pollution in the Rhône, Isère and Gier regions of France. Recent work by Dr Catinon's for the Minapath Laboratory has focused on dust buil-up in human organs (such as lungs, kydneys, ovaries and blood). Dr Catinon joined the SILICOIS ERC Grant team for 2 years (2013 September until 2015). He is the principal writer of article about Minasarc Study project seeking to find an inorganic cause of sarcoidosis by comparing dust exposure by specific questionnaire and mineralogical analysis of bronchoalveolar lavage in two populations (20 sarcoïdosis cases and 20 matched healthy subjects). Since 2015 September he works full time in Minapath Development team.

CAVALIN Catherine

Catherine Cavalin is a researcher at Sciences Po's Center for European Studies (SILICOSIS project financed by the European Research Council, directed by Paul-André Rosental) and associate researcher at the Centre d'études de l'emploi (Ministry of Research, Ministry of Labor and Employment). Following interdisciplinary studies in social sciences (aggregation in social sciences, postgraduate studies in contemporary history and health economics) and a dozen years teaching economics and business studies in preparatory colleges, Catherine Cavalin coordinated the study Evènements de vie et santé (EVS-Life Events and Health) in the Direction de la Recherche, des Etudes, de l'Evaluation et des Statistiques (DREES) in the French Ministry of Health. This study examines the relationship between violence suffered and state of health. This was the theme of her doctorat in sociology of 2016 October. In all her work she crosses multiple statistical sources to question the construction of categories and tends towards a qualitative interpretation of quantitative data.

CHEMARIN Cécile

Cécile Chemarin is Scientific Director of the mineralogical analysis section of the biology laboratory at the Centre Hospitalier St Joseph St Luc in Lyon, France and she works with Minapath Development company. She holds a master of Advanced Studies in materials science from the Claude Bernard University, Lyon, France and she completed her Ph.D. in materials science on "Nanostructures in silicate glasses" in 1998 at the same institution. Teaching at the Institut Universitaire Technologique (IUT) de Grenoble from 1998-2001, she was also being a research assistant at the Laboratoire d'Electrochimie et de Physicochimie des Matériaux et des Interfaces (LEPMI) associated with the Phelma school (school of engineering in Physics, Applied Physics, Electronics & Materials Science). In 2002, she joined Michel Vincent team to develop physical and chemical analysis in biological samples and to create the mineralogy section which is associated to the Silicosis and Minasarc projects. Her publications include :

CHOUVET Martine

La scierie 42500 Usson en Forez
Tel : 0613312412
E mail: martine.chouvet@itga.fr
Date of birth: November 5th, 1966 French citizen

Proficiency: Industrial Hygiene, Chemical risk assessment, Development and validation of tests methods, chemical samplings, Chemicals analysis, physical modelling, reglations on classification of hazardous substances and prevention of chemical risks. French and European standardisation.

Professional positions : From September 2013

ITGA France. Technical Director: Management of a group of 10 engineers, technical assistance for the company (900 collaborators/14 agencies laboratories)

Field : Industrial hygiene, indoor quality air. Asbestos.Developpement and validation of test methods. Project manager for specific case.

From 2000 to 2013 : Technical manager. Industrial Hygiene Department

Definition of working method about chemical risk assessment, sampling strategy, chemical sampling, chemical analysis. Development of progiciels concerning chemical risk assessment. Project manager for specific case

From 1997 to 2006 ITGA Saint Etienne Laboratory Manager : Management. Development and validation of test methods. Survey in industrial hygiene.

From 1995 to 1997 : Houillères du bassin centre–midi (charbonnages de France) Saint Etienne : Laboratory and Quality Manager : accreditation of the laboratory

Background

1994 : Chemical Engineer-Option materials

Toulouse-France: National School of Chemistry Toulouse

Collaborations

French Standardisation : Since 1996. Participation in Commission X43C : Workplace

European standardisation : Since 2012. Participation in the technical Committee 1 : measurement of exposure by inhalation to chemical agents. Strategy for testing exposure limit values

INRS: Since 2009. Member of the scientific committee for the department Pollution

CHU Montreal : Collaboration from 2015 on the development of Bayesian tools for the industrial hygiene

DE LA HOZ Rafael

Rafael de la Hoz, MD, MPH, MSc, Associate Professor of General Internal Medicine, and Occupational Medicine in the Department of Environmental Medicine and Public Health and practices at the Selikoff Centers for Occupational Health, at the Mount Sinai Medical Center, in New York City. Dr. de la Hoz has had a longstanding career interest in research in clinical occupational respiratory toxicology and occupational obstructive lung diseases. He has been particularly interested in obstructive airway diseases that escape classification into the accepted nosological entities. Since 2002, Dr. de la Hoz has followed and characterized the types of bronchial disease associated with occupational toxicant exposures at the WTC disaster site. He is the principal investigator of a research grant correlating symptoms, pulmonary functional, and qualitative and quantitative chest CT scan findings in the WTC occupational cohort followed at Mount Sinai. Dr. de la Hoz received his medical degree at the Universidad del Rosario, in Bogotá, Colombia, his Master's in Public Health at the Yale School of Public Health, and his Master of Science at the NYU Graduate School of Arts and Sciences. He is board-certified internist, pulmonologist, and occupational physician.

FIREMAN Lizy, Ph.D

Place of work : Tel-Aviv Sourasky Medical Center.
Faculty/Hospital : Pulmonary and Allergic Diseases
Position : Head Laboratory Pulmonary and Allergic Diseases
Head National Laboratory Service for Interstitial Lung Diseases
Chair Department of Environmental and Occupational Health Tel Aviv University

EDUCATION

1969-1973	Faculty of mathematics, physics and natural sciences University of Bologna, Italy Awarded degree of Dottore in Science Biologiche -Laurea - Title from the above mentioned institution (equivalent to M.Sc.) in July,1973
1985-1990	Started Ph.D. in Immunology at Tel-Aviv University Awarded Ph.D. Degree in May, 1990

Title of Master's Thesis

"The coupling factor in photosinthesis in the anaerobic bacteria Rhodopseudomonas Capsulata"
Name of supervisors: Dr. Assunta Baccarini-Melandri - Dr. Bruno Andrea Melandri

Title of Doctoral Dissertation : "Suppressor cell activity of human alveolar macrophages in interstitial lung diseases"

Name of Supervisors: Prof. S. Ben Efraim - Prof. M. Topilsky - Prof. Z. Spirer

FURTHER STUDIES

1994-1996	Ramot - Tel Aviv University Medical Management Graduate; September 1996

ACADEMIC AND PROFESSIONAL EXPERIENCE

Academic Experience

1994 - 1998	Postgraduate courses Laboratory graduates
1997 - 2000	Pulmonary diseases in occupational medicine Postgraduate course
2000	Second, third, year medical students - Immunology, Sackler School of Medicine Tel-Aviv (4 and New York courses)
2009	Sixth year medical students Non invasive methods for the assessment of Occupational Lung Diseases. Epidemiology and Preventive Medicine
2012	Immunology in Occupational /Environmental Lung Diseases MPH program Department of Occupational Environmental Health

Professional Experience

1985 - present	Head, Laboratory Pulmonary and Allergic Diseases
1999 - present	Head, National Laboratory Service for Interstitial Lung Diseases
1999 - present	Editorial Board, Sarcoidosis Vasculitis and Diffuse Lung Diseases

2000 - present	Reviewer for International Journal CHEST, ERJ and Sarcoidosis Vasculitis and Diffuse Lung Diseases, European J Clin Inv, Respiratory Medicine, American Journal Industrial Medicine, Respirology, Mediators of Inflammation, American Journal Respiratory and Critical Care Medicine
2012- present	Israel National Delegate in the European Respiratory Society
2004 -	Senior Lecturer in Occupational and Preventive Medicine, Sackler School of Medicine
2010 -	Associated Professor in Occupational Environmental Health Sackler School of Medicine
2016 -	Chair Department of Environmental and Occupational Health - Tel Aviv University

Research experience

9 - 11/1993	Visiting Investigator Department of Neurobiology, Cell Biology and Anatomy Loyola University of Chicago, Illinois USA
9-11/2009	Visiting Professor Department of Occupational Lung Diseases and Preventive Medicine University of Parma Italy
9-10/2011	Visiting Professor Pulmonary and Critical Care New York University USA

More than 100 pier reviewed papers, 90 presentations in scientific meetings

GATTI Antonietta

Dr. Gatti is an International Fellow of the Union of the Societies of Biomaterials and Engineering. At present she is Associate Professor at the National Council of Research of Italy, Institute of Science and Technology of Ceramic materials; has a contract as lecturer at the University of Urbino (Italy), is Visiting Professor at NUARI (Washington, USA) and consultant of the European Science Foundation.

Dr. Gatti is the discoverer of "nanopathologies", i.e. pathologies due to the exposure to engineered or incidental nanoparticles, responsible of nano-bio-interactions, that cause pathologies like cancer and mysterious diseases.

She has an interdisciplinary background that ranges from physics, chemistry, biology, physiology, medicine and pathology. Dr. Antonietta Gatti has a 30-year experience in research in the field of biomaterials and biocompatibility at national and international level in various capacities. In 2002-05 Dr Gatti was appointed coordinator of the European project called Nanopathology through which a new diagnostic clinical tool was developed. The results of the project are described in her book Nanopathology, published by Pan Stanford Publishing (Singapore) 2008. In 2006 she coordinated the European Project called DIPNA (Nanotoxicology) (Development of an integrated platform for the nanoparticles toxicology assessment) and the project of nanoecotoxicity called INESE. In 2004 until 2012 she was appointed consultant of the Italian Governmental Commission on the Depleted Uranium and related diseases and member of the Technical Committee for the Prevention and Control of the soldiers Diseases by the Minister of Defense. In 2005 she was invited at the House of Lords in London foe an Audition. She coordinated Italian Projects of the Ministry of Defense (VENAM, BATNAN). Author of about 220 articles in peer reviewed journals and she published in 2015 a new book called Case Studies in Nanotoxicology and Particle Toxicology, (Elsevier, USA) 2015. From 1981 up to 2012 she has created and directed the Laboratory of Biomaterials at the University of Modena where he was Professor of Biomaterials at the Faculty of Biotechnology.

She was member of some European Commissions and worked at ISO and OECD for the Nanomaterials Standardization.

GRUNEWALD Johan

Grunewald J has a PhD in basic immunology (1993) and became certified doctor (MD) in 1997. In 2005 he was appointed Professor at the Department of Medicine, Karolinska Institutet, where he also was Deputy Chair 2003-2014. Since 2007 he is Head of Respiratory Medicine Unit there.

Grunewald has had a focus on immunological, genetic, proteomic and clinical aspects of sarcoidosis and authored > 200 publications, including > 160 original articles in international scientific journals, ~ 45 reviews, editorials, clinical guidelines etc. Grunewald has been invited opponent (Faculty opponent) at several occasions and supervised 20 students to their PhD. Since 2008, he is General Secretary of the WASOG organisation.

KAMBOUCHNER Marianne

Dr Marianne Kambouchner is a pathologist in the Department of Pathology of the Avicenne University Hospital Bobigny. She initially trained as a general practitioner, and then completed her residency at the pathology department of Strasbourg University Hospitals (1986-9) and then a fellowship in the Department of Pathology of Avicenne Hospital (1989-93), where she became interested by pulmonary pathology. So far, she is mostly involved in thoracic pathology (surgical and non-surgical). In 1993 , she had the possibility to train in the field of interstitial lung disease in the Department of Mayo Clinic, Rochester. She is consultant for the editorial board of the Revue des Maladies Respiratoires and has participated to numerous articles and book chapters in this field. She has had the great opportunity to train with Pr JF Bernaudin (Tenon university Hospital, Paris).

MORNEX Jean-François

Professor Jean François Mornex (Claude Bernard University) is chief of pulmonary department in Louis Pradel Hospital in Lyon : reference center for pulmonary orphan disease, Pulmonary Hypertension, Thoracic Oncology and pulmonary and cardiopulmonary transplantation. He has a focus on interstitial lung disease and authored more than 100 publications.

MOTTO-ROS Vincent

Vincent Motto-Ros is a 39 yo Associated Professor in University of Lyon. He graduated with a M.S. in "Laser and Spectroscopy" in the University of Lyon (France) in 2002 and continued to complete his Ph.D. in the 'Laboratoire de Spectrométrie Ionique et Moléculaire' (Lasim, Lyon) working on "high-precision and high-sensitive spectroscopy of gaseous molecular species (O2, H2O, NO2) using high finesse cavities pumped by CW laser diode". He starts his research on Laser-induced Breakdown Spectroscopy in 2007 with a post-Doc position in the Canadian Space Agency for which he demonstrated the potential of artificial neural network (ANN) in LIBS data processing for material identification. He was recruited in 2008 by the Lyon 1 University where he developed since advanced experimental setups for fundamental research as well as applications related to biology or medicine. His panel covers the fundamentals of laser-induced plasmas, the application of laser spectroscopies such as LIBS, Fluorescence and Raman, as fundamental diagnostics as well as sensing techniques for industrial, environmental, geological and biomedical applications.

MULLER-QUERNHEIM Joachim

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Short CV

1974 - 80 Medical School, University of Frankfurt and University of Mainz, Germany

1980 - 82 Post-Doc in Immunology, collaborative research center 107, University of Mainz

1982 - 90 Home staff training appointment for internal medicine, pneumology and allergology, IIIrd Medical Department, University of Mainz

1984 - 86 Post-Doc (sponsored by German Research Foundation), Pulmonary Branch of the National Institutes of Health, Bethesda, USA

Since 1987 Extramural funding of research in interstitial lung diseases by German Research Foundation and Federal Minister of Research and Technology

Since 1990 Scientific advisor of the support group "Deutsche Sarkoidosevereinigung"

1990 Habilitation (Ph.D.Thesis)

1990 - 93 Senior staff member, IIIrd Medical Department, University of Mainz

1993 - 02 Senior investigator, Research Center Borstel; and Senior staff member, Medical Hospital Borstel, Germany

Since 2002 Full Professor of Internal Medicine / Pneumology, University of Freiburg Five most important clinical trials published in 2011 – 2016

1. Fischer A, Ellinghaus D, Nutsua M, Hofmann S, Montgomery CG, Iannuzzi MC, Rybicki BA, Petrek M, Mrazek F, Pabst S, Grohe C, Grunewald J, Ronninger M, Eklund A, Padyukov L, Mihailovic-Vucinic V, Jovanovic D, Sterclova M, Homolka J, Nothen MM, Herms S, Gieger C, Strauch K, Winkelmann J, Boehm BO, Brand S, Buning C, Schurmann M, Ellinghaus E, Baurecht H, Lieb W, Nebel A, Muller-Quernheim J, Franke A, Schreiber S. Identification of Immune-relevant Factors Conferring Sarcoidosis Genetic Risk. Am J Respir Crit Care Med. 2015: 192: 727
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3. Balmes JR, Abraham JL, Dweik RA, Fireman E, Fontenot AP, Maier LA, Muller-Quernheim J, Ostiguy G, Pepper LD, Saltini C, Schuler CR, Takaro TK, Wambach PF. An official american thoracic society statement: diagnosis and management of beryllium sensitivity and chronic beryllium disease. Am J Respir Crit Care Med. 2014 Nov 15;190(10):e34-59.
4. Hofmann S, Fischer A, Nothnagel M, Jacobs G, Schmid B, Wittig M, Franke A, Gaede KI, Schürmann M, Petrek M, Mrazek F, Pabst S, Grohe C, Grunewald J, Ronninger M, Eklund A, Rosenstiel P, Hohne K, Zissel G, Müller-Quernheim J, Schreiber S. Genome-wide association analysis reveals 12q13.3-q14.1 as new risk locus for sarcoidosis. Eur Respir J. 2013 Apr;41(4):888-900.
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NEMERY Benoit

Benoit Nemery is at the head of Occupational and Environmental Medecine Department in Leuven University Bruxelles and a research unit in toxicology. He is author in over 400 publications. His main scientific focusses are on animal experimental studies and clinical epidemiologic studies about mechanisms of pulmonary diseases in relation with occupational or environmental agents.

Member of many national and international scientific societies and several editorial boards, among them the American Journal of Respiratory and Critical Care Medicine.

PACHECO Yves

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Retired Pulmonology Professor. Currently working in research Unit EA 7426. Chief of French research program SARCFAM. Genetic studies “whole exome sequencing” on families sarcoidosis cases. In charge development model of murine granulomatosis pulmonary disease induced by nanoparticles inhalation.

PAVAN Cristina

Dr. Cristina Pavan is a postdoc research fellow in the Toxicology and Biocompatibility of Material group, Dept. of Chemistry, and Interdepartmental Centre “G. Scansetti” for Studies on Asbestos and Other Toxic Particulates, University of Torino. She graduated in Drug Chemistry and Technology in 2012 and obtained a PhD degree in Pharmaceutical and Biomolecular Sciences, under the supervision of Prof. Bice Fubini in 2016 and in collaboration with the Louvain Centre for Toxicology and Applied Pharmacology, headed by Prof. Dominique Lison, Université catholique de Louvain, with a thesis on the role of the surface properties of silica particles in the interaction with membranes and in activation of inflammatory pathways. Her research interests concern the interaction of materials with living matter, with particular emphasis on the toxicity mechanisms of cell responses to particulates.

SANYAL Soma, MD

Dr. Sanyal is currently an Assistant Professor of Pathology and Fellow in Environmental / Occupational Pathology at SUNY Upstate Medical University at Syracuse, NY.

Dr. Sanyal was born in Kolkata, India where she also completed her medical school training at University of Kolkata Medical College in 2000. This was followed by a residency in Medical Microbiology at PGIMER, Chandigarh, India from 2001-2004. After moving to the US, she worked in Dr. Abraham's Environmental/Occupational Pathology laboratory in 2007-2008 when she was introduced to microanalysis of inorganic particles in human tissue. At this time she worked and published on Gd and Nephrogenic Systemic Fibrosis. She then completed a residency in Anatomic/Clinical Pathology at SUNY Upstate Medical University from 2008 to 2012. She worked as a general pathologist for three years in Iowa, US and then returned to work with Dr. Abraham again in 2015. She has been involved in multiple ongoing projects in the laboratory involving occupational pathology including rapidly progressive pneumoconiosis, accelerated silicosis, metal working fluid related disease and welding particles. A few articles authored by Dr. Sanyal related to environmental/occupational pathology are mentioned.

(in preparation) **Sanyal, S**, Abraham JL. Pathology of accelerated silicosis.

(in preparation) Kristin J. Cummings, Marcia L. Stanton, Leopoldo N. Segal, Randall J. Nett, Jerrold L. Abraham, Thomas V. Colby, Francis H.Y. Green, Angela D. Franko, **Soma Sanyal**, et al. B-cell lymphocytic bronchiolitis and alveolar ductitis: novel disease in metal machining workers.

Abraham JL, Crawford JA, **Sanyal S**, Scalzetti E, Kramer L. Analysis of Lung Particle Burden in a 28 Year Old Man with Rapidly Progressive Coal Worker's Pneumoconiosis (Accelerated Silicosis). Am Thoracic Soc meeting May 2016, San Francisco, Am J Respir Crit Care Med 2016.193:A7074.

Sanyal S, Marckmann P, Scherer S, Abraham JL. Multiorgan gadolinium (Gd) deposition and fibrosis in a patient with Nephrogenic systemic fibrosis - an autopsy based review. Nephrol Dial Transplant. 2011; 26(11): 3616-26.

Boyd AS, **Sanyal S**, Abraham JL. Tissue Gadolinium deposition and fibrosis mimicking nephrogenic systemic fibrosis (NSF)-subclinical nephrogenic systemic fibrosis? J Am Acad Dermatol. 2010 Feb; 62(2):337-342.

SONG Yuguo

Yuguo Song works as a chief-physician and as the deputy director at the Department of Occupational Medicine & Clinical Toxicology, Beijing Chaoyang Hospital, Capital Medical University (Beijing, China). He received his B.S degree in clinical medicine from the University of Tsingdao Medical College, Shandong Province in 1990, and then he got his MD and Ph.D degree in Capital Medical University. He is the recipient of several research achievement awards including Wu Zhizhong Prize in occupational medicine (China) and International Travel Award from the American Academy of Clinical Toxicology. He ever worked as a visiting scholar in 2010 at West Virginia University, USA. His research focus is occupational lung disease, clinical toxicology and nanotoxicology.

THIVOLET- BEJUI Françoise

Françoise Thivolet-Béjui, M.D, is Coordinator of the College of Pathology at the Hospices Civils de Lyon and Director of the Center for Biological Resources "Cardiobiotec" based at the same institution. She completed her doctoral thesis for the title of Medical Doctor in 1978, and a doctoral thesis in human biology in 1990 (earning the title of Physical Doctor), the year in which she was also appointed Professor of Anatomy and Cytological Pathologies in Lyon. From 1995-1997 she served as President of the French Society of Clinical Cytology (SFCC). The following year she was axarded the title of "First Class Professor". Since 2007 Professor Thivolet-Béjui has also served as the Chief of the Pathology Department, Groupement Hospitalier Est of the Hospices Civils de Lyon. She is an author of over 120 publications listed in Pub Med.

TRUNFIO-SFARGHIU Ana-Maria

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Research Associate - CNRS (CR1, section 9), LaMCoS, UMR 5259 CNRS - INSA Lyon (since 2009)

ATER Teaching and Research at the Laboratoire de Physique de la Matière Condensée et Nanostrucutres (LAMCOS), (UCB Lyon-CNRS UMR 5586) (2006-2009)

Master in Engineering of Protein Materials, University of Iasi, Romania (2001)

Bio-Enginner, Specialization : Biomaterials and Prosthetic Technology, University of Iasi, Romania

33 publications in international journals with reviewing committee

VELTKAMP Marcel

(1976) graduated in both Medical Biology (1999) and Medicine (2003). During his training in pulmonology he obtained his PhD with a thesis entitled *"Sensing of pathogens by Toll-like receptors in Sarcoidosis"* (2011). At the end of his pulmonology training he worked as a fellow-pulmonologist specializing in Interstitial Lung Disease (ILD) for 6 months at the University Hospital Gasthuisberg in Leuven, Belgium (2012). Since 2013 he works as a pulmonologist at the ILD center of excellence in the St. Antonius Hospital Nieuwegein (The Netherlands).

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VERGNON Jean-Michel

MD, Ph D, Pr JM Vergnon (Jean Monnet Medicine University Saint Etienne) is chief of department of chest diseases and thoracic oncology. Competence center for orphan diseases, neuro-muscular disease and bronchoscopy. He authored more than 142 articles

VINCENT Michel

Dr Michel Vincent is a pulmonologist and thoracic oncologist. He was based at the Centre Hospitalier Saint Joseph –Saint Luc in Lyon where from 1993 –Avril 2013 he was Chief of Pulmonology and Thoracic Oncology and from 2013- 2015 , Coordinator of the Federation of Oncology and of the Innovation and Research Commission . In 2015 he created Minapath development a Business social society for buying a scanning electron microscopy. Since 1988 until 2015, he has been the President of the Association contre les Cancers Thoraciques et les maladies pulmonaires (ALCTMP). He is an author of over 70 publications listed in Pub Med.

ASSESSING THE MINERAL EXPOSOME

Chairs : Benoit Nemery, Department of Public Health University of Leuven, Belgium

Françoise Thivolet, Department of Cytology and Pathology Pole Est HCL, Lyon

Measuring methods for assessing exposure to micron and nanoparticles in workplaces and in environment

(Martine Chouvet / Technical director of ITGA)

Airborne particles are a major component of pollution both in the environment and in workplaces.

Epidemiological evidence indicates a clear relationship between exposure to particulate matter and effects on health, particularly smaller particles that can reach the deep regions of the lungs. Only particles, smaller than about 10 microns will reach the alveoli. Larger particles are deposited higher up in the respiratory system and can be removed by the mucocilliary escalator, but may then be swallowed and subsequently absorbed through the gastro-intestinal tract. The particles can also be solubilised in the lung, reach other organs and produce diseases.

In practice, every individual will inhale a different fraction of a given dust cloud, and this can also vary depending on the particular environment and activity undertaken during exposure. However it is useful to define size selection criteria to use when sampling for airborne particulates, and this task has been undertaken by the International Organization for Standardization (ISO).

Conventional fractions express the efficiency of penetration into the body as a function of the aerodynamic diameter of the particles. For simplification, they can be represented by the median diameter in size.

Conventional fractions have been defined for two population types:

- The working population which contains people aged about 18-65 years in good health, and who are exposed height hours a day, five days a week, eleven months a year, for fourty years

- The general population which contains all people, including children, the elderly, the ill people and the working population, who may be exposed twenty four hours a day, seven days a week, for seventy years.

In the field of workplace atmospheres, the standard ISO 7708 (Air quality - particle size definitions for health-related sampling) define three conventional fractions :

- The inhalable fraction: mass fraction of the particles contained in the ambient air which is inhaled by the nose or by the mouth. The diameter is between 0 and 100 µm.

- The Thoracic fraction: mass fraction of particles that penetrate beyond the larynx. The diameter is between 0 and 30 µm; The median diameter is 10 µm.

- The respirable (alveolar) fraction: mass fraction of the particles which can reach the pulmonary alveoli and are deposited there. The diameter is between 0 and 10-15 µm. The median diameter is 4 µm.

In the field of environmental pollutants, two other fractions are defined by the standard ISO 23210 (Determination of PM10/PM2,5 mass concentration):

PM10 : particles which pass through a size-selective inlet with a 50 % efficiency cut-off at 10 µm aerodynamic diameter. PM10 corresponds to the “thoracic convention” as defined in ISO 770

PM2,5 : particles which pass through a size-selective inlet with a 50 % efficiency cut-off at - 2,5 µm aerodynamic diameter. PM2,5 corresponds to the “high-risk respirable convention” as defined in ISO 7708.

In addition, we have to consider the definition of a nanoparticle (ISO TR/27628) as a particle with a nominal diameter (such as geometric, aerodynamic, mobility, projected-area or otherwise) smaller than about 100 nm.

For the exposure risk assessment to micronic particles, limit value or target value are defined for a given population (workers or whole population), for a given conventional fraction and for a given reference exposure duration (8h, 15min, 24 hours, whole life) and sometimes for a given effect.

For nanoscale particles, knowledge is limited and only a few number of limit values is available (like for titanium dioxyde). Moreover, the metric (mass, number, surface) to be taken into consideration remains to be specified.

Workplace exposure to an aerosol (airborne particulate) is characterized quantitatively by the time-weighted average concentration in air (mg / m3 of air) of a fraction of the health-related particles that enter the different regions of the respiratory tree (inhalable, thoracic and alveolar fractions or PM10 and PM 2,5).

By the effect of the gravity, the aerosol granulometry decrease when the distance of the source increase, so in the field of workplace atmospheres, it is important to evaluate the concentration in the breathing zone of the workers by using individual samplers worn by the worker. The breathing zone is define as a hemisphere of 30 cm of radius extending in front of human face, centred on the midpoint of a line joining the ears. The base of the hemisphere is a plane through this line, the top of the head and the larynx.

When considering environmental pollution, sources are remote from people. So, the particulate contamination is more homogeneous, it is therefore possible to use ambient sampling methods to characterize exposures.

In all cases, the exposure assessment is based on a measurement strategy to determine the mean exposure but also its variation and a selection of measurement method taking into account the fraction of interest and the population concerned.

Among the main categories of method, we can mention:

- Selective sampling systems of the fraction of interest (inhalable, respirable, PM10...) allowing to collect particles on filter which are then analysed in laboratory with specific analytical techniques (ICP, ion chromatography, gravimetry…) of the compounds or the group of compounds (metals, acid, etc.). These techniques generally offer good selectivity, precision and optimum sensitivity.

- Real-time measuring instruments such as particle counter which can either have at their input a selector of the fraction of interest or either perform selection during the treatment of the signal. These instruments often act as granulometers (measure of particle size) and determine the mass of the substance from modeling based on hypothesis about the shape factor and the density of the particle. These techniques permit to estimate the particle size of the aerosol concerned, they can also give an

overview of the exposure profiles (peak of exposure, constant background contamination, etc.) but give more approximate results not always specific of the substance of interest. They were used in exploration phase.

Concerning nanoparticles, it seems increasingly clear that for nanomaterials made up of insoluble or poorly soluble substances, exposure cannot be assessed by the two indicators: mass and chemical composition. It is then necessary to complete mass measurements by number measurements (particles / cm3) or by surface measurements (µm2 / m3).

The particle size range of the aerosol to be considered extends from a few nm to approximately 10 µm because although their individual size is smaller, the nanoparticles in their free form, agglomerated or aggregated form must be considered. Indeed, nanoparticles in free form can diffuse by heterogeneous coagulation on the particles of submicron and micron size constituting the background aerosol. Thus, the alveolar fraction remains in a first approximation the fraction of interest.

In general, the measurement methods implemented in exploratory phase are:

Real-time methods for measuring particle concentrations in air in the 10 nm - 1 to 10 µm range, such as condensation nuclei counter (CNC), or optical particle counter (COP).

While providing interesting elements, real-time measurements do not distinguish the aerosol of interest from the ambient aerosol, composed of particles in the nanometric to micronic scale range that can mask the target aerosol.

Methods for collecting aerosol samples for observation of elementary particles (eg, electron microscopy) or for analyzing the chemical composition (eg, by mass spectrometry).

When these methods prove insufficient, it is possible to use more advanced methods to detect lower or higher concentrations and to better discriminate particles, but a high level of expertise is required. Technics based on the electric mobility (ELPI, SMPS) and /or on sampling with impactor associated with chemical analysis can then be carried out.

In conclusion, the assessment of particulate exposure must take into account the chemical nature of the particles, the health effects, the routes of exposure and the target populations concerned. It is then essential to define the conventional fraction of interest and to use methods of measurements adapted to the problematic.

A suitable measurement strategy and sufficient sensitivity and accuracy methods provide representative samples and ensure reliable results.

The characterization of exposure to aerosols of nanoparticles still require experts.

MINASARC : a case-control study using an exposure questionnaire to assess the mineral exposome[1] in sarcoidosis

(Catherine CAVALIN – Michel VINCENT)

Catherine Cavalin[1,2,3,] Mickaël Catinon[1,4,5,] Cécile Chemarin[4], Michel Vincent[1,4,5], Odile Macchi[1], Stéphane Rio[4], Élisabeth Roux[4], Mathieu Pecquet[4], Anne-Sophie Blanchet[4], Sylvie Vuillermoz[4], Audrey Natalizio[6], Christophe Pison[7], François Arbib[7], Vincent Bonneterre[8], Dominique Valeyre[9], Olivia Freynet[9], Jean-François Mornex[10], Nathalie Freymont[11], Yves Pacheco[11], Françoise Thivolet[12], Marianne Kambouchner[13], Jean-François Bernaudin[14], Paul-André Rosental[1,2,15]

[1]Centre for European Studies (Sciences Po, Paris, SILICOSIS team); [2]Laboratory for Interdisciplinary Evaluation of Public Policies; [3]Centre for Employment Studies (Noisy-le-Grand); [4]Saint-Joseph Saint-Luc Hospital (Lyon); [5]Minapath Développement (Villeurbanne) ; [6]Dermscan (Villeurbanne); [7]Pneumology Department, Teaching Hospital (Grenoble); [8]Occupational Medicine Department, Teaching Hospital (Grenoble); [9]Pneumology Department, Avicenne Teaching Hospital (Bobigny); [10]Pneumology Department, Louis Pradel Teaching Hospital (Bron); [11]Pneumology Department, Lyon Sud Teaching Hospital (Lyon); [12]Cytology and Pathology Department, Lyon Hospices civils, Teaching Hospital (Bron); [13] Cytology and Pathology Department, Avicenne Teaching Hospital (Bobigny); [14]Cytology and Pathology Department, Tenon Teaching Hospital (Paris); [15]National Institute for Demographic Studies (INED, Paris)

Introduction

For the last twenty years, exposures to inorganic agents and particularly to crystalline silica particles have been highlighted as possible risk factors of the onset and/or the symptomatology of several autoimmune/systemic/inflammatory diseases[2,3]. All these diseases share complex and largely unknown etiology. Sarcoidosis is among them, with a particular suspicion of the involvement of genetic factors, organic antigens and inorganic particles[4,5]. MINASARC is a prospective case-control study in which patients (Pts) diagnosed with sarcoidosis and healthy volunteers (HVs) answered an exposure questionnaire (EQ) and were subjected to broncho-alveolar lavages (BALs) the mineralogical content of which was analyzed in transmission electron microscopy.

Material and Methods

20 Pts and 20 HVs matched on sex, age-range and smoking habits were telephone-interviewed (Fig. 1) with an EQ focused on a thorough exploration of exposure to crystalline silica, and endeavouring to tackle exposure to some other inorganic particles (e.g. asbestos, metal particles, welding fumes) more

[1] Wild C, 2012. The Exposome: From Concept to Utility. Int J Epidemiol, 41: 24-32.

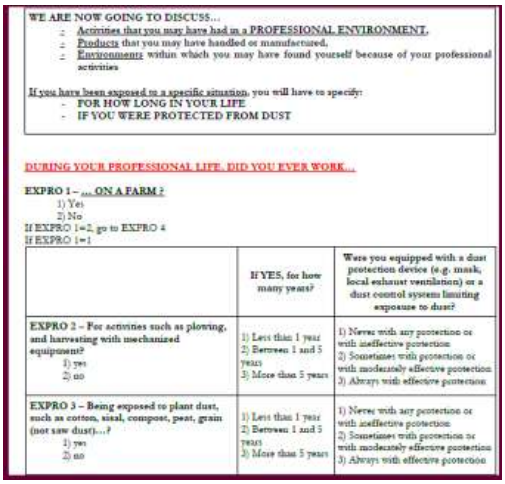
[2] Parks CG, Conrad K, Cooper GS, 1999. Occupational Exposure to Crystalline Silica and Autoimmune Disease. Environ Health Perspect, 107(Suppl. 5):793–802.

[3] Webber M, Moir W, Crowson C, Cohen H, Zeig-Owens R, Hall C et al., 2016. Post-September 11, 2001, Incidence of Systemic Autoimmune Diseases in World Trade Center – Exposed Firefighters and Emergency Medical Service Workers. MayoClinics Proc., 91(1):23-32.

[4] Newman KL, Newman LS, 2012. Occupational Causes of Sarcoidosis. Curr Opi, Allergy Clin Immunol, 12: 145-50

[5] Vincent M, Chemarin C, Cavalin C, Catinon M, Rosental P-A. From the Definition of Silicosis at the 1930 Johannesburg Conference to the Blurred Boundaries Between Pneumoconioses, Sarcoidosis, and Pulmonary Alveolar Proteinosis (PAP). Am J Ind Med. 2015;58:S31–8.

briefly. About 130 questions compose the exposure modules of this EQ, with about 90 questions on occupational activities and 40 questions on non-occupational activities.



WE ARE NOW GOING TO DISCUSS…

- Activities that you may have had in a PROFESSIONAL ENVIRONMENT,
- Products that you may have handled or manufactured,
- Environments within which you may have found yourself because of your professional activities

If you have been exposed to a specific situation, you will have to specify:

- FOR HOW LONG IN YOUR LIFE
- IF YOU WERE PROTECTED FROM DUST

DURING YOUR PROFESSIONAL LIFE, DID YOU EVER WORK…

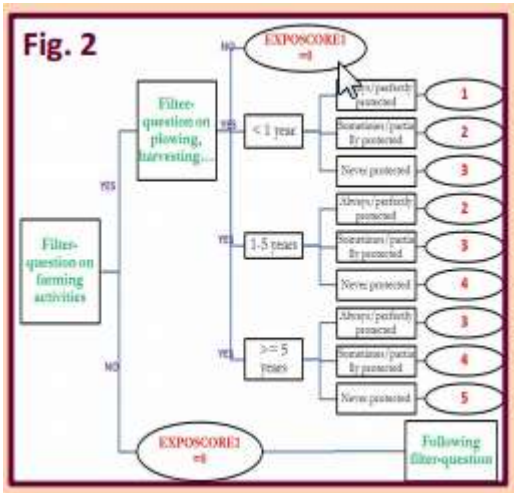
EXPRO 1 – … ON A FARM ?
1) Yes
2) No
If EXPRO 1=2, go to EXPRO 4
If EXPRO 1=1

	If YES, for how many years?	Were you equipped with a dust protection device (e.g. mask, local exhaust ventilation) or a dust control system limiting exposure to dust?
EXPRO 2 – For activities such as plowing, and harvesting with mechanized equipment? 1) yes 2) no	1) Less than 1 year 2) Between 1 and 5 years 3) More than 5 years	1) Never with any protection or with ineffective protection 2) Sometimes with protection or with moderately effective protection 3) Always with effective protection
EXPRO 3 – Being exposed to plant dust, such as cotton, sisal, compost, peat, grain (not saw dust)…? 1) yes 2) no	1) Less than 1 year 2) Between 1 and 5 years 3) More than 5 years	1) Never with any protection or with ineffective protection 2) Sometimes with protection or with moderately effective protection 3) Always with effective protection

The wording of the questions avoids to be restricted to "formal" labels of occupations or sectors. It addresses exposure through many different approaches including also: technical and lay vocabulary on chemical substances, descriptions of activities, equipments, types of contexts, professional or non-professional environments, etc. The aim is to reach a satisfactory level of sensibility and a high level of specificity on the screening of exposure, and to stir memories, even for short working periods that could have occurred during the whole life course.

Respondents are not only questioned about exposure itself but also about its cumulative duration in life (most of the time assessing duration brackets: < 1 year, [1;5 years[, ≥ 5years) and their evaluation of the presence and efficiency of individual and collective protections against inorganic dusts (e.g. masks, gloves, glasses, dampening, ventilation).

From the collection of these data on the occurrence of exposure, its cumulative duration and protection against dust, we compute a dust exposure score (DES) following the algorithm shown in Fig. 2 (the algorithm applies to 95% of the questions of the EQ). As an exhaustive metrological approach is unrecheable (impossible ex post reconstruction of an individual's mineral exposome taking into account all the physical characteristics of all exposures met in life), we propose to neutralize the scoring as much as possible by graduating it according to the scale described in Fig. 2. As a whole, the more, the longer and the less protected one has been exposed, the higher his/her DES will be.



We have used the EQ in a survey (ELIPSSilice) in the general population. We present our results benchmarking them against the ELIPSSilice's DES.

Results

In ELIPSSilice, respondents (n=825) self-complete the EQ on tablets. « Patients » are « self-reported patients » (SRPs, several systemic diseases) and « healthy subjects », « self-reported healthy subjects ».

The EQ's sensibility in exposure screening is certainly lower in ELIPSSilice. The average and median levels of the ELIPSSilice's DES are lower (Fig. 3) – but not significantly different (Wilcoxon signed-rank test for patients: p=0.27; for healthy subjects: p=0.44) – from the MINASARC's DES.

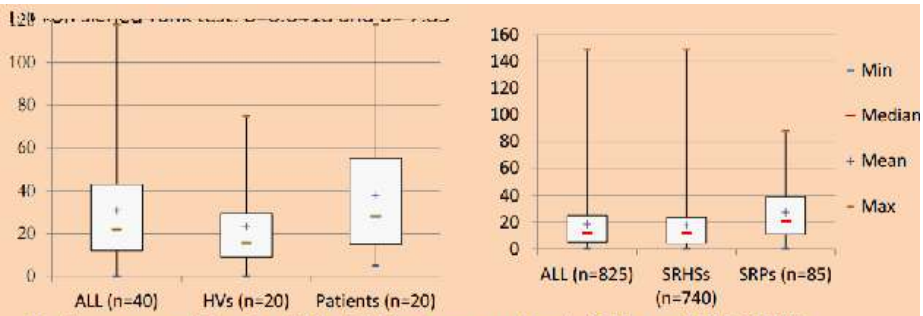


Fig. 3: compared levels of the DES between patients/SRPs and HVs/SRHSs

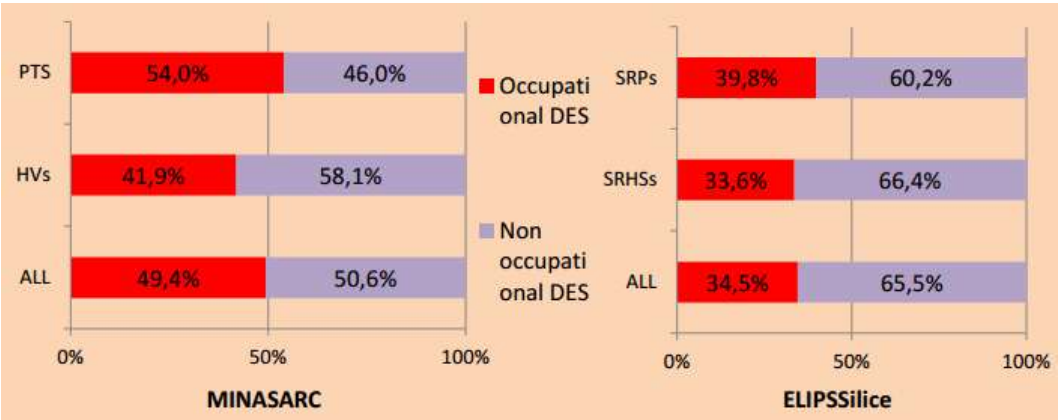
In MINASARC and in ELIPSSilice, the difference between the patients'/SRPs' and HVs'/SRHSs ' DES is significant (paired Wilcoxon signed-rank test, p=0.0418 and p= 7,857.10-6 respectively.

Table 1: Modeled probability of being a patient in MINASARC

Nationality/National origin	
French and born as French	1
Born as a foreigner and having acquired the French nationality or foreigner living in France	11.772* [1.787;77.565]
Pro. and extra-pro. construction activities	
"Construction score" below the median construction score	1
"Construction score" above the median construction score	7.489* [1.506;37.247]

Being a foreigner of foreign origin AND having a DES subscore related to –professional and non-professional – construction activities increases the probability of having sarcoidosis.

Fig. 4: respective shares of the occupational and non-occupational subscores within the DES in MINASARC compared with ELIPSSilice



The share of the occupational subscore within the DES is higher for patients (MINASARC) and SRPs (ELIPSSilice). In both studies, the non-occupational subscore is never lower than 46.0% of the DES.

Discussion and conclusion

By benchmarking the results of MINASARC against ELIPSSilice, we get a well-reasoned confidence on the metrological properties (sensibility, specificity) of our EQ in screening exposure to silica and other inorganic particles.

The DES which summarizes this mineral exposome allows to draw a dividing line between sick people (sarcoidosis in MINASARC) and healthy subjects.

We cannot directly conclude that silica is the etiological key of sarcoidosis (MINASARC) and other systemic or autoimmune diseases (ELIPSSilice) but : a) the EQ gives hints on the involvement of inorganic particles in these diseases; b) the DES highlights the relevance of health surveillance for environmental contexts of exposure, beside occupational ones and for some specific activities such as construction; c) the national origin evidenced as a risk factor for sarcoidosis in MINASARC will be further examined to differentiate between what it reflects of sarcoidosis as an unequally global distributed disease and a possible underrepresentation of foreigners among the MINASARC's HVs. On the whole, larger clinical samples on other diseases (e.g. systemic lupus or scleroderma, rheumatoid arthritis) and ELIPSSilice2 (n=3000, Autumn 2016) will soon help strengthen the statistical power of our observations.

Mineral exposome and sarcoidosis : Sarcoidosis and occupational exposures : lessons from the French National network for occupational disease surveillance and prevention network (rnv3p)

Vincent Bonneterre[1,2], Lynda Larabi[1,3], Isabelle Berta-Vanrullen[3] (MSc) on behalf of rnv3p.

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France has a network of 32 occupational diseases clinics (OD clinics) located in teaching hospitals. These are "resources centers" to which occupational physicians, GP and organ specialists send their patients in order to get advices regarding work-relatedness of their patient's disease mainly, but also for some other issues such as medical screening after exposure to carcinogenic compounds, work-fitness, etc. At the national level, these OD clinics are gathered within an expert network known as rnv3p, which is supervised by the French Agency for Health Safety in Environment, Work and Food (ANSES). The asset of rnv3p is that the OD clinics record since 2001, in a standardized way all their consultations in a common database (today through a web-based information system allowing nearly real time updating of the database). The variables diseases, occupation, activity sector, but also exposure, are coded with hierarchical standardized codes (international for the first three variables, national for the last one), which allow analyses to be performed at different precision levels. Work-attributability (imputability) is rated by the expert through a probability scale (4 levels : no relation, weak, intermediate, strong). The rnv3p main objectives are to create knowledge on work-related diseases, and especially describe occupational situations "at-risk" for specific diseases, but also highlight new emerging risks. RNV3P collaborate with EU partners also involved in surveillance systems or data collection in the field of work-related diseases (comparisons of trends and exchange about signals).

From 2001 till today, more than 300,000 consultations have been realized in the French OD clinics, and registered within rnv3p information system. Among them, 336 cases of sarcoidosis (ICD-10 code D86*). Men account for 78% of the cases, and women for 22% (which means referral is more often done by physicians for men, due to their occupational exposures). Mean ages are respectively 46 and 47 years old for men and women. Interest in seeking an occupational origin mostly concern a limited number of OD clinics (In decreasing order : Grenoble n=48 , Bordeaux n=40 , Lyon n=33, followed by Paris OD clinics of Cochin, Creteil, Garches with 23, 21, and 20 cases).Nevertheless, there seem to be a rising interest in the last years (increasing number of cases referred to OD clinics in the last years).

Finally 28% of the cases (n=95) have been considered as potentially related to work by experts, with different work-attributability levels, usually weak as the etiology of sarcoidosis remains unknown. Silica is by far the most often reported *exposure* (n=63) among all cases of sarcoidosis investigated in OD clinics, whatever the final rating for work-relatedness. Beryllium is the second most cited exposure (n=17; most of other cases being recorded as chronic berylliosis so not highlighted here). Some other

mineral dust are reported (welding fumes, hard-metals dusts, other metals, etc), more often than organic dusts or chemicals do (wood dust n=17 being the most reported).

Overall the 336 cases belong to 241 different activity sectors and 167 occupations. The most often cited *activity sector* is construction work (n=60), and the most often cited *occupation group* is related to construction activities (n=65), including masons/bricklayers (n=14), carpenters (n=10), etc.

The second most often cited occupation group is related to metallurgy and metal work (n=44), including welders (17).

The information registered is richer, thanks to the code and information registered as free text. This will be summarized for the workshop.

Finally, rnv3p data highlight the importance of mineral dusts exposure, especially silica, among sarcoidosis patients referred to one of the French OD clinics for work-relatedness assessment.

Novel toxicity related to nanomaterials ? Silica nanoparticles cause pleural effusion and pericardial effusion in workers and in rats

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With the rapid development of nanotechnology and the extensive use of nanoproducts, the potential hazards of nanomaterials to the environment and human health were widely concerned[1],[2] Nanomaterials introduce novel risk factors and potentially lead to novel hazards within the workplace or through environmental contamination. In vitro and in vivo studies show that the toxicities nanomaterials posed include damage to lungs, heart, liver, kidney and nerve, as well as reproductive and immune systems and they also have carcinogenicity[3,4]. Additionally, some studies reported the specific toxicity of nanomaterials which appears due to their unique physicochemical properties[5]. However, it is still controversy in regarding to the nano-specific toxicity[6,7], and some scientists regard that there is no evidence of novel 'nano-specific hazard' comparing to micro –materials.

We previously reported that a group of patients exposed to nanomaterials presented with an unusual disease with pleural and pericardial effusion, pulmonary fibrosis and granuloma[8,9]. And our further rodent study shows that silica nanoparticles that were isolated in patients can also cause pleural effusion and pericardial effusion- a rare and unusual symptom- which may be the novel toxicity related to nanomaterials[10]. Here we introduce our study in the nanoexposed workers and animal experiment, further information on the novel toxicity related to the silica nanoparticles was collected and the potential mechanisms were discussed.

1 - An unusual disease of a group of workers

Seven patients, 18–47 years old female workers, were found in almost the same time frame with the same symptoms of shortness of breath and the same clinical findings of pleural effusion and pericardial effusion. They all worked at the same workplace in a print plant for 5–13 months with poor personal protective equipment. These females previously underwent treatment in local hospitals including

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[3] Duan J, Yu Y, Shi H et al. Toxic effects of silica nanoparticles on zebrafish embryos and larvae. PLoS One. 2013. 8(9): e74606.
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[5] Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005. 113(7): 823-39.
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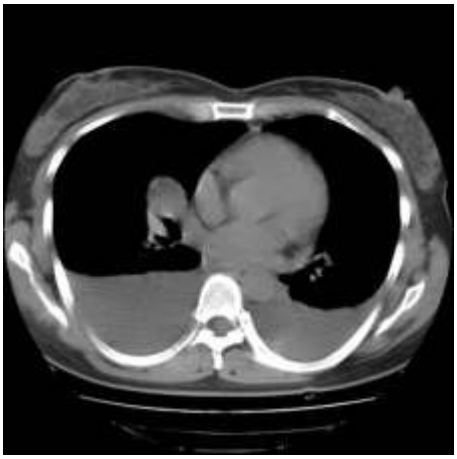
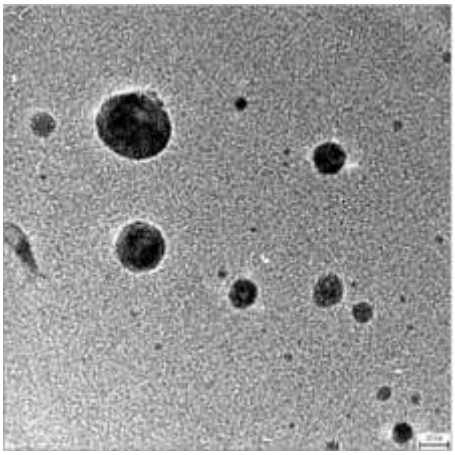
[8] Song Y, Li X, Du X. Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. Eur Respir J. 2009. 34(3): 559-67.
[9] Song Y, Li X, Wang L, et al. Nanomaterials in humans: identification, characteristics, and potential damage. Toxicol Pathol. 2011. 39(5): 841-9.
[10] Zhu X, Cao W, Chang B, et al. Polyacrylate/nanosilica causes pleural and pericardial effusion, and pulmonary fibrosis and granuloma in rats similar to those observed in exposed workers. Int J Nanomedicine. 2016. 11: 1593-605.

multiple thoracentesis attempts with recurrence of effusion, antibiotics, anti-tuberculosis drugs, and methylprednisolone or prednisolone.

In our hospital repeated examinations of chest radiographs, CT scanning and ultrasound examinations showed that all the patients were characterized with pleural effusion consisting of an amber exudates ; five out of the seven patients had pericardial fluid at the depth of 4–8mm. One 19-year-old patient was performed fenestration of the pericardium, and 170mL of light-colored fluid was drained from the pericardial cavity. Also, these seven patients had pulmonary interstitial inflammation and pulmonary fibrosis by medical imaging examinations.



Pleural effusion in a patient



nanoparticles in a patient's pleural effusion (scale bar = 10nm)

These effusions recurred repeatedly after thoracentesis, and multi-methods treatments were rendered ineffective. Their pathological examinations displayed nonspecific pulmonary inflammation, inflammatory infiltration, pulmonary fibrosis, and foreign-body granulomas of the pleura. After seven months observation, rapid progressive pulmonary interstitial fibrosis was observed in two patients and both patients died of pulmonary failure, the pulmonary fibrosis in other five patients developed very slowly. Five years follow-up shows that the left five patients' condition has improved, and they have no apparent shortness of breath.

In all of these cases, clinical observations, examinations and long-term follow-up have excluded infections, malignant tumors, immune-related disorders and other diseases.

2 - Nanoparticles isolated and detected in patients

The paste coating material which these workers used in the workplace is an ivory white soft coating mixture of polyacrylic ester (polyacrylic ester stated by the paste producer), it was analyzed by gas chromatography/mass spectrometry (GC/MS), and was found containing 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. But these patients presented with none of the symptoms and signs related to these irritant or asphyxiant gases. In addition, the powder component in the raw materials used in the workplace was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) and was found containing: Si, Ba, Ca, Mg, Cu, Fe, K, Na, Zn, and P.

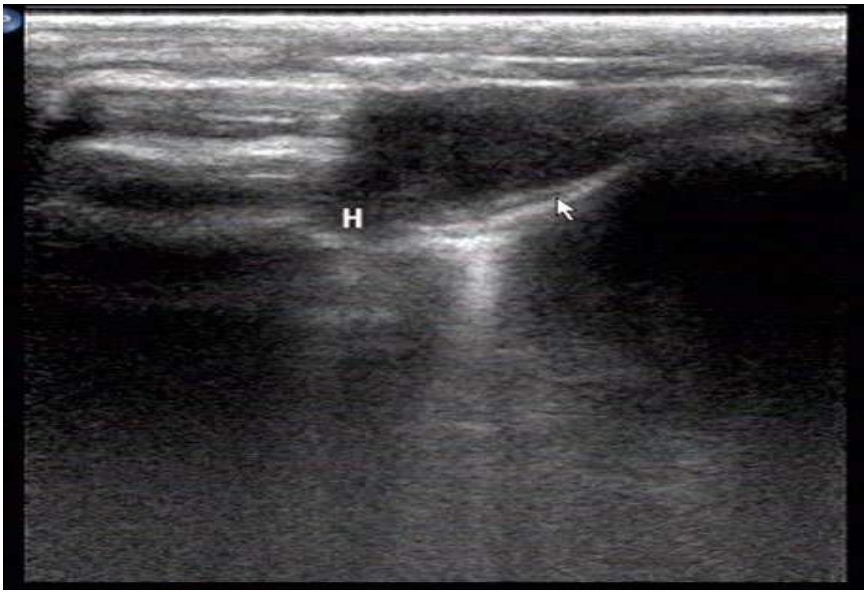
Importantly, nanoparticles (20-30 nm in diameters) were observed in the raw materials and in the dust materials that accumulated at the local ventilation in the workplace. Similar nanoparticles were also found in the patients' pulmonary tissues and chest effusion. They were largely found in macrophages (cytoplasm, mitochondria, lysosomes, and nuclei), pulmonary microvascular vessels, vascular endothelial cells, microlymphatic vessels, and pleural effusion. By using transmission electron microscopy and energy dispersive X-ray analysis, element of silica of nanoparticles was identified in biopsies and chest fluid. In addition, nanosilicates, as well as microscale silicates, were observed in a patient pleural effusion and contained elements of Si, Ca, Mg, K, Na, Ba, Al, P, S, Cl, and O.

Given the well - documented toxicity of micro-silica that can result in or contribute to a series of respiratory diseases, including silicosis, interstitial fibrosis, industrial bronchitis, small airway disease, emphysema, and vascular diseases, as well as immunologic reactions[11], it is reasonable that the patients' disease was related to their heavy exposure to silica nanoparticles.

3. Toxicity of silica nanoparticles in rats was found similar to those observed in nanoexposed workers

As silica nanoparticles were found in the raw materials used at workplace and isolated in patients' pulmonary biopsy, we speculate that the patients' disease is related to their heavy exposure to silica nanoparticles.

Giving rats polyacrylate/nanosilica (made by order) that the workers were exposed to by intratracheal instillation[10], we found that polyacrylate/nanosilica caused toxicity of pleural and pericardial effusion and the pulmonary and pleural fibrosis, similar to those observed in exposed workers. Sonographic findings, CT images and dissection of rats revealed that polyacrylate/nanosilica caused pleural effusion and pericardial effusion. Pleural effusion occurred on day 3 post-administration, peaked on days 7-10, slowly decreased and dismissed on day 14. Additionally, with the rise of polyacrylate/nanosilica concentrations, pleural effusion produced more in volume and generated more rapidly. Meanwhile, silica nanoparticles were also detected in rats' pleural effusion.



[11] Ding, M., Chen, F., Shi, X., Yucesoy, B., Mossman, B., and Vallyathan, V. Diseases caused by silica: Mechanisms of injury and disease development. Int Immunopharmacol. 2002, 2: 173–82.

An apparent gap (arrow) between the two layers of the pericardial membrane indicates pericardial effusion in a rat given silica nanoparticles.

Similarly, giving rats only silica nanoparticles by intratracheal instillation, we also observed the toxicity in rats with pleural and pericardial effusion. Furthermore, these toxicities are also similar to those in rats given the raw materials that workers used at the workplace at doses of 5–10 mg/kg. Moreover, we found that silica nanoparticles resulted in elevation of TGF-β1 and VEGF-C, activation of VEGF-C/VEGFR-3 signaling pathway in rats' lungs and parietal pleura.

Conclusion

Our study shows that the death and damage of the reported workers is closely related to the heavy exposure of silica nanoparticles.

Silica nanoparticles can cause unusual symptoms of pleural effusion and pericardial effusion.

Our study indicates that pleural and pericardial membrane is another target of injury of silica nanoparticles, as the specific toxicity related to pleural damage is asbestos.

These findings of the novel toxicities highlight the awareness that some nanomaterials like nanosilica may cause unusual toxicity upon heavy exposure, which should be taken seriously in the development of nanoscience and nanotechnology.

Discussion

1 - The mechanisms of silica nanoparticles resulting in pericardial effusion and pleural effusion.

Our study shows that silica nanoparticles cause novel toxicity of pleural and pericardial effusion, as well as fibrosis of lung and parietal pleura. These toxicities in rats are almost identical to those observed in reported patients presenting with pleural and pericardial effusion, pulmonary fibrosis and granuloma [8,9]. Also, they are almost identical to those observed in rats by intratracheal instillation of polyacrylate/nanosilica [10]. The present results in turn reveal that the death and damage of the reported workers is closely related to the heavy exposure of silica nanoparticles in polyacrylate coatings.

We had speculated that the pleural effusion may be related to the mechanical obstruction of draining lymphatics, which may give us a clue from the asbestos- induced benign pleuritis and effusion[12]. Due to the key role of the parietal pleural lymphatics in terms of reabsorption of effusion, asbestoic body can obstruct parietal pleural microlymphatics and cause pleural effusion. Similarly, a great number of silica nanoparticles may penetrate into pleural cavity, flow into microlymphatics, mechanically block microlymphatics and damage its function of reabsorption, and then cause effusion. Unfortunately, by TEM we did not find any obstruction of parietal pleural microlymphatics by silica nanoparticles. However, pleural fluid in our patients persisted for months and ~1000ml in volume was drained by closed thoracic drainage every day, and was not effective to glucocorticoids and antibiotics [8,9], these characteristics of the pleural effusion made it very difficult to be explained just simply by the mechanisms of inflammation and production of reactive oxygen species (ROS). The impairment of fluid

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clearance by damaging the draining lymphatics may have involved in the accumulation of the pleural fluid, more studies related to the lymphatic system injury due to silica nanoparticles are needed.

In addition to excessive plasma leakage through the hyperpermeable pleural vasculature and the potential impairment of pleural lymphatic drainage, an increasing oncotic pressure due to nanosilica deposition in plural cavities, which we found both in our animal experiments and reported patients[8,11], may also have contributed to the accumulation of the fluid in pleural space. Moreover, as silica nanoparticles increase ROS concentrations, induce inflammatory production, cause mitochondrial depolarization and reduce glutathione levels both in vivo and in vitro[3-4], the increased interstitial fluid in the lung or increased permeability of the pleural capillaries because of inflammation and ROS production should have promoted the formation of pleural effusion.

2. Nano specific toxicity ?

There is still controversy whether there is nano-specific toxicity comparing to micro- materials [6,7]. Our study shows that silica nanoparticles can cause novel symptoms of pleural effusion and pericardial effusion. There are many reasons that can cause pleural effusion and pericardial effusion such as lung infections, tuberculosis, breast cancer, lung cancer, or autoimmune diseases. However, no reports on the toxicity due to exposure to nanoparticles have been made until now. These novel symptoms related to silica nanoparticles, in my oppinion, should be regarded as the nano-specific toxicity which appears due to their unique physicochemical properties.

These findings of the novel toxicities highlight the urgent need and importance of nanosilica safety for workers and its release to the environment, and they also highlight the awareness that some nanomaterials like nanosilica may cause unusual toxicity upon heavy exposure, which should be taken seriously in the development of nanoscience and nanotechnology. More researches and collaborative efforts on nanosafety are required in order to prevent and minimize the potential hazards of nanomaterials to humans and the environment.

Acknowledgments

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Exposure to particles : from the workplace to the urban space

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During their entire life, people are continuously exposed to particles that are in suspension in the air. Particles may be emitted in the air by mechanical processes or following combustion of various materials. Many occupations involve exposure to particulates or fibrous dusts produced by extracting, crushing, drilling or polishing rocks or other solid materials, or when manufacturing, handling or transporting substances of mineral, synthetic or biologic origin. Many jobs also entail exposures to smoke or fumes resulting from the combustion (and subsequent condensation) of organic or inorganic materials. However, exposure to particles is not restricted to the occupational setting: tobacco smoking leads to a massive intake of particles by the smoker ("do-it-yourself air pollution"); household air pollution caused by burning biomass affects a large proportion of the world's population, especially women and children; outdoor exposure to ambient particles, caused by industrial pollution and motor vehicles, also represents a significant cause of ill health for the general population.

The degree to which exposure may affect human health depends on the amount and nature of the inhaled particles, on the one hand, and on individual characteristics, on the other hand. The amount of particles that are deposited and retained in the respiratory tract depends on their concentration in the air, the duration of the exposure, and importantly their size and shape. The deposition of particles in the respiratory tract is governed by the interaction of particle-specific factors, air velocity and structural features of the airways. Particles with a large aerodynamic diameter (> 5 µm) are more likely to deposit by inertial impaction in the upper airways (i.e. above the larynx); particles with aerodynamic diameters between 1 and 5 µm are more likely to deposit by sedimentation in the conducting airways; particles smaller than 1 µm are more likely to reach the alveolar spaces by diffusion; particles in the nanometer range may also translocate to some extent across the air-blood barrier and reach organs beyond the lungs. Fortunately, particles that have deposited in the respiratory tract can also be cleared from the lungs and airways, either via the mucociliary escalator (fast clearance) or via phagocytosis by macrophages (slow process). However, the clearance of these particles may be insufficient (in the case of high cumulative exposures) or inefficient (for some types of dusts or fibres). Moreover, the nature and extent of the cellular and tissue responses to the presence of particles in the lung also depend on host-specific immune mechanisms.

The most studied condition associated with the inhalation of particles is "pneumoconiosis", which is defined as focal or diffuse fibrotic disease of the lung parenchyma resulting from the host reaction to an excessive accumulation of mineral dust or fibres. Various types of pneumoconiosis can be considered based on the type of exposure: silicosis [caused by free crystalline silicon dioxide (SiO2)], coal worker's pneumoconiosis (often labelled as anthraco-silicosis), asbestosis, talcosis, etc. In clinical practice, the diagnosis of pneumoconiosis depends essentially on radiologic imaging, which in rich countries nowadays mainly consists of computed tomography, and a compatible occupational exposure history. The incidence of typical pneumoconioses has declined considerably in industrially developed countries because of the closure of mines and improved working conditions. However, isolated cases of silicosis,

asbestosis or other pneumoconiosis are still diagnosed (sometimes after having been missed because occupational exposures are not sufficiently taken into consideration by doctors) and even outbreaks of severe forms of silicosis have recently been described, e.g. among workers employed in sandblasting denims or in the manufacture of quartz-based countertops. Moreover, inhaling dust particles at work does not lead only to pneumoconiosis: occupational dust exposure has also been demonstrated to be a risk factor for common conditions, such as chronic rhinosinusitis, chronic obstructive pulmonary disease (COPD) and, to some extent, also idiopathic pulmonary fibrosis and bronchopulmonary cancer.

However, in some patients and for some agents, lung disease may be found even after "not so high" particle exposure. In these cases, host susceptibility – rather than cumulative exposure – appears to be the most important determinant in the pathogenesis of the disease in individual subjects. This is well-known for hypersensitivity pneumonitis, which occurs in people who become sensitized to particles, generally of biologic origin. This is also well-established for chronic beryllium disease (or berylliosis), which is characterized by the formation of granulomas (mainly in the lung and draining lymph nodes) in people who are sensitized to beryllium. Such cell-mediated sensitization can be demonstrated in individuals by the ex vivo proliferation of lymphocytes incubated with a beryllium salt. The similarity between chronic beryllium disease and sarcoidosis, a relatively common immunological disease characterized by the formation of granulomas (in the lung and other organs) for which no causal agent has been identified, has led to the hypothesis that some cases of sarcoidosis are in fact undiagnosed cases of chronic beryllium disease. However, it is plausible that beryllium is not the only agent that can lead to the formation of granulomas in susceptible persons. Various agents, including some metals and silica, have been suspected to do so. The potential role of silica or silicates in the etiology of (some cases of) sarcoidosis still rests on indirect evidence: silica particles can cause granulomas in experimental animals, silica-containing particles can be identified in relevant areas in lung or lymph node biopsies of patients with sarcoidosis, patients with sarcoidosis appear to be more likely to have had occupational exposure to silica than controls. However, unlike for chronic beryllium disease, no method is available at present to demonstrate immune sensitization to silica (or a similar immune-mediated process) in patients with sarcoidosis and occupational dust exposure. Nevertheless, it is noteworthy that silicosis – and even exposure to silica without overt silicosis – is recognized as a risk factor for autoimmune connective tissue disease, most notably scleroderma, although no proof exists for silica-induced or silica-mediated mechanisms in the autoimmune process.

Finally, although particle-induced lung disease has been studied mostly in the context of occupational exposures, research into the effects of ambient pollution has also shown that traffic-related particles exert deleterious effects on human health. It should be realized that the concentrations of particulates found in ambient air are much lower, even in polluted cities, than those that are found – and tolerated – in workplaces. Overt pulmonary disease can, therefore, rarely, if ever be attributed to urban air pollution in individual patients, even though epidemiological studies have clearly demonstrated adverse pulmonary effects (asthma, lower pulmonary function, exacerbations of COPD, lung cancer) of such pollution at population level. It is conceivable that particles also contribute to other conditions, including those that are still labelled as idiopathic.

INORGANIC PARTICLE AND ORGANIC TISSUE : A CHEMICAL APPROACH

Chairs : A. Auroux, French Chemical Society, IRCELYON CNRS, ALCTMP

M Kambouchner, Cytology and Pathology Department, Avicenne Teaching Hospital, CHU Paris

Revisiting the paradigm of the pathogenicity of crystalline silica : crystallinity or surface disorder?

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[§]Presenting author

Exposure to crystalline silica dusts in respirable size is commonly associated to severe lung diseases, but not all the silica sources were found equally pathogenic. Several physico-chemical features of the silica surface are implied in the pathogenic process and a number of interactions between particle surface and the cells/molecules of the respiratory system are established, which account for the huge variability of silica toxicity. Crystallinity has always been indicated as the prerequisite feature of hazardous silica dusts. However, recent findings reveal that the biological reactivity of quartz is not related to crystallinity per se. In the effort to find out the key surface features explaining the reactivity of silica, we propose that surface disorganization of silanol groups is at the origin of the inflammatory response induced by silica particles, which in turn is the crucial step of most of the silica-related diseases.

In situ Mineralogical Analysis (MA) by Scanning Electron Microscopy and Microanalysis on paraffin specimen biopsies of patients with infiltrative pulmonary disease : Feasability study and some preliminary results from the Minasarc Study

Trunfio-Sfarghiu AM, Catinon M, Thivolet F, Malicier D (Forensic Institute Lyon), Chemarin C, Vincent M

Introduction

The observation by optical microscopy (under polarized or natural light) of samples embedded in paraffin from patients with microndular and interstitial lung disease, can reveal opaque or anisotropic particles but not their chemical composition. Our aim is to compare optical and SEM images and relate these data to the chemical composition determined by EDS.

Patients and Methods

We observed specimens from five patients whose diagnoses were : pulmonary varicella sequellae, two cases of silicosis (one dental technician and one construction worker) and two cases of sarcoidosis but with dust exposure.

We also report results from five of the sarcoidosis cases in the Minasarc Study : four from mediastinal node biopsies and one case with granulomas observed in bronchial and skin biopsies.

Finally we report SEM-EDS analysis of two mediastinal nodes from control autopsied subjects provided by the Forensic Institute in Lyon.

Results

We observed many calcium phosphate particles inside the varicella scarring nodule typical of an endogenous reaction (probably calcification) (Visscher D et al. Modern Pathol 1988;1:415-419).

Surprinsigly , the inorganic particles found inside the silicotic nodule of one of the silicosis patient were also found to be calcium phosphate . However many silica particles were found around the nodule.

Many silica and silicates particles were also observed for the two patients diagnosed with sarcoïdosis but exposed to dust.

Among the five cases from the minasarc study, we observed for one patient (artifical nail worker) aluminosilicate and silica which may according with questionnaire data and BAL fluid's MA results. For all five patients we observe also steel and Ti-Ni alloys particles probably realted to needle used for EBUS (Gounant and al Chest 2011, 139: 138-43).

On five mediastinal node specimen coming from Forensic Insitute, we observe essentially alumino silicate and silicium oxide and no steel, and TiNi particles.

Conclusion

Mineralogic analysis by SEM-EDS reveals 1/ the location and the chemical composition of the observed particles 2/ their probable origin (exogenous or endogenous), and 3/ facilitates the interpretation of the optical images and provides a better understanding of the underlying physiopathological mechanisms.

Tissue Elemental Imaging with LIBS (Laser-Induced Breakdown Spectroscopy) : recent advances and medical applications

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Elemental imaging is very useful to image either labeled-free nanoparticle's, or trace element, metals, and organic ions that play major roles in physiological and pathological processes. We developed an all-optical method for the multi-elemental imaging of biological tissues that is fully compatible with standard microscopy systems. Our instrument is based on Laser Induced Breakdown Spectroscopy (LIBS) technology and allows the imaging and quantification of the elements from the periodic table in biological tissues, with ppm-scale sensitivity and a pixel size of up to 10x10 μm^2 [(1)].

Samples of interest are scanned boustrophedonically. Each laser shot induces a plasma at the surface of the sample. The emitted light is collected (optic fibers) and analyzed (spectrometers). The obtained spectrum contains data regarding the nature of the elements contained in the few ng of abated matter. Elemental maps are obtained after the complete scan of the sample and each pixel corresponds to a single laser shot. We generally create elemental images of 100.000 to 500.000 pixels, that are superimposed with the adjacent HES pathology images.

We successfully applied this method to image and quantify the distribution of various metal-nanoparticles in different organs from animals. As an example, we studied the renal elimination of gadolinium-based nanoparticles[2]. Nanoparticles containing gold, platinum, silver, or calcium were observed in various organs or tumors after injection to rodents. Recently, we successfully created elemental images of human samples of medical interest. As an example, LIBS elemental imaging allowed the identification of Aluminum in a cutaneous granuloma, Tungsten and Copper in pigmented lymph nodes, Silicon or Titanium in pulmonary granulomas.

The LIBS instrument we developed is highly versatile because almost any element can be quantified with high sensitivity. Besides, this technique is fully complementary with standard optical microscopy, in particular with conventional gold-standard pathology analysis for diagnostic purposes. Here, we will describe our technology and show an overview of the main results obtained with LIBS for multi-elemental imaging of biological tissues for preclinical and medical applications.

Acknowledgements
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GRANULOMATOSIS, HYPERSENSITIVITY : FROM BERYLLIUM TO METAL AND SILICA

Chairs : J Muller Quernheim, Medicine Unit University Hospital, Freiburg, Germany

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The use of lymphocytes proliferation test in the assessment of occupational diseases

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Occupational and environmental factors make an important contribution to the global burden of disease. Work-related morbidity and mortality not only results in suffering and hardship for the worker and his or her family, but also it adds to the overall cost to society through lost productivity and increased use of medical and welfare services. The cost to society has been estimated at 2-14% of the gross national product in different studies in different countries[1]. The severity of the disease is related to the material inhaled and the intensity and duration of the exposure. Even individuals who do not work in the industry can develop occupational disease through indirect exposure. Although these diseases have been documented as far back as ancient Greece and Rome, the incidence of the disease increased dramatically with the development of modern industry.

An idiopathy is any disease with unknown pathogenesis or apparently spontaneous origin[2]. For some medical conditions, one or more causes are somewhat understood, but in a certain percentage of people with the condition, the cause may not be readily apparent or characterized. Advances in medical science improve etiology (the study of causes of diseases) and nosology (the classification of diseases); thus, regarding any particular condition or disease, as more root causes are discovered, and as events that seemed spontaneous have their origins revealed, the percentage of cases designated as idiopathic decreases. With the introduction of new materials and changes in manufacturing practices, occupational health investigators continue to uncover associations between novel exposures and chronic forms of diffuse parenchymal lung disease and terminal airways disease. In order to discern exposure disease relationships, clinicians must maintain a high index of suspicion for the potential toxicity of occupational and environmental exposures.

The possibility of an occupational etiology should always be considered in the differential diagnosis of ILDs, particularly for conditions such as sarcoidosis and idiopathic pulmonary fibrosis, because 'occult' exogenous causes are easily missed if a thorough occupational and environmental history is not taken. There are epidemiological reasons to believe that occupational and environmental factors may be involved in these conditions[3,4,5].

Exploring causality in patients who develop an acute parenchymal process immediately after a high-intensity exposure is usually straightforward; however, inferring causality when chronic lower-level

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exposures occur over many months to years is challenging. Inferring such associations requires a high index of suspicion, a careful exposure history in individual patients and a meticulous evaluation of respiratory surveillance data for larger worker cohorts[6].

Under-reporting of occupational disease is most likely to occur in older patients who are no longer at work but whose condition may well be due to their previous job. In addition, there may be no incentive to report occupational diseases, and insufficient awareness among physicians may also contribute.

Metals are known to cause a number of different pathological conditions, including pulmonary disease[7,8]. The inhalation of metal dust can cause a variety of lung diseases, such as parenchymal lung fibrosis and granulomatous lung disorders[9,10]. Granulomatous inflammation and hypersensitivity pneumonitis are associated with the inhalation of metal dust and fumes as well as with mycobacterial or fungal infections [10].

It was recently shown that lymphocyte proliferation tests can be useful in assessing occupational sensitization[11]. Several case reports demonstrated increased lymphocyte proliferation to titanium[12], aluminum[13], chromium and nickel[14], the latter as a side effect following hip arthroplasty[15].

We conducted a pilot study to determine whether MELISA® (MEmory Lymphocyte Immnuno Stimulation Assay) is also effective in identifying sensitization to a number of selected metals in a cohort of exposed sarcoid patients with lung granulomatous diseases who had been exposed to various substances at the workplace and in the environment[16] and we proposed that this test can be exploited to identify specific sensitization in individuals exposed to inhaled particles from a variety of metals.

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Identification of metal sensitization in sarcoid-like metal-exposed patients by the MELISA® lymphocyte proliferation test - a pilot study.

Netherland experience

Marcel VELTKAMP

Sarcoidosis is a systemic disorder of unknown etiology. Many potential organic/anorganic substances or microorganisms have been suggested to trigger sarcoidosis, such as mycobacteria, propionibacteria, aluminum, beryllium, silica and zirconium. Routine testing for possible triggers in sarcoidosis is not daily clinical practice. However, published data suggest that possible triggers could be identified in 74% of sarcoidosis patients. Conformation of these data in Dutch sarcoidosis patients can lead the way for randomized controlled trials in distinct subgroups of patients assessing the efficacy of antimycobacterial or antipropionibacterial treatment. In addition, it has been demonstrated previously that in a well characterized group of biopsy proven sarcoidosis patients, approximately 6% could be diagnosed as berylliosis after a thorough re-evaluation of metal exposure Termination of exposure to triggers such as beryllium could protect patients with sarcoidosis from developing progressive disease.

In our center 2 major studies are currently underway to address these issues. The first study is a retrospective cohort study in which a group of 1000 biopsy proven sarcoidosis patients is studied. By using exposition questionnaires we will try to identify patients with a high risk of metal or silica exposition. The patients with a high exposition risk will be invited to donate blood for a lymphocyte proliferation test.

The second study is the prospective Dutch IGRASAR study. In this study, 200 new sarcoidosis patients will be included .As controls, patients presenting on the pulmonary outpatient clinic with problems other than interstitial lung diseases will be included. The prevalence of sensitization against antigens of mycobacteria, propionibacteria, silica, beryllium, aluminum and zirconium will be determined in sarcoidosis patients and non-sarcoidosis patients using interferon gamma release assay (IGRA). Both the classical test in diagnosing metal sensitization, the lymphocyte proliferation test (LPT), as well as an IGRA set up to detect metal sensitization will be used, and a comparison between those tests will be made. After two and four years of follow up, clinical outcome status in sarcoidosis patients will be defined. If trigger-related phenotypes in Dutch patients can be identified these subgroups can be used for further treatment-intervention studies.

Nanopathology : the body reactions to micro and nanosized inorganic foreign bodies

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Back in the early Nineties, Dr. Gatti and Montanari had a chance to study the case of a patient with strange symptoms (periodic fever, chronic fatigue symptoms, ear ache and tears from only one eye) no diagnosis and no therapy. And after 8 years of this calvary he developed also splenomegaly and cryptogenic granulomatosis of the liver and of the kidneys.

After an interdisciplinary anamnesis made by pathologists and bioengineers and innovative investigations of Scanning Electron Microscopy supported by an Energy Dispersive spectroscopy they verified the presence of wear debris of worn-out dental bridges inside the granuloma of both organs. From this first case, further investigations were performed under the European projects Nanopathology (FP5-QOL-2002-141), and DIPNA (FP6 NMP-2006-02132) and of which Dr Gatti was the international coordinator, showed that inorganic, biopersistent particles with a size ranging from a few microns down to a few tens of nanometers can enter the organism mainly (though not exclusively) through inhalation and ingestion, get in the blood circulation and reach virtually any organ where they behave like foreign bodies. (1,2,3)

While they are in the blood, those particles can induce thrombosis and, once they are trapped in the internal organs and agglomerate, they can cause the formation of inflammatory tissue that can revert to cancer. A further possibility is that they enter the cell nucleus, as demonstrated (4). The cancerogenicity of nanoparticles was experimentally shown by inducing rhabdomyosarcoma in rats through the implantation of such particles in the animals' dorsal. Other experiments completed by different Authors confirmed the phenomenon and a recent Chinese paper described cases of cancer in workers in professional contact with nanoparticles. It must be observed that the physiological barriers look ineffective and that no elimination mechanisms seem to exist once nanoparticles have entered an organ or, which is even more important, cells and cell nuclei. (5)

An interesting point in Gatti and Montanari's research is that they did not start with laboratory experiments on cells and tissues but with observations in people affected by what were called "cryptogenic" illnesses (granulomatosis and sarcoidosis, at the beginning), and in a number of them found the then unexpected presence of inorganic micro and nanoparticles. The investigation system used is based on the examination of bioptic or autoptic samples taken from pathological tissues, an examination carried out though a method of electron microscopy devised by Dr Gatti.

Nowadays more and more products contain engineered nanoparticles (e.g. clothes, paints, food, drugs, cosmetics) or release them (e.g. air conditioners and washing machines) and their interaction with the living organisms has been studied only very superficially, often without considering that nanoparticles (e.g. asbestos') may take decades after exposure before showing their pathogenicity, or, not too rarely, not studied at all, taking for granted their never-proved harmlessness. Their size is comparable to a virus' or a protein's and, though no study has been carried out so far specifically on engineered nanoparticles, the impact of unintentionally-produced particles of comparable dimensions has been

observed in thousands of pathological cases. High temperature combustive processes as well as explosions of weapons generate a fraction of submicronic and nanosized particles that are dispersed in the environment. They can contaminate air, soil and water exposing humans, animals and plants to this pollution extremely dangerous especially if it is composed by heavy metals.

So, tT.Iannitti, S. Capone, A.M. Gatti, et al, Intracellular heavy metal nanoparticle storage: progressive accumulation within lymph nodes with transformation from chronic inflammation to malignancy International Journal of Nanomedicine 2010: 5; 955 – 960

The concept of toxicology needs to be extended and a new expression must be invented to describe specific behaviours over time. In fact, a side-effect can occur due to indirect effects. One of the factors to be kept in mind is that most inorganic nanoparticles are insoluble either in water or in fats, are not biodegradable and are bodies foreign to animal (and, of course, human) organisms with a size and a consequent behaviour that are considerably different either from molecules and ions and from larger objects. It is a well-known fact that the surface/volume ratio has an important influence on the physical and chemical properties of nanoparticles (a phenomenon advantageously exploited in nanotechnology), and that too must be considered. In some cases inorganic nanoparticles, interacting physically and chemically with elements or compounds of the biological substrate, even directly inside cells, can corrode, releasing ions and the corrosion products have an obvious classical toxicological impact on the host tissue. But many other aspects need to be studied in depth or even discovered. Mobility inside the organism, affinity for different tissues, types of cells, proteins, enzymes, etc, and capability to enter cell nuclei are all sides of the problem that need careful investigation.

Future researches will be developed in the field of nanotoxicology, personalized medicine and forensic nanopathology.

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MINERAL EXPOSOME, SARCOIDOSIS, GENOME AND IMMUNOLOGY

Chairs : JF Mornex, Louis Pradel Hospital, CHU Lyon

M Vincent, ALCTMP and Minapath Development

Sweden experience with genome and sarcoidosis

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and Karolinska Institut, Sweden.

Sarcoidosis is characterized by an accumulation of CD4+ T cells (T helper cells, Th) in the lungs i.e. at the focus of the inflammatory process. Such Th cells have been shown to be highly activated, to produce cytokines, and to show evidence of previous antigen recognition. Sarcoidosis has been regarded as an archetypical Th1 driven disease, with exaggerated production of IFNγ, TNF and IL-12. Recent studies pointed out Th17 (producing IL-17) as well as T regulatory (Treg) cells to be of importance too. A majority of Th17 lung cells of sarcoidosis patients were recently shown to be able to produce high levels of IFNγ [1], and our own studies revealed elevated numbers of Th17 cells expressing transcription factors typical for Th1 (T-bet) and Th17 (RORgT) simultaneously, i.e. Th17/Th1 cells[2]. This may reflect a process called "T-cell plasticity", where one distinct T cell phenotype e.g. Th1 can transform into another specific subtype e.g. Th17 cells. It was shown that such Th1/Th17 cells were significantly more activated, proliferated to a higher degree, produced both IFNγ and IL-17 and a wider range of cytokines (2). Such hybrid cells were identified more often in patients with Löfgren´s syndrome (LS), characterized by an acute onset with fever, bilateral hilar lymphadenopathy, erythema nodosum and bilateral ankle arthritis, and in general a good prognosis (2).

It is still not known whether sarcoidosis is an autoimmune disease or not. Interestingly, patients treated by blocking CTLA4 (e.g. because of metastatic melanoma) are at higher risk of developing sarcoidosis, possibly by stimulating Th17 cells and impairing Treg-mediated suppression[3] and thereby activating underlying or pre-existent sarcoidosis. Also treatment with IFNα, a known Th1 promotor, increase the risk for developing sarcoidosis. These are arguments for sarcoidosis, or variants of sarcoidosis, being an autoimmune disorder. In spite of increased knowledge of the different Th cell populations, such as the seemingly dysregulated Treg cells, and Th17 cells producing other interleukins in addition to IL-17, we still do not know much about the etiology of the disease. In fact, there might be several different causes of the disease, or possibly diseases.

Detailed analyses of the T cell receptor (TCR) for antigen already in the late 1980:ies showed a preferential expression of distinct TCR variable (V) gene segments in sarcoidosis. This data suggested that T cells of patients with sarcoidosis had been stimulated by a specific antigen, inducing T cell activation and proliferation by selected clones that responded to such antigens. Our own observations of T cells expressing the TCR Vα2.3 gene segment in the lungs of HLA-DRB1*03pos (HLA-DR3pos) sarcoidosis patients but not controls, further strengthened the hypothesis of specific antigens driving the inflammatory process in sarcoidosis. Subsequent studies on Vα2.3pos T cells showed a strong

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association with disease activity, and that they typically produce Th1 cytokines. The Vα2.3pos lung accumulated T cells were shown to be oligoclonal, i.e. derived from a limited number of original T cells[4]. When we applied endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) to compare T cell phenotypes in lymph nodes (LN), BAL fluid and blood from the same individual patient, we found support for specific antigen recognition in the alveoli i.e. in line with an inhaled antigen[5]. Moreover, the Vα2.3pos T cells associated with clinical features of pulmonary sarcoidosis[6], and they expressed activation markers to a high degree.

More recent data show Vα2.3pos cells to preferentially express the Vβ22 gene segment. Sequencing the α and β chains of the lung accumulated Vα2.3pos T cells showed 1) in each patient a dominance of 1-4 different α or β chain sequences (clones) and 2) different nucleotide sequences that coded for identical amino acids at distinct positions presumably interacting with an antigenic peptide, and 3) identical TCR α-chain sequences in lung accumulated T cells from different patients. These findings are in line with exposure to an identical antigenic protein/peptide[7]. Interestingly, one of the repeated amino acid sequences (motifs) of the part of the TCR that interact with the antigenic peptide was found more or less identical in our previous analyses some 20 years ago of the TCR α chain of Vα2.3pos lung accumulated cells T (4).

Creating a molecular model of the interaction between the TCR harboring repeated motifs, the HLA-DR3 molecule and the antigenic peptide, showed a perfect match for a vimentin peptide, interacting with both the antigen binding cleft of the DR molecule and the Vα2.3/Vα22+ TCR. Interestingly, this particular vimentin peptide (Vim429-443) was previously recognized applying a new technique to identify HLA presented peptides[8]. The same peptide was able to stimulate CD4+ T cells of patients with sarcoidosis, but in general not controls, provided they were HLA-DR3pos, and thus already suggested as an auto-antigen in sarcoidosis. Anti-vimentin antibody titers has been suggested to correlate with disease severity in human tubulointerstitial lupus nephritis, and therefore vimentin was suggested to be a dominant target of in situ humoral immunity in that disease[9]. To understand whether vimentin could act as an autoantigen also in sarcoidosis, anti-vimentin antibodies are being analysed and preliminary found at higher levels, and in more patients, compared to controls. However more studies in particular on the adaptive immune system are required before one could designate vimentin as an antigen involved in sarcoidosis, e.g. T cell stimulatory capacity of vimentin needs to be more extensively investigated. Any cross-reactivity to external antigens needs to be investigated as well.

The role of lung accumulated Vα2.3/Vβ22+ T cells in HLA-DR3pos sarcoidosis patients should also be investigated more in detail. They appear in patients that are HLA-DR3pos, indicating recognition of

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specific antigens presented by the HLA-DR3 molecules. Since they appear in more or less only HLA-DR3pos patients, that most often are of the Löfgren´s subtype with an excellent prognosis[10], one could speculate that their function is to efficiently eliminate an offending antigen. These are questions to be answered by future studies of this enigmatic disease.

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Genetic analysis of inherited forms of sarcoidosis

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Sarcoidosis (OMIM 181100) is an enigmatic multisystem disease characterized by the development and accumulation of granulomas, a compact collection of macrophages which have differentiate into epithelioid cells and fuse to form multinucleated giant cells. The disease might be considered as the consequence of unregulated granulomatous reaction after exposure to various antigens and/or mineral particles in individuals who have a genetic predisposition. Sarcoidosis may be a chronic, benign or fatal disease affecting mainly lungs, skin, eyes and various other organs. We might expect that 2 to 5% of cases occur in the context of familial predisposition with an autosomal dominant trait and moderate to high penetrance. A national network (Group Sarcoidosis France) focusing this disease has been established in France since 2008 and allowed us to collect clinical data and DNA samples on ≈ 800 patients, 460 of them belonging to a cohort of 190 families (SARCFAM) predisposed to sarcoidosis with at least two first-degree related affected individuals. We recently published a genetic screening of one of the gene previously described as a putative predisposing factor, BTNL2, a co-factor of CD86 in the T-cell activation process (Pacheco and al, in press). The rs2076530 SNP of BTNL2 gene leads to a splicing defect and a truncated form of the BTNL2 protein. As shown by others, we confirmed that this variant induced a low risk of occurrence of the disease (OR=2), but we demonstrated that this putative mutation may not be considered as major gene explaining the dominant inheritance of the disease in SARC families.

Most of the genes which have been previously published as related to sarcoidosis have been characterized by GWAS, a SNP-based genome-wide method which allows the identification of genetic association between SNP's and an odd-ratio parameter. As for BTNL2, specific closely linked HLA class II haplotypes have been suggested to play a synergistic role in the disease. Many other genes such as ANXA11, CCDC88B, XAF-1, NOTCH-4, and loci involved in the IL23/Th17 signaling pathway have been published in the last few years. Nevertheless, none of them have been related to inherited presentation of the disease. In order to address this question, we performed WES (Whole Exome Sequencing) on a subset of our SARCFAM cohort and tried to identify genetic variants which might be considered as putative minor/moderate or high risk factors segregating inside each family and explaining the high penetrance and the 'mendelian' inheritance of sarcoidosis. A partial analysis of our WES data has been done on 4 families and allowed us to propose the following observations :

1. Even in inherited forms, sarcoidosis has a very heterogeneous genetic background and we might expect that each family carry one or a few number of gene variants which explain the risk of the disease.

2. As expected for a multi-genic and multi-factorial disease, the genetic risk of sarcoidosis seems to be based on the synergistic effect of several genes, affected either by high MAF (Minor Allele Frequency) and low MAF variants.

3. We did not find a single gene which mutations occur in different families

4. Comparisons of WES data in distinct SARCFAM pedigrees allowed the characterization of pathways which may be related to the crucial role of environment in the occurrence of sarcoidosis. One of them may impact the intracellular trafficking and endosomal / lysosomal process involved in the elimination of external contaminating mineral or microbial elements.

5. Finally, we have the clear impression that sarcoidosis inheritance must not be related to specific gene variants or haplotypes, but probably to what we may defined as: “a functional panel type” including between 5 to more than 30 genes affected by defective or dominant negative mutations, which accumulation in a single patient might be further related to very high odd-ratios (OR>10), greater the OR is, more frequently the familial presentation of the disease is.

Immunology, genome and sarcoïdosis

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The genetic background of sarcoidosis has been scrutinized over decades but a genetic signature of clinical usefulness has not been found. However, numerous gene variants associated with sarcoidosis have been identified and the gene products of most are involved in immunological mechanisms. Network analyses of these genetic variants provide the option to identify those with a pivotal function. Among those are toll-like receptors (TLR) which are molecules involved in the innate host defense and genetic variants of these molecules associate with sarcoidosis. Since a role of microbial factors in the pathogenesis of sarcoidosis is assumed and TLRs are involved in the initiation of a first immune response a search for bacteria of pathogenetic relevance in sarcoidosis is warranted. This is supported by the fact that TLR9 expression by sarcoid bronchoalveolar lavage (BAL) cells is elevated and that they release elevated amounts of cytokines after *P.acnes* stimulation was measured.

Next generation sequencing of the 16S DNA of sarcoid samples showed marked differences of the microbial composition compared with controls. *Atopobium spec*. and *Fusobacterium spec*. were detected in 68% of the sarcoidosis samples, but not in controls. Most interestingly, *Mycobacteria* and *Propionibacteria* were not found to be imbalanced between sarcoidosis samples and controls. Host genotype analysis revealed an association of the *BTNL2* risk allele with a decrease in bacterial burden.

In the context of the literature these findings support the hypothesis of a defect in immune tolerance against bacteria or non-animated exogenous agents as underlying pathogenetic mechanism which is modified by genetic variants.

Detection and quantification of non-fibrous particulates *in situ* in tissues : methodology and applications

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SUMMARY

Analysis of the retained tissue burden of particulate material provides important clues to exposures in individual cases as well as in epidemiological studies. Since 1980 we have developed and utilized microanalytical techniques to identify and quantify the inorganic particles retained in lungs (and other tissue). These results and the cumulative data in the database have had far-ranging diagnostic and research applications. We discuss our method of Scanning Electron Microscopy (SEM)/ Energy Dispersive Spectroscopy (EDS) to analyze the lung tissue particulate burden. This is a non-destructive, powerful tool for correlating tissue histology with the distribution of particles. The disadvantages include lower sensitivity (compared to true 'trace element' techniques -- e.g. ICP-MS) and inability to detect very low atomic number elements (e.g., Be). We herein share our experience applying this technique in various clinical and epidemiological scenarios. We will discuss our investigation of an accelerated silicosis outbreak, hard metal lung disease, aluminum lung disease, and so-called 'idiopathic' lung disease-including granulomatous disease related to silica and other exposures, and cases possibly misdiagnosed as 'sarcoidosis.' Pitfalls in routine diagnostic pathology practice are also highlighted.

Introduction and Methodology

Abraham and Burnett (1983) described the in situ method for quantitative analysis of inorganic particles in lung. This is basically a morphometric point counting approach using standard 5 um thick paraffin sections mounted on carbon planchets, resulting in concentrations in particle numbers per volume of lung (roughly equivalent to number concentrations per gram of wet lung). The in situ method detects particles which may be lost or altered during destructive analytic approaches such as ashing or digestion. Correlations with other analytical techniques and laboratories have validated this methodology. Detection limits are usually 1 x 106 particles/cm3 of tissue [ppcm]. Individual particle analysis also yields elemental composition and particle size. In control lungs (Abraham, 2007) total concentrations range up to 1 to 2 x 106 ppcm; aluminum silicates, silica and metal particles are the types found, with Fe and Ti the only commonly found metal particles. The results from all the analyses are contained, along with demographic and diagnostic information, in our pneumoconiosis database, described in detail in 1991, and updated in 2002). This data has been mined for various projects to date. Currently the database contains > 1000 analyses of > 800 cases for non fibrous particles, and > 1200 analysis from > 500 cases for fibrous particles.

Examples of results and analyses

Silica particles are frequently undetected by light microscopy (LM), as routine use of polarized light microscopy (PLM) often is technically inadequate to observe the very weakly birefringent silica particles. A case series of sandblasters using pure silica in the oil drilling industry (Abraham, 1997) illustrates how high concentrations of silica particles in lungs correlate with the diagnosis of silicosis. The database

shows the sandblasters have among the highest concentrations of silica observed. Many cases are associated with mixed exposures including aluminum silicates, talc and metal particles. Some are classified as accelerated silicosis which can overlap with mixed dust pneumoconiosis (MDP). A detailed description of the histopathology of accelerated pneumoconiosis is lacking and is in preparation (Sanyal). Another potentially powerful use of the database in this case series grouped the workers by employer and, using only the concentrations data of silica, aluminum silicates, iron and chromium particles, found that discriminant analysis grouped the workers very well by their different employers, consistent with differing work practices and exposures among workers basically all doing sandblasting.

Rapidly progressive coal worker's pneumoconiosis (RPCWP) is being recognized in the USA [Cohen, 2015]. The histopathology was described as likely showing the role of mixed silica and silicate exposure, and the first case which has had quantitative SEM/EDS analysis confirmed high concentrations of silica and aluminum silicate particles in the lungs, associated with MDP/accelerated silicosis (Abraham, 2016).

Aluminum metal particles are only rarely detected in controls. We examined the database for cases with aluminum welding exposure resulting in pulmonary fibrosis and found such cases stand out by virtue not only of their Al particle concentrations but also by the fraction of total particles comprised of Al particles (Hull, 2002). Exposures to differing forms of aluminum compounds, such as Al trihydroxide and Al oxide have also been studied (Raghu, 2014). Such exposures may require additional forms of particle analysis, such as Raman spectroscopy, to identify compounds instead of just the elements detectable by SEM/EDS.

Giant Cell Interstitial Pneumonia (GIP) is the rare histopathologic pattern seen in many cases of hard metal disease (HMD) from exposure to cobalt (Co), most often associated with tungsten carbide (WC) grinding tools, etc. Analysis of the database showed the nearly unique association of HMD/GIP with detection of W in the lungs (Naqvi, 2006). GIP is felt to be partially a hypersensitivity reaction; cases of hypersensitivity pneumonia and of granulomatous disease are potential candidates for tissue microanalysis. Of course, some granulomatous cases are related to organic antigens, not detectable by SEM/EDS. Some elements, like Beryllium, also are not readily detectable by SEM/EDS but may be detected and quantified using techniques like secondary ion mass spectroscopy (SIMS) (Abraham, 1980; Sawyer, 2005).

Metals and 'Idiopathic' pulmonary fibrosis. The detection of various metal particles in lungs as evidence of welding or other exposures is under-reported by LM (Sanyal, 2017) but is greatly enhanced by SEM/EDS analysis (Abraham, 1997). The diagnosis of Idiopathic pulmonary fibrosis (IPF) is often made without full investigation of occupational/environmental exposures, despite epidemiologic reports of such exposures as risk factors. A study (Zhao, 2013) of 30 cases diagnosed as IPF showed that by LM, many did not fit histopathologic criteria of usual interstitial pneumonia (UIP), and showed evidence by LM and/or SEM/EDS of a variety of exposures (such as asbestos, silica and various metals) capable of causing lung injury and fibrosis.

Histo-compartmental analysis of metal particles in autopsy lung samples from the London smog of 1952 [Hunt, 2002] allowed observation of different types of particles in physiologically (temporally) distinct compartments (airspace macrophages [most recent exposure], interstitium [older exposure] and lymph nodes [representing particles cleared from the lung]. This unique study supported the importance of differing solubility of inhaled metal particles (e.g., Pb, Zn) in evaluating epidemiologic and toxicologic

factors in air pollution, of contemporary concern with severe particulate air pollution in different parts of the world.

Dose-response is difficult to evaluate in human tissues, as exposures are not controllable or frequently measured during any individual's lifetime. In a study of racing dogs exposed to dust from the dirt tracks on which they ran (Schoning, 1996), a strong correlation was seen between the age of the dog and the amount of dust in the lung, and the composition of the dust in the lung matched that of the dirt tracks. This type of analysis is also powerful in that if the lung dust burden does not fully match with similar analyses of putative source(s) of exposure, further investigation to try to identify additional source(s) of exposure may be indicated.

Recommendations for future: There is a paucity of pathologists, epidemiologists and occupational medicine specialists training in this field, and results such as ours over several decades demonstrate that the incorporation of particle analysis as part of diagnostic practice and inclusion of this in epidemiologic investigations may make important contributions to understanding effects of both heavy and cryptic exposures into the future -- important for prevention and public health. As a consequence of lack of interest and awareness, there remains substantial under-diagnosis of diseases caused by exposure to inorganic particulates.

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NANOPARTICLE AND GRANULOMA AND FIBROSIS

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Nanoparticles analysis in lung and bronchi during various Pulmonary Interstitial diseases and relationships with their etiology (NanoPi trial)

Saint-Etienne experience

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Introduction

Despite their widespread use, the true impact of nanoparticles (NP) on human health remains unknown and poorly studied. NP exposure in humans primarily occurs via inhalation through the respiratory system. The cause of sarcoidosis or idiopathic pulmonary fibrosis (IPF) remains unknown. We postulate that inhaled toxic NP could play a role in these diseases.

Aim of the study

To measure the NP load (qualitative and quantitative) in bronchoalveolar lavages (BAL), bronchial washings (BW) or exhaled air condensates (EAC) both in patients with either idiopathic interstitial diseases (such as sarcoidosis or IPF) or in patients with interstitial lung diseases of known etiology.

Methods

Prospective mono-centric study including 100 patients suffering from an interstitial lung disease. The patients were recruited in 2 years by the department of chest diseases of the university hospitals of St Etienne. The accurate diagnosis of the disease has been determined in accordance to the latest international guidelines, including the past history of each patient, the cursus laboris with focus on potential NP exposure, environmental studies, tobacco or drug use and exhaustive research of collagen or vascular diseases. Dynamic light scattering (DLS) has been used to assess quantitative analysis of nanoparticles. The elemental compositions of both the particulate (pellet) and the soluble (supernatant) fractions of each sample have been measured by means of inductively coupled plasma optical emission spectroscopy (ICP-OES). All these analysis were conducted in the dedicated lab of the “Ecole Nationale supérieure des mines de St Etienne”.

Results

34 patients were in the « idiopathic group (sarcoidosis 14, IPF : 8, other idiopathic interstitial diseases 12). 59 were in the known etiology group (drug induced interstitial lung diseases: 12, infectious cause: 12, hypersensibility pneumonitis : 7, lymphangitidis : 4, miscellaneous 21.) Today, 3 diagnoses remain unclear and will be clarified in our multidisciplinary committee. In DLS analysis, only 2 patients had a significant detection of NP in BAL. In contrast, in BW, a large distribution of NP was observed from less than the threshold to 250 times this threshold. No significant difference was observed between idiopathic and known etiology group. ICP-OES analysis showed different metals including Al, Be, Co, Cr, Cu, Fe, Ni, Ti , W, Zr and Zn but none seems associated with a clinical diagnosis.

Comments

This NanoPi trial shows the feasibility of the research of nanoparticles in human lungs on BAL and bronchial washings. The interpretation of the results is very complex : first quantitative results in DLS should be combined with qualitative results in ICP-OES. Electron microscopy should confirm the presence of NP. Moreover, the level of NP in the lung at each time is the result of a clearance process and combine inhalation and exhalation of the NP both with a probable systemic passage through the alveolo-capillary membrane. Today, it's impossible to find a typical profile of nanoparticles associated with sarcoidosis or IPF.

The Challenge of Characterizing the Diseases Associated with Occupational Exposures at the WTC Disaster Site

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The terrorist attack on September 11, 2001, and subsequent rescue, recovery, and service restoration of the World Trade Centre, created an unprecedented and unique occupational and environmental exposure that affected a large group of workers and residents of New York City. The collapse of the towers, immediate and subsequent 3-month smoldering files, created a complex mixture of dust, fumes, and smoke that has not been fully characterized, but is thought to have included alkaline dust particles of very diverse size, polyaromatic hydrocarbons and other volatile and non-volatile organic and inorganic combustion products, silica, silicates, talc, gypsum, asbestos, metals, and aeroallergens[1,2,3]. A variety of acute[4,5] and chronic respiratory illnesses[6,7] have been reported among the workers, and is the subject of large scale ongoing investigation and follow up. It seems clear that the toxicant exposures affected the entire range of upper and lower airway tract[8,9]. The predominant WTC related chronic lower airway disorders have been clinically characterized as irritant-induced asthma, nonspecific chronic bronchitis, chronic bronchiolitis, and aggravated pre-existent chronic airway disease [4317; 4545]. In addition to those, several often single case reports have suggested associations with granulomatous pneumonitis[10], sarcoid-like granulomatous disease or sarcoidosis[11], eosinophilic pneumonitis[12], and giant cell interstitial pneumonia[13]. Besides those studies on occupational cohorts, studies on

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community residents have suggested increased respiratory morbidity[14]. Asthma incidence appeared to peak within months of the disaster with subsequent return to population expected rates[15]. Lung function surveillance (particularly among firefighters, who most consistently had data predating the episode), demonstrated an exaggerated one time expiratory flow loss (about 500 ml), followed by an average decline in subsequent years that seems to follow age-related rates[16,17].

Lung function studies have consistently identified nonspecific reduction of forced vital capacity (FVC) as the overwhelmingly dominant abnormality in these workers [(6, 7)], while obstruction, bronchodilator response and bronchial hyperreactivity are all much less frequent findings[(7)]. Small airway disease was suggested, however, by chest CT imaging studies demonstrating end-expiratory air trapping[18], and impulse oscillometry findings in a study of group of local residents and workers residing near the WTC with normal routine spirometry[19]. A more detailed study demonstrated some restrictive features in basically obstructive lung disease[20]. Histopathological studies have been scarce. Cases of constrictive bronchiolitis have been reported[(7,21)]. A case report of granulomatous pneumonitis (excluded as sarcoidosis because the Kveim test was negative) did not report birefringent particles, but X-ray dispersive radiography found silica, silicates, and calcium oxalate[(10)]. A study of 12 cases with predominance of either interstitial pulmonary abnormalities, or airway abnormalities, reported histological features of interstitial fibrosis, emphysema, and small airway abnormalities. All cases had opaque and birefringent particles within macrophages, and X-ray dispersive radiography in 6 of the cases indicated that those particles contained silica, aluminum silicates, titanium dioxide, talc, and metals[22].

We examined the lungs of two patients with high WTC dust exposure, who required lung transplant in 2014. Both cases had chest CT imaging patterns and histopathological findings classified as nonspecific interstitial pneumonitis (NSIP). There were no asbestos bodies, but after excluding elements associated with normal and inflamed tissues, X-ray dispersive radiography revealed numerous particles in both lymph nodes and lung parenchyma containing silica, aluminosilicates, and talc. A third case, with

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documented mild constrictive bronchiolitis, and progressive pulmonary functional improvement on follow up is pending examination.

Although epidemiological studies have not provided evidence of increased incidence of interstitial pulmonary fibrosis in the WTC workers, the potential has been well recognized[23,24], and surveillance needs to remain in place to study and detect the cases. The three studies summarized in this report, including 9 cases, are starting to suggest the role of silica and silicates in the etiology of those processes.

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Characterization of an experimental mouse model of lung exposure to nanoparticles. Relevance to human sarcoidosis-like disease.

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Abstract

Background

Nanoparticles are increasingly suspected as an etiologic factor of granuloma formation in sarcoidosis. The aim of our study was to compare lung inflammatory response and histology changes following exposure of mice to two widely used nanoparticles: carbon nanotubes (MWCNT) and cadmium-based nanoparticles (QDOT) in an attempt to better our understanding of granulomatous inflammation.

Material and methods

Various groups of mice were included: control mice received PBS or Complete Freund's adjuvant (CFA) and treated mice received PBS or CFA followed by intranasal exposure to MWCNT or QDOT. At day 30 post-challenge, alveolar lavages (BAL) and lung tissues were collected for analyses of inflammatory and histologic changes in response to nanoparticles.

Results

Analyses of lung BAL fluids and tissues derived from nanoparticle-challenged mice by comparison to controls found (1) higher BAL cellularity, (2) increased numbers of neutrophils, macrophages and lymphocytes, (3) heterogeneous areas with increased cellular infiltrates and granuloma surfaces, (4) macrophages, CD3+ T, Treg and B cell infiltration in inflammatory areas, (5) altered regulation of key inflammatory mediators and receptors, and (6) increase of total protein concentration, LDH activity and proteolytic activity in BAL fluids. Importantly, these changes were nanoparticle type-dependent. Finally, the observed lung changes in response to nanoparticles mimic closely characteristic features of human sarcoidosis-like disease.

Conclusions

Our work enhances our understanding of nanoparticles-induced lung inflammatory and histological changes that result in granuloma formation. It also suggests that this experimental model is clinically relevant for both investigating mechanisms that mediate the initiation and/or progression of sarcoidosis-like disease as well as testing anti-inflammatory strategies against granuloma formation.

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Automatic detection and characterization of particles through EDS-SEM.

Benefit of their use as control group in a context of highlighting a professional or environmental exposure

CHEMARIN Cécile (for the Minasarc Study team)

Cécile Chemarin, Mickaël Catinon, Catherine Cavalin, Stéphane Rio, Elisabeth Roux, Matthieu Pecquet, Anne-Sophie Blanchet, Sylvie Vuillermoz, Christophe Pison, François Arbib, Vincent Bonneterre, Dominique Valeyre, Olivia Freynet, Jean-François Mornex, Nathalie Freymond, Yves Pacheco, Françoise Thivolet, Marianne Kambouchner, Jean-François Bernaudin, Audrey Nathalizio, Paul-André Rosental, Michel Vincent (for author origin see p 33)

In order to diagnose dust-induced diseases, the assessment of an occupational or environmental exposure to dust is necessary. Different diagnostic tools such as clinical data, radiology, histology, questionnaires, and immunological tests are used. The difficulties encountered in this assessment have led some clinicians and pathologists to try and highlight the presence of particles in biological samples. In particular the mineralogical analysis of broncho alveolar lavages (BAL) helps to determine the physicochemical nature of the particles. In order to interpret a patient outcome it is necessary to compare it with reference values. The Minasarc study is a prospective, multicentric, diagnostic and physiopathological blind study. It aims to describe the exposure to inorganic particles from a new questionnaire “whole life” and mineralogical analyses. Hence it helps to establish a database on the presence of airborne non fibrous particles among 19 healthy subjects BALs.

Our laboratory performs electron microscopy analyses on digested BAL. We use a Low Vacuum Scanning Electron Microscope (LV-SEM) which enables the automatic detection and characterization of particles through Energy Dispersive Spectroscopy (EDS). More than 1000 particles per BAL are analyzed and classified by mineral particles classes. Particles concentrations in classes are determined and a mineralogical profile can be described for each subject. These profiles are consistent with the exposures highlighted by the questionnaire results. The acquisition of a control population and an easy and low-cost implementation of electron microscopy make it possible to explore the link between exposure and pathologies for which environmental risk factors are suspected.

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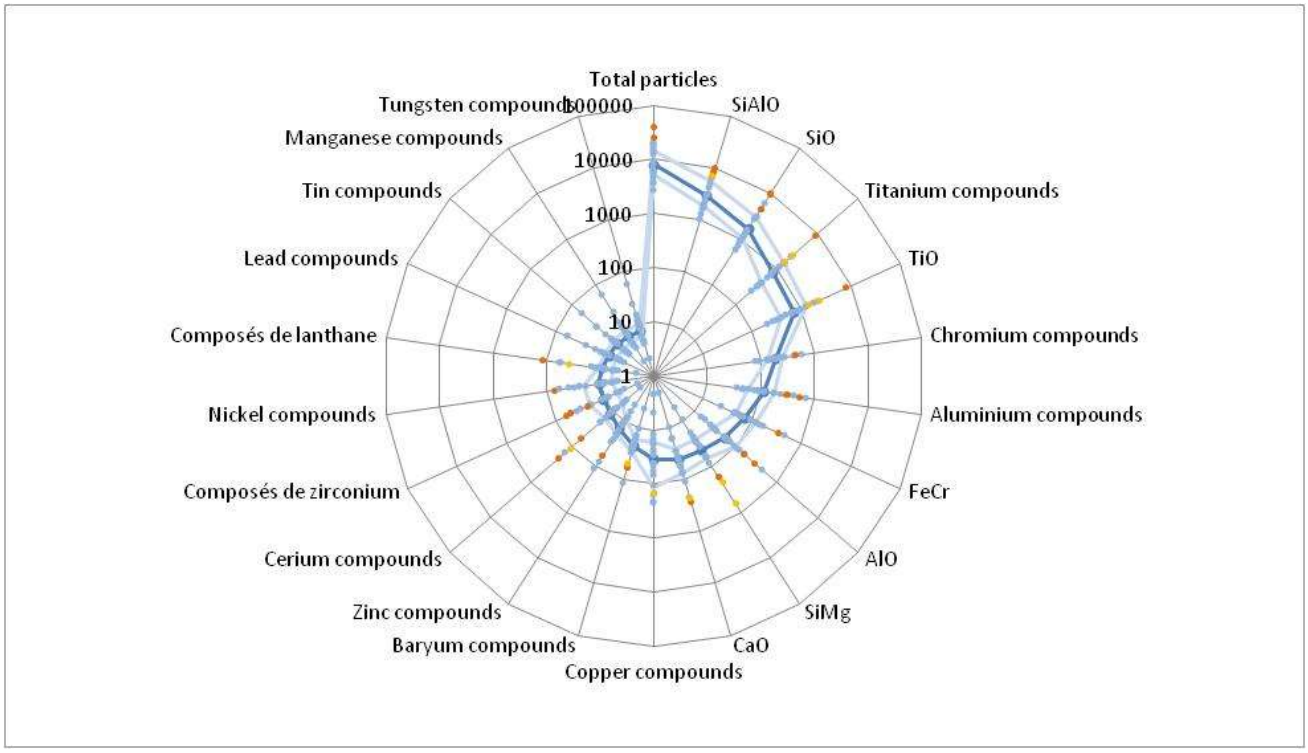

Figure : BAL fluid's mineralogical profils from healthy subjects : particles content for each class (number of particles per ml of BAL fluids).

Sarcoidosis and inorganic dust exposure in the MINASARC (MIneralo-NAno-SARCoidosis) study

CATINON Mickaël

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Introduction

MINASARC study is a prospective case-control study measuring mineral exposome by a Specific Questionnaire (SQ) and Mineralogical Analysis (MA) of Broncho-Alveolar Lavages (BALs) by Transmission Electron Microscopy (TEM) in 20 sarcoidosis cases (SarC) compared to 20 healthy volunteers (HV).

Objectives

To compare MA results between SarC and HV and to evaluate the correlation between MA and SQ.

Methods

Every SarC is matched to a HV by sex, age and smoking habit. Each BAL is treated by a digestion-filtration method. One hundred inorganic particles are analyzed blindly by TEM-Energy-dispersive X-Ray (EDX) and classified according to their elemental composition. MA results were considered as “suspect” when the rate of dust accumulation is among the highest in, at least, one particle class.

Results

SQ shows a significantly higher level of inorganic dust exposure in SarC compared to HV. TEM-EDX analysis of BALs identifies a high particle burden in eight SarC (silica, aluminum, titanium, iron, chromium compound, sulfur) and in five HV (chromium, titanium and aluminum compounds). While a good correlation is shown between MA and SQ, especially for building activities, there is no statistical difference in geometric mean of global load in inorganic particles between SarC and HV.

Conclusion

In the hypothesis of sarcoidosis related to mineral exposure, the granulomatosis disease of the eight SarC with a “suspect” MA could be related to an airborne inorganic dust exposure and, for the twelve other SarC, other associations (cutaneo-mucous contamination, nanoparticle exposition and genetically determined hypersensitivity) have to be explored in larger SarC cohorts using more sensitive MA.

Inorganic Particles and causal relationship in Sarcoïdosis from Hill's criterias

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The balance between health and disease is governed by the relationship between our exposome throughout life, our genetic background, the passage of time and random chance.

The predominance of these factors varies between diseases : Genetic factors (BRCA mutation) dominate for some breast cancers. For severe asbestosis on the other hand, occupational exposure is the main factor. For example, we know of a textile factory in the 1930s handling asbestos in which all the workers died before the age of 40 from pulmonary fibrosis. For lung cancer, smoking and the inhalation of other inorganic substances (asbestos, silica, chromium, cadmium, etc.) are also more important than the genetic background.

What is the relative importance of mineral exposure and genes in sarcoidosis ? This is the principal subject of this colloquium.

There are three ways of measuring mineral exposome. A personal detector can be worn but only for a short period. To measure lifetime exposures, the only two options are mineralogical analysis and precise questioning. We are going to think about what information these two tools can provide, based on Hill's criteria and some historical data.

Mineralogical analysis (in comparison with healthy subjects) is a good way to attribute a disease to a particular inorganic exposure. However, mineralogical analysis is difficult for a number of reasons :

1. The need for healthy subjects (controls).
2. The cost of the studies, which can nonetheless be reduced by using automated analysis.
3. Quantification. How many particles is a lot?

Questionnaires are rarely used for historical reasons. The risk posed by silica and other inorganic particles has been underestimated since the Johannesburg conference in 1930, which established a truncated definition of silicosis.

Many of Hill's criteria are satisfied in relation to mineral exposure for a large number of sarcoidosis cases, which we would describe as inorganic particle-induced granulomatosis. We will focus on the analogy between sarcoidosis and podoconiosis and asbestos-induced disease. We will propose a pathophysiological model for sarcoidosis. We suggest that the burden of proof when diagnosing sarcoidosis should be reversed: the disease should only be described as idiopathic if neither questioning nor mineralogical analysis are suspect.