Letter to the Editor

On Beryllium Lymphocyte Transformation Test and Beryllium Levels in Work Environment

Dear Editor:

Yoshida et al. approach an important topic in 'A Study on the Beryllium Lymphocyte Transformation Test and Beryllium Levels in Working Environment, Industrial Health 1997, 35. 374-379. We wish to comment on five matters in this paper.


These investigators employ methodology quite different from that commonly used in assessing sensitivity to beryllium (authors' ref. 10). They employ a high concentration of serum, 25%, that is partially inhibitory with regard to lymphocyte division. They utilize autologous rather than pooled complement inactivated human serum, known to support thymidine incorporation (by testing prior to use). Using autologous plasma introduces the potential for individual variation in cytokine levels, proliferation inhibitors, hormones, etc., which might markedly influence the results in either direction. Their procedures use triplicates instead of octuples; they sample at only one time point instead of two, and use concentrations of beryllium cation that have not been shown to be (but may be) reflective of sensitization as validated by chronic beryllium disease determined by lung biopsy. Thus, the authors may have missed the optimal assay time or beryllium concentration for demonstrating sensitization. Furthermore, the established stimulation index for sensitization is 3.0, not 2.0 as was used in this study. While the data are most interesting, it is very difficult to compare them to other studies.

2. Biologic stability of the beryllium lymphocyte proliferation test over time.

The authors relate the mean and percent positive (>|2.00) values on the Be LTT to general area exposures encountered over a four year (1992-1995) period. The data presented indicate that many of the persons surveyed have been employed in the beryllium industry for a much longer period. Experience in other surveys of beryllium workers indicates that work-related patterns of Be-LTT positivity may persist for greater than a decade. We suggest the data be reanalyzed to attempt to relate beryllium lymphocyte proliferation test results to total work history to determine whether recent or remote exposures or both are influencing the results.

3. General area samples

The authors use general area samples as the measure of worker exposure to beryllium. Studies comparing general area sample data to exposure data obtained in or relative to the breathing zone of the worker clearly demonstrate that general area monitoring is an inadequate representation of the air to which a worker is being exposed. Therefore the general area air sample data presented cannot be used to evaluate or determine a dose response relationship to beryllium sensitivity (lymphocyte proliferation test result).

4. Statistical analysis of serial measures on a population

In Table 2 multiple observations on the same individuals are added in the column labeled “1992-1995” and a Student’s t test is performed. As the data points do not represent independent samples, this is an incorrect application of the Student’s t test. The vertical comparisons in the rest of the table are comparable using an appropriate statistical test. However, due to the high degree of skew resulting in a non-Gaussian distribution of the data, the Student’s t test is not an appropriate choice. As explained below, even if the data were transformed to achieve a Gaussian distribution, this would be inappropriate for other reasons.

5. Parameters chosen for statistical testing

The skewness in lymphocyte proliferation test data reflects the intrinsic biologic meaning of the test. Persons who are biologically sensitized to beryllium have a significant elevation in the SI, and those that are not, do not. Therefore the test data are most appropriately analyzed in binomial form by comparing the percent of independent samples with elevated (over some pre-determined cut-point) Be-LTT values.

We suggest that since the Be-LTT results may reflect the presence of sub-clinical chronic beryllium disease this
diagnosis should be evaluated in persons with confirmed positive blood Be-LTT through bronchoscopy with LTT testing on lavage-obtained lymphocytes and biopsy for the presence of non-caseating granulomas. Information on the incidence of clinically significant chronic beryllium disease in the population of workers with beryllium exposures in plants A and B should be provided.

In summary, the data in this paper on Be-LTT patterns would be most useful if summarized as elevated/not elevated and analyzed relative to lifelong work exposure to beryllium. Conclusions about the relation of Be-LTT to exposure levels of individuals can not be based on data from area samples, as these do not adequately reflect personal exposures.

Sincerely,

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References

The Authors’ Reply:

We would like to thank Dr. Deubner and his colleagues for the opportunity to comment to Be-LTT values.

Our methodology actually differs from your “current” concept of the lymphocyte proliferation test. We have been performing the test in accordance with the method of Williams et al.1. When PPD (purified protein derivative) is used as an antigen in accordance with the current concept of Dr. Deubner, is it possible to obtain the SI of 3.0?
Be-LTT obtained by our method showed a fairly high correlation between patients who were diagnosed as CBD (chronic beryllium disease) in accordance with the diagnostic criteria of Hardy, Williams, and Kriebel2–4, and healthy beryllium-exposed workers, in terms of its sensitivity, specificity, and predictive values.
With Be-LTT of 200% or higher defined as positive (sensitized), all patients in Japan who were accurately diagnosed as CBD, excluding those who had been spontaneously remitted, exhibited Be-LTT of 400% or higher. Specifically, when Be-LTT is 400% or higher, it reflects a high likelihood of CBD, and therefore symptoms, laboratory findings, chest X-ray findings, and findings of the pulmonary function test, including DLco, CT, TBLB, and BALF-LTT, should be considered to ensure that a definitive diagnosis is made.
In addition to Be-LTT values were determined in 353 male workers not exposed to beryllium in accordance with the percentile method, they ranged from 54% to 195%. Furthermore, the use of autologous plasma also increases the viability of the lymphocytes, thereby making it possible to obtain more stable Be-LTT values. Naturally, we examined the point you commented on. We would like to recommend that our methodology be examined further.

2. The time course of the biological stability of beryllium in exposed workers determined using the lymphocyte proliferation test.
In this study, we selected these workers who had been engaged in the same job in a beryllium factory, excluding those involved in producing beryllium ceramics and
beryllium oxide.

Needless to say, a past history of beryllium exposure, particularly the types and doses of beryllium compounds, would be extremely useful predictive data in the attempt to prevent the occurrence of CBD. These workers are currently being followed up. Subsequent data obtained from them should be valuable in determining the sensitization and onset of CBD.

3. Samples from general area

These samples must be measured by general area method in working environment measurement law in Japan. In our study, therefore, samples from general area were used to determine Be-LTT values. Data obtained from the breathing zone will be reported in the near future.

4. Statistical analysis of serial measurement values for the population, and

5. Parameters for statistical analysis

If CBD occurs due to the accumulation of dust in the lung the same as silicosis, the influence of beryllium compounds accumulated in the lungs must be considered. However, in view of the fact that allergy is involved in the occurrence of CBD, we conclude the Be-LTT value should be set as an independent parameter each year. Be-LTT dramatically increases with the occurrence of CBD. Therefore, when the population of beryllium-exposed workers contains CBD patients, Be-LTT values naturally have a logarithmically normal distribution. In this study, however, since the subjects were healthy beryllium-exposed workers, we performed a statistical analysis after confirming that Be-LTT values were distributed normal distribution.

Lastly, we would like to mention that a Be-LTT value of 180–200% have been adopted as the standard for assessing persons positive to drug or metal allergies in representative laboratories in Japan.

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References