Published in final edited form as:

J Occup Environ Hyg. 2014 ; 11(12): 809–818. doi:10.1080/15459624.2014.922689.

A Novel Alternative to Environmental Monitoring to Detect Workers at Risk for Beryllium Exposure-Related Health Effects

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Abstract

The purpose of this study was to describe a methodology for surveillance and monitoring of beryllium exposure using biological monitoring to complement environmental monitoring. Eighty-three Israeli dental technicians (mean age 41.6 ± 1.36 years) and 80 American nuclear machining workers (54.9 ± 1.21 years) were enrolled. Biological monitoring was carried out by analyzing particle size (laser technique) and shape (image analysis) in 131/163 (80.3%) induced sputum samples (Dipa Analyser, Donner Tech, Or Aquiva, Israel). Environmental monitoring was carried out only in the United States (Sioutas impactor, SKC, Inc., Eighty Four, Pa.). Pulmonary function testing performance and induced sputum retrieval were done by conventional methods. Sixty-three Israeli workers and 37 American workers were followed up for at least 2 years.

Biological monitoring by induced sputum indicated that a >92% accumulation of $<5 \mu\text{m}$ particles correlated significantly to a positive beryllium lymphocyte proliferation test result (OR 3.8, 95% CI 1.2–11.4, $p = 0.015$) among all participants. Environmental monitoring showed that beryllium particles were $<1 \mu\text{m}$, and this small fraction ($0.1\text{--}1 \mu$) was significantly more highly accumulated in nuclear machining workers compared to dental technicians. The small fractions positively correlated with induced sputum macrophages ($r = 0.21$ $p = 0.01$) and negatively correlated with diffusion lung carbon monoxide single breath (DLCO-SB $r = 0.180$ $p = 0.04$) in all subjects. Years of exposure were positively correlated to the number of accumulated particles $2\text{--}3 \mu$ in diameter ($r = 0.2$, $p = 0.02$) and negatively correlated to forced expiratory volume in one second/forced vital

capacity findings ($r = -0.18$, $p = 0.02$). DLCO was decreased in both groups after two years of monitoring.

Biological monitoring is more informative than environmental monitoring in the surveillance and monitoring of workers in beryllium industries. Induced sputum is a feasible and promising biomonitoring method that should be included in the surveillance of exposed workers.

Keywords

induced sputum; exposure assessment; biological monitoring; particulate matter; hazardous dust

INTRODUCTION

Chronic beryllium disease (CBD) is an immune-mediated granulomatous lung disease detected in some workers exposed to beryllium. In 1949, the Atomic Energy Commission recommended exposure limits of $2 \mu\text{g}/\text{m}^3$ for occupational exposure and $0.01 \mu\text{g}/\text{m}^3$ for non-occupational exposure in an effort to prevent both the acute and chronic forms of the disease.⁽¹⁾ This level was adopted by the U.S. Occupational Safety and Health Administration (OSHA) as a permissible exposure limit (PEL) for U.S. workplaces in 1970.⁽²⁾

However, many studies indicate that $2 \mu\text{g}/\text{m}^3$ in the workplace is grossly inadequate to prevent disease from occurring.^(3,4) CBD and beryllium sensitization (BeS) have been identified in workers whose average Be exposure levels ranged between 0.02 – $0.10 \mu\text{g}/\text{m}^3$ ^(2,3) values that are 20–100 times lower than the current PEL. Most of those epidemiological studies have reported elevated prevalence of BeS and CBD among employees in specific work processes, and have related this excess risk to various exposure metrics predicated on airborne Be mass concentration.⁽⁵⁾

Exposure-response relationships have, however, been inconsistent.⁽⁶⁾ possibly due to factors such as exposure misclassification and lack of accurate and/or biologically relevant exposure metrics.^(7,8) The established particle size-selective criteria are based on the probability of an aerosol penetrating into the lung rather than depositing particulate matter. Penetration for sub-particulate matter is a constant 100%, while the extent of deposition fluctuates. The deposited particulate criterion has been suggested as a more appropriate measure of exposure and may be of significance for the development of CBD.⁽⁹⁾ The aerodynamic diameter of particles influences the site of aerosol penetration and deposition throughout the respiratory tract. As such, different particle size distributions of the same contaminant will result in differences in deposition among regions of the respiratory tract. Therefore, particle size is an important exposure consideration relevant to the development of pulmonary disease.⁽¹⁰⁾ Inhalable particles (20 – $100 \mu\text{m}$) are those with sizes capable of entering the head airways region and, therefore, may be relevant to the development of BeS. Respirable particles (1 – $10 \mu\text{m}$) are the fraction of the aerosol that penetrates the tracheobronchial region and the elements that may enter the non-ciliated alveolar region, therefore making them more relevant to the development of inflammation and CBD.^(8,9)

A limited number of studies have reported size-fractionated Be exposures for jobs or processes in workplaces.^(10,11) Other studies suggest that exposure to Be particles $<6\text{ }\mu\text{m}$ and $<1\text{ }\mu\text{m}$ in aerodynamic diameter may be associated with BeS or CBD,⁽⁸⁾ and that the CBD risk may be better correlated to exposure metrics based either on total mass or on the estimated number of Be particles in the size fraction most likely to deposit in the alveolar region of the lung.⁽¹²⁾ However, to date, data on the direct measurement of deposited particulate matter from airways of workers exposed to Be in different industries have not been reported in the literature.

The focus of this study is on alternative exposure metrics using a direct measurement of deposited particles in the airways sputum as a form of biological monitoring of workers exposed to Be for their possible role of particle size in risk prediction of BeS and CBD. According to the definition of American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Diseases (ATS/ERS/WASOG),⁽¹³⁾ similar to sarcoidosis, CBD is characterized by the T helper-1 response, with accumulation of positive T lymphocytes and activated macrophages in the lung, resulting in the typical alveolitis response. In this context, induced sputum (IS) was used to fulfill the same criteria as bronchoalveolar lavage (BAL) as a diagnostic tool in sarcoidosis CBD by virtue of its ability to identify a high percent of lymphocytes with CD4 predominance.

MATERIALS AND METHODS

Study Population

The present study reflects a binational collaborative study between research teams in Israel and National Jewish Health (NJH) in the United States. Active dental technicians (DTs) in Israel were invited to participate in the study by phone calls to their workplaces. NJH is a national referral center for Be-related health effects in the United States, with one of the largest cohort of workers diagnosed as having BeS or CBD, and workers exposed to Be but not sensitized to it (Be-NS). Subjects were identified from the clinical cohort as well as from a precision machining plant for whom NJH provides ongoing medical and environmental surveillance. The final diagnosis (FDx) of Be-related health effects was characterized in both populations as those with Be-NS, BeS, or CBD. Specifically, evidence of BeS was demonstrated by two abnormal blood Be lymphocyte proliferation tests (BeLPT) or one abnormal bronchoalveolar lavage (BAL) LPT and no evidence of granulomatous inflammation on transbronchial lung biopsy. Those diagnosed with CBD showed evidence of BeS as well as proven granulomatous inflammation, as demonstrated by pathological or radiographic evidence. Individuals employed at the precision machining plant who had at least one normal blood BeLPT were enrolled as Be-NS subjects.

All subjects gave written informed consent to be enrolled and the studies were ethically approved by the Tel-Aviv Sourasky Medical Center Institutional Ethics Committee and the National Jewish Health Institutional Review Board. Subjects completed demographic, clinical, and occupational questionnaires, clinical assessment pulmonary function testing (PFTs), induction of sputum, and BeLPT.

PFTs and IS Induction and Processing

PFTs were performed in Israel by a Masterlab spirometer (Masterlab, E. Jaeger, Wurzburg, Germany) and in the United States by a MasterScreen spirometer (Jaeger, Wurzburg, Germany) using standard protocols according to American Thoracic Society guidelines.⁽¹⁴⁾ Sputum induction and processing was done as described in our previous studies.^(15,16)

BeLPT

BeLPT was performed within 24 hr of venipuncture on all subjects according the method of Mroz et al.⁽¹⁷⁾ It was done at the same time as IS and PFT in both Israel and in the United States, but previous BeLPT data were also available for the U.S. subjects. An abnormal BeLPT was required to have two or more elevated SIs occurring at any of the BeSO₄ concentrations tested. An SI >2.5 was considered abnormal.

Particle Size Distribution Analysis and Image Analysis

Particle Size Analysis and Image Analysis were performed using induced sputum samples according to a laser technique based on the time of transition theory (TOT) using an DAPI Analyzer (Donner Technologies Ltd., Or Aquiva, Israel).⁽¹⁸⁾ Additional information is available on an online supplemental file.

Environmental Monitoring

In Israel, individual samples of airborne particles were collected for each DT using personal samplers (SKC, Inc.) and 37-mm cassettes (SKC, Inc.) loaded with membrane filters (mix cellulose ester membrane filters, 0.8 µm pore size) within 24 hr of the procedure. A primary calibration source (BIOS DryCal DC-Lite, Brandt Instruments, Inc., Prairieville, La.) was used to calibrate the airflow rate prior to and upon termination of sampling, and the average between the two was taken for calculation. Samples were analyzed for their Be content by atomic absorption spectroscopy, and the time-weighted average (TWA) of Be exposure was calculated. Particle size selective sampling in the United States was performed for each participant within 48 hr of his or her IS procedure. Each particle size sampling was conducted for 8 hr using the Sioutas Impactor (SKC, Inc.) measuring particles on different cutoffs of 0.25, 1.0. and 2.5 µm). Additional information is available on an online supplemental file.

Statistical Analysis

Comparison of all the tested parameters between the American and Israeli workers was by the two-sample t-test. Since the groups were not comparable with regard to age, body mass index (BMI), and smoking, an additional comparison was performed to adjust for these parameters by way of a two-way analysis of variance (ANOVA) with age as covariate. A similar analysis was performed to compare between diagnosis categories. Since there were only a few smokers among the BeS and CBD patients, they were excluded from this analysis, and the healthy workers were divided into two groups, smokers versus non-smokers. An ANOVA with age and BMI as covariates was performed. PFT parameters were compared between countries and diagnosis categories, adjusting for smoking only. A receiver operating curve (ROC) curve was calculated to find the optimal cutoff point of

accumulated particles $<5 \mu\text{m}$ that differentiates between negative/positive BeLPT subjects. The subjects were then categorized according to whether the number of their particles $<5 \mu\text{m}$ totaled less or more than 92% of all the particles.

Logistic regression models were applied to the data to locate risk factors (predictors) associated with positive BeLPT or those with BeS or CBD. The first model included all subjects, and allowed examination of whether different types of industry (DT versus NM) are associated with a positive BeLPT after adjustment for age, gender, smoking, particles $<5 \mu\text{m}$, FEV1/FVC, and -percent lymphocytes. Additional models were constructed separately for each country/group of workers to identify specific risk factors associated with positive BeLPT in each of them. Three model building methods were applied for each logistic regression model: forced entry, forward selection, and backward elimination. The change between two time points in all parameters was calculated for each worker. Differences were compared between countries and between diagnosis categories using ANOVA adjusted for smoking, age, and BMI as detailed above. All statistical analysis was performed using Statistical Analysis Software (SAS) for Windows, version 9.2.

RESULTS

The combined U.S. and Israel study population included 163 individuals: 83 DTs were recruited in Israel (mean age (SE Standard Error) 41.6 (1.36 years); 71 males and 12 females) and 80 nuclear machine workers (NMs) in the United States (mean age 54.9 (1.21) years; 67 males and 13 females). There were no differences in gender and years of exposure between both populations, but the U.S. population were significantly older than the Israeli dental technicians ($p < 0.0001$), with significantly higher BMIs ($p = 0.001$) and differences in smoking habits ($p = 0.0003$). Final diagnosis (FDx) of the participants was done by clinical parameters and BeLPT results and the results were significantly different, as would be expected with different enrollment schemes. Specifically, 66/83 (79%) Israeli DTs had no sensitization to Be, 10/83 (10%), had BeS, and 7/83 (8.4%) were defined as having CBD. In the U.S. population, 24/80 (30%) of the NMs had no BeS, 28/80 (35%) others had a borderline response, and 28/80 (35%) were defined as having CBD ($p = 0.007$ between all groups) (Table I).

Since there was a significant difference in smoking status, all further analyses were done after dividing the study population into four groups: Be No sensitization non-smoking (Be NS NSm), Be No sensitization smoking (Be NS S), BeS, and CBD. PFT was done in all subjects and showed that the forced expiratory volume in one second/forced vital capacity (FEV1/FVC) ratio and total lung capacity (TLC) was significantly different between both populations in Israel and the United States. When subjects were stratified by their FDx results, the CBD patients were observed to have worse PFT outcome data, with significant differences for FEV1/FVC and DLCO-SB for the Be-NS, NSm, and BeNS S groups (Table II).

Differential counts and T cells subset analysis by FACS (Fluorescent Analyzer Cell Sorter, Becton, Dickinson and Company, Franklin Lakes, NJ) were done on 78/83 (94%) subjects in Israel and 53/80 (66.2%) in the United States. The differential cell counts were significantly

different between the United States and Israel, as follows: macrophages 25(1.9) versus 59.1(3.5) $p = 0.001$; neutrophils 60.8(2.2) versus 36.5(3.3) $p = 0.001$; eosinophils 2.1(0.4) versus 1.1(0.3) $p = 0.03$; and lymphocytes 11.9(0.9) versus 4.5(0.6) $p = 0.001$, respectively. (Table IIIa). Particle size distribution analysis and image analysis were performed for all IS samples from Israel and for 53/80 (66.2%) samples from the United States. The samples that were measured by the laser channel for particle size distribution analysis showed a significantly higher proportion of particles in the small fraction (0.1–1 μm) in the IS of the U.S. population compared to the Israel population [21.5(9.4) versus 25.4(11.3), respectively, $p = 0.03$].

A further analysis was done using the Image Analysis channel where the size is measured by average ferret (AF) that is the average of 36 diameters taking into account that the particles are not spheres. The AF was larger in the U.S. group compared to the Israeli group (5.8(0.2) μm versus 5.3(0.2) μm , $p = 0.005$, Table II). When the subjects were stratified solely by FDx results, the AFs of the CBD subjects were significantly larger compared to the other groups: Be-NS NSm, Be-NS S and BeS (6.1(0.3) for CBD versus 5.3(0.2), 5.4(0.3), 5.7(0.2), respectively, $p = 0.03$) (Table II). The fraction of particles (0–5 μm) for the entire cohort was negatively correlated with DLCO-SB parameters ($r = -0.22$, $p = 0.01$), but only the smallest fraction (0.1–1 μm) was positively correlated with the cellular fraction of macrophages ($r = 0.21$, $p = 0.04$, data not shown).

A total of 33 impactor samples had been collected over two years of sampling in the United States. Of these samples, 23 had detectable levels of Be on at least one stage of the impactor, with 6 detecting Be on all stages. It appeared that the majority of the Be particles were <1 μm in diameter, and there was probably a bimodal distribution consisting of small (<1 μm) general room air particles and large particles produced directly by the process the participant was working on during the sampling period (data not shown).

The biological and environmental monitoring results in Israel were compared by performing environmental measurements in 5 of the 15 laboratories, testing for particle size distribution and total Be exposure. Be was not found in any of the laboratories tested. Figure 1 shows a representative measurement of environmental monitoring from one dental laboratory (pink line) and biological monitoring performed from sputum of four DTs working in that laboratory (brown, yellow, green, and blue lines) using Dipa (Or Aquiva, Israel) laser technology. The data illustrate that the environmental monitoring shows a higher proportion of larger particles while the small particles were retained in the DTs' airways.

In this context, we demonstrated that the size of the particles retained in the airways decreased over the years of exposure ($r = -0.2$, $p = 0.02$), and only the small-sized fraction (range of 2–3 μ) had significantly accumulated over time ($r = 0.2$, $p = 0.02$). Moreover, long exposure induced low FEV1/FVC ratios ($r = -0.18$, $p = 0.02$) (Figures 2a – c).

The study populations in both countries were followed up after two years. Recruitment in Israel was higher than in the United States (68/83 (82%) versus 34/80 (42%), respectively). Only the diffusing capacity for carbon monoxide single breath (DLCOSB) decreased in both countries, with higher levels of negative change in the U.S. population (–0.86(0.3)

-2.33(6.0), $p = 0.06$), while a significant accumulation of CD4 T lymphocytes was observed (1.9(0.9) and 1.5(2.1) $p = 0.004$).

Examination of the particulate matter according to the image analysis revealed that the shape composition of the accumulated particles was significantly different between the groups from the two countries. The retained fraction showed a significant lower average ferret (AF) for the particles (5.3(0.17) μm versus 3.6(0.16) μm in Israel and 5.8(0.2) μm and 4.3 (0.2) μm in the United States, $p = 0.01$), with intense opacity in both countries (73.4 (2.1) versus 76 (2.3) in Israel and 72 (2.3) versus 100 (2.4) in the United States, $p = 0.0002$) (Table IIIa).

Association between group/work area and risk for a positive BeLPT test was assessed via multiple logistic regression model. It included all subjects, and it was controlled for variables that were significantly associated in the bivariate analysis: age, gender, smoking, BMI, accumulation of particles $<5 \mu\text{m}$, FEV1/FVC, DLCOSB, and lymphocytes. The American NMs had an increased risk to have a positive BeLPT compared with the Israeli DTs (Odds Ratio 23.4 (95% CI, 5.6–98.8), $p < 0.001$). An accumulation over 95% of particles $<5 \mu\text{m}$ was significantly correlated to a positive BeLPT (Odds Ratio 3.8 (95% CI 1.2–11.4), $p = 0.0154$). A significant association was found for FEV1/FVC as well (Table IIIb).

DISCUSSION

Historically, the available data on the deposition of Be particles in the airways had been derived from indirect extrapolations of measurements taken by monitoring exposure at the workplace by personal impactors and fixed-airhead area samples. This article describes the first exposure characterization done by means of exposure metrics related to particulate matter deposited in the airways that may be more biologically relevant. This characterization was performed comparing active DTs in Israel to current and former workers involved in the fabrication of Be parts for the nuclear, defense, and aerospace industries in the United States. This comparison elucidates the crucial correlation between industry type and biological responses. The physical characteristics of deposited particulate matter, including particle size and shape, were examined in relation to the risk of BeS and CBD, as were a number of physiological and immunological parameters related to BeS and CBD.

In the United States, the largest cohorts of CBD cases came from a variety of industries, ranging from nuclear weapons manufacture to Be machining to recycling.^(12,19–21) In Israel, the first case of a DT with CBD was published in 2001⁽²²⁾ followed by a series of 12 DTs subsequently diagnosed as having CBD.⁽²²⁾ All workers exposed to Be in Israel were then tested, and a significant correlation was found between being a DT and the risk of developing CBD.⁽²³⁾

The analyses revealed significant differences in the rate of BeS and CBD among DTs and NMs according to BeLPT results, based on different enrollment patterns. While the prevalence of BeS and CBD is inarguably highly dependent on type of industry,^(12,24,25) those differences cannot be taken into account in our study population since the recruited

American workers had already been diagnosed as having BeS or CBD based on previous BeLPT findings.

The workers underwent further screening by routine PFTs and IS studies. The U.S. group showed an obstructive pattern compared to the Israeli population, although this was likely related to differences in diagnosis and smoking status. PFTs are usually included in an evaluation to rule out CBD, but they are non-specific in CBD, which has a range of abnormalities, including obstruction, restriction, a mixed process, and/or a reduced DLCO, with obstruction being the most common abnormality.⁽²⁶⁾ The CBD patients in the current study had significantly worse lung function parameters than the BeS and Be-NS workers. It is already known that CBD often results in progressive loss of respiratory function and early abnormalities, including airflow obstruction and restriction, with decreased diffusing capacity for carbon monoxide (DLCO).⁽²⁷⁾ Taking into account the years of exposure for the entire cohort, the FEV₁/FVC ratio was negatively correlated to long exposures as well.

In contrast with the findings of previous studies,⁽¹⁸⁾ the current results did not reveal any differences in the CD4/CD8 ratio between the study groups in showing that a high variability in CD4/CD8 T lymphocyte may be present in CBD, as in sarcoidosis.⁽²⁸⁾ Although the change in CD4/CD8 ratio was also not significant after follow-up, a clear accumulation of CD4 T lymphocytes in IS samples was observed over time for all individuals tested. Be-specific CD4 T cells in peripheral blood were recently found to highly secrete IFN gamma and could be used as a biomarker of disease progression.⁽²⁹⁾

Several authors speculated that CD4 T lymphocytes in sputum samples may be used to monitor Be-exposed workers.^(30–32) This supports the concept of an uninterrupted recirculation and dynamic balance of lymphocytes in general, and of the CD4 T lymphocytes in particular, from mucosa-associated lymphoid tissue with its activated lymphoid follicles to effectors sites in the integrated human mucosal immune system.

Particle size and shape analysis was performed for particulate matter in all available sputum samples. The particles derived from both groups were <10 µm in aerodynamic diameter, but the U.S. samples contained a significantly higher fraction of small particles (0–1 µm) than the Israeli samples, as measured by a laser beam (under the assumption that all particles are spheres), with a higher general mean size measured by image analysis (AF, under the assumption that not all particles are spheres). These data are in agreement with previous reports that size-fractionated Be exposure is dependent on jobs or processes in different workplaces. The aerosol particles generated during the milling of Be silicate ores, Be oxide, and Be hydroxide showed specific surface areas which decreased in size with processing from feedstock ores to drumming final product of Be hydroxide.⁽³³⁾ Moreover, some Be processes, especially machining, are already known to be associated with an increased risk of BeS and CBD, generating more than 50% of particles with an aerodynamic diameter <10 µm.⁽²⁰⁾

The particle aerodynamic diameter influences the site of aerosol penetration and deposition throughout the respiratory tract. As such, different particle size distributions of the same contaminant will result in differences in deposition among various regions of the respiratory

tract. Therefore, particle size is an important exposure consideration relevant to the development of pulmonary injury or disease.⁽¹⁰⁾ Inhalable particles are those with sizes capable of entering the head airways region and, therefore, are highly likely to be relevant to the development of BeS and, subsequently, CBD.⁽²⁰⁾ Indeed, it was possible to demonstrate in the current study that the percentage of particles between 0 and 5 μm in IS samples was higher in the BeS and CBD individuals, and that particles with the specific fraction of 2–3 μm are retained in the airways and accumulated over the years of exposure for the entire sample population in both countries.

The retention of particles and their accumulation over the years of exposure was already demonstrated by us in our previous work on welders⁽³⁴⁾ and subsequently in the firefighters cohort on duty during the WTC (World Trade Center) collapse.⁽³⁵⁾ In our recent study on a dental technicians cohort we demonstrated that high content of particles (92%) in IS >5 μm in size is correlated to a positive BeLPT risk (odds ratio (OR) = 3.4, 95% confidence interval (CI): 0.9–13).⁽³⁶⁾

Several studies have shown an association between *environmental* particulate size and risk for BeS and CBD development. A case control study of workers in a precision machine shop demonstrated that exposure to Be particles measuring either <6 μm or >1 μm was associated with BeS or CBD and was higher among BeS or CBD cases than controls, albeit not significantly.⁽¹²⁾ Others reported significant associations between CBD and BeS and exposure metrics based either on mass or on estimated number of *environmental* Be particles in the size fraction most likely to deposit in the alveolar region of the lung.⁽¹³⁾ However, to date, there is no report on an association between particulate size with the development of BeS and CBD as revealed by direct biological relevant exposure metrics or assessment of particulate matter in airways.

There was an accumulation of particles in the fraction between 0 and 5 μm for both countries during the follow-up of the studied workers. This was confirmed when the shape of the particles measured by an AF diameter was taken into consideration. There was a negative correlation between this diameter and the accumulation of small particles over the years of exposure. Fractions between 0 and 5 μm had not accumulated among CBD patients over the 2-year follow-up. That might be explained by the changes that may occur in the diseased lung. For example, it is known that human lung parenchyma retains small fraction particles,⁽⁶⁾ while particles >5 μm and <10 μm reach the proximal airways and are eliminated by mucociliary clearance. The finding of a positive correlation with the percentage of macrophages in sputum for both studied populations might also support this lack of particle accumulation, since macrophages may assist in their clearance. In fact, a recent study demonstrated the availability of dissolved Be to immune-competent cells during mechanical clearance from the conducting airways in a model of artificial lung alveolar macrophage phagolysosomal fluid.⁽³⁷⁾

Particles in the small-size range (0–5 μm) were inversely correlated to the DLCO parameters but positively correlated with the percentage of macrophages in sputum for all study participants. The association between particle size and decreasing DLCO values may reflect

the highly sensitive ability of these parameters to show early changes in interstitial lung disease.⁽³⁷⁾

The particle size in the environment of a dental laboratory was directly compared to the size of the particles present in the IS of the DTs working in that space. The inhalable particles in the airways were smaller than those present in the air. This finding was compatible with those of previous studies on foundry workers⁽³⁹⁾ and on the exposed firefighters at the site of the WTC collapse.⁽³⁵⁾

A logistic regression model showed that an over 95% accumulation of particles <5 µm was the most significant parameter that can predict an abnormal BeLPT. This may be due to the fact that the aerodynamic size of the particles in Be aerosol affects the site of deposition and alters the ability of the particles to cause sensitization and disease. Small particles deposited below the ciliated region of the conducting airways result in greater Be retention and greater opportunity for contact between Be and cells of the immune system, potentially resulting in increased sensitivity and disease. This was also demonstrated in an animal model in which the immunologic effects of two different particle sizes of Be metal, <2.5 µm (fine Be) and <10 µm (inhalable Be), on C3H/HeJ mice showed that fine particles may induce more pronounced immunological effects than inhalable particles.⁽⁴⁰⁾

Although the main route of exposure is by inhalation, attention has been drawn to the control of exposure via skin contact with beryllium materials, including salts and particles.⁽⁴¹⁾ This possibility could be relevant to the development of beryllium sensitization. We recently demonstrated a validated case of skin sensitization to beryllium through shrapnel that had penetrated the skin of the chest wall of a patient with no history of occupational exposure and finally correctly diagnosed as having chronic beryllium disease.⁽⁴²⁾ Moreover, it was demonstrated by others that fine particles (0.5–1.0 µm) are able to penetrate the stratum corneum and reach the immunologically active epidermis, and that flexing motion (e.g., at the wrist) *or the motion associated with machining and grinding* provides the kinetic energy necessary to move particles into the skin. These data are consistent with the development of a hapten-specific, cell-mediated immune response following topical application of beryllium and suggest a mechanistic link between the persistent rate of beryllium worker sensitization and skin exposure to fine and ultrafine beryllium particles.⁽⁴³⁾

The main limitation of this study is the retention of relatively few NWs during the 2-year follow-up, leaving smaller numbers for long-term comparisons. This may be the reason that some of the results at baseline and at 2 years turned out not to be significant. Moreover, the different recruitment protocols of the U.S. and Israel subjects should be taken in consideration since the NWs had already been diagnosed as having BeS or CBD based on previous BeLPT.

CONCLUSION

The information gleaned from traditional environmental exposure assessment is incomplete for adequate surveillance and monitoring of workers in Be industries. The novel biological

approach of supplementing the investigation of exposed workers with data on the biological effects of size and shape on particle lung deposition retrieved by induced sputum holds promise to fill some of the gaps in the older methodology. Biological monitoring findings raise the possibility that the ability of particles to penetrate into the respiratory system may be one of several factors relevant to the development of BeS and CBD. This is important for understanding the elevated risk posed by certain industrial processes as well as for developing novel exposure metrics that incorporate these biologically relevant factors for use in future epidemiologic investigations.

ACKNOWLEDGMENT

Esther Eshkol, the institutional medical and scientific copyeditor, is thanked for editorial assistance.

REFERENCES

1. Eisenbud M. Origins of the standard for control of beryllium disease (1947–1949). *Environmental Research*. 1982; 27:79–88. [PubMed: 7040071]
2. “The Occupational Safety and Health Act, a National Consensus Standard for Beryllium and Beryllium Compounds.”. Code of Federal Regulations Title. 1970; 29 Part 1910.1000, Table Z-2. [ANSI Standard Z37.29].
3. Van Dyke MV, Martyny JW, Mroz MM, et al. Risk of chronic beryllium disease by HLA-DPB1 E69 genotype and beryllium exposure in nuclear workers. *Am. J. Respir. Crit. Care. Med.* 2011; 68:842–848.
4. Kelleher PC, Martyny JW, Mroz MM, et al. Beryllium particulate exposure and disease relations in a beryllium machining plant. *J. Occup. Environ. Med.* 2001; 43:238–249. [PubMed: 11285872]
5. Kreiss K, Day GA, Schuler CR. Beryllium: A modern industrial hazard. *Annu. Rev. Public Health.* 2007; 28:259–277. [PubMed: 17094767]
6. Kreiss K, Mroz M, Zhen B, Wiedemann H, Barna B. Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occup. Environ. Med.* 1977; 54:605–612. [PubMed: 9326165]
7. Day GA, Stefaniak AB, Weston SA, Tinkle SS. Beryllium exposure: Dermal and immunological considerations. *Int. Arch. Occup. Environ. Health.* 2006; 79(2):161–164. [PubMed: 16231190]
8. McCawley MA, Kent MS, Berakis MT. Ultrafine beryllium number concentration as a possible metric for chronic beryllium disease risk. *Appl. Occup. Environ. Hyg.* 2001; 16:631–638. [PubMed: 11370941]
9. McCawley MA. Rationale for sampling deposited submicrometer beryllium particulate matter. *J. Occup. Environ. Hyg.* 2009; 12:789–793. [PubMed: 19894182]
10. Thorat DD, Mahadevan TN, Ghosh DK. Particle size distribution and respiratory deposition estimates of beryllium aerosols in an extraction and processing plant. *Am. Ind. Hyg. Assoc. J.* 2003; 64:522–527.
11. Chauhan S, D’Cruz R, Faruqi S, Singh KK, Varma S, Singh M. Chemical warfare agents. *Environ. Toxicol. Pharmacol.* 2008; 26:113–122. [PubMed: 21783898]
12. Kent MS, Robins TG, Madl AK. Is total mass or mass of alveolar-deposited airborne particles of beryllium a better predictor of the prevalence of disease? A preliminary study of a beryllium processing facility. *Appl. Occup. Environ. Hyg.* 2001; 16:539–558. [PubMed: 11370934]
13. American Thoracic Society/European Respiratory Society/World Association Sarcoidosis other Granulomatous Diseases Statement. Statement on Sarcoidosis. *Am. J. Respir. Crit. Care. Med.* 1999; 160:736–755. [PubMed: 10430755]
14. Miller MR, Hankinson J, Brusasco V. Standardization of spirometry. *Eur. Respir. J.* 2005; 26:319–338. [PubMed: 16055882]

15. Popov T, Gottschalk R, Kolendowicz R, Dolovich J, Powers P, Hargreave FE. The evaluation of a cell dispersion method of sputum examination. *Clin. Exp. Allergy*. 1994; 24:778–783. [PubMed: 7982128]
16. Fireman E, Topilsky I, Greif J, et al. Induced sputum compared to bronchoalveolar lavage for evaluating patients with sarcoidosis and non-granulomatous interstitial lung disease. *Respir. Med*. 1999; 93:827–834. [PubMed: 10603633]
17. Mroz MM, Kreiss K, Lezotte D, Campbell PA, Newman LS. Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *J. Allergy Clin. Immunol*. 1991; 88:54–60. [PubMed: 2071785]
18. Fireman E, Lerman Y. Induced sputum in occupational lung diseases. *Breathe*. 2005; 2:50–59.
19. Martyny J, Hoover M, Mroz M, et al. Aerosols generated during beryllium machining. *J. Occup. Environ. Med*. 2000; 42:8–18. [PubMed: 10652683]
20. Sackett HM, Maier LA, Silveira LJ, et al. Beryllium medical surveillance at a former nuclear weapons facility during cleanup operations. *J. Occup. Environ. Med*. 2004; 46:953–961. [PubMed: 15354061]
21. Fireman E, Kramer MR, Kaufman J, Müller-Quernheim J, Lerman Y. Beryllium disease: First case reported in Israel. *Isr. Med. Assoc. J*. 2001; 3:224–225. [PubMed: 11303384]
22. Fireman E, Kramer MR, Priel I, Lerman Y. Chronic beryllium disease among dental technicians in Israel. *Sarcoidosis Vasc. Diffuse Lung Dis*. 2006; 23:215–221. [PubMed: 18038921]
23. Fireman E, Mazor O, Kramer M, Priel I, Lerman Y. Non-invasive diagnosis of chronic beryllium disease in workers exposed to hazardous dust in Israel. *Occup. Environ. Med*. 2010; 67:631–635. [PubMed: 19955573]
24. Schuler CR, Kent MS, Deubner DC. Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. *Am. J. Ind. Med*. 2005; 47:195–205. [PubMed: 15712254]
25. Aronchick JM, Rossman MD, Miller WT. Chronic beryllium disease: Diagnosis, radiographic findings, and correlation with pulmonary function tests. *Radiology*. 1987; 163:677–682. [PubMed: 3575713]
26. Kriebek D, Sprince NL, Eisen EA, Greaves IA, Feldman HA, Greene RE. Beryllium exposure and pulmonary function: A cross sectional study of beryllium workers. *Br. J. Ind. Med*. 1988; 45:167–173. [PubMed: 3258161]
27. Rossman MD. Chronic beryllium disease: Diagnosis and management *Environ. Health Perspect*. 1996; 104:945–947.
28. Kantrow SP, Meyer KC, Kidd P, Raghu G. The CD4/CD8 ratio in BAL fluid is highly variable in sarcoidosis. *Eur. Respir. J*. 1997; 10:2716–2721. [PubMed: 9493649]
29. Martin AK, Mack DG, Falta MT, et al. Beryllium-specific CD4+ T cells in blood as a biomarker of disease progression. *J. Allergy Clin. Immunol*. 2011; 128:1100–1106. [PubMed: 21943943]
30. Fireman Z, Osipov A, Kivity S, et al. The use of induced sputum in the assessment of pulmonary involvement in Crohn's disease. *Am. J. Gastroenterol*. 2000; 95:730–734. [PubMed: 10710066]
31. Scuster M, Tschernig T, Krugand N, Pabst R. Lymphocytes migrate from blood onto bronchoalveolar lavage and lung parenchyma in the asthma model of a brown Norway rat. *Am. J. Respir. Crit. Care Med*. 2000; 161:558–566. [PubMed: 10673200]
32. Pabst R, Tschering T. Lymphocyte dynamics: Caution in interpreting BAL numbers. *Thorax*. 1997; 52:1078–1080. [PubMed: 9516903]
33. Stefaniak AB, Chipera SJ, Day GA, et al. Physicochemical characteristics of aerosol particles generated during the milling of beryllium silicate ores: Implications for risk assessment. *J. Toxicol. Environ. Health*. 2008; 71:1468–1481.
34. Fireman E, Lerman Y, Stark M, et al. Detection of occult lung impairment in welders by induced sputum particles and breath oxidation. *Am. J. Ind. Med*. 2008; 51:503–511. [PubMed: 18459140]
35. Fireman EM, Lerman Y, Ganor E, et al. Induced sputum assessment in New York City firefighters exposed to World Trade Center dust. *Environ. Health Perspect*. 2004; 112:1564–1569. [PubMed: 15531443]
36. Stark M, Lerman Y, Kapel A, et al. Biological exposure metrics of beryllium-exposed dental technicians. *Arch. Environ. Occup. Health*. 2014; 69:89–99. [PubMed: 24205960]

37. Stefaniak AB, Virji MA, Day GA. Dissolution of beryllium in artificial lung alveolar macrophage phagolysosomal fluid. *Chemosphere*. 2011; 83:1181–1187. [PubMed: 21251696]
38. Mukerjee D, George CSt, Knight J. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology*. 2004; 43:461–466. [PubMed: 15024134]
39. Fireman E, Greif J, Schwarz Y, et al. Assessment of hazardous dust exposure by BAL and induced sputum. *Chest*. 1999; 115:1720–1728. [PubMed: 10378572]
40. Salehi F, Zayed J, Audusseau S, et al. Immunological responses in C3H/HeJ mice following nose-only inhalation exposure. *J. Appl. Toxicol.* 2009; 29:61–68. [PubMed: 18980269]
41. Curtis GH. Cutaneous hypersensitivity due to beryllium: A study of thirteen cases. *Arch. Dermat. Syph.* 1951; 64:470–482.
42. Fireman E, Shai ABar, Lerman Y, et al. Chest wall shrapnel-induced chronic beryllium disease. *Sarcoidosis Vasc. Diffuse Lung Dis.* 2012; 29:147–150. [PubMed: 23461078]
43. Tinkle SS, Antonini MJ, Rich BA, et al. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ. Health Perspect.* 2003; 111:1202–1208. [PubMed: 12842774]

FIGURE 1.

Comparison between particle size distributions in the induced sputum of dental technicians and environmental monitoring using the laser particle analyzer. (Particle size and shape were done by a DAPI scanner as describe in Methods. Samples of airborne particles were collected for each dental technician using personal samplers [SKC, Inc.] as described in Methods). (color figure available online).

FIGURE 2.

Correlation between years of exposure and (A) size of particles by average ferret diameter (B) % particles 2–3 μ , and (C) FEV₁/FVC. (Particle size and shape were done by DAPI scanner, and pulmonary function testing was done as described in Methods). (color figure available online).

TABLE I

Demographic Parameters and BeLPT Test Results

Country	Gender (M/F)	Age (years) Mean ($\pm$ SE)	BMI w/h ² Mean ($\pm$ SE)	Exposure (years) Mean ($\pm$ SE)	Smoking (y/nv)	Final Diagnosis		
						Be-NS N (%)	BeS N (%)	CBD N (%)
Israel (N = 83)	71/12	41.6 (1.36)	26.9 (0.6)	18.9 (1.21)	24/59	66(79)	10(12)	7(8.4)
United States (N = 80)	67/13	54.9 (1.21)	32.2 (0.63)	19.3 (1.25)	5/65	24(30)	28(35)	28(35)
p-value	0.71	<0.0001	0.0001	0.8	0.0003		0.007 ^A	

Notes: y, present or ever smoker; nv, never smoker; BMI, body mass index; w, weight; h, height; Be-NS, no sensitization to beryllium; BeS, beryllium sensitization; CBD, chronic beryllium disease. FDx was done by BeLPT and clinical parameters.

^A p = 0.007 between the BeLPT distribution in the Israeli and U.S. populations.

TABLE II

PFT Findings of the Entire Study Population (n = 157) by Country and Diagnosis

	Israel	United States	p-value	Be-NS NSm (1)	Be-NS S (2)	BeS (3)	CBD (4)	p-value
FEV1/FVC	81.8 (0.77)	78.8 (0.74)	0.009	81.1 (0.9)	81.7 (1.0)	79.1 (0.8)	78.7 (1.6)	0.01 (1-4)
Mean (SE)	N = 83	N = 80		N = 58	N = 32	N = 36	N = 31	0.05 (2-4)
TLC (%)	98.4 (1.9)	108.3 (1.6)	0.002	102.7 (18)	102.3 (12.2)	103 (14.7)	100 (18.5)	NS
Mean (SE)	N = 82	N = 54		N = 45	N = 21	N = 36	N = 29	
DLCOSB (%)	86.4(2.2)	83.1 (2.1)	0.3	88.8 (2.8)	88.4 (3.5)	88.1 (2.2)	73.5 (3.7)	0.002 (1-4)
Mean (SE)	N = 82	N = 54		N = 45	N = 21	N = 36	N = 29	0.002 (2-4)
								0.02 (3-4)
Macro (%)	25 (1.9)	59.1 (3.5)	0.001	33.5 (3.8)	35.4 (5.6)	48 (5.3)	41.5 (4.7)	NS
Mean (SE)	N = 78	N = 53		N = 53	N = 32	N = 27	N = 25	
Neut (%)	60.8 (2.2)	36.5 (3.3)	0.001	54.6 (3.5)	53 (5.2)	46.6 (4.6)	50.9 (4.1)	NS
Mean (SE)	N = 78	N = 53		N = 58	N = 32	N = 27	N = 25	
Eos (%)	2.1(0.4)	1.1 (0.3)	0.03	2.1 (4.0)	1.6 (3.8)	1.3 (1.9)	1.4 (2.5)	NS
Mean (SE)	N = 78	N = 53		N = 58	N = 32	N = 27	N = 25	
Lymph (%)	11.9 (8.1)	4.5 (4.5)	0.001	9.4 (0.6)	9.8 (0.8)	5.8 (0.4)	8.3 (0.5)	NS
Mean (SE)	N = 78	N = 53		N = 45	N = 21	N = 27	N = 25	
CD4/CD8	3.8 (0.4)	4.6 (0.5)	0.9	4(0.6)	3.4 (0.5)	4.1 (0.6)	4.8 (0.9)	NS
Mean (SE)	N = 68	N = 49		N = 45	N = 21	N = 25	N = 24	
0.1-1 µm (%)	21.5 (1.05)	25.4 (1.5)	0.03	21.6 (1.3)	24.2 (2.1)	24.7 (2.0)	25.1 (1.9)	NS
Mean (SE)	N = 81	N = 53		N = 53	N = 28	N = 27	N = 25	
0-5 µm (%)	87.8 (1.1)	89.5 (1.1)	0.5	85.7 (1.6)	88 (1.8)	91.4 (1.3)	91.2 (1.0)	0.08 (1-3,4)
Mean(SE)	N = 81	N = 53		N = 53	N = 28	N = 25	N = 27	
5-10 µm (%)	7.8 (0.6)	6.9 (0.7)	0.5	8.7 (0.9)	8.1 (1.1)	5.9 (0.8)	5.4 (0.7)	NS
Mean (SE)	N = 81	N = 53		N = 53	N = 28	N = 25	N = 27	
AR µm	0.61 (0.01)	0.58 (0.01)	0.9	0.6(0.02)	0.6 (0.01)	0.6(0.02)	0.6 (0.01)	NS
Mean (SE)	N = 81	N = 53		N = 53	N = 28	N = 27	N = 24	
AF µm	5.3 (0.2)	5.8 (0.2)	0.005	5.3 (0.2)	5.4 (0.3)	5.7 (0.2)	6.1 (0.3)*	0.01 (1-4)
Mean (SE)	N = 81	N = 49		N = 53	N = 28	N = 4	N = 27	

Notes: Values are expressed as mean ± standard error; p values are adjusted to smoking, age, and body mass index.

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PFT, pulmonary function tests; Be-NS, no sensitization; CBD, chronic beryllium disease; BeS, beryllium sensitization; FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; TLC, total lung capacity; DLCO SB, diffusion lung CO in a single breath; NSm, non smoking. Neu, neutrophils; Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Be-NS, no sensitization; BeS: beryllium sensitization; CD4, cluster of differentiation 4 helper T cells; CD8, cluster of differentiation 8 suppressor T cells; AF, average ferret diameter, AR, aspect ratio; NS = not significant. A p-value <0.05 was considered significant.

TABLE IIIParameters in the Follow-up of Israel-United States Populations^A

	DLCOSB (%) Mean (SE)	CD4/CD8 Mean (SE)	AFμm Mean (SE)	GL Mean (SE)
Israel				
Time 1	86.4 (2.2) N = 82	3.8 (0.42) N = 68	5.3 (0.17) N = 81	73.4 (2.1) N = 81
Time 2	85.1 (1.83) N = 68	4.7 (0.72) N = 62	3.6 (0.16) N = 62	76 (2.3) N = 62
United States				
Time 1	83.1 (2.1) N = 54	4.6 (0.5) N = 49	5.8 (0.2) N = 49	72 (2.3) N = 49
Time 2	79 (3.9) N = 17	10 (4.3) N = 17	4.3 (0.2) N = 17	100 (2.4) N = 17
Israel	-0.86 (3.0)	1.9 (0.9)	-1.7 (0.3)	2.5 (3.5)
United States	-2.33 (6.0)*	1.5 (2.1)	-2.3 (0.3)*	38.7 (4.5)*
p-value	p = 0.06*	p = 0.004*	p = 0.01*	p = 0.0002

Notes: Israel and **United States** values were calculated for individuals who were recruited in the 2 years follow-up; DLCOSB, diffusion lung CO single breath; CD4, cluster of differentiation 4 helper T cells; CD8, cluster of differentiation 8 suppressor T cells.; AF, average ferret; GL, gray level. Negative values indicate decrease and positive values indicate increase. Particle size and shape were done by Eyethec scanner as described in the Methods section.

^AValues and expressed as mean ($\pm$ standard error) p-values are adjusted to smoking, age, and body mass index and <0.05 was considered significant.

Table IIIb

Logistic Regression for Variables in Bivariate Analysis in the Entire Population (N = 163)

Parameter	Coefficient Estimate	OR	95% Confidence Limits	p-value ^A
Age	0.0360	1.037	0.985 1.091	0.1701
Gender	0.5184	1.679	0.358 7.877	0.5110
Smoking	-0.7635	0.466	0.107 2.038	0.3104
Group ^B	3.1546	23.444	5.562 98.824	<.0001
0–5 µm	1.3497	3.856	1.294 11.489	0.0154
FEV1/FVC	0.0802	1.084	0.988 1.188	0.0875
BMI	0.0487	1.050	0.953 1.157	0.3245
Exposure (years)	0.007	1.007	0.939 1.081	0.8410

Note: FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; Lymph, lymphocytes; BMI, body mass index

^A A p-value <0.05 was considered significant.^B Group, Israel vs. United States; 0–5µm, particles <5 µ in diameter with an upper quartile range of 92%.