



Safety in Mines Research Advisory Committee

SIMRAC PROJECT SIM 03-08-03

MARKERS FOR PREDICTION AND EARLY DETECTION OF PNEUMOCONIOSIS

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March 2004

EXECUTIVE SUMMARY

The South African gold mining industry recently joined the World Health Organisation Global Campaign to Eliminate Silicosis. Subsequently, a number of research projects are being focused on the evaluation of silica dust levels and dust allaying measures. However, the debate regarding the risk of developing silicosis at various exposure levels continues. The assessment of the efficacy of these dust-allaying measures will depend on an assessment of the incidence of adverse health outcomes due to silica dust exposure, rather than the prevalence of silicosis.

Silicosis, a late (and irreversible) manifestation of disease, is the currently used health outcome for silica dust dose-response assessments and clinical detection is dependent on radiology. Early evaluation of the health outcomes of dust allaying interventions is not currently performed. The use of biomarkers can change this and greatly enhance the process of risk assessment. In the past few years, many aspects of the previously unknown pathological mechanisms in the pathway from silica exposure to the development of silicosis have been elucidated.

Silica-induced inflammation and fibrosis result from complex interactions between the particles and lung macrophages, alveolar epithelial cells, fibroblasts, neutrophils, and lymphocytes. There are complex networks stimulating the production of oxidants, chemokines and cytokines. The surface characteristics of the silica particles determine their redox potential and ability to react with hydrogen, oxygen, and nitrogen. In biomarker studies, an event, which can be measured, is selected from this cascade.

If scientifically acceptable existing biomarkers for silica dust exposure can be identified, industry could utilise these for the early detection of adverse health effects, rapid evaluation of dust-allaying projects that may be introduced in the near future, and timely implementation of intervention strategies.

The objectives of the project were to:

1. Undertake a comprehensive literature survey to identify biomarkers for the early detection and / or prediction of silicosis
2. Develop a systematic framework for the evaluation of studies on biomarkers
3. Conduct a meta-analysis of data, if appropriate
4. Hold a workshop of international experts, primarily to evaluate the potential for conducting a Phase II study.
5. Develop an outline of a proposal for a Phase II evaluation of any promising markers(s) identified.

The relevant literature was identified, using, primarily, occupational health websites. Each study was scrutinised, using a systematic review form. A total of 171 papers and articles related to biomarkers for silicosis were identified; these were reviewed by international experts. These summary reviews formed the discussion document for a workshop, which was attended by both local and international experts in the fields of silicosis and biomarkers.

Although the literature on silicosis-specific biomarkers is fairly extensive, no definitive conclusions have been reached. Previous studies have been cross sectional rather than prospective in design, and many of the studies have used animals or cell

systems. Furthermore, often only one or two biomarkers have been evaluated in any one study, precluding a comparative assessment. By analysing several biomarkers from a single individual at a single point in time, more information may be obtained about the nature of the exposure than from use of a single biomarker.

Recommendations

Based on these factors, an outline for a Phase II proposal has been developed for further evaluation of 10 of these markers, viz.

- ? Glutathione peroxidase (GPx) and glutathione-S transferases (GSTs)
- ? Glutathione (GSH)
- ? 8-Isoprostane
- ? Total Antioxidant Capacity
- ? Reactive Oxygen Species
- ? Tumour necrosis factor- α (TNF α)
- ? Interleukin-8 (IL8)
- ? Platelet derived growth factor (PDGF)
- ? Clara cell protein (CC16)
- ? TNF α polymorphism and dust induced TNF-release

In selecting markers for the Phase II proposal, attention was paid to:

Biological relevance
Temporal relevance towards effect
Background variability
Confounders
Reproducibility and predictive value
Practicability

The primary objective of the Phase II proposal is to determine which of the 10 biomarkers has/have the highest sensitivity and/or specificity in detecting changes in response to silica exposure, or susceptibility to silicosis? A prospective cohort study is necessary to answer this question, with annual follow up for at least five years. The biomarkers chosen can be measured in serum or whole blood.

ACKNOWLEDGEMENTS

This research project was funded by the Safety in Mines Research Advisory Committee (SIMRAC) of the Mines Health and Safety Council. Guidance, stimulation and intellectual support were provided by Prof Mary Ross throughout the project.

Prof David Rees, Head of the National Institute for Occupational Health (NIOH) enabled the researchers to devote considerable time and effort to this project.

To the many persons who have made valuable contributions to this study, we gratefully acknowledge our debt. In particular we wish to thank:

- ✍ Prof. Brendan Girdler-Brown, Head: Department Community Health, School of Health Systems and Public Health, University of Pretoria
- ✍ Dr Belinda Dias, Occupational Medicine Practitioner, Environmental Control, Occupational Health, CSIR Miningtek
- ✍ Dr Michelle Wong, Principal Specialist and Head: Respiratory Unit, Chris Hani Baragwanath Hospital.

STUDY TEAM

As the field of research into biomarkers of occupational lung disease is relatively new, and progressing at a fast rate, it was essential to have direct and rapid access to the latest developments in this field. This was facilitated by the inclusion in the study team of scientists, abreast of the latest developments in their fields, with expertise in toxicology, biochemistry, pathology, genetics and immunology.

Dr Mary Gulumian is presently Chief Specialist Scientist and also head of Toxicology and Biochemistry Research at the NIOH, and an honorary lecturer in the Haematology and Molecular Medicine Department at the University of the Witwatersrand. Since her employment at the NIOH, she has been involved in elucidating mechanisms implicated in mineral particle-induced toxicity, and is author or co-author of numerous publications on the subject.

Dr Jill Murray is head of the Pathology Directorate of the National Institute for Occupational Health (National Health Laboratory Service). She is a specialist pathologist with extensive experience in lung pathology, and is also on the staff of the School of Public Health of the University of the Witwatersrand. Dr Murray has researched silicosis, amongst other respiratory diseases, for almost two decades and has published extensively on the subject.

Prof Vince Castranova is presently Chief: Pathology and Physiology Research Branch at NIOSH. He is also Professor at the School of Pharmacy and School of Medicine, West Virginia University, as well as at the Dept. of Environmental and Occupational Medicine, University of Pittsburgh. Prof Castranova has been involved in research on silica and silicosis since his employment at NIOSH and has published over 300 papers on the subject.

Prof Val Vallyathan has been working on the elucidation of mechanisms involved in silica-induced diseases for the last two decades at NIOSH and he has published extensively on this subject. He has previously worked on a SIMRAC project on the respiratory health of coal miners in South Africa, and has visited South Africa. Through his work, Prof Vallyathan has proposed a number of biomarkers that may be used in the identification of silicosis.

Prof Ken Donaldson has carried out research into the effects of particles in the lungs for 20 years. He has acted as a consultant to the Department of Health (UK) and the American Environmental Protection Agency and as a consultant on silica carcinogenicity to the Industrial Minerals Association of Europe. Prof Donaldson was, until recently, the Transco British Lung Foundation Fellow in Air Pollution and Respiratory Health and is currently co-Director of the Edinburgh Lung and the Environment Group Initiative (ELEGI), a collaborative venture aimed at focusing research on the mechanisms of lung disease caused by airborne particles. Other posts he holds are Professor of Respiratory Toxicology, University of Edinburgh Medical School, and toxicologist on the Department of Health Committee on the Medical Effects of Air Pollution.

Prof Paul Borm is the head of the Particle Research Core at the Institut für Umweltmedizinische Forschung in Dusseldorf. His current area of research is the development of biomarkers to screen human populations for early effects and or susceptibility to airborne agents, such as particles and fibres. Current interests include the role of cytokines, their activation and release by intracellular pathways, and the genotoxicity of particles through inflammatory pathways. Prof Borm is a Member of the Dutch Evaluation committee on Occupational Substances (DECOS), part of the Dutch Health Council, and a Member of the Dust Sub-group of the German MAK-Commission. He is also on the Editorial Board of Inhalation Toxicology and Human Experimental Toxicology.

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Glossary

- A** **ACE** (angiotensin converting enzyme) is a peptidyl dipeptide hydrolase in the rennin-angiotensin system that converts angiotensin I into the potent vasopressor angiotensin II and also inactivates the vasodilator bradykinin, which is the product of kallikrein-kinin enzyme system.
- ACGIH** (American Conference of Governmental and Industrial Hygienists) is a community of professionals that advances worker health and safety through education and the development and dissemination of scientific and technical knowledge
- B** **BAL** (bronchoalveolar lavage) is a semi-invasive procedure, performed when a patient undergoes bronchoscopy. Fluid is introduced into the lung tissue through a tube that is pushed into the bronchi (air passages). The fluid is then suctioned out and its contents (cells, chemicals native to the lung, etc.) can be analysed.
- C** **CC16** (Clara cell protein) is a homodimer consisting of 70 amino acid subunits and has a molecular mass of 15, 840 kDa hence the CC16 abbreviation. CC16 protein is secreted by the nonciliated (Clara) cells of the tracheobronchial epithelium and by some reproductive system organs such as the prostate. The exact physiological function of CC16 remains unknown but there are several lines of evidence indicating that it is an immunosuppressive and anti-inflammatory protein protecting the airways from undue activation of the immune system that might cause tissue injury.
- CL** (chemiluminescence) is the production of light generated from chemical sources. Generally the light so produced is in the visible range (400 – 600 nm) but can also be in the ultraviolet region. With this method the production of free radicals can be assessed using compounds such as lucigenin that can be activated by these reactive species to produce light.
- CA 19-9** (Carbohydrate antigen) 19-9 is a cancer cell surface antigen, the elevation of which has been shown in the serum and in BAL of patients with a number of fibrotic lung diseases.
- G** **GPx** (glutathione peroxidase) is an antioxidant enzyme which converts reduced glutathione (GSH) into an oxidised form (GSSG) at the expense of hydrogen peroxide.
- GSH** (glutathione) is a small molecular weight peptide that scavenges free radicals and is a substrate for hydrogen peroxide-removing enzyme GPx.
- GSTs** (glutathione-S transferases) are enzymes that conjugate GSH to other substrates and aid in their detoxification.
- H** **HLAs** (human leukocyte antigens) are linked with the immune response capability
- I** **IL-8** (interleukin-8) is a member of the chemokine family with a molecular weight of ~ 8 kDa and possesses a conserved 4 cysteine motif in its mature protein sequence. Chemokines are secreted by a variety of cell types including fibroblasts in response to cytokines.

IL-1 (interleukin-1) is a highly pleotropic cytokine released primarily from activated monocytes or macrophages and many other cell types.

IL-1RA (interleukin-1 receptor antagonist)

IFN- γ (interferon-gamma) is a lymphokine with broad biologic functions including antifibrotic effect

M **Meta-analysis** is the statistical analysis of the results of several independent studies for the purposes of integrating the findings

N **NBAC** National Bioethics Advisory Commission

O **Oxidative stress** is the disturbance in the prooxidant/antioxidant balance in favour of the former leading to potential damage to biological cells.

P **PDGF** (platelet derived growth factor) is a small molecular weight polypeptide growth factor that may act on a variety of cell types and alter their proliferative properties.

Polymorphism is a sequence variation in the genes encoding certain enzymes and other proteins that may accumulate in a population. If the frequency of a specific variant reaches 1 % or more in a population, it is referred to as polymorphism.

R **Redox potential** is a measure of the ability of a substance to donate electrons.

S **SOD** (superoxide dismutase) catalyses the dismutation of superoxide anion radical ($O_2^{\cdot-}$) into hydrogen peroxide.

T **TEAC** (trolox equivalent antioxidant capacity) assay that measures total antioxidant content of a biological solution.

TLV (threshold limit value) – a guideline designed for use by industrial hygienists in making decisions regarding safe levels of exposure to various chemical substances and physical agents found in the workplace.

TNF- α (tumour necrosis factor- α) is a pro-inflammatory cytokine important in the early onset of inflammation, development and progression of several diseases including pulmonary fibrosis. **TNF- α** can be produced by a number of cell types including macrophages, monocytes and polymorphonuclear cells.

U **Upregulation** is the increased biosynthesis of an enzyme protein leading to its increased levels in biological fluids.

INTRODUCTION

The South African gold mining industry has a legacy of high levels of silica dust exposure and of silicosis.¹⁻³ Recently, the industry has engaged in the World Health Organisation Global Campaign to Eliminate Silicosis. As a result, a number of research projects concerned with the evaluation of silica dust levels and innovative dust allaying measures have been instituted under the auspices of SIMRAC. However, it is well known that there is still debate with regard to the risk of developing silicosis at various exposure levels,⁴⁻⁷ and also with regard to the safe occupational exposure level for silica dust.⁸ Hence, the assessment of the efficacy of these dust-allaying measures will depend on an assessment of the actual incidence of adverse health outcomes due to silica dust exposure.

Silicosis, a late (and irreversible) manifestation of disease, is the currently used health outcome for silica dust dose-response assessments. Clinical detection of silicosis is dependent on radiology, which is not 100% sensitive or specific.⁹⁻¹¹

Early evaluation of the health outcomes of dust allaying interventions is not currently performed. However, the use of biomarkers can change and greatly enhance the process of risk assessment. Recently, considerable attention in the scientific literature has been given to the utilisation of biomarkers in the prevention of occupational disease.¹²⁻¹⁶

In the past, toxicological methods were not able to identify and characterise the events leading from exposure to disease; these events were a "black box" (Figure 1).

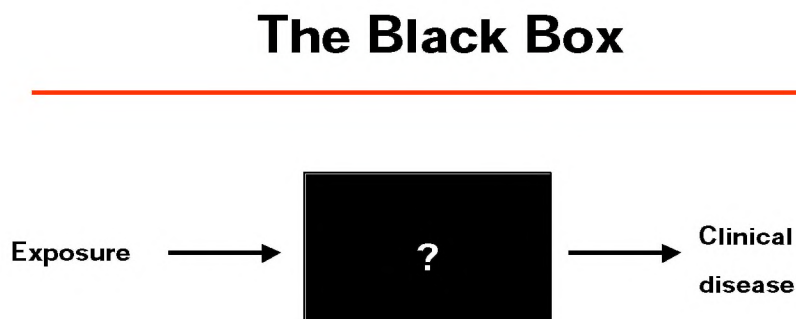


Figure 1. The Black Box

However, many aspects of the pathological mechanisms in the pathway from silica exposure to the development of silicosis have now been elucidated.

An excellent review of this topic summarises the processes whereby silica produces inflammation and fibrogenesis in the lung.¹⁷ Silica-induced inflammation and fibrosis result from complex interactions between the particles and lung macrophages, alveolar epithelial cells, fibroblasts, neutrophils, and lymphocytes. There are complex networks stimulating the production of oxidants, chemokines and cytokines. The surface characteristics of the silica particles determine their redox potential and ability to generate active species of oxygen and nitrogen.¹⁸⁻²¹ In biomarker studies, an event, which can be measured, is selected from this cascade.

Although the major determinant of silicosis is the level of exposure to silica-containing dust, individual susceptibility to the disease may play a role.²² It was recently reported, in a study of South African miners, that polymorphisms in the TNF- α gene promoter might predispose workers to severe silicosis.²³

Traditionally, biomarkers are classified as biomarkers of exposure, effect, and susceptibility. Biomarkers can be used to: (1) assess the occurrence of exposure; (2) identify various effects as a result of this exposure by measuring changes at a molecular or cellular level; (3) estimate the presence of pathological changes prior to disease state; and (4) predict underlying susceptibility of persons to disease. In addition, biomarkers can be used to predict the progression of disease.

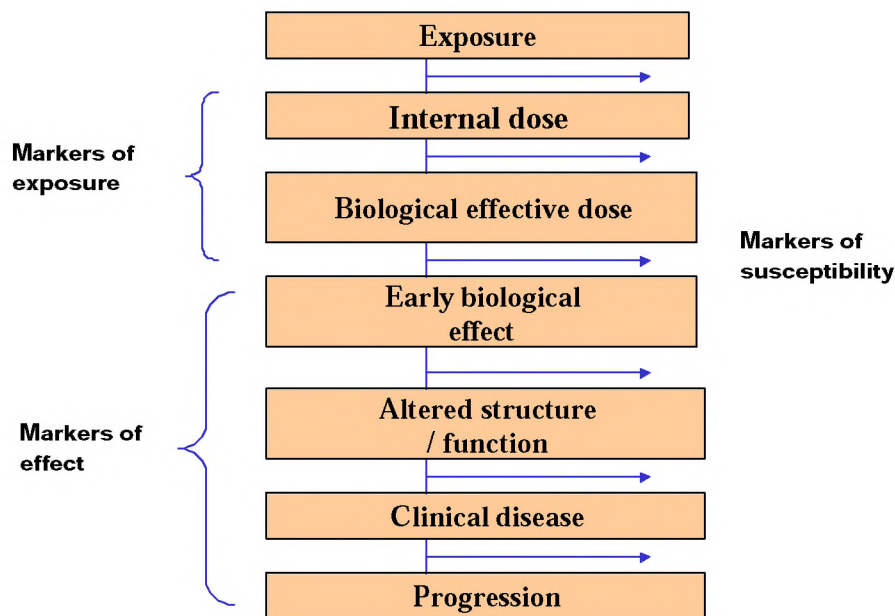


Figure 2. Categories of biological markers that could help identify processes within the "Black Box"

If scientifically acceptable existing biomarkers for silica dust exposure can be identified, industry could utilise these for the early detection of adverse health effects, rapid evaluation of dust-allaying projects that may be introduced in the near future, and timely implementation of intervention strategies.

OBJECTIVES

1. To undertake a comprehensive literature survey to identify biomarkers for the early detection and / or prediction of silicosis
2. To develop a systematic framework for the evaluation of studies on biomarkers
3. To conduct a meta-analysis of data, if appropriate
4. To hold a workshop of international experts
5. To develop an outline proposal for Phase 2 evaluation of any promising markers(s) identified.

IDENTIFICATION OF THE LITERATURE

The key facility for the identification of the literature was the NIOH Technical Advisory Division databases. As one of the collaborating centres for the WHO/ILO Joint Effort on Occupational Health and Safety, NIOH has extensive access to literature in the occupational health arena, and access to an international network of over 120 institutions, using the latest information technology.

Research pertaining to biomarkers for silicosis was identified, using the following sources:

1. <http://www-apps.niehs.nih.gov/centers/public/res-core/ctr300-2029.htm>
2. <http://www.cdc.gov/niosh/02-129J.html>
3. <http://www.niehs.nih.gov/centers/res-core/nyu-res5.htm>
4. <http://www.nap.edu/books/0309051878/html/37.html>
5. <http://www-apps.niehs.nih.gov/centers/public/res-core/ctr300-2029.htm>
6. <http://cebp.aacrjournals.org/cgi/content/abstract/3/6/471>
7. <http://www.uml.edu/Dept/WE/people/faculty/derrico.htm>
8. <http://ehpnet1.niehs.nih.gov/docs/1999/107-7/niehsnews.html>
9. <http://wornhlth.home.mindspring.com/PSC/BIOPLAUS.HTM>
10. <http://www.rsc.org/pdf/books/ftoxictc.pdf>
11. <http://www.tdh.state.tx.us/epitox/definitions.htm>
12. http://www.miu.uni-duesseldorf.de/mit/borm/borm80_97.pdf
13. <http://www.md.ucl.ac.be/toxi/99.html>
14. http://acadprojwww.wlu.edu/vol4/BlackmerH/public_html/xliberty/blung/isi.html
15. http://www.med.nus.edu.sg/cof/resourcectr_book1.htm
16. <http://www.amazon.com/exec/obidos/ISBN%3D0309051878/vermontsiriA/002-9069222-3620840>
17. <http://www.urbanfischer.de/journals/intjhyg/content/2000/issue56/4410057a.pdf>
18. <http://www.cuhk.edu.hk/med/cmd/ytspublications.html>
19. <http://www.pitt.edu/~biostat/landsittel.htm>
20. <http://www.acoh2002.org.tw/DailyProgramme-P.htm>
21. <http://www.ias.ac.in/jbiosci/feb2003/61.pdf>
22. <http://elvira.ingentaselect.com/vl=6429729/cl=24/nw=1/rpsv/catchword/tandf/1354750x/v4n5/s5/p361>
23. <http://www.njc.org/faculty/rose.html>
24. http://www.niih.go.jp/jp/indu_hel/2001/pdf/IH39_15.pdf
25. <http://www.dqaum.med.uni-rostock.de/abstr1.htm>
26. <http://www.ccohs.ca/who/beijrep.htm>
27. <http://www.sfr-europe.org/Content.asp?CID=30>
28. <http://www.insp.mx/biblio/alerta/al0100/03.pdf>
29. <http://www.osh-council.dk/epicohmonday.html>
30. <http://www.textbookx.com/browse.php?code=MED061000>
31. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>
32. <http://www.rivm.nl/bibliotheek/rapporten/640070002.pdf>
33. <http://www.atsdr.cdc.gov/toxprofiles/tp161.pdf>
34. <http://www.ymed.lu.se/papers/publications/publ93.html>
35. <http://www.rivm.nl/bibliotheek/rapporten/640070002.pdf>
36. <http://www.aiha.org/abs02/ps504.htm>
37. <http://www.chestjournal.org/content/vol109/issue3/index.shtml>
38. http://www.zoey.med.howard.edu/2005/lec_material/m&s_2/walker%20unit4/m&n/Medicine%20and%20Society2.doc

Silicosis-specific biomarker references, which were identified, included not only those biomarkers measured in humans but also those in animal studies, and those that elucidated mechanisms involving a particular biomarker.

The international collaborators perused this list carefully, and identified any references that had been omitted, or any that were irrelevant or inappropriate.

In this way, 192 references were identified. These included general reviews on biomarkers, silicosis biomarker reviews, articles on mechanisms of the development of silicosis and papers on silicosis-specific biomarkers. A total of 171 papers and articles related to biomarkers for silicosis were identified.

Electronic Reference Manager

An electronic reference manager (EndNote version 6) was used to create libraries of the different categories (general biomarker reviews, specific biomarkers, etc.). (appendix 1). Using this software, it is possible to search online databases for references and to create reference lists that can be stored in libraries. References do not have to be typed, but appear automatically in a predetermined layout. At the same time, the abstracts can be stored.

DEVELOPMENT OF THE SYSTEMATIC REVIEW FORM / PROCESS

Each study was scrutinised by one of the expert panel, using a form developed by the study team. The systematic review form (appendix 2) was developed locally and evaluated by the international participants. Each report could then be evaluated for, amongst other things, its validity, the sensitivity and specificity of the biomarker in question, the methods employed and, ultimately, its "value" to this project.

The systematic review form took into account the following issues:

- ? Classification as the biomarker in the assessment of exposure, early outcome or susceptibility
- ? Source in the body (tissue or cell type producing the biomarker)
- ? Mechanism of action (processes investigated through which silica particles are shown to produce pathological effects, and the identification of molecules during these processes that can be used as biomarkers)
- ? Hypothesis being tested / objective of study
- ? Biological material tested
- ? Study population and number of samples/ subjects tested
- ? Methods used (including statistical methods)
- ? Practicability
- ? Study design
- ? Specificity and sensitivity of the biomarker
- ? Results of the study

SYSTEMATIC REVIEW OF THE LITERATURE

The majority of the 149 silicosis-related biomarker references which were identified were reviewed. Those references that were not reviewed were unobtainable in South Africa, the USA, the UK and Germany, or were in a foreign language* that could not be translated into English by members of the expert group.

The international experts reviewed the references, using the systematic review form (appendix 2). The allocation of references to each person was based on their relevant expertise in the field.

Dr Mary Gulumian	40 references
Prof Paul Borm	35 references
Prof Vince Castranova	27 references
Prof Ken Donaldson	23 references
Prof Val Vallyathan	24 references

The reviewers attempted to classify each biomarker in terms of its appropriateness as a biomarker of exposure, effect or susceptibility (appendix 3).

A short summary review was written by each person (appendix 4).

Note: A paper entitled, "Biomarkers of exposure, effect and susceptibility for silicosis: a comprehensive review", is being written, and will be submitted to the Journal of Toxicology and Environmental Health. This paper will be added to the report as an addendum, once it has been published.

WORKSHOP

The summary reviews formed the discussion document for the workshop. In addition, the potential for meta-analysis of data was discussed, controversies in the literature were clarified, and potential biomarkers were identified for further study (phase 2). An outline proposal was then developed for further evaluation of these markers.

META-ANALYSIS

Meta-analysis refers to the statistical analysis of a large collection of results from individual studies. The result is potentially more objective and exact than the results of the individual studies. However, consensus was reached at the workshop that meta-analysis was not warranted for a number of reasons. The different studies on each biomarker were too dissimilar; there were too few reports on each biomarker for meta-analyses to be performed; and the numbers of subjects used in each study were too small.²⁴

* Chinese, Italian, Russian, Polish

MOTIVATION FOR PHASE II STUDY

If scientifically acceptable, existing biomarkers for silica dust exposure can be identified, industry could utilise these for the early detection of adverse health effects, rapid evaluation of dust-allaying projects that may be introduced in the near future, and timely implementation of intervention strategies.

The literature on silicosis-specific biomarkers is fairly extensive, but no definitive conclusions that can be put into practice have been reached. Some of the limitations of the current research are discussed under meta-analysis. Previous studies have been cross sectional rather than prospective in design, and many of the studies have been in animals or have used cell systems. Furthermore, often only one or two biomarkers have been evaluated in any one study, precluding a comparative assessment. By analysing several biomarkers from a single individual at a single point in time, more information may be obtained about the nature of the exposure than from use of a single biomarker.

A comprehensive prospective study would provide a unique opportunity to contribute to the global campaign to eliminate silicosis. South Africa is ideally suited for such a study to be conducted.

SELECTION OF BIOMARKERS FOR PHASE II STUDY

Of the numerous biomarkers identified in the literature review, only a limited number were considered to be worth pursuing.

In selecting markers for Phase II, attention was paid to:

- Biological relevance
- Temporal relevance towards effect
- Background variability
- Confounders
- Reproducibility and predictive value
- Practicability

By the end of the workshop, 10 biomarkers were identified as having potential for the next phase (appendix 5). These markers were selected not only because they appeared to have sound scientific merit, but also on their practicability. There were no biomarkers that could be assessed in the urine. Any biomarker that required tissue biopsy or bronchiolar/ alveolar lavage was excluded. Thus, only those requiring blood tests were considered.

RATIONALE FOR SELECTION OF BIOMARKERS FOR PHASE II STUDY

I. BIOMARKERS OF EARLY EFFECT

A. OXIDATIVE STRESS MARKERS

- ? Glutathione peroxidase (GPx) and glutathione-S transferases (GSTs)
- ? Glutathione (GSH)
- ? 8-Isoprostane
- ? Total Antioxidant Capacity
- ? Reactive Oxygen Species

Free radicals generated either by the surface activity of crystalline silica or by the inflammatory response invoked by crystalline silica and the ensuing oxidative stress, feature prominently in the early stages of pathogenesis of silicosis.^{25,26} A substantial number of oxidants, antioxidants and antioxidant enzymes (GPx, GSTs, GSH) have been tested in human red blood cells, serum, BAL fluid and BAL cells to assess the degree of oxidative stress following exposure to silica, or in silicotic patients to validate the premises resulting from *in vitro* and *in vivo* animal investigations. Since the anti-oxidant system is composed of many mutually dependent components, integrative approaches have also been implemented, using alternative assays. Most of the important studies to assess oxidative stress have been conducted on peripheral blood components.

There is sufficient evidence in the literature with substantial *in vitro* and *in vivo* animal investigations followed by validation studies with human subjects, to conclude that oxidative stress is a major process involved in silicosis. Results have consistently indicated that, during the first stages of pneumoconiosis, oxidative stress is present in the lung. This is reflected by the changes in concentration of the antioxidants and antioxidant enzymes in the peripheral blood, reflecting exposure to dust or different stages of silicosis.^{27,28}

B. LUNG INJURY MARKER, CLARA CELL PROTEIN-16 (CC16) LEVELS IN SERUM

- ? Clara cell protein (CC16)

Concentration of this protein in plasma could be a measure of integrity of Clara cells and thus serve as a specific biomarker of lung injury.²⁹ Decreased concentration of CC16 has been associated with increased recruitment of fibroblasts in fibrosing lung disorders³⁰ and also in workers exposed to crystalline silica.^{31,32}

It was therefore suggested that a significant reduction of serum CC16 in workers inhaling silica-rich dust could be used as a biomarker of early toxicity due to exposure to silica. CC16 concentrations in serum correlate well with those in BAL fluid in humans^{33,34} and, since almost exclusive expression of this protein is originated in the respiratory tract,³⁵ this low-molecular weight protein would make an excellent biomarker of early effects of silica toxicity, especially when its decrease is associated with exposure to silica *per se* rather than with silica-induced lung impairment.

II. BIOMARKERS OF LATE EFFECT

A. BIOMARKERS OF LUNG INFLAMMATION

- ? Tumour necrosis factor- α (TNF α)
- ? Interleukin-8 (IL8)

Note: TNF α polymorphism is also a marker for susceptibility

TNF- α is important in the early onset of inflammation, development and progression of several diseases, including pulmonary fibrosis, and has been demonstrated to play a key-role in particle-induced lung fibrosis and, more specifically, in quartz-induced lung fibrosis.^{36,37}

Overwhelming literature with *in vitro* and *in vivo* animal experiments as well as epidemiological studies on human subjects for validation of inflammatory cytokines (especially TNF- α and IL-8) makes these ideal biomarkers to assess exposure to silica, or to predict the progression of disease. The relative ease with which these measurements can be achieved in blood and plasma³⁸ makes it all the more practical to consider them as biomarkers of choice in silica dust exposures.

B. BIOMARKERS OF FIBROSIS

- ? Platelet derived growth factor (PDGF)

PDGF acts on a variety of cell types including lung fibroblasts, and alters their proliferative and secretory properties^{39,40}

Elevation of serum PDGF levels has been detected in advanced silicosis patients. This, and the fact that PDGF-positive patients have progressed in their disease, indicate that elevated levels of this growth factor may be a marker for the development of severe and progressive silicosis.⁴¹

A diagrammatic representation of the toxicological mechanisms on the pathway from silica dust exposure to silicosis is shown in appendix 6, indicating the associated biomarkers selected for further study.

PROPOSAL FOR PHASE II SILICOSIS BIOMARKERS STUDY

All workshop participants expressed an interest in playing a role in the phase II study. It is apparent that several biomarkers (as discussed above) need to be measured, simultaneously.

Research question

Which of the 10 biomarkers has/have the highest sensitivity and/or specificity in detecting changes in response to silica exposure, or susceptibility to silicosis?

Secondary research questions

1. Is there a difference in the presence/ level of the different biomarkers in silica exposed and unexposed groups?
2. Is there a difference in the presence/ level of the different biomarkers in silica exposed individuals with and without silicosis?
3. Does the level / presence of the different biomarkers change, over time, as silica exposure continues, in both those with and without silicosis at baseline?
4. Does the level / presence of the different biomarkers change, over time, as silica exposure continues, in those who develop silicosis?
5. What is the change in biomarkers, in relation to exposure (measured in quartiles)?
6. How do confounders (e.g. smoking, tuberculosis, altitude) affect biomarkers?

Study design

It was agreed that a prospective cohort study is necessary. Cohort members could be followed up annually for 5 years. Interim analysis could be performed at a fairly early stage. Cross-sectional studies could be conducted at baseline; nested case-control studies could be performed at intervals.

Study subjects

1. New employees – radiologically asymptomatic (non silicotics) with no previous exposure
2. Current and ex-workers – radiologically asymptomatic (non-silicotics)
3. Current and ex-workers – radiologically symptomatic (silicotics)
4. Reference group – radiologically asymptomatic (non silicotics, non miners)

Measurements

The biomarkers will be measured in serum or whole blood. In addition, spirometry, chest radiography and dust sampling will be undertaken.

Implementation

The tests and assays to measure the various biomarkers will be established and validated in South African laboratories. Normal ranges in the South African population will be established. The effects of potential confounders such as smoking and tuberculosis will be assessed. This phase could take from nine to 12 months. Sample sizes will be calculated. Thereafter, the prospective cohort study will be initiated.

PROPOSED STAGES OF THE PHASE II STUDY

Stage 1

Stage 1 will involve the identification of laboratories within South Africa that have the facilities and resources to perform the tests required for this study. Quality assurance will be of paramount importance in selecting laboratories, including appropriate standard operating procedures, normal ranges, calibration of equipment, reporting of results, etc. Costs will also be taken into consideration, as will the practicability of collecting, storing, and transporting the samples.

The methodology will be tested on a sample of appropriate men (i.e. new employees, current employees, and a control group).

Stage 2

Appropriate gold mines, that are willing to participate in the long-term study, will be identified. Baseline levels for each biomarker will be measured in each of the four study subject groups, viz. new employees, current and ex-workers with and without silicosis, and a reference group. These baseline levels will be compared in the four groups (cross-sectional analysis). Other tests to be taken will include spirometry and radiography. All measurements will be correlated with dust measurements. Potential confounders will be included in the analysis.

Stage 3

The number of men required in each study subject group, and the follow up time for the cohort will be determined from the results obtained in stage 2. The cohort will then be established and followed up for the pre determined length of time.

Measurements (biomarker levels, spirometry, radiology, dust levels) will be taken at regular intervals, and interim analyses will be performed.

Stage 4

Final analysis of the results obtained from follow up the cohort will be performed. Nested case-control analyses may also be performed.

TECHNOLOGY TRANSFER

Workshop on biomarkers in occupational health

In March 2003, Dr Mary Gulumian attended an international workshop on "Applying Biomarkers to Occupational Health Practice" in Sante Fe, New Mexico, USA (Appendix 7) where she made contact with other experts in the field of biomarker research and familiarised herself with the latest developments in this field. Her expenses were partially covered by SIMRAC.

SIMRAC presentations

- ? April 2003. SIM 030803 Start-up Presentation. Offices of the Health and Safety Mining Council, Braamfontein.
- ? November 2003. SIM 030803 Progress Report Presentation. Offices of the Health and Safety Mining Council, Braamfontein.
- ? 11 November 2003. SIMRAC Workshop to Seek Research Proposals for Project 03-06-03 Phase 2: The Control of Silicosis in the SA Mining Industry. South African National Museum of Military History, Saxonwold, Johannesburg. Approximately 40 people attended this presentation.

Other Presentations

- ? 28 June to 2 July 2003. Poster presentation. 43rd Conference of the Federation of South African Societies of Pathology, Faculty of Health Sciences Wits Medical School. Approximately 300 delegates attended the conference.
- ? 8 Aug 2003. University of the Witwatersrand Medical School. Introduction to occupational and environmental toxicology biomarkers: lecture to seven post-graduate students, by Dr Gulumian, during a week on "Occupational and Environmental Toxicology" which she coordinated.
- ? 1 September 2003. National Health Laboratory Service, Hillbrow. Seminar on biomarkers in occupational health. Four international collaborators presented topics with an emphasis on silicosis, to occupational health care workers, primarily mine medical officers (approximately 45 people).
- ? 2 to 5 September 2003. Shibula Lodge, Waterberg Game Reserve. Workshop to discuss silicosis biomarker literature and possibility of a phase II study. Twelve people attended, including the four international co-authors and collaborators from the UK, the USA and Germany.

Other

- ? June 2003. Letter to mine medical officers sent via the Mine Medical Officers Association. The letter summarised the objectives of the project and asked interested parties, and those with experience in this field to contact the project leader for more information.
- ? July/August 2003. Notice entitled, Project to identify markers for prediction and early detection of silicosis, published in Occupational Health South Africa (ISSN 1024-6274). Vol 9 (no. 4).

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Appendix 1 References identified pertaining to silicosis biomarkers

A document with the abstracts, where available electronically, can be requested from the investigators.

Libraries:

1. Angiotensin converting enzyme (ACE)
2. Oxidative stress, free radicals, antioxidant enzymes, oxidative damage markers
3. Clara cells and CC16
4. CA19-9
5. Apoptosis, Fas, sFas, FasL
6. C-reactive Protein and Sialic acid
7. Elastin
8. Growth Factors
9. Review on Biomarkers in silicosis and Occupational Diseases
10. IL-1
11. IFN-?
12. Mechanism
13. General
14. PIIIP (Procollagen type III peptide) and Type I Procollagen
15. T-Lymphocytes
16. TNF-?
17. HLA and Immunoglobulins
18. Kinin aminopeptidase
19. Arachidonic acid Metabolites
20. Alkaline phosphatase
21. Alveolitis/Inflammation
22. Fibronectin
23. Haptoglobins
24. Lymphotoxin-?
25. Neopterin
26. Unclassified

Angiotensin Converting Enzyme (ACE)

No	Author	Year	Title	Source	Vol	Issue	Pages
1.	Ashutosh, K et al	1976	Diagnostic value of serum Angiotensin converting enzyme activity in lung diseases.	Thorax	31		552-557
2.	Battesti, JP, D. Sandron, et al.	1982	[Characteristics, assay and semeiologic value of angiotensin converting enzyme ACE].	Ann Biol Clin	40	3	199-203
3.	Beneteau-Burnat BB et al	1991	Angiotensin-converting enzyme clinical applications and laboratory investigations on serum and other biologicals fluids.	Crit Rev Clin Lab Sci	28	5-6	337-356
4.	Brice, E. A., W. Friedlander, et al.	1995	Serum angiotensin-converting enzyme activity, concentration, and specific activity in granulomatous interstitial lung disease, tuberculosis, and COPD.	Chest	107	3	706-10.
5.	Brown, R. C., D. E. Munday, et al.	1983	Angiotensin converting enzyme in the serum of rats with experimental silicosis.	Br J Exp Pathol	64	3	286-92.
6.	Bucca, C., F. Veglio, et al.	1984	Serum angiotensin converting enzyme ACE in silicosis.	Eur J Respir Dis	65	7	477-80.
7.	Calabro, S., G. Arcangeli, et al.	1990	[The evaluation of serum angiotensin-converting enzyme in silicosis and silicotuberculosis].	Med Lav	81	4	283-9.
8.	D'Andrea, F., C. Lechi, et al.	1979	[Behavior of plasma ACE angiotensin-converting enzyme in pulmonary silicosis].	Med Lav	70	5	369-74.

9.	Fernandez Jorge, M. A, E. Alonso Mallo	1994	[Angiotensin-converting enzyme ACE in sarcoidosis, tuberculosis, silicosis, and coal mining workers].	An Med Interna	11	12	588-90.
10.	Gronhagen-Riska, C	1979	Angiotensin-converting enzyme. I. Activity and correlation with serum lysozyme in sarcoidosis, other chest or lymph node diseases and healthy persons.	Scand J Respir Dis	60	2	83-93.
11.	Gronhagen-Riska, C., K. Kurppa, et al.	1978	Angiotensin-converting enzyme and lysozyme in silicosis and asbestosis.	Scand J Respir Dis	59	4	228-31.
12.	Hiwada, K et al	1987	Direct Radioimmunoassay of Angiotensin-converting enzyme in sera from patients with pulmonary diseases.	Lung	165		27-35
13.	Lin, J.	1990	[Studies on serum angiotensin-I-converting enzyme activity of experimental silicosis in rats].	Zhonghua Yu Fang Yi Xue Za Zhi	24	5	274-6.
14.	Nordman, H., H. Koskinen, et al.	1984	Increased activity of serum angiotensin-converting enzyme in progressive silicosis.	Chest	86	2	203-7.
15.	Rohatgi, PK	1982	Serum Angiotensin converting enzyme in pulmonary disease.	Lung	160		287-301
16.	Romano, C., F. Sulotto, et al.	1985	Serum angiotensin-converting enzyme level in silicosis.	Med Lav	76	5	366-70.
17.	Romer, F. K.	1985	Angiotensin-converting enzyme activity in sarcoidosis and other disorders.	Sarcoidosis	2	1	25-34.
18.	Serbescu, A. and E. Paunescu	1992	[The importance of assessing angiotensin-converting activity in silicosis patients].	Pneumoftiziologia	41	1	17-20.
19.	Shi, Z. C.	1986	[Serum angiotensin-converting enzyme, ceruloplasmin and lactic dehydrogenase in anthracosilicosis and anthracosilico-tuberculosis].	Zhonghua Jie He He Hu Xi Xi Ji Bing Za Zhi	9	1	16-8, 61-2.
20.	Szechinski, J., A. Skoczynska, et al.	1986	Serum angiotensin-converting enzyme levels in patients with silicosis.	J Toxicol Environ Health	17	1	73-9.
21.	Thompson, A. B., W. F. Cale, et al.	1991	Serum angiotensin-converting enzyme is elevated in association with underground coal mining.	Chest	100	4	1042-5.
22.	Wallaert et al	1985	Letter to the editor: Serum Angiotensin-converting enzyme in coal worker's pneumoconiosis.	Chest	87		844-845
23.	Yano, E., K. Takeuchi, et al.	1987	Serum angiotensin converting enzyme activity in silicosis.	Ind Health	25	1	11-8.
24.	Zhicheng S et al	1986	Serum angiotensin converting enzyme, ceruloplasmin, and lactic dehydrogenase in anthracosilicosis	Br J Indust Med	43		642-643
25.	Zhu, D. S.	1984	[Measurement of angiotensin-converting enzyme in lung diseases].	Zhonghua Jie He He Hu Xi Xi Ji Bing Za Zhi	7	1	15-7.

Oxidative Stress, Free Radicals, Antioxidant Enzymes, Oxidative Damage Markers

No	Author	Year	Title	Source	Vol	Issue	Pages
26.	Barett EG et al.	1999	Antioxidant treatment attenuates cytokine and chemokine levels in murine macrophages following silica exposure.	Toxicol Appl Pharmacol	158		211-220
27.	Borm PJA et al	1986	Red blood cell anti-oxidant parameters in silicosis.	Int Arch Occup Environ Health	58		235-244
28.	Borm, P. J., A. Bast, et al.	1987	Red blood cell anti-oxidant parameters in healthy elderly subjects versus silicosis patients.	Free Radic Res Commun	3	1-5	117-27.
29.	Castranova V et al	?	Oxidant release from pulmonary phagocytes.	In: Silica and silica-induced Lung Diseases			185-195

30.	Engelen JJM, Borm PJA, et al	1990	Blood Ant-Oxidant parameters at different stages of pneumoconiosis in coal workers	EHP	84		165-172
31.	Evelo, C. T., R. P. Bos, et al.	1993	Decreased glutathione content and glutathione S-transferase activity in red blood cells of coal miners with early stages of pneumoconiosis.	Br J Ind Med	50	7	633-6
32.	Ghio AJ et al.	1990	Hypothesis: is lung disease after silicate inhalation caused by oxidant generation.	Lancet	336		967-969
33.	Goodman GB et al.	1992	Acute silicosis responding to corticosteroid therapy	Chest	101	2	366-370
34.	Gossart S et al.	1996	Reactive oxygen intermediates as regulators of TNF- α production in rat lung inflammation induced by silica.				
35.	Hubbard, A. K., C. R. Timblin, et al.	2002	Activation of NF-kappaB-dependent gene expression by silica in lungs of luciferase reporter mice.	Am J Physiol Lung Cell Mol Physiol	282	5	L968-75.
36.	Jesch NK, Dörger M, et al	1997	Expression of iNOS and formation of NO by alveolar macrophages: an interspecies comparison	Env Health Persp	105		1297-1300
37.	Kang, J. L., Y. H. Go, et al.	2000	Silica-induced nuclear factor-kappaB activation involvement of reactive oxygen species and protein tyrosine kinase activation.	J Toxicol Environ Health	60	1	27-46.
38.	Maly, E. R.	1988	Generation of free oxygen radicals from human polymorphonuclear granulocytes by cytokines from human mononuclear cells, treated with quartz dust DQ12 or coal mine dust TF-1--new aspects in pathogenesis of pneumoconiosis.	Zentralbl Bakteriол Mikrobiol Hyg [B]	187	2	142-65.
39.	Perrin-Nadif et al	1996		Occup Environ Med	53		41-45
40.	Perrin-Nadif, R., J. M. Porcher, et al.	1998	Erythrocyte antioxidant enzyme activities in coal miners from three French regions.	Int Arch Occup Environ Health	71	4	257-62.
41.	Pilger, A., D. Gemadnik, et al.	2000	8-Hydroxydeoxyguanosine in leukocyte DNA and urine of quartz-exposed workers and patients with silicosis.	Int Arch Occup Environ Health	73	5	305-10.
42.	Rom, W. N., P. B. Bitterman, et al.	1987	Characterization of the lower respiratory tract inflammation of nonsmoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts.	Am Rev Respir Dis	136	6	1429-34.
43.	Schins RPF et al	1994	Oxidative DNA damage in peripheral blood lymphocytes of coal workers	In: Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			57-64
44.	Schins, R. and e. a. Derhaag T	1994	Serum total radical-trapping antioxidant parameter (trap) in coal workers.	In Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			53 - 56
45.	Schins, R., K. S, et al.	1996	Blood antioxidant status in coal dust induced respiratory disorders: a longitudinal evaluation of multiple biomarkers.	In Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			65 - 75.
46.	Voisin, Wallaert, et al	1984	Oxidant and anti-oxidant activities of alveolar macrophages in sarcoidosis. and pneumoconiosis	In Mucosal Immunity IgA and PMN, Paris,			220-233
47.	Wallaert B et al	1990	Superoxide anion generation by aleolar inflammatory cells in simple pneumoconiosis and in progressive massive fibrosis of nonsmoking coal workers.	Am rev respir Disease	141		129-133

Clara Cells and CC16

No	Author	Year	Title	Source	Vol	Issue	Pages
48.	Albrecht C et al	2001	Clara-cell hyperplasia after quartz and coal-dust instillation in rta lung.	Inhal Toxicol	13	3	191-205
49.	Bernard A	1998	The Clara Cell Protein, CC16: A Biomarker of Pulmonary Toxicity	In: Biomarkers: Medical and Workplace Applications			273-83
50.	Bernard, A. M., J. M. Gonzalez-Lorenzo, et al.	1994	Early decrease of serum Clara cell protein in silica-exposed workers.	Eur Respir J	7	11	1932-7

CA19-9

No	Author	Year	Title	Source	Vol	Issue	Pages
51.	Totani, Y., Y. Demura, et al.	2000	[Silicosis characterized by increasing serum CA 19-9 in parallel with progression of lung fibrosis].	Nihon Kokyuki Gakkai Zasshi	38	2	137-42.

Apoptosis, Fas, sFas, FasL

No	Author	Year	Title	Source	Vol	Issue	Pages
52.	Borges, V. M., H. Falcao, et al.	2001	Fas ligand triggers pulmonary silicosis.	J Exp Med	194	2	155-64.
53.	Iyer, R., R. F. Hamilton, et al.	1996	Silica-induced apoptosis mediated via scavenger receptor in human alveolar macrophages.	Toxicol Appl Pharmacol	141	1	84-92.
54.	Lim, Y., J. H. Kim, et al.	1999	Silica-induced apoptosis in vitro and in vivo.	Toxicol Lett	108	2-3	335-9.
55.	Otsuki, T., H. Sakaguchi, et al.	2000	Detection of alternatively spliced variant messages of Fas gene and mutational screening of Fas and Fas ligand coding regions in peripheral blood mononuclear cells derived from silicosis patients.	Immunol Lett	72	2	137-43.
56.	Otsuki, T., H. Sakaguchi, et al.	1998	Soluble Fas mRNA is dominantly expressed in cases with silicosis.	Immunology	94	2	258-62.
57.	Otsuki, T., K. Ichihara, et al.	1999	Evaluation of cases with silicosis using the parameters related to Fas-mediated apoptosis.	Int J Mol Med	4	4	407-11.
58.	Tomokuni, A., T. Aikoh, et al.	1997	Elevated soluble Fas/APO-1 CD95 levels in silicosis patients without clinical symptoms of autoimmune diseases or malignant tumours.	Clin Exp Immunol	110	2	303-9.
59.	Tomokuni, A., T. Otsuki, et al.	1999	Serum levels of soluble Fas ligand in patients with silicosis.	Clin Exp Immunol	118	3	441-4.
60.	Ueki, A.	2000	[Fas-Fas ligand system in silicosis patients].	Nippon Eiseigaku Zasshi	55	2	474-80.

C-reactive Protein and Sialic acid

No	Author	Year	Title	Source	Vol	Issue	Pages
61.	Cojocaru, M.	1997	Relationship between serum total sialic acid and C-reactive protein in silicosis.	Rom J Intern Med	35	1-4	77-82.

Elastin

No	Author	Year	Title	Source	Vol	Issue	Pages
62.	Mariani, T. J., E. Crouch, et al.	1995	Increased elastin production in experimental granulomatous lung disease.	Am J Pathol	147	4	988-1000.
63.	Mariani, T. J., M. C. Arian, et al.	1999	Fibroblast tropoelastin and alpha-smooth-muscle actin expression are repressed by particulate-activated macrophage-derived tumor necrosis factor-alpha in experimental silicosis.	Am J Respir Cell Mol Biol	21	2	185-92.

Growth Factors

No	Author	Year	Title	Source	Vol	Issue	Pages
64.	Absher M M. Sjostrand, et al.	1993	Patterns of secretion of transforming growth factor-alpha TGF-alpha in experimental silicosis. Acute and subacute effects of cristobalite exposure in the rat.	Reg Immunol	5	3-4	225-31.
65.	Brandt-Rauf, P. W., S. Smith, et al.	1992	Serum oncoproteins and growth factors in asbestosis and silicosis patients.	Int J Cancer	50	6	881-5.
66.	Chen, F., H. Y. Deng, et al.	1994	Excessive production of insulin-like growth factor-I by silicotic rat alveolar macrophages.	Apmis	102	8	581-8.
67.	Guoping, C., P. Fan, et al.	1997	Purification and characterization of a silica-induced bronchoalveolar lavage protein with fibroblast growth-promoting activity.	J Cell Biochem	67	2	257-64.
68.	Hamada, H. V. Vallyathan et al.	2000	Mast cell basic fibroblast growth factor in silicosis.	Am J Respir Crit Care Med	161	6	2026-34.
69.	Jagirdar, J., R. Begin, et al.	1996	Transforming growth factor-beta TGF-beta in silicosis.	Am J Respir Crit Care Med	154	4 Pt 1	1076-81.
70.	Kumar, R. K., G. M. Velan, et al.	1994	Epidermal growth factor-like activity in bronchoalveolar lavage fluid in experimental silicosis.	Growth Factors	10	3	163-70.
71.	Lesur, O., B. Melloni, et al.	1992	Silica-exposed lung fluids have a proliferative activity for type II epithelial cells: a study on human and sheep alveolar fluids.	Exp Lung Res	18	5	633-54.
72.	Liu, K., R. Situ, et al.	1995	[The effect of anti basic fibroblast growth factor on the development of experimental silicosis bacillus].	Zhonghua Jie He He Hu Xi Za Zhi	18	6	351-3, 383.
73.	Melloni, B., O. Lesur, et al.	1994	Partial characterization of the proliferative activity for fetal lung epithelial cells produced by silica-exposed alveolar macrophages.	J Leukoc Biol	55	5	574-80.
74.	Ohta, K., J. Nakano, et al.	1997	Suppressive effect of antisense DNA of platelet-derived growth factor on murine pulmonary fibrosis with silica particles.	Chest	111	6 Suppl	105S.
75.	Olbruck, H., N. H. Seemayer, et al.	1998	Supernatants from quartz dust treated human macrophages stimulate cell proliferation of different human lung cells as well as collagen-synthesis of human diploid lung fibroblasts in vitro.	Toxicol Lett	96-97		85-95.
76.	Williams, A. O. and U. Saffiotti	1995	Transforming growth factor beta1, ras and p53 in silica-induced fibrogenesis and carcinogenesis.	Scand J Work Environ Health	21	Suppl 2	30-4.
77.	Williams, A. O., K. C. Flanders, et al.	1993	Immunohistochemical localization of transforming growth factor-beta 1 in rats with experimental silicosis, alveolar type II hyperplasia, and lung cancer.	Am J Pathol	142	6	1831-40.

Review on Biomarkers in silicosis and Occupational Diseases

No	Author	Year	Title	Source	Vol	Issue	Pages
78.	Borm, P. J.	1994	Biological markers and occupational lung disease: mineral dust-induced respiratory disorders.	Exp Lung Res	20	5	457-70
79.	Borm, P. J.	2002	Particle toxicology: from coal mining to nanotechnology.	Inhal Toxicol	14	3	311-24

80.	Borm, P. J. and R. P. Schins	2001	Genotype and phenotype in susceptibility to coal workers' pneumoconiosis. The use of cytokines in perspective.	Eur Respir J Suppl	32		127s-133s
81.	CDC, Department of Health and Human Services, NIOSH	2002	Biomarkers	In: Health Effects of Occupational Exposure to respirable Crystalline Silica	DHH S 9NI OSH) Publication No 2002 -129		80-88
82.	Schins R	1995	Summary and General Discussion	Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			145-154
83.	Schins, R. P. and P. J. Borm	1999	Mechanisms and mediators in coal dust induced toxicity: a review.	Ann Occup Hyg	43	1	7-33.
84.	Spitsyn, V. A., S. V. Makarov, et al.	2000	[Genetic polymorphism and occupational diseases results of 10-years studies].	Vestn Ross Akad Med Nauk	5		27-32.
85.	Vanhée D et al.	1995	Cytokines and cytokine network in silicosis and coal worker's pneumoconiosis	Eur Respir J	8		834-842

IL-1

No	Author	Year	Title	Source	Vol	Issue	Pages
86.	Huau, F., J. Louahed, et al.	1998	Role of interleukin-10 in the lung response to silica in mice.	Am J Respir Cell Mol Biol	18	1	51-9.
87.	Schmidt, J. A., C. N. Oliver, et al.	1984	Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1. A potential role for interleukin 1 in the pathogenesis of silicosis.	J Clin Invest	73	5	1462-72.
88.	Srivastava K et al.	2002	Crucial role of interleukin-1? and nitric oxide synthase in silica-induced inflammation and apoptosis in mice.	Respire Crit Care Med	165	4	527-533
89.	Struhar, D. and R. J. Harbeck	1989	Anti-Ia antibodies inhibit the spontaneous secretion of IL-1 from silicotic rat alveolar macrophages.	Immunol Lett	23	1	31-3.
90.	Struhar, D. J., R. J. Harbeck, et al.	1989	Increased expression of class II antigens of the major histocompatibility complex on alveolar macrophages and alveolar type II cells and interleukin-1 IL-1 secretion from alveolar macrophages in an animal model of silicosis.	Clin Exp Immunol	77	2	281-4.
91.	Yucesoy, B., V. Vallyathan, et al.	2001	Polymorphisms of the IL-1 gene complex in coal miners with silicosis.	Am J Ind Med	39	3	286-91.
92.	Yucesoy, B., V. Vallyathan, et al.	2002	Cytokine polymorphisms in silicosis and other pneumoconioses.	Mol Cell Biochem	234-235	1-2	219-24.

IFN-?

No	Author	Year	Title	Source	Vol	Issue	Pages
93.	Davis, G. S., CE. Holmes, et al.	2001	Lymphocytes, lymphokines, and silicosis.	J Environ Pathol Toxicol Oncol	20	Suppl 1	53-65.
94.	Davis, G. S., L. M. Pfeiffer, et al.	1999	Expansion of interferon-gamma-producing lung lymphocytes in mouse silicosis.	Am J Respir Cell Mol Biol	20	4	813-24.

95.	Davis, G. S., L. M. Pfeiffer, et al.	2000	Interferon-gamma production by specific lung lymphocyte phenotypes in silicosis in mice.	Am J Respir Cell Mol Biol	22	4	491-501.
96.	Li, W., R. K. Kumar, et al.	1992	Role of lymphocytes in silicosis regulation of secretion of macrophage- derived mitogenic activity for fibroblasts.	Int J Exp Pathol	73	6	793-800.

Mechanism

No	Author	Year	Title	Source	Vol	Issue	Pages
97.	Arcangeli G V, Cupelli, et al.	2001	Effects of silica on human lung fibroblast in culture	Sci Total Environ	270	1-3	135-9.
98.	Barrett, EG, C. Johnston, et al.	1999	Antioxidant treatment attenuates cytokine and chemokine levels in murine macrophages following silica exposure.	Toxicol Appl Pharmacol	158	3	211-20
99.	Castranova, V. and V. Vallyathan	2000	Silicosis and coal workers' pneumoconiosis.	Environ Health Perspect	108	Suppl 4	675-84.
100.	Castranova, V., D. Porter, et al.	2002	Effect of inhaled crystalline silica in a rat model time course of pulmonary reactions.	Mol Cell Biochem	234-235	1-2	177-84
101.	Ding, M., F. Chen, et al.	2002	Diseases caused by silica mechanisms of injury and disease development.	Int Immunopharmacol	2	2-3	173-82.
102.	Driscoll KE	1995	The toxicology of crystalline silica studied <i>in vitro</i>	Appl Occup Environ	10		1118-1125
103.	Ghio, A. J., T.P Kennedy et al.	1990	Hypothesis is lung disease after silicate inhalation caused by oxidant generation?	Lancet	336	8721	967-9.
104.	Lapp, N. L. and V. Castranova	1993	How silicosis and coal workers' pneumoconiosis develop--a cellular assessment.	Occup Med	8	1	35-56.
105.	Piguet, P. F.	1993	Cytokines involved in pulmonary fibrosis.	Int Rev Exp Pathol	34	Pt B	173-81.
106.	Rojanasakul, Y., J. Ye, et al.	1999	Dependence of NF-kappaB activation and free radical generation on silica-induced TNF-alpha production in macrophages.	Mol Cell Biochem	200	1-2	119-25.
107.	Schins R	1966	Design of the studies	Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			47-50
108.	Schins, R.	1996	Coal dust induced lung disorders: Mechanisms	Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers.: PhD Thesis.			25-45

General

No	Author	Year	Title	Source	Vol	Issue	Pages
109.	Aitio, A	1999	Biomarkers and their use in occupational medicine	Scand J Work Environ Health	25	6	521-8
110.	Bennet DA and Waters MD	2000	Applying biomarker research.	Environ Health Perspect	108	9	907-910

111.	Bingham E	1998	Ethical issues of genetic testing for workers	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			415-422
112.	Cantor CR et al	1995	Implications of large scale DNA analysis for the development and application of biomarkers.	Biomarkers and Occupational Health: Progress and Perspectives. Mendelsohn ML et al Eds. Joseph Henry Press: Washington DC.			257-263
113.	Committee	1987	Biological markers in environmental health research. Committee on Biological Markers of the National Research Council	Environ Health Perspect	74		3-9
114.	Gochfeld M	1998	Susceptibility biomarkers in the workplace: Historical perspective.	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			3-22
115.	Grandjean, P.	1995	Biomarkers in epidemiology	Clin Chem	41	12 Pt 2	1800-3
116.	Grandjean, P., S. S. Brown, et al	1995	Biomarkers in environmental toxicology: state of the art	Clin Chem.	41	12 Pt 2	1902-4
117.	Harrison MC	1998	Implications of genetic testing for medical examinations in the workplace	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			387-395
118.	Henderson RG	1998	Finding a biomarker is a first step	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			33-39
119.	Howe GR	1998	Practical uses of biomarkers in population studies	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington			41-49
120.	Hulka, B. S. and T. Wilcosky	1988	Biological markers in epidemiologic research	Arch Environ Health	43	2	83-89
121.	Keman, S.	1997	Biomarkers of chronic non-specific airway diseases. An application of molecular epidemiology in occupational settings.	Keman PhD Thesis			
122.	Keman, S., J. M, et al.	1997	Relation between inflammatory markers in serum and nasal lavage.	In Keman 1997 PhD Thesis			99 – 103

123.	Kernan, S., S. R, et al.	1997	Blood cytokines in coal dust induced respiratory disorders.	In Kernan 1997 PhD Thesis			73 – 84
124.	Kreps, S. E., N. Banzet, et al	1997	Molecular biomarkers of early responses to environmental stressors: implications for risk assessment and public health	Rev Environ Health	12	4	261-80
125.	Maltby L	1995	Biomarkers: the down side.	Biomarkers and Occupational Health: Progress and Perspectives. Mendelsohn ML et al Eds. Joseph Henry Press: Washington DC.			52-57
126.	McCunney RT		Use of biomarkers in Occupational Medicine	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			377-386
127.	Merlo, F	2002	3rd International Symposium on Silica, Cancer and Other Diseases, Italy	La Medicina del Lavoro			
128.	Rom WN	1995	Biomedical research ethics related to biomarkers.	Biomarkers and Occupational Health: Progress and Perspectives. Mendelsohn ML et al Eds. Joseph Henry Press: Washington DC.			48-51
129.	Schulte PA	1995		Biomarkers and Occupational Health: Progress and Perspectives. Mendelsohn ML et al Eds. Joseph Henry Press: Washington DC.			1-6
130.	Schulte PA and Rothman N	1998	Epidemiological validation of biomarkers of early biological effect and susceptibility.	In: Biomarkers: Medical and Workplace Applications. Mendelsohn et al eds. Joseph Henry Press: Washington DC			23-32
131.	Trosko JE	1995	Epigenetic biomarkers: Potentials and limitations	In: Biomarkers and Occupational Health: Progress and Perspectives. Mendelsohn ML et al Eds. Joseph Henry Press: Washington DC.			264-274

PIIIP (Procollagen type III peptide) and Type I Procollagen

No	Author	Year	Title	Source	Vol	Issue	Pages
132.	Janssen, Y. M., J. J. Engelen, et al.	1992	Serum type III procollagen N-terminal peptide in coal miners.	Exp Lung Res	18	1	1-8
133.	Jorna THJM, Borm PJA, et al	1994	Respiratory effects and serum type III procollagen in potato sorters exposed to diatomaceous earth	Int Arch occup Environ Health	66		217-222
134.	Mariani, T. J., J. D. Roby, et al.	1996	Localization of type I procollagen gene expression in silica-induced granulomatous lung disease and implication of transforming growth factor-beta as a mediator of fibrosis.	Am J Pathol	148	1	151-64.
135.	Schins, R. P. and P. J. Borm	1994	Serum procollagen type III peptide in coal workers' pneumoconiosis: a five year follow-up study.	Exp Lung Res	20	5	445-55
136.	Schins, R. P., R. J. Lamers, et al.	1995	Evaluation of serum type III procollagen peptide as an exposure marker in retired coal workers.	Int Arch Occup Environ Health	66	6	413-9.

T-Lymphocytes

No	Author	Year	Title	Source	Vol	Issue	Pages
137.	Davis, G. S., K. O. Leslie, et al.	1993	Altered patterns of lung lymphocyte accumulation in silicosis in cytokine-sufficient C3H/HeN and cytokine-deficient C3H/HeJ-LPSd mice.	Chest	103	2 Suppl	120S-121S.
138.	Garn, H., A. Friedetzky et al.	1997	T-lymphocyte activation in the enlarged thoracic lymph nodes of rats with silicosis.	Am J Respir Cell Mol Biol	16	3	309-16.
139.	Hubbard, A. K.	1989	Role for T lymphocytes in silica-induced pulmonary inflammation.	Lab Invest	61	1	46-52.
140.	Surcel, D., A. Ossian, et al.	1991	[The effect of smoking on immunological parameters in silicosis].	Pneumoftiziologia	40	1	47-50.
141.	Watanabe S et al	1987	Alterations in lymphocyte subsets and serum immunoglobulin levels in patients with silicosis.	J Clin Lab Immunol	23		45-51

TNF-?

No	Author	Year	Title	Source	Vol	Issue	Pages
142.	Bissonnette, E. and M. Rola-Pleszczynski	1989	Pulmonary inflammation and fibrosis in a murine model of asbestosis and silicosis. Possible role of tumor necrosis factor.	Inflammation	13	3	329-39
143.	Borm PJA, Palmen N, et al	1988		Am Rev Respir Dis	138		1589-1594
144.	Corbett, E. L., N. Mozzato-Chamay, et al.	2002	Poly morphisms in the tumor necrosis factor-alpha gene promoter may predispose to severe silicosis in black South African miners.	Am J Respir Crit Care Med	165	5	690-3.
145.	Davis, G. S., L. M. Pfeiffer, et al.	1998	Persistent overexpression of interleukin-1beta and tumor necrosis factor-alpha in murine silicosis.	J Environ Pathol Toxicol Oncol	17	2	99-114.
146.	Gossart, S., C. Cambon, et al.	1996	Reactive oxygen intermediates as regulators of TNF-alpha production in rat lung inflammation induced by silica.	J Immunol	156	4	1540-8.
147.	Hadnagy W, Idel H	1998	Role of soluble Tumor necrosis factor receptor in TNFa mediated pathogenesis of mineral dust induced pneumoconiosis	In relationships between respiratory disease and exposure to air pollution (Mohr, U, editor-in-chief), ILSI Monographs, Washington DC			334-337
148.	Jorna THJM, Schins RPF, et al	1994	Airflow obstruction and monocyte TNF release in coal workers.	Exp Lung Res	20		421-431

149.	Keman S et al	1997	Blood cytokines in coal dust induced respiratory disorders.	In: Biomarkers of chronic non-specific airway diseases. Phd Thesis			73-84
150.	Keman S, et al	1997	Blood interleukin-8 production is increased in chemical workers with bronchitic symptoms.	Am J Ind Med	32		670-673
151.	Kim KA, et al	1999	Potential biomarker of coal workers' pneumoconiosis	Toxicology Letters	108		297-302
152.	Lee, H. G., I. Choi, et al.	1995	Peritoneal lavage fluids stimulate NIH3T3 fibroblast proliferation and contain increased tumour necrosis factor and IL-6 in experimental silica-induced rat peritonitis.	Clin Exp Immunol	100	1	139-44.
153.	Lim Y et al	1998	The measurement of IL-1, IL-8, TNF for the diagnosis of pneumoconiosis.	In: advances in the prevention of occupational respiratory disease, Elsevier Amsterdam.			845-848
154.	Mohr, C., D. Gemsa, et al.	1991	Systemic macrophage stimulation in rats with silicosis enhanced release of tumor necrosis factor-alpha from alveolar and peritoneal macrophages.	Am J Respir Cell Mol Biol	5	4	395-402.
155.	Morfeld, P. and e. a. Borm PJA	2001	Cross sectional study on cytokine production (TNF-alfa, IL-8) in German coal miners with progressive massive fibrosis and in control miners using a rapid whole-blood assay.	Biomarkers	6	6	428 – 439
156.	Ohtsuka, Y., M. Munakata, et al.	1995	Increased susceptibility to silicosis and TNF-alpha production in C57BL/6J mice.	Am J Respir Crit Care Med	152	6 Pt 1	2144-9.
157.	Orfila, C., J. C. Lepert, et al.	1998	Immunocytochemical characterization of lung macrophage surface phenotypes and expression of cytokines in acute experimental silicosis in mice.	Histochem J	30	12	857-67.
158.	Ortiz, L. A., J. Lasky, et al.	2001	Tumor necrosis factor receptor deficiency alters matrix metalloproteinase 13/tissue inhibitor of metalloproteinase 1 expression in murine silicosis.	Am J Respir Crit Care Med	163	1	244-52.
159.	Piguet, P. F.	1990	Is tumor necrosis factor the major effector of pulmonary fibrosis?	Eur Cytokine Netw	1	4	257-8.
160.	Piguet, P. F. and C. Vesin	1994	Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice.	Eur Respir J	7	3	515-8.
161.	Piguet, P. F., G. E. Grau, et al.	1991	Tumor necrosis factor and immunopathology.	Immunol Res	10	2	122-40.
162.	Piguet, P. F., M. A. Collart, et al.	1990	Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis.	Nature	344	6263	245-7.
163.	Porcher JM, Oberson D, et al	1994	Evaluation of Tumor Necrosis Factor-Alpha (TNF) as an exposure or risk marker in three French coal mining regions.	Exp Lung Res	20		433-443
164.	Rom WN	1991	Relationship of inflammatory cell cytokines to disease severity in individuals with occupational dust exposure	Am J Industr Med	19		15-27
165.	Savici, D., B. He, et al.	1994	Silica increases tumor necrosis factor TNF production, in part, by upregulating the TNF promoter.	Exp Lung Res	20	6	613-25.
166.	Schins, R. P. and P. J. Borm	1995	Plasma levels of soluble tumour necrosis factor receptors are increased in coal miners with pneumoconiosis.	Eur Respir J	8	10	1658-63.
167.	Schins, R. P. and P. J. Borm	1995	Epidemiological evaluation of release of monocyte TNF-alpha as an exposure and effect marker in pneumoconiosis: a five year follow up study of coal workers.	Occup Environ Med	52	7	441-50.

168.	Schins, R., G. P, et al.	1996	Multiple cytokines as biomarkers in coal dust exposure and pneumoconiosis: TNF-alfa, IL-6, TGF-beta.	Biomarkers of Mineral Dust Induced Lung Disorders. Molecular epidemiologic studies in coal workers. PhD Thesis.			109 - 119.
169.	Vanhee D et al	1995		Am J Respir Crit Care Med	152		298-306
170.	Yucesoy, B., V. Vallyathan, et al.	2001	Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis.	Toxicol Appl Pharmacol	172	1	75-82.
171.	Zhai et al	1998		Am J Industr Med	34		318-324

HLA and Immunoglobulins

No	Author	Year	Title	Source	Vol	Issue	Pages
172.	Callhoun WJ et al	1986	Raised immunoglobulin concentrations in bronchoalveolar lavage fluid of healthy granite workers.	Thorax	41		266-273
173.	Gáliková E	1982	Immunoglobulin levels in colliers and miners in central Slovakia.	Prac Lék	34	3	83-85
174.	Gulade N et al	1977	HLA and silicosis.	Am Rev Respir Dis	116		334-336
175.	Honda K et al.	1993	Immunogenetic analysis of silicosis in Japan.	Am J Respir Cell Mol Biol	8	1	106-111
176.	Karnik AB et al	1990	Humoral immunoglobulin dysfunction in silicosis.	Indian J Med Res [B]	92		440-442
177.	Koskinen H et al	1983	Increased prevalence of HLA-Aw19 and of the phenogroup Aw19, B18 in advanced silicosis.	Chest	83	6	848-852
178.	Kreiss K et al.	1989	Histocompatibility antigens in a population based silicosis series.	Br J Ind Med	46		364-369
179.	Pevnitskiy LA et al.	1978	Some immunogenetic indexes in silicosis.	Gig Trud Prof Zabol		5	52-54
180.	Polzik, E. V., M. Kochneva, et al.	1988	[Antigens of the HLA system and silicosis].	Gig Tr Prof Zabol		8	28-31.
181.	Sluis-Cremer GK and Maier g	1984	HLA antigens of the A and B locus in relation to the development of silicosis.	Br J Ind Med	41		417-418

Kinin aminopeptidase

No	Author	Year	Title	Source	Vol	Issue	Pages
182.	Szechinski, J.	1986	[Kinin aminopeptidase in lung tissue and activity of kininase II in the serum of patients with silicosis].	Pol Tyg Lek	41	19	635-7.

Arachidonic acid Metabolites

No	Author	Year	Title	Source	Vol	Issue	Pages
183.	Bissonnette, E., B. Carre, et al.	1990	Inhibition of alveolar macrophage cytotoxicity by asbestos possible role of prostaglandins.	J Leukoc Biol	47	2	129-34.
184.	Mariani, T. J., S. Sandefur, et al.	1998	Collagenase-3 induction in rat lung fibroblasts requires the combined effects of tumor necrosis factor-alpha and 12-lipoxygenase metabolites a model of macrophage-induced, fibroblast-driven extracellular matrix remodeling during inflammatory lung injury.	Mol Biol Cell	9	6	1411-24.
185.	Mohr, C., G. S. Davis, et al.	1992	Reduced release of leukotrienes B4 and C4 from alveolar macrophages of rats with silicosis.	Am J Respir Cell Mol Biol	7	5	542-7.

186.	Mohr, C., G. S. Davis, et al.	1992	Enhanced release of prostaglandin E2 from macrophages of rats with silicosis.	Am J Respir Cell Mol Biol	6	4	390-6.
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Alkaline phosphatase

No	Author	Year	Title	Source	Vol	Issue	Pages
187.	Capelli, A., M. Lusuardi, et al.	1997	Lung alkaline phosphatase as a marker of fibrosis in chronic interstitial disorders.	Am J Respir Crit Care Med	155	1	249-53.

Alveolitis/Inflammation

No	Author	Year	Title	Source	Vol	Issue	Pages
188.	Begin, R. O., A. M. Cantin, et al.	1987	Spectrum of alveolitis in quartz-exposed human subjects.	Chest	92	6	1061-7
189.	Donaldson, K., G. M. Brown, et al.	1992	Epithelial and extracellular matrix injury in quartz-inflamed lung role of the alveolar macrophage.	Environ Health Perspect	97		221-4.
190.	Rom WN et al.	1987	Characterization of the lower respiratory tract inflammation of non-smoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts	Am Rev Respir Dis	136		1429-1434
191.	Sjostrand, M., P. M. Absher, et al.	1991	Comparison of lung alveolar and tissue cells in silica-induced inflammation.	Am Rev Respir Dis	143	1	47-52.

Fibronectin

No	Author	Year	Title	Source	Vol	Issue	Pages
192.	Zhestkov, A. V.	2000	[Immunological changes in dust-induced lung diseases].	Gig Sanit		6	30-3.

Haptoglobins

No	Author	Year	Title	Source	Vol	Issue	Pages
193.	Morozova, O. A., A. Gorbatsovskii la, et al.	2001	[Hereditary polymorphism features in patients with silicosis associated with chronic bronchitis].	Med Tr Prom Ekol	4		9-13.

Lymphotoxin-?

No	Author	Year	Title	Source	Vol	Issue	Pages
194.	Nadif R et al.	2003	Effect of TNF and LTA polymorphism on biological markers of response to oxidative stimuli in coal miners: a model of gene-environment interaction. Tumour necrosis factor and lymphotoxin alpha.	J Med Genet	40	2	96-103

Neopterin

No	Author	Year	Title	Source	Vol	Issue	Pages
195.	Altindag ZZ et al	2003	Neopterin as a new biomarker for the evaluation of occupational exposure to silica.	Int Arch Occup Environ Health	76		318-322

Unclassified

No	Author	Year	Title	Source	Vol	Issue	Pages
196.	Izmerov NF et al.	2002	Genetic-biochemical criteria for individual sensitivity in development of occupational bronchopulmonary diseases	Cent Eur J Public Health	10		35-41
197.	Kuz'mina LP et al.	1998	Genetic aspects of silicosis: polymorphic gene distribution frequency	Vestn Ross Med Nauk	5		7-10
198.	Selvaggio G et al.	1967	The haptoglobins and Gc group specific components in silicotic patients	Zacchia	3		418-425
199.	McCanlies E et al.	2002	Significance of genetic information in risk assessment and individual classification using silicosis as a case model	Ann Occup Hyg	46		375-381

Appendix 2 Systematic review form

Process of Systematic Analysis

1. Reference	
2. Biomarker – Classify according to whether the biomarker is for: a. Assessing exposure. b. Early outcome. c. Susceptibility. d. Source in the Body.	
3. Mechanism of action	
4. Hypothesis/Objective.	
5. The biological material tested.	
6. Study population and number.	
7. Methods implemented.	
8. Practicability.	
9. Study design. a. Cohort. b. Case-Control. c. Cross Sectional.	
10. Results.	
11. Statistical Methods.	
12. Our evaluation of results/Evaluation of biomarker.	
13. Evaluation of biomarker(s). a. Biomarker of exposure. b. Biomarker of effect. c. Biomarker of susceptibility.	
14. Assessing the specificity and accuracy to predict silicosis.	

Appendix 3 Classification of biomarkers identified in the literature

MARKERS OF EFFECT

1. Clara cell protein-16 (CC16) in serum and in bronchoalveolar lavage (reviewed by Mary Gulumian)
2. Markers of oxidative stress (reviewed by Paul Borm and Vince Castranova)
 - a. Glutathione peroxidase (GPx)
 - b. Glutathione (GSH)
 - c. Glutathione-S transferases (GSTs)
 - d. Silica-induced ROS (Reactive Oxygen Species) – measured using chemiluminescence
 - e. 8-isoprostane
 - f. Total Antioxidant Capacity - measured by Trolox Equivalent Antioxidant Capacity (TEAC)
3. Procollagen type III N-terminal peptide (PIIIP) in serum (reviewed by Paul Borm)
4. Angiotensin Converting Enzyme (ACE) in serum (reviewed by Mary Gulumian)
5. Platelet Derived Growth Factor (PDGF) in serum/Other possible growth factors (reviewed by Ken Donaldson)
6. Tumour Necrosis Factor (TNF-?) and IL-8 release by peripheral monocytes (reviewed by Paul Borm)
7. TNF-? polymorphism (reviewed by Val Vallyathan)
8. sFas in serum/Other possible markers of apoptosis (reviewed by Mary Gulumian)

MARKERS OF SUSCEPTIBILITY

9. Interleukin-1 (IL-1) and IL-1RA polymorphism in autopsy lung samples (reviewed by Val Vallyathan)
10. TNF-? polymorphism in whole blood or in autopsy lung samples (reviewed by Val Vallyathan)
11. Lymphotoxin alpha (LTA) polymorphism (reviewed by Val Vallyathan)

OTHER MARKERS

12. CA19-9 (reviewed by Vince Castranova)
13. C-reactive Protein (reviewed by Ken Donaldson)
14. Elastin (reviewed by Vince Castranova)
15. IFN-? (reviewed by Vince Castranova)
16. HLA (reviewed by Ken Donaldson)
17. Immunoglobulins (reviewed by Vince Castranova)
18. Kinin aminopeptidase (reviewed by Ken Donaldson)
19. Arachidonic acid metabolites (reviewed by Mary Gulumian)
20. Alkaline phosphatase (reviewed by Ken Donaldson)
21. Haptoglobins (reviewed by Val Vallyathan)
22. Alveolitis/inflammation (reviewed by Ken Donaldson)
23. Fibronectin (reviewed by Vince Castranova)
24. Lymphotoxin-? (reviewed by Val Vallyathan)
25. T-lymphocytes (reviewed by Mary Gulumian)

Appendix 4 Summary reviews of biomarkers

A. Oxidative stress, Free Radical (induced damage) and anti-oxidant response as a pool for biomarkers in CWP.

Paul Borm:

The concept that oxidative stress either by reactive oxygen species from the particle surface or from the inflammatory response invoked by inhaled particles has been elaborated very strongly in the past decade. This was done by experimental animal studies showing upregulation of ROS-formation or anti-oxidant enzyme induction as well as numerous cellular studies looking at mechanisms of chemokine induction in various lung target cells. The elucidation of several redox-sensitive transcription factors and receptors (e.g. NFkB, EGF-R) connecting oxidative stress to downstream events such as inflammation, proliferation, DNA-damage and degradation of tissue components and extracellular matrix have provided a large pool of evidence to elaborate this concept for further use as a biomarker in particle induced lung disease. There is little question on the biological relevance of this mechanism and recently it has even been suggested to use differences between animal species with regard to this system (Jesch et al, 1997) in the risk assessment of particle effects to humans (Driscoll et al, 2002).

As a result of oxidative stress a number of events can occur such as upregulation of anti-oxidant enzymes, oxidant damage to DNA/proteins or breakdown of extracellular matrix. These events can be used as smaller fishing pools for useful biomarkers. Early clinical work has been published on **increased radical generation by BAL cells** recovered by lavage from coal miners. This data confirms animal studies that in humans, coal dust exposure obviously upregulates the capacity of the AM to produce ROS such as H₂O₂ and O₂-anion. In addition, at similar exposure, patients with progressive massive fibrosis seem to have more active cells in their lungs. Although the number of papers is low, and the effort for these biomarkers is highly invasive, the evidence is consistent and it supports the concept of oxidative stress in the lung even in mild disease such as sCWP. Care should be taken however to extrapolate animal findings (such as on NO-generation) directly to humans since especially iNOS generated NO is much lower in humans than in rats.

A fair amount of epidemiological studies among coal miners have been performed between 1985 and 2000, on the subject of **anti-oxidant response in peripheral blood** as an easy tool in occupational studies. This work was mainly generated in two or three labs that at some time also had collaborative projects, i.e. the University of Maastricht (Paul Borm) in the Netherlands and the INRS/INERIS in France (drs Nadif, Porcher and Auburtin). The cross-sectional work has shown that in early stages of CWP (0/1- 1/1) a number components of the systemic anti-oxidant network are affected. Most constant from independent studies are induction of total SOD and a reduction of GSH as well as GSH-dependent enzymes GST and GPX. Recently (Nadif et al, 2003) it was also shown that there is a connection between the TNF-308 genotype and effects of coal dust on GPX activity in coal miners which opens up further investigations between inflammation and oxidative stress. Since the anti-oxidant system is composed of many mutually dependent components, also integrative approaches were done trying to use alternative assays (TRAP, TEAC) or alternative analyses (sums of anti-oxidants) as predictors of effect. So far only one longitudinal study has been published with respect to the predictive power of AOE response to respiratory effects in coal miners and discriminative power was found to be low (Schins et al, 1997). The total of data seems ready for re-analysis and pooling although meta-analysis seems to be a big word for such an effort. In addition, as

validation it would be strongly advisable to look at the concomitant changes in anti-oxidants in lung tissue or lining fluid and peripheral blood.*

Apart from responses on anti-oxidants, an alternative is to look at damage done by ROS escaping from the anti-oxidant defence system. Two *in vivo* studies have been reported on **oxidative DNA damage** in peripheral blood lymphocytes of quartz exposed workers. Data suggests that particle exposure *per se* already caused an increase in oxidative DNA damage, irrespective of the induction or presence of CWP by inhaled particles. This set of data is too small for any meta-analysis but instead requires additional work, especially in relation to quartz induced lung cancer.

In Particle Toxicology the role of the neutrophil (PMN) is discussed extensively especially with regard to its being indicative of overload as well as causing lung damage (Driscoll et al, 2002). Human evidence is now emerging that PMN counts are predictive in lung interstitial disease (Cullen et al, 1992; Kuempel et al, 2003). Since the PMN is the primary source of ROS in the lung upon inflammation, it is important to connect biomarker studies on oxidant effects to neutrophil influx and activity.

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Vince Castranova:

Premise:

1. Freshly fractured silica has siloxyl radicals on the fracture planes, which can generate hydroxyl radicals in aqueous medium.
2. Aged silica can absorb iron from body fluids and participate in the Fenton reaction to generate hydroxyl radicals.
3. Silica can stimulate ROS production (O_2^- , H_2O_2 , CL) by alveolar macrophages.
4. ROS can activate the transcription factor NFkB.
5. NFkB induces gene expression for TNF- α , cytokines, and growth factors, which lend to inflammation, damage, fibrosis, and cancer.
6. In response to oxidant stress, antioxidant levels may initially be depleted or may later be elevated as a compensation.

* An effort was made to relate AOE response in blood and lung of rats exposed to cristobalite as reported by Janssen et al (1992) but technical difficulties were met in the storage and haemolysis of rat blood samples.

Biomarkers of effect:

1. ROS from AM increases with CWP and silicosis.
2. Mediator production (fibronectin and growth factors) by AM increases in CWP and silicosis.
3. NFkB is activated by in vitro and in vivo exposure to silica.
4. Total radical trapping antioxidant level in serum is elevated in CWP.

Problems:

1. BAL to obtain AM is invasive.
2. Exposure to asbestos, metals, coal dust, as well as silica causes oxidant stress in the lung. Thus, not specific.

Val Vallyathan:

Neopterin, D-erythro-trihydroxypropylpterin, is a stable, low-molecular mass molecule that is produced in humans and primates by monocytes/macrophages upon stimulation with interferon. Several studies have shown that in diseases related to immunity, rheumatoid arthritis, and malignancies, neopterin is elevated and may be used as a valuable biomarker. The production of neopterin by monocytes/macrophages is reported to be closely related with the capacity of the cells to release toxic metabolites, especially reactive oxygen species (ROS). Neopterin was also shown to induce apoptosis in alveolar epithelial cells.

In a study of 22 silica exposed workers and 20 healthy volunteers, serum and urine neopterin levels were reported to be increased in exposed population. Authors claim that neopterin monitoring is a new and useful early marker for the prediction of some disorders related to occupational silica exposure.

Neopterin was also shown to be a valuable test in conjunction with lymphocyte proliferation test in chronic beryllium disease with a high positive predictive value of 92% in identifying cases. Increased production of neopterin from alveolar macrophages in patients with interstitial lung diseases was also reported as a marker of disease. It may be useful as a biomarker combined with others but no specificity can be ascertained without validation studies in experimental animal models and a case control study in humans.

B. Collagen synthesis-degradation network as a source of biomarkers for CWP

Paul Borm:

The connective tissue of the lung consists of collagen, elastic fibres and proteoglycans. Collagens are the most abundant molecules of the extracellular matrix and they are subject to continuous synthesis and degradation. Collagens are produced by a variety of lung cells and a number of these collagens or components (collagen I, III and VI) have chemotactic activity on other cells as well. Among those cells fibroblasts are the most important but also epithelial and endothelial cells can contribute to collagen synthesis, which is mainly controlled by a large set of cytokines including TGF β , TNF α , PDGF and IL-1 (review Schins & Borm, 1999). Degradation is controlled by many enzymes including elastases, cathepsins and gelatinase, and some of these with ingenious controlling enzymes such as α_2 -macroglobulin, α_1 -

antitrypsin or tissue inhibitor of metalloproteinases (TIMP). In this way a complex network of factors is apparent each with different kinetics and control on synthesis-degradation equilibrium of collagen in the lung. *Since the amount and types of collagen determine the elasticity and physiological performance of the lung, the biological background of using this network to find and validate biomarkers for exposure or early effects is obvious.*

Therefore it is somewhat surprising that relatively few studies have been published on biomarkers generating from this domain. A small set of studies have been published on the potential use of **N-terminal propeptide of the pro-collagen type III (PIIIP)** which can be detected in BALF and serum. In an initial cross-sectional study Janssen et al (1992) found increased serum PIIIP in coal miners with early stage CWP. However, subsequent work on the same and extended study groups (Schins et al, 1994; 1995) showed that PIIIP did not predict for the progression of CWP over a five year period and that its use as an exposure marker should also be questioned. Additional work by us in another occupational cohort revealed no additional power in comparison to (negative) HRCT diagnosis of abnormalities or lung function indices that indicate abnormalities in the small airways and interstitium (Jorna et al, 1994). These findings are in contrast to positive studies reported in subjects exposed to asbestos (Cavallieri et al, 1991) and sarcoidosis progression (Pohl et al, 1992). Recently, we also evaluated PIIIP in combination with Type VI collagen, which is suggested to allow for parallel assessment of synthesis and degradation (Schins and Borm, 1999). This ratio was lower in health control miners, and suggests that collagen accumulation is reduced in miners in the absence of disease.

Complementary work has been published on the activity of **neutrophil elastases** in coal miners, showing elevated levels of neutral metalloendopeptidases (NMEP) as well as human leukocyte elastase (HLE) in blood of coal miners (Porcher et al, 1993). Since coal dusts have been found to have potent effects *in vitro* on several elastases or anti-proteases (Huang et al, 1993) this pathway deserves to be pursued in relation to epidemiological studies. It is recommended to perform a qualitative evaluation of all clinical work with collagen degrading factors in relation to coal dust to start new studies on biomarkers in this area. Either they can be used to assess the harmfulness of coal dusts (*in vitro*) or the effect of these dusts *in vivo*.

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C. Carbohydrate antigen 19-9 (CA 19-9)

Vince Castranova:

Premise:

1. Silica can stimulate lung epithelial cell hyperplasia and hypertrophy.
2. Stimulated lung epithelial cells will produce CA 19-9, raising serum levels.

Biomarker of effect:

1. Reported to be more sensitive than a chest radiograph in detecting lung fibrosis.

Problems:

1. Reported in only a single silicotic patient.
2. Many exposures that cause oxidant stress would cause type II cell hyperplasia and hypertrophy. Therefore, question specificity for silicosis.
3. Patient already was symptomatic. Question of sensitivity.

D. Elastin

Vince Castranova:

Premise:

1. Silica stimulates TNF- α production by AM.
2. TNF- α decreases tropoelastin and elastin from fibroblasts in vitro.
3. Silica increases TNF- α and TGF- β in granulomatous lesions, while decreasing elastin in these lesions.
4. Silica increases tropoelastin and elastin in non-granulomatous regions.

Biomarkers of effect:

1. Elastin and tropoelastin in non-granulomatous regions of the lung.
2. TNF- α and TGF- β in granulomatous regions of the lung.

Problems:

1. Invasive, need lung tissue.
2. TNF- α is upregulated under numerous exposure conditions.
3. TGF- β is upregulated in lung fibrosis due to asbestos not only silica. Thus, non-specific.
4. Elastin is inversely related to granulomatous lesions.

E. Interferon γ (IFN- γ)

Vince Castranova:

Premise:

1. Silica stimulates IL-12 and IL-18 production from AM.
2. These cytokines stimulate IFN- γ production by T_{H1} lymphocytes.
3. IFN- γ activates AM to produce growth factors for fibroblasts.
4. IFN- γ knockout mice are less responsive to silica exposure.

Biomarkers of effect:

1. Assay AM by BAL and lung lymphocytes from lung parenchyma in mice.
2. Assay IFN- γ production by lung lymphocytes.

Problems:

1. Invasive.
2. Many particles result in a T_{H1}-like reaction. Not specific for silica.
3. Question of sensitivity, since this animal model develops fibrosis.

F. Fibronectin

Vince Castranova:

Premise:

1. Silica stimulates fibronectin production from AM.
2. Results in elevated fibronectin in blood.

Biomarkers of effect:

1. Rom et al. measured increased fibronectin production by lavaged human AM from patients with CWP and silicosis. Not elevated in asymptomatic workers.
2. Zhestkov reported increased blood fibronectin in patients with various dust-induced lung diseases.

Problems:

1. Not specific to silica.
2. Sensitivity – positive subjects were symptomatic.

G. HLA and Immunoglobulins

Vince Castranova:

Premise:

1. Silica exposure results in an immune inflammatory response.
2. Increased number of lymphocytes in the bronchoalveolar lavage.
3. Polyclonal activation of B cells.
4. Increased production of immunoglobulins (IgG, IgM, and IgA).
5. Increased BAL immunoglobulins; increased blood immunoglobulins.
6. Silica increases expression of HLA-linked genes and B-antigens from the HLA series (glycoproteins).

Biomarker of exposure but not response:

1. IgG and IgA increased in BAL of asymptomatic workers.
2. IgG, IgM, and IgA increased in serum – not related to severity of disease.
3. BAL immunoglobulins more sensitive than serum.
4. PCR of blood granulocytes showed association of HLA-Bw54 gene (susceptibility); increased levels of HLA-88 and HLA-B13 in silicotics.

Problems:

1. Sensitivity of serum immunoglobulins.
2. BAL is invasive.
3. Not related to severity, which is related to cumulative dose. Question of dose-response relationship.
4. Serum IgA increased with pneumoconiosis, not just silicosis. Question of specificity.
5. Serum IgM and IgG increased with bronchitis. Problem of smoking.
6. HLA antigens elevated in silicotics. Question of sensitivity and specificity.

H. Angiotensin converting enzyme (ACE)

Mary Gulumian:

ACE is a key enzyme in the rennin-angiotensin system, converting angiotensin I into the potent vasopressor angiotensin II and also inactivating the vasodilator bradykinin. These last two are highly potent regulatory peptides, angiotensin II being hypertensive and bradykinin hypotensive. ACE is a peptidyl dipeptide hydrolase that is located mainly on the luminal surface of vascular endothelial cells but also in cells derived from the monocyte-macrophage system. ACE is found in the endothelium of blood vessels in liver, kidney, and brain. ACE activity in serum is only a very small fraction of the ACE activity of the total organism and therefore the serum activity of ACE in pulmonary diseases is of interest owing to its principal localisation in the large capillary bed of the lungs.

1. Serum ACE activity and silicosis:

a. Human serum

Jorge and Mallo 1994 have studied serum ACE activity in 25 control, 19 patients with sarcoidosis, 14 tuberculosis, 16 silicosis and 15 workers of coal mining without silicosis or other concomitant diseases in order to assess the usefulness of ACE as a parameter for differential diagnosis of these diseases. The results have shown significantly increased values with respect to the control group in all of these diseases: Control group (40.84 ± 13.06 U/l), sarcoidosis (57.77 ± 16.47 U/l), tuberculosis (46.85 ± 10.25 U/l), silicosis (61.50 ± 21.40 U/l), and miners (71.50 ± 10.25 U/l). No significant difference were noted between different disease states except the miners and tuberculosis ($p=0.004$). The authors concluded that serum ACE activity is not a useful parameter to differentiate between these diseases (lack of specificity to a disease).

Serbescu and Paunescu (1992) measured serum ACE levels in 116 silicotic patients revealed over 60 % higher levels than in normals. The authors concluded that on the present evidence, it can be appreciated that increased serum levels of ACE activity could be the expression of an active progressive state of silicosis.

Hiwada et al (1987) have measured ACE concentration with direct immunoassay in sera of 47 normal subjects and 107 patients with various pulmonary diseases. The mean concentration of ACE in normal serum were $320.9 \pm$ ng/ml, with 21 silicosis patients 569.5 ± 183.8 ng/ml, in 11 active pulmonary tuberculosis 508.5 ± 159.4 ng/ml

Yano et al (1987) have measured serum ACE activity in 107 male silicosis patients. In agreement with previous reports, the mean activity of ACE in silicosis patients was elevated (47.4 ± 14.2 U/ml; normal 30.5 ± 8.6). Among the various characteristics of the patient analysed in this study, only the progression of radiographical perfusion category showed a statistically significant association with the elevation of ACE, and severity of dyspnea was inversely associated with ACE. No association was found between ACE and age of the patient, duration of exposure to dust, and smoking habits.

Szechinski et al (1986) have attempted determine the relationship between serum ACE levels and nodular silicosis and between serum ACE levels and progressive massive fibrosis. The authors also examined whether serum ACE levels could be used to distinguish between silicosis and other pulmonary diseases with similar clinical and radiographic characteristics, e.g., silicosis versus sarcoidosis.

The data presented indicate that silicosis patients either in the nodular or progressive massive fibrosis stage, have only moderately elevated serum ACE levels, which are not statistically different from the serum ACE level in normal subjects. However, their data did indicate that serum ACE level determinations may be used to assist in distinguishing between silicosis and certain other pulmonary diseases such as silicotuberculosis but not silicosis and sarcoidosis as both these other diseases had very significantly increased serum ACE levels as compared to the silicosis patients. The Mean serum ACE levels for controls were 38.1 ± 10.6 U/ml while in patients with silicosis was 45.2 ± 16 U/ml and patients with silicotuberculosis was 69.

Nordman et al (1984) have analysed the serum activity of ACE in 135 silicosis patients was analysed 28 of which had referents matched for silica dust exposure and age but without roentgenographic signs of silicosis. An overall reference group

not exposed to silica dust comprised 34 lumberjacks. Results have indicated that the serum mean activity of ACE was higher in silicosis patients (46.6 ± 12.1 U/L) than in referents exposed to silica (38.5 ± 8.1 U/L) or in the lumberjacks (36.6 ± 9.7 U/L). An association could be seen between the serum ACE level and the roentgenographic severity of fibrosis. A retrospective side-by-side assessment of roentgenographic progression was made in 49 silicosis patients. The ACE was found to be higher in the 15 patients with progression (50.5 ± 16.4 U/L) than in those with no progression (41.5 ± 9.5 U/L). According to the multivariate regression analysis, progression of fibrosis explained the elevation of ACE better than profusion. The results confirmed that the serum ACE activity is elevated in silicosis and suggest that the elevation is associated with progression of the disease. According to the present results, smoking *per se*, chronic bronchitis, and age did not affect the level of serum ACE, which is in agreement with earlier observations; however, age dependence was claimed by Lieberman (1975) and Ashutosh and Keighley (1976) in healthy control subjects. It is obvious that serial measurements in relation to established parameters (e.g., chest roentgenograms) are needed to assess the usefulness of ACE as a means to monitor the progression in individual patients (Nordman et al 1984).

Grönhagen-Riska (1979) has determined the mean serum ACE activity in 90 healthy adults was 32.1 ± 15.4 U/ml. The mean level of 70 women, 31.9 U/ml did not differ significantly from that of 20 men, 32.5 U/ml. The ACE mean of 15 smokers in the control group was 31.1 U/ml and that of 59 non-smokers 32.4 U/ml; the difference is not significant. Eight of the 19 patients with silicosis had increased activity (46.1 ± 20.2 U/ml). In silicosis, ACE activity did not clearly reflect the severity of the disease as determined from chest x-ray changes and respiratory function tests. The same author in an earlier work (**Grönhagen-Riska 1978**) when compared serum ACE activity in silicosis patients with control and 36 treated sarcoidosis patients, he found that in all groups, there were significantly raised ACE values compared to control. He therefore concluded that raised ACE activity in both these diseases weakens the differential diagnostic importance of this enzyme determination in any of these diseases although very high values still may indicate sarcoidosis.

b. Rat serum

Brown et al (1983) have investigated the effects of experimental silicosis on serum ACE in laboratory rats in order to explore the possibility that the serum levels of this enzyme could be used to follow the development of the lung lesions. This represents the only work conducted on experimental animals.

Results have indicated that following intratracheal instillation of quartz (DQ12), there was fibrosis and a concomitant increase in the serum level of ACE. However, the changes in the activity of this enzyme were variable and only poorly correlated with the observed histological changes. Therefore, the conclusion was that serum ACE level was not a useful parameter in monitoring the response of individual rats to lung damaging agents.

2. Serum ACE activity and Coal Workers Pneumoconiosis (CWP):

Thomson et al (1991) have found that the ACE activity in miners without recent exposure was not elevated (39.8 ± 1.3 U/ml) compared with the normal controls. No increase in serum ACE activity was found when the miners were grouped according to the presence or absence of coal worker's pneumoconiosis (CWP). They have therefore concluded that underground coal mining but not CWP, is associated with elevations in serum ACE and that after removal from the exposure to mixed coal

mine dusts, the levels of serum ACE was normalised. Therefore, exposure to the mixed dusts found with underground coal mining was associated with elevated serum ACE, and that after the exposure ceased, serum ACE normalised. In contrast, the correlation of the presence of CWP with elevated serum ACE did not reach statistical significance.

Wallaert et al (1985) have also measured serum ACE activity in 141 pneumoconiotic coal miners, 40 healthy coal miners and 30 controls. The results demonstrated a high proportion (45 %) of elevated serum ACE level in coal worker's pneumoconiosis (CWP). The mean serum ACE activities were significantly higher in CWP (33.8 ± 13.0) than in controls (25.2 ± 4.7) and non-pneumoconiotic coal workers (29.5 ± 9.5). Serum ACE levels did not vary according to the roentgenographic profusion categories (1, 2 or 3). Serial measurements of serum ACE in 50 CWP patients did not demonstrate correlations at six-month intervals. In addition, when the progression of both profusion and type of lung opacities was assessed since the beginning of occupational risk by double-blind- examination of annual roentgenogram, no significant correlation was found between the progression of the disease and serum ACE activity. Therefore, serum ACE activity was not found to be related to progression of fibrosis in CWP but rather related to the acute forms of CWP and silicosis.

3. Serum ACE activity and Anthracosilicosis:

Zhicheng et al (1986) have determined the activities of serum ACE anthracosilicosis and anthracosilicotuberculosis in order to see if any biochemical changes take place and to find laboratory indices for the early diagnosis and evaluation of the treatment in patients with anthracosilicosis and anthracosilicotuberculosis. Ninety male anthracosilicotic patients were recruited with 24 suspected cases, 25 cases in category 1, 24 in category 2, and 22 in category 3. In addition, the study included 20 cases of anthracosilicotuberculosis, 24 male workers with mixed dust exposure and 26 health men with no dust exposure. The levels of serum ACE measured were for controls 33.44 ± 12.84 , exposed to mixed dust 43.29 ± 16.65 , suspected cases 44.22 ± 17.94 , category 1 patients 53.14 ± 18.65 , category 2 patients 53.53 , category 3 patients 63.32 ± 23.98 , and anthracosilicotuberculosis patients 48.39 ± 17.00 . It was therefore concluded that since the levels of serum ACE are also increased in patients with anthracosilicotuberculosis, the serum ACE levels cannot be used to differentiate anthracosilicosis from anthracosilicotuberculosis.

4. Source of increased serum ACE activity:

a. Silicosis

In all the investigations reviewed, source of serum ACE elevation in silicosis remains hypothetical. Both Nordman et al (1984) and Brown et al (1983) have argued that as the endothelial cells in the capillary bed have a high ACE content, the fibrotic involvement of new tissue including capillaries may give rise to an ACE release. Also, the cytotoxic effect of silica particles on macrophages leading to their rupture and loss of the cytoplasmic contents seem to be crucial in the development of silicosis and therefore can be another source of increased serum ACE in silicotics. The involvement of both endothelial cells and macrophages in the increased serum ACE levels in silicotics and other lung diseases seem to be confirming earlier discussions (Grönhagen-Riska 1979; Grönhagen-Riska 1978).

b. CWP

The source of serum ACE in coal workers was also hypothetical and with a similar argument that pulmonary macrophages, activated by ingestion of mixed coal mine dust, are the source of serum ACE. In addition, it was also proposed that since alveolar macrophages could be a potential source of serum ACE, elevation of serum ACE activity in underground miners may reflect alveolar macrophage activation caused by increased pulmonary mixed coal mine dust burden. If serum ACE elevation in coal workers is a marker of alveolar macrophage activation, then the data will suggest that with time, the number of dust-activated alveolar macrophages diminishes, consistent with the benign natural history of simple CWP that does not progress on cessation of exposure (Thomson et al 1991).

c. Anthracosilicosis

Once again, Zhicheng et al (1986) consider that the rise in serum ACE levels is a signal both of the continuing harm done to the pulmonary macrophages and of the progression of pulmonary fibrosis. Therefore, serum ACE may be used clinically as an index to show the progression of pulmonary fibrosis as well as to evaluate the effects of treatment.

Conclusions:

1. Increased serum levels of ACE activity could be the expression of an active progressive state of silicosis.
2. ACE activity could be expression of exposure to mixed dust in CWP.
3. ACE activity is not a useful parameter to differentiate between silicosis and other diseases (lack of specificity to a disease).
4. ACE determination was hampered by a rather low sensitivity because serum ACE remained normal in about 30-40 % of the patients.

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I. Apoptosis, sFas, mFas, FasL, sFasL in macrophages and in T-lymphocytes.

Mary Gulumian:

The role of apoptosis in the development of silicosis as well as in immunological disorders in silicotic patients were studied by a number of investigators in humans (Iyer et al 1996; Tokomuni 1997; Otsuki et al 1998; Lim et al 1999; Tomokuni et al 1999; Otsuki et al 1999; Otsuki et al 2000) and animal experiments (Borges et al 2001) *in vivo* and *in vitro*.

Apoptosis, programmed cell death, can be triggered by the binding of membrane bound Fas (mFas) surface protein to its ligand (FasL).

mFas is a well known cell surface molecule involved in the apoptosis pathway which belongs to the TNF-receptor family. Several alternative spliced variants of the Fas gene have been reported previously. Among those variants the deletional form of exon 6 in the transmembrane domain is generally known as **sFas**. This sFas inhibits membrane Fas (mFas)/FasL binding by competition, subsequently preventing the apoptosis of cells. Tomokuni et al (1997) and Otsuki et al (1998) have observed elevation of serum sFas levels in cases of silicosis and have proposed that elevation of serum sFas levels suggest that the disruption of the competition between sFas and mFas to bind FasL in T-lymphocytes may be one of the most important mechanisms behind the acquisition of autoimmunity in silicosis patients. Otsuki et al (1999) have applied factor analysis to evaluate new parameters related to Fas-mediated apoptosis; i.e., membrane Fas expression on peripheral blood lymphocytes (mFas), serum sFas, serum sFasL levels, and the sFas/mFas mRNA expression ratios in PBMC. The results clearly showed that these should be good tools for detecting immunological impairment independent of respiratory disorders in cases of silicosis. Therefore, On the other hand, dysregulation of apoptosis of T-lymphocytes is proposed to be correlated to predisposition to developing of immunological disorders in silicotic patients (Tokomuni 1997)

Fas ligand (**FasL**) is a membrane bound and shed protein belonging to the TNF gene family, and the natural counter-receptor for the death-promoting Fas molecule expressed by a variety of lymphoid and nonlymphoid tissues. **sFasL** is converted to a soluble form by a metalloproteinase-like enzyme. Tomokuni et al (1999) observed an increase in the levels of sFasL in serum of silicotic patients with slight dyspnoea or normal PCO₂. Therefore, fibrotic potential of a particulate depends upon its ability to cause apoptosis of alveolar macrophages (Iyer et al 1996). Animal studies using Fas-ligand deficient generalised lymphoproliferative disease mutant (*gld*) mice have shown that they did not develop silicosis. Administration of neutralising anti-Fas ligand antibody *in vivo* blocked induction of silicosis. Thus, it was shown that FasL played a central role in induction of pulmonary silicosis (Borges et al 2001). As locally produced sFasL can induce bystander neutrophil apoptosis and blocks further neutrophil extravasation in mice, sFasL could play a role in modulation of silica-induced inflammation in humans. The finding of a central role of FasL in experimental silicosis could provide clues for the pathogenesis and treatment of this common and life-threatening occupational disease. An important issue is whether the role of FasL can be extended to human silicosis. Increased levels of sFasL were reported in patients with silicosis (Tomokuni et al 1999).

Conclusions:

Increased sFasL levels in serum could be a good biomarker of effect.

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J. Clara cell protein: CC16

Mary Gulumian:

Clara cell protein is a potentially immunosuppressive protein secreted by nonciliated cells of the tracheobronchial epithelium and by some reproductive system organs such as the prostate. It is a homodimer consisting of 70 amino acid subunits and has a molecular mass of 15, 840 kDa hence the CC16 abbreviation.

The exact physiological function of CC16 remains unknown but there are several lines of evidence indicating that it is an immunosuppressive and anti-inflammatory protein protecting the airways from undue activations of the immune system that might cause tissue injury.

Bernard et al (1994; 1998) have shown that the concentration of CC16 in the serum from exposed workers to silica averaged 12.3 µg/l against 16.3 µg/l in control with no change in the respiratory symptoms and lung function tests. It was therefore concluded that a significant reduction of serum CC16 in workers inhaling silica-rich dust for less than 2 years and with no radiographic or functional signs of lung impairment. It can therefore be used as a biomarker of early toxicity of exposure to silica and subsequently improve the capability to detect groups at risk. Further studies are however needed to assess the health significance and the validity (specificity and sensitivity) of this new marker.

Conclusions:

Decreased levels of CC16 in sputum and serum can be a good biomarker of early effect.

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K. Cytokine growth factors

Ken Donaldson:

Both human and experimental data suggest that the growth factors TGF α and PDGF have the most potential as biomarkers of ongoing silicotic fibrosis. Data reveal that PDGF and/or TGF- β are elevated during silicotic fibrogenesis in animal models [19] [23] [11] and in human silicotic blood [2] and BAL or produced by alveolar macrophages from silicotics [13] (note that Rom et al describe increased in AMDGF, later discovered to be predominantly PDGF activity) [16]. BAL IGF also appears to be elevated [20] also serum bFGF [7] and BAL EGF [22] in sporadic studies.

Intuitively PDGF and TGF β would appear to be good biomarkers since their role in fibrosis is confirmed by key proof of concept studies such as presence of TGF β in histological sections of silicotic nodules and hyperplastic silicotic epithelium [8,17] and the ability of anti-PDGF and antisense PDGF DNA to down-regulate the histopathological changes and collagen content of silica-exposed mouse lungs [24]. The role of these two growth factor cytokines is recognised as central to the molecular pathogenesis of other types of fibrosis [29, 30]

Markers such as CRP, sialic acid [21] alkaline phosphatase [3] and inflammation generally [13] are not sufficiently specific to silicotic fibrosis to be considered. In addition, as was detailed in the IARC silica monograph' BAL in silicosis does not reliably demonstrate any increase in PMN that would indicate inflammation [31].

L. HLA

Ken Donaldson:

This has been examined in several studies [5, 9, 15, 27, 10] and although some associations have been demonstrated, there is no specificity in these associations; meta-analysis may assist with this.

M. C-reactive protein

Ken Donaldson:

Although one study shows this to be related to degree of silicosis [21] this is a very non-specific measure that is elevated in all types of inflammation, infection and in cardiovascular disease.

N. Kinin aminopeptidase

Ken Donaldson:

The single paper dealing with its this biomarker in silicosis was in Polish. No other papers could be identified linking this with fibrosis in any other situation.

1. Materials reviewed:

Of the 28 papers that were identified for review only 18 were available in their full form [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18] whilst 7 were available only in abstract form [19, 20, 21, 22, 23, 24, 25] and 3 were in a foreign language except for the abstract [26, 27, 28].

Attempts are ongoing to obtain full papers of all of these references

2. Potential for meta-analysis:

Few of these studies have the potential for meta-analysis.

In the case of growth factors, human population studies were represented in the following studies; however, as shown by the growth factor studied, it is doubtful whether there are enough studies with any single biomarker to warrant meta-analysis :- [1] fibronectin, procollagen, [2] PDGF, [3] alkaline phosphatase, [8] TGFb, [7] bFGF, [23] PDGF, acidic FGF, [13] AMDGF, [16] TNF, IL-6, IGF, PDGF, TGFb .

With regard to HLA haplotype, the potential is better since all of the following have large(ish) populations studied for the same or similar endpoints [5, 9, 10, 15].

The remainder of the studies are rat , mouse or human *in vitro* experimental studies that do not involve silicotics and are not suitable for meta-analysis.

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O. TNF α , TNF-receptors and related cytokines as biomarkers in CWP

Paul Borm:

Since the original findings of Heppleston & Stiles (1964) that silica-exposed AM produce factors that stimulate the production of collagen by fibroblasts, the field of growth factors and cytokines has developed tremendously and several cytokines have now come forward as crucial in particle-induced fibrosis (Reviews: Kelly, 1990; Gauldie et al, 1993; Vanhee et al, 1995). A body of research has concentrated on tumour necrosis factor- α (TNF), which is a pro-inflammatory cytokine that has been demonstrated to play a key-role in particle-induced lung fibrosis, an more specifically in quartz-induced lung fibrosis. Crucial experiments were reported by Piquet et al (1990) demonstrating that silica-induced lung fibrosis could be ameliorated using a specific anti-TNF antibody and that the infusion of soluble-TNF receptors which complexes free TNF could prevent and reduce existing fibrosis (Piquet et al, 1994). These animal experiments and many to follow (cf. Brody, Driscoll, Gauldie) showed the importance of TNF in the cytokine-network leading to inflammation, tissue remodelling and (interstitial) collagen synthesis. Also clinical evidence has demonstrated increased levels of TNF and TNF-receptors in BAL, tissue specimens of subjects with various inflammatory lung diseases. More specifically, in patients with coal workers pneumoconiosis (CWP) or PMF increases of TNF, but also changes in interleukin-6, TGF- β , MCP-1 and PDGF were noted (Rom, 1991; Vanhee et al, 1996).

Validation studies: TNF α is by far the best investigated in epidemiological studies. The validity of monocyte or whole blood TNF release and the two soluble TNF-receptors as markers for exposure, effect and or susceptibility have been studied by number of research groups. A series of case-control studies have shown that TNF α release from blood monocytes discriminates between coal miners with pulmonary and respiratory effects of coal dust (Borm et al, 1988, Kim et al, 1998; Jorna et al, 1994). Most extensive epidemiological work has been published by Borm and colleagues culminating in a 5-year follow-up study to show the stability of this biomarker over the years, the effect of retirement on monocyte priming and the predictive power of abnormal TNF-release for 5-years progression of CWP (Borm et al, 1988; Schins et al, 1995). Later work in the same cohort of coal miners showed that also the TNF A-308 genotype was associated to CWP, but since no relation was present between this genotype and the monocyte TNF-phenotype, genotyping of TNF was considered less predictive (Zhai et al, 1998). Collaborative studies on TNF as a biomarker for CWP in other cohorts from Germany (Morfeld et al, 2001) and

France (Porcher et al, 1993) confirmed the validity of monocyte derived TNF as a marker for CWP, and more specifically in progressive massive fibrosis. In the meantime Borm and co-workers developed a whole blood assay (Schins et al, 1996) that is much more easy to apply in occupational settings than monocytes and delivers similar outcomes (Morfeld et al, 2001). In this assay system as well as in isolated monocytes one should realise that the incubation time, when optimal for TNF, may not be so for other cytokines and comparisons between power of different cytokines are to be made at optimal time points. It is recommended to perform a review on all studies that have used monocyte (or whole blood) TNF-release in relation to stage and progression of CWP as well potential confounders such as age, smoking, medication. At first glance, it appears that baseline monocyte TNF-release is not affected by confounders as well as exposure to coal dust. Stimulated TNF-release which is used as the biomarker needs further standardisation to warrant long-term, broad use in different settings.

Although mediators of the TNF-effect, TNF-receptors - apart from experimental studies in KO-mice and with infusion of cloned receptor- have proven of little extra value versus TNF-release. However, it is recommended to measure the soluble receptor of TNFR75 as a potential effect modifier of free TNF? (Hadnagy & Idel, 1998)

Other cytokines such as IL-6 and TGF- β have been shown to be valuable potential biomarkers worthwhile to investigate with regard to their validity towards onset and progression of CWP and PMF. TGF- β has been suggested as anti-inflammatory and was found to be decreased in alveolar macrophages of subjects with PMF (Vanhee et al, 1996). Based on recent studies on the balance of TNF and TGF- β (Borm & Schins, 2001) imbalance of these cytokines is related to CWP, and this hypothesis merits further follow-up in epidemiological studies.

P. Genetic Polymorphism

Val Vallyathan:

1. Cytokines:

Cytokines produced by mononuclear phagocytes are called monokines, and those produced by activated lymphocytes are called lymphokines.

DISEASES-GENE ASSOCIATIONS	
Beryliosis	HLA-DPB1
COPD	mEH, TNF- α -308, α -1 antitrypsin, Vitamin D binding protein, TNF- α
IPF	TNF, lymphotoxin- α , TNF-receptor II, IL-6
TB	NRAMP1
EOP	IL- α , IL-1?
J. arthritis	IL-1?
C. polyarthritis	IL-1?
Asthma	IL-4RA, IL-13
Periodontitis	IL-1?, IL-1?
Psoriasis	IL-1?
Silicosis	IL-1RA, TNF- α

Chemokines are cytokines that share the ability to stimulate movement of leukocytes (chemokinesis) and direct movement (chemotaxis) which are important in inflammation.

IL-1 and TNF- α and TNF- β are two closely related cytokines sharing similar and synergistic effects in respect to inflammation and immunology. IL-1 and TNF- α are produced by activated macrophages and has a wide spectrum of biologic actions on immune and non-immune target cells. TNF- β is produced by activated T cells. TNF- α and TNF- β bind to the same receptor on target cells.

Examples of cytokine SNPs found to effect expression levels and modify disease	
Cytokine	Polymorphic locus
IL-1?	-889, +4845
IL-1?	-511, +3953
IL-1RA	VNTR, +2018
IL-4	-590, +33
IL-6	-174, -572
IL-10	-627, -1082, -819, -592
IL-13	-1-55, -1111
TNF-?	-3-8, -238
TGF-?1	-509, codon 10, 25

The cytokines receiving the most attention to date in relation to pulmonary diseases include: Interleukin-1 (IL-1), Tumor necrosis factor- α (TNF- α), Platelet-derived growth factor (PDGF), Transforming factor- β (TGF- β), Insulin-like growth factor I (IGF-I), and interleukin-6 (IL-6).

2. IL-1 and IL-1RA polymorphisms:

IL-1 is a highly pleiotropic cytokine released primarily from activated monocytes or macrophages and many other cell. Three genes located on the long arm of chromosome 2 encode for IL-1 α , IL-1 β , and IL-1 receptor antagonist (RA). Each of these genes possesses exonic polymorphisms resulting in changes in the cytokine expression which are known to play important roles in certain inflammatory diseases.

IL-1 is involved in the induction of chemotaxis in PMN, and macrophages, induction of endothelial cell proliferation, pro-coagulant activity, type IV collagen production, osteoblast proliferation, tissue repair, lymphokine release by T cells, NK-mediated cytotoxicity, release of factors associated with growth and differentiation, induction of prostaglandin release, induction of fever and many others.

Previous studies have shown that IL-1 is involved in mechanisms that underlie the cascade of events leading to the development of pulmonary fibrosis, such as chemotaxis, inflammation, proliferation and secretion of connective tissue components and various other steps promoted by it. IL-1 appears to be intimately associated with the evolution of the silicotic lesions and its profusion.

Recent studies focused on differences in cytokine levels among individuals and inheritable SNPs contained within the regulatory elements of cytokine genes support these observations in silicotics. Although there are no studies showing genetic associations between silicosis and cytokines it is likely that IL-1RA polymorphism

make a good candidate susceptibility gene and its variants may shed some light on high incidence of silicosis and its severity in exposed population. It is therefore considered a good candidate for biological monitoring in silicosis.

3. TNF- α Polymorphism:

TNF- α is a pro-inflammatory cytokine important in the early onset of inflammation, development and progression of several diseases including pulmonary fibrosis. TNF is reported to play a central role in the development of silicotic-like granulomas in experimental animals and several studies have shown consistent dose-dependent release of TNF- α after silica exposure. It was also shown that TNF- α mRNA and protein expression localised directly within the silicotic lesions co-expressed with IL-6. In experimental studies on silicosis treatment of animals with anti-mouse TNF- α reduced the development of silicosis and treatment with exogenous recombinant TNF- α enhanced the development of lesions. It has been also demonstrated that TNF- α deficient mice resist to the development of fibrosis by silica exposure. The mechanisms responsible for the sustained release of these cytokines probably play a major role in the development of silicosis because exposure to inert particles such as titanium dioxide or carbonyl ion may trigger only a transient release of cytokines including TNF- α without promoting fibrosis. Additional support for the role of TNF involvement in silicosis comes from the studies of MMP-13. TIMP-1 an inhibitor of MMP-13 degradation is enhanced in silicosis. TNFR deletion modifies MMP-13 and TIMP-1. Furthermore MMP-13 and TIMP-1 activation is directly correlated with AP-1 and NF- κ B. Activation of AP-1 is mediated via the p55 TNF receptor.

In several experimental studies it is well documented that repeated cycle of macrophage injury and release of cytokines after silica exposure is a major contributing factor in the development of silicosis. It is now well established that there is a strong link between overexpression of TNF- α during the genesis and progression of pulmonary fibrosis in humans exposed to silica and silicate minerals. A number of studies also provide support for the involvement of a genetic component as a determinant of susceptibility and development of pulmonary fibrosis in animal models and humans. In humans, the gene encoding TNF- α is located on chromosome 6 between HLA-B and DR within the class III region of the major histocompatibility complex. Any changes including single nucleotide polymorphism (SNPs) would affect the TNF- α production. SNPs containing G ? A substitutions have been described in the promoter region at positions -308 and -238 in a number of inflammatory diseases. In a study of miners with moderate and severe silicosis it was shown recently that a minor variant, TNF- α (-238) was markedly higher in severe silicosis and significantly lower for moderate silicosis. It was also shown that regardless of disease severity IL-1RA (+2018) or TNF- α (-308) variants were elevated. These studies suggest that gene-environment interactions are involved in TNF polymorphisms which may play important role on susceptibility and extent of silicosis severity in exposed population. It was also recently reported in a study of South African miners that polymorphisms in the TNF- α gene promoter may predispose the workers to severe silicosis. In this study miners with severe silicosis were shown to have -238A and -376A alleles in linkage equilibrium. In addition, severe silicosis patients were also reported to have -308 allele. These studies demonstrate reasonably good specificity and possible predictability as a good biomarker if combined with other cytokines such as IL-1. TNF cytokine polymorphisms may therefore provide valuable information on disease susceptibility and severity in workers exposed to crystalline silica.

4. Haptoglobins polymorphism:

Haptoglobin (Hp) is an acute phase protein capable of binding hemoglobin forming a stable Hp-Hb complex and thereby prevent iron-induced oxidative tissue damage. Clearance of Hp-Hb is mediated through a macrophage scavenger receptor CD163. Hp also acts as an antioxidant, has antibacterial activity and plays a role in modulating many aspects of the acute phase response. Hp influences the T-lymphocyte function and specifically interacts with both resting and activated CD4+ and CD8+ T cells. Binding of Hp strongly suppresses the T cell proliferation. Hp inhibits Th2 anti-inflammatory cytokines such as IL-1, IL-10, and IL-13 and plays a role in modulating pro-inflammatory cytokines such as IL-1, TNF- α , IL-18, and IFN γ .

There are three major phenotypic forms Hp-1-1, Hp 2-1, Hp2-2 associated with distinct clinical manifestations of different disease conditions of microvascular, macrovascular and diabetic complications. Several studies have demonstrated that functional allelic polymorphism in Hp gene acts as a major determinant of susceptibility for the development of diabetic microvascular complications and a risk factor for cardiovascular disease in individuals with diabetes. Hp polymorphism and its influence on iron metabolism in hereditary hemochromatosis is also well characterised.

In studies on occupational respiratory diseases 7 highly polymorphic genetic variants including Hp were investigated by scientists in Russia as potentially useful in assessing individual risk of occupational disease. Use of Hp as a biomarker for silicosis based on available evidence is unwarranted.

5. Lymphotoxin- α polymorphism:

The TNF- α and lymphotoxin- α (LT- α) genes are located adjacent to each other in the major histocompatibility complex class III region, on chromosome 6p21.3. TNF- α and LT- α act via two receptors 55-kD TNF-R1 and 75-kD TNF-R2. It was demonstrated that the surface expression of these receptors are necessary for the development of fibrosis in silica and bleomycin exposed double knockout mice. TNF- α /LT- α double gene knockout studies have also shown that mice are resistant to bleomycin-induced pulmonary fibrosis.

In a recent report a hypothesis was tested to evaluate whether TNF -308 and LTA *Nco*I polymorphisms modify the pulmonary responses to oxidants in coal miners differently exposed to cigarette smoke and coal mine dust. Overall findings showed severity of silicosis with TNF -308 at various stages of disease progression. Results also showed an association of CWP prevalence with *Nco*I polymorphism in LTA in individuals with low catalase activity.

Appendix 5 Biomarkers selected for further study

MARKERS OF EFFECT

Measures of oxidative stress:

- Glutathione peroxidase (GPx) and glutathione-S transferases (GSTs)
- Glutathione (GSH)
- 8-Isoprostane
- Total Antioxidant Capacity
- Reactive Oxygen Species (Chemiluminescence)

Tumour necrosis factor- α (TNF α)

Interleukin-8 (IL8)

Platelet derived growth factor (PDGF)

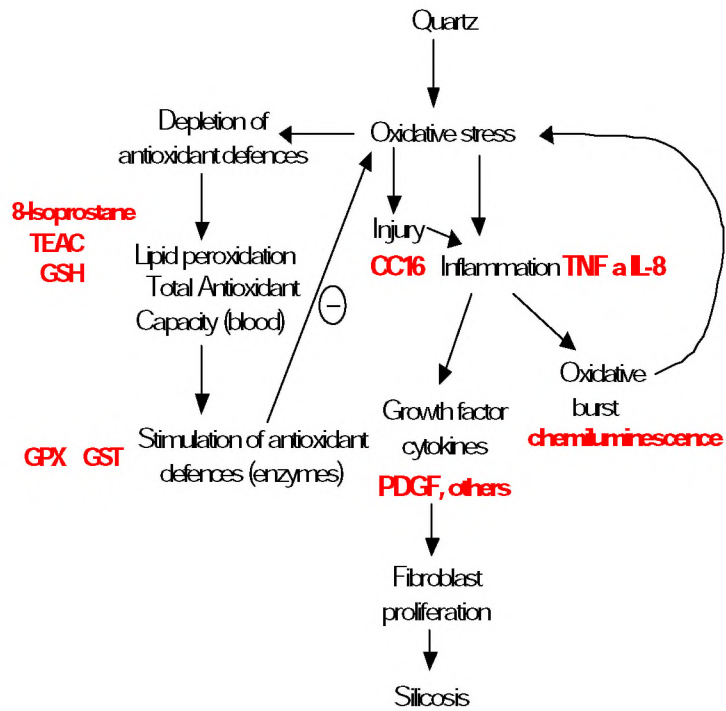
Clara cell protein (CC16)

These assays require either whole blood or serum and, in some instances, silica particle stimulation.

MARKERS OF SUSCEPTIBILITY

TNF α polymorphism and dust induced TNF-release

Appendix 6 Diagrammatic representation of responses to silica exposure and associated measurable biomarkers selected for further study



Appendix 7 Report back on international workshop on "Applying Biomarkers to Occupational Health Practice"

Santa Fe, New Mexico, 24-25 March 2003

The purpose of the workshop, which was convened by the National Institute for Occupational Safety and Health (NIOSH), was to bring together leading researchers who develop, validate, and use biomarkers, and occupational health practitioners, to foster collaboration and promote effective application of biomarkers in occupational health practices. Dr M Gulumian attended this workshop to strengthen contact with those who develop, validate and use biomarkers in occupational health.

The presenters at the workshop included John Howard, Head of NIOSH/CDC (Centers for Disease Control and Prevention) who indicated that biomarkers should be seen foremost as tools for prevention of occupational diseases. He also emphasised disadvantages of such an exercise, including the invasiveness of some test systems and the ethical issues involved. The next speaker, Claude Viau, University of Montreal, emphasised the points to be considered when choosing a biomarker for application. These included specificity, knowledge of toxicokinetics, and the establishment of the reference baseline values of the identified biomarker. The difficulty, however, arises when exposure is to a mixture of toxicants rather than to a single toxic compound as there will be additive, synergistic or antagonistic effects between the components of the mixture. In this instance, physiologically based toxicokinetic (PBTK) models may be established.

On the Development of Biomarkers, John Groopman of Johns Hopkins University stressed the point that development and validating of biomarkers is not an overnight process. There are strategies to be followed where the route from population to individual can be implemented. Michael Morgan of the University of Washington, on the other hand, spoke about Use of Biomarkers. Being a member of Committee on TLV and BEI guidelines developed by ACGIH, USA, he also spoke about Biological Exposure Indices (BEIs) and the Threshold Limit Values (TLV) relationship. He indicated that BEI can be the bioequivalent to TLV. However, BEI means an indication of biological effect and consequently, biological effect means the absorbed dose and not the TLV. Ethical, legal and social issues in using biomarkers in Occupational Safety and Health were discussed by Paul Schulte of NIOSH/CDC. He indicated the importance of the rights of participants, validity and clinical utility, action resulting from biomarker information, social implications, and the right of participants emphasising the point that the National Bioethics Advisory Commission (NBAC) in 1999 has agreed on: Autonomy, respect for person, beneficence, privacy, and confidentiality. As far as ethics on genetic polymorphism is concerned, he indicated that the NBAC decision on this matter included the validity and clinical utility (scientific attributes of a biomarker) and action resulting from biomarker information such as interpretation and communication, identification and labelling of individual and groups at risk, psychological distress, discrimination (insurance, employment), early warning, policy and regulation, genetic exceptionalism: treat genetic information if or as if it were different.

Workshops conducted on the next day of the workshop have indicated that future of biomarker research should concentrate on molecular markers of exposure, susceptibility and effect with the incorporation of future advances in analytical capability, better sampling techniques, additional data on toxicokinetics and pathophysiology and International cooperation.