

Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease

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The T cell response to beryllium, measured in bronchoalveolar lavage by the lymphocyte transformation test (LTT), is a critical diagnostic test for discriminating between chronic beryllium disease (CBD) and other granulomatous diseases. We examined the sensitivity, reproducibility, and methods of a less invasive, peripheral blood LTT in 17 patients with CBD and in 18 beryllium-exposed control subjects. Ninety-four percent of CBD cases (16/17) had abnormal blood LTT results, and all 18 beryllium-exposed control subjects had normal blood LTT results. Split samples for 10 beryllium disease cases and eight control subjects demonstrated that the blood LTT was reproducible between two separate laboratories. The LTT was equally sensitive with 10% and 20% serum in the culture medium. We conclude that an abnormal blood LTT can be used to diagnose CBD in patients with compatible lung pathology. (J ALLERGY CLIN IMMUNOL 1991;88:54-60.)

Key words: beryllium, chronic beryllium disease, lymphocyte transformation, stimulation index, bronchoalveolar lavage

Industrial exposure to beryllium dust continues to cause CBD in subjects who develop beryllium-specific, cell-mediated immunity. The beryllium LTT with BAL lymphocytes is an in vitro measure of this cellular immune response¹⁻⁵ and has become the principal diagnostic tool in differentiating CBD from other granulomatous diseases.^{3, 4} Although the same assay can be performed less invasively with peripheral blood

Abbreviations used

LTT:	Lymphocyte transformation test
BAL:	Bronchoalveolar lavage
CBD:	Chronic beryllium disease
MNC:	Mononuclear cell
Con A:	Concanavalin A
PHA:	Phytohemagglutinin
BeSO ₄ :	Beryllium sulfate
SI:	Stimulation index
CV:	Coefficient of variation

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lymphocytes,^{4, 6-12} some investigations have found the blood LTT to be considerably less sensitive than the BAL LTT.^{3, 5, 13} In contrast, recent studies have demonstrated the blood LTT to be useful in screening for beryllium sensitization and for identifying subclinical beryllium disease.^{4, 6, 8, 9} We have reexamined the role of the blood LTT in the diagnosis of CBD by (1) comparing the blood LTT to the BAL LTT in a series of biopsy-confirmed cases of CBD, (2) examining the reproducibility of the blood LTT between laboratories, and (3) proposing methods for the blood LTT that allow for broader application of this tool in the diagnosis of CBD and in workplace surveillance.

TABLE I. Demographic data for study subjects

	No. tested (N)	Sex (M/F)	Mean age (yr) (range)	Smoking status	Mean years since first exposure (range)
Beryllium-exposed subjects*					
CBD cases	17	17/0	50.1 (32-70)	8 NS† 8 FS‡ 1 CS§	17.7 (7-31)
Subjects with other diseases	18	18/0	50.8 (30-73)	2 NS 8 FS 8 CS	15.6 (2-31)
Unexposed subjects					
Research laboratory blood LTT control subjects	31	23/8	32.3 (21-44)	27 NS 3 FS 1 CS	—
Clinical laboratory blood LTT control subjects	20	12/8	34.8 (23-46)	18 NS 1 FS 1 CS	—
Research laboratory BAL LTT control subjects	17	6/11	43.6 (21-61)	10 NS 5 FS 2 CS	—

NS, Nonsmoker; FS, former smoker; CS, current smoker.

*All subjects had respiratory exposure to beryllium fume and/or dust.

†Nonsmoker has smoked <20 packs of cigarettes or 12 ounces of tobacco in a lifetime or <1 cigarette a day for 1 year.

‡Former smoker has not smoked in the month before evaluation.

§Current smoker has smoked cigarettes or tobacco in the month before evaluation.

MATERIAL AND METHODS

Subjects

CBD cases. We studied 17 patients with CBD documented by a history of exposure to beryllium, histologic evidence of disease (noncaseating granulomas and/or MNC infiltrates on lung biopsy specimen),¹⁴ and evidence of beryllium-specific, cell-mediated immunity in the lung, as demonstrated by an abnormal BAL LTT.^{3,4} These patients included 11 newly diagnosed cases of CBD. Results of the other six cases have been previously reported with an alternate method for the LTT.^{4,6} Only two of the 17 cases were being treated with oral corticosteroids at the time of testing. The other 15 cases had milder CBD. Demographic information for all study subjects is presented in Table I.

Beryllium-exposed subjects without CBD. We evaluated 18 working-age male subjects with documented beryllium exposure from occupations similar to those of CBD cases. All subjects completed clinical evaluation that included blood LTT and BAL LTT, physiologic and radiographic assessment, and lung biopsy. None of these subjects had histologic evidence of CBD. Six subjects had no sign of any lung disease on lavage or biopsy specimen. Twelve subjects had other lung diseases, including four with chronic obstructive pulmonary disease, four with idiopathic pulmonary fibrosis (one of whom also had chronic obstructive pulmonary disease), three with asthma, and one subject with asbestosis.

Unexposed subjects. Two separate groups of unexposed subjects were studied to establish normal values in two

independent laboratories. Normal values for the blood LTT in the research laboratory were based on testing 31 healthy employees from the National Jewish Center. Twenty volunteers from a local industry were tested to establish normal values for the clinical immunology laboratory. The control subjects had no history of beryllium exposure.

Comparison values for the BAL LTT were established by testing 17 individuals with other diseases causing lymphocytic alveolitis and granuloma formation who had no history of beryllium exposure. This group included 13 subjects with sarcoidosis and four with hypersensitivity pneumonitis.

For all subjects, informed consent was obtained before any procedures were performed, in accordance with Institutional Human Subjects Review Board requirements.

Blood LTT

A 50 ml sample of venous blood was collected in sodium heparin vacutainers from each subject at the time of their bronchoscopy. The blood LTT was performed within 24 hours of venipuncture as follows: Peripheral blood MNCs were isolated from whole blood by Ficoll-Paque (Pharmacia, Piscataway, N.J.)—density centrifugation and washed three times in balanced salt solution. The MNCs were then suspended in complete medium, consisting of RPMI 1640 (Irvine Scientific, Santa Ana, Calif.), supplemented with 2 mmol/L of glutamine, 10 mmol/L of *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid buffer, 5×10^{-4} mol/L of 2-mercaptoethanol, 50 µg/ml of gentamicin sulfate, and 10%

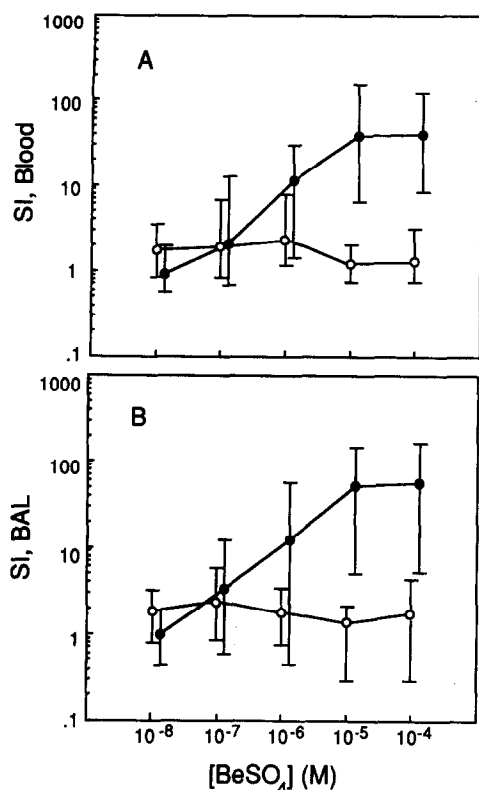


FIG. 1. A, Mean blood LTT responses. B, Mean BAL LTT responses. Patients with CBD (●) and beryllium-exposed control subjects (○). Blood results were omitted for one case with normal blood LTT. Results from day of peak response were used in analysis. Bars illustrate the ranges of responses.

pooled AB-positive human serum (Flow Laboratories, McLean, Va.). Blood MNCs were cultured at a concentration of 2.5×10^5 cells per well in flat-bottom, 96-well microtiter plates (Corning, Inc., Corning, N.Y.). Cell groups were cultured alone and in the presence of $BeSO_4$ at five concentrations (1×10^{-8} to 1×10^{-4} mol/L) (Fisher Scientific Co., Fairlawn, N.J.). Two mitogens, Con A ($10 \mu\text{g/ml}$) and PHA ($30 \mu\text{g/ml}$) (Sigma Chemical Co., St. Louis, Mo.), served as positive controls for nonspecific lymphocyte proliferation. All mitogen- and beryllium-exposed cell groups were run in quadruplicate. Unstimulated cell controls were run in groups of 12 because stability of this control is critical to the assay. When adequate cell numbers were available, duplicate assays were performed substituting 20% pooled human serum in the media as a comparison method.

Plates were incubated at 37°C in 10% CO_2 in air¹⁵ for 3, 5, and 7 days. During the last 4 to 6 hours of incubation, cells were pulsed with $0.1 \mu\text{Ci}$ of tritiated-thymidine deoxyriboside, a DNA precursor, to measure blastogenesis. After cells were pulsed, they were then either harvested or frozen at -20°C for later harvesting onto glass-fiber filters (Gelman Sciences Inc., Ann Arbor, Mich.) with a PHD harvester (Cambridge Technologies, Cam-

bridge, Mass.). Incorporated radiolabel was then counted in an LS 1801 liquid scintillation counter (Beckman Instruments, Irvine, Calif.). Data, expressed as counts per minute, were transferred directly into a buffer linked to a personal computer for analysis.

Each cell group was tested for within-group variability because extreme variations in replicate-stimulated cultures are often indicative of technical problems.¹⁵ If the CV for a quadruplicate was $>25\%$, the most distant outlier was discarded and the CV was recalculated. If the CV of the remaining values was still $>25\%$, the quadruplicate group was discarded from further analysis. The cell control group was discarded from analysis if seven outliers existed.

BAL LTT

BAL cells were collected, prepared, and counted by methods reported earlier.^{4, 16} Cells for the BAL LTT were resuspended in complete medium, as described above, and cultured at a concentration of 1×10^5 cells per well in flat-bottom, 96-well microtiter plates and then incubated, harvested, and counted, as described above for the blood LTT. The test was duplicated, substituting 20% serum in complete medium, when sufficient numbers of cells were available.

Definition of abnormal tests

These data were normalized by dividing the mean counts per minute for beryllium-stimulated cells by the mean counts per minute of cells cultured in the absence of beryllium¹⁵ after subtracting background counts from each. This calculation results in the SI. Peak SI, the highest ratio among the five beryllium concentrations on any of the 3 harvest days, was used to analyze lymphocyte transformation results. The upper limits of the normal range for blood and BAL LTT are laboratory dependent and based on the mean peak SI plus 2 SD for unexposed subjects. SIs higher than these values were considered to be elevated. An abnormal BAL LTT was required to have two or more elevated SIs occurring at $BeSO_4$ concentrations 1×10^{-4} to 1×10^{-6} mol/L.³ We used the same criteria to define an abnormal blood LTT.

Assessment of test reproducibility

To evaluate interlaboratory reproducibility of the blood LTT, blood samples obtained from 10 patients with CBD and from eight beryllium-exposed control subjects were split between our research laboratory and the clinical immunology laboratory of National Jewish Center. The technicians performed the assay using identical methodology but with separate reagents and equipment. The same lot of serum was used by the two laboratories.

Statistical analyses

The Wilcoxon's sign-rank test¹⁷ was used to compare blood and BAL LTT results, 10% and 20% serum methods, and LTT reproducibility. Student's *t* test, with corroboration by the Wilcoxon's sum-rank test,¹⁷ was used to compare the LTT results for the beryllium-exposed control subjects to results from the unexposed population.

TABLE II. Blood and BAL LTT results for patients with CBD*

Subject No.	Blood LTT				BAL LTT			
	Mean cell control (cpm)	Mean maximum response (cpm)	Peak SI	Day of peak response	Mean cell control (cpm)	Mean maximum response (cpm)	Peak SI	Day of peak response
1	429.8	6398.3	14.9	5	484.4	54584.0	112.7	5
2	211.8	11987.8	56.6	7	137.7	7731.4	56.2	5
3	524.2	29493.0	56.3	5	278.8	53569.5	192.2	3
4	1300.1	68542.3	52.7	5	672.6	14017.0	20.8	7
5	575.8	5504.3	9.6	5	494.3	20870.0	42.2	3
6	658.2	106958.7	162.5	5	1596.0	52987.5	33.2	3
7	532.9	15813.3	29.7	7	1005.4	9703.0	9.7	7
8	258.3	406.0	1.6	7	624.0	30400.0	48.7	3
9	285.0	8544.0	30.0	7	125.3	8456.8	67.5	3
10	224.3	4004.5	17.9	7	229.9	1873.3	8.0	7†
11	586.3	6663.0	11.4	5	462.2	5198.3	11.3	7
12‡	369.9	20354.3	55.0	7	553.1	24957.0	45.1	3
13	514.7	62559.3	121.6	5	406.3	24388.6	60.0	3†
14	1277.0	41110.5	32.2	5	539.0	3283.3	6.1	3
15	590.3	16787.3	28.4	5	226.9	20547.0	90.6	3
16	360.2	3950.3	11.0	5	527.5	99152.0	188.0	5
17§	127.8	4390.8	34.4	7	709.1	84342.0	118.9	5
Mean	519.2	24321.6	42.7		533.7	30356.5	65.4	

cpm, Counts per minute.

*Serum method, 10%, unless it is otherwise noted.

†Serum method, 20%.

‡Receiving 10 mg of prednisone, alternate days, at time of lavage.

§Receiving 30 mg of prednisone, alternate days, at time of lavage.

RESULTS

Unexposed subjects that were tested in the research laboratory had a blood LTT mean peak SI of 2.1 (SD, 0.7). We defined a blood SI >3.5 to be abnormal. Unexposed subjects who had BAL LTT performed demonstrated a mean peak SI of 2.1 (SD, 1.5), resulting in an upper limit of normal of 5.1. The mean peak blood SI for unexposed subjects that were tested in the clinical immunology laboratory was 1.0 (SD, 0.4).

Beryllium-exposed control subjects (Fig. 1) demonstrated a mean peak SI of 2.5 (SD, 1.7) on blood LTT and a mean peak SI of 2.6 (SD, 1.5) on BAL LTT. Values were not significantly different from the unexposed control groups ($p = 0.4$ [with a correction for unequal variances] and 0.5, respectively). Two subjects had blood SIs above the cutoff of 3.5, but neither case met the criteria for an abnormal test. One subject had an elevated blood SI of 7.3 occurring at BeSO₄ concentrations of 1×10^{-6} mol/L, and the other subject had values of 6.5 and 4.7 at BeSO₄ concentrations of 1×10^{-6} and 1×10^{-7} mol/L. The elevated SIs appeared to be caused by low cell-

control counts per minute values, which can falsely inflate the SI. All SIs on repeat blood LTTs were <3.5 for both subjects.

Blood LTT results were significantly elevated in 16 of 17 patients (94%) with CBD. As indicated in Table II, only patient 8 had a normal blood LTT result. The 16 cases with abnormal tests had an average of 4.8 elevated SIs (range, 2 to 9) during the 3 harvest days with at least two of the elevated SIs occurring at BeSO₄ concentrations of 1×10^{-4} and/or 1×10^{-5} mol/L. Only two patients had elevated values at BeSO₄ concentrations of 1×10^{-7} or 1×10^{-8} mol/L. BAL LTT results were abnormal in all 17 patients with CBD, as expected, given the case definition (Table II). The concentration-response pattern for blood LTTs was similar to the pattern of BAL LTTs, as illustrated in Fig. 1.

The relationship between blood and BAL LTT results for CBD cases and beryllium-exposed control subjects is illustrated in Fig. 2. There was no significant difference between peak blood and BAL LTT ($p = 0.5$), although the mean peak SI for blood LTTs of CBD cases (42.7, SD, 41.9) was lower than the

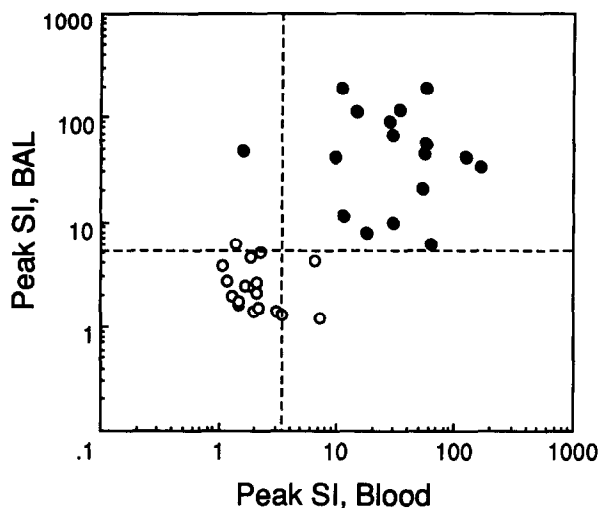


FIG. 2. Relationship between peak blood and peak BAL LTT responses for patients with CBD (●) and beryllium-exposed control subjects (○). The upper limit of the normal ranges are indicated by dashed lines.

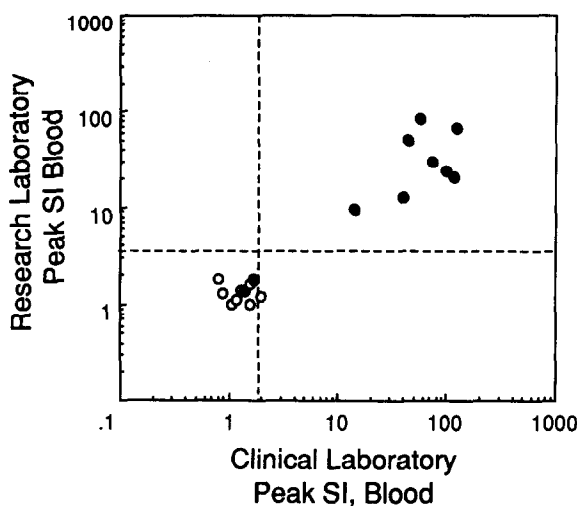


FIG. 3. Comparison of peak SI for blood samples split between laboratories to assess test reproducibility; patients with CBD (●) and beryllium-exposed control subjects (○). Upper limits of the normal range for each laboratory are indicated by dashed lines.

BAL LTT mean peak SI (65.4, SD, 58.1), as has been reported previously.^{1, 3, 4}

No significant difference existed in results of blood samples that were split between the research laboratory and clinical immunology laboratory, as demonstrated in Fig. 3. The typical BeSO₄ concentration-response pattern was found in both laboratories for CBD cases. One patient with CBD, who had abnormal blood results in 1988 (SI, 30.0), converted to normal in 1990 (SI, 1.6) and had decreased mitogen responses as well (1988 Con A, 58.3; 1990 Con A, 7.2; 1988 PHA, 53.2; and 1990 PHA, 21.2). Interestingly, the patient had developed metastatic prostatic cancer in the interim. An anergy panel performed on this patient revealed negative or minimal responses to four standard recall antigens.

The relationship between 10% and 20% serum methods is illustrated in Fig. 4. Two individuals, the CBD case with a normal blood LTT and a beryllium-exposed control subject, had isolated elevated SIs with the 20% serum method. Blood peak SI was higher with the 10% serum method in nine of 13 patients with CBD who had testing performed with both methods. These blood results were similar to results obtained in BAL LTT for 23 subjects, including 13 patients with CBD. BAL LTT peak SI was higher with the 10% serum method for eight of 13 patients in which the two methods were compared.

DISCUSSION

The present study demonstrates that the blood LTT identifies beryllium-specific, cell-mediated immunity in most individuals with CBD. Peripheral blood LTT

results were predictive of BAL LTT results in 94% of CBD cases. Furthermore, the blood LTT was reproducible between laboratories, making it a potentially useful clinical tool.

These data suggest that if our methods are applied, the blood LTT is a reliable test that can be used in lieu of BAL LTT in the diagnosis of CBD in certain circumstances. Specifically, if patients have pathologic alterations consistent with CBD,¹⁴ our data suggest that the blood LTT can confirm the diagnosis. An abnormal blood test by itself, however, is not sufficient for diagnosis. Some individuals with abnormal blood LTTs are beryllium-sensitized but do not have CBD.^{4, 6} Conversely, a normal blood test should not be used to exclude a diagnosis of CBD. Special circumstances may result in falsely negative blood results, as observed in one patient with CBD who developed metastatic cancer.

We found the blood LTT to be more sensitive in identifying CBD than have other researchers. In correlating blood LTT to BAL LTT, Rossman et al.³ found abnormal blood results in only four of 14 (28.6%) CBD cases. However, they speculated that this lack of blood response may have been due to selection bias, since several patients were referred to their laboratory because the peripheral blood studies had been negative. A more recent study by Stokes and Rossman¹³ found blood results to be abnormal in 10 of 27 (37%) CBD cases. Saltini et al.⁵ reported only one of eight patients with CBD (12.5%) as having an abnormal blood LTT. Other researchers, using morphologic methods to measure blastogenesis, found abnormal blood results in 57% and 71% of cases.^{11, 12}

Differences in blood LTT sensitivity may be related to methodology. Our increased blood LTT sensitivity might be due to using 2.5 times more blood MNCs per well than either Rossman et al.,³ Saltini et al.,⁵ or Stokes and Rossman.¹³ The elevated cell number may increase the number of stimulated T cells in our assay. The use of flat-bottom plates instead of round-bottom plates also might influence cellular responses. Some investigators have linked blood LTT sensitivity to the concentration of serum used in the assay.^{7, 8} However, data presented here suggest that the percent serum used in the culture media does not affect the identification of beryllium-specific, cell-mediated immunity. The use of 10% serum reduces the expense of the test without reducing its sensitivity.

The beryllium concentration at which an abnormal response occurs is another difference among published studies. For example, Williams and Jones Williams⁷ found peak responses at 1×10^{-6} and 1×10^{-7} mol/L of BeSO_4 . However, only 41% of our cases were abnormal at these lower concentrations, and higher BeSO_4 concentrations were nearly always accompanied by higher SIs. These data demonstrate the importance of including the higher beryllium concentrations in the assay.

Tissue culture can produce variable results even when technicians are well trained and familiar with the procedure. Our use of CV and the discarding of a single high or low outlier help control for technical problems, such as bacterial contamination, cell death, or omission of tritiated-thymidine deoxyriboside pulse, which can cause extreme variations in replicate cultures.¹⁵ In addition, the cell control is set up in groups of 12. The stability of the cell control is critical, since it enters into the calculation for each SI. Our earlier use of quadruplicate cell controls resulted in having to discard some entire tests when variability in the quadruplicate was high.

Streamlining the blood LTT would reduce its cost and increase its acceptability for clinical use in diagnosis and for surveillance. Elevated SIs rarely occurred at BeSO_4 concentrations of 1×10^{-7} and 1×10^{-8} mol/L. Eliminating these concentrations does not affect test results. Since peak blood responses for CBD cases occurred on either day 5 or day 7, the blood LTT can be further simplified by eliminating the BeSO_4 concentrations on day 3. However, we still advocate the testing of mitogen control responses that are best detected on day 3. This reduced assay requires approximately 15 ml of peripheral blood and saves labor and supplies. Normal values differed for the research and clinical immunology laboratories and should be independently established by each laboratory conducting the assay to account for differences in reagents, technicians, and control subjects.

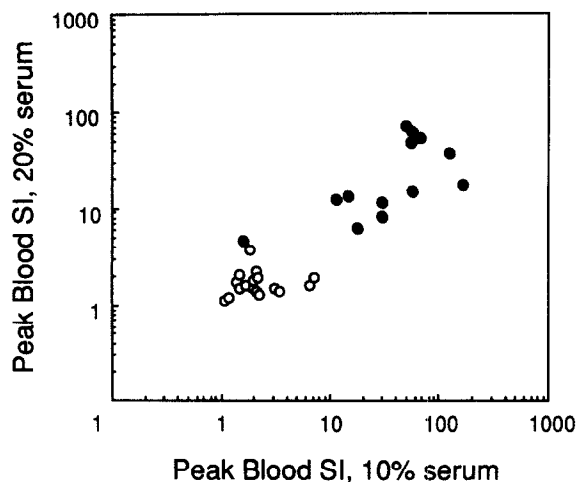


FIG. 4. Comparison of 10% serum and 20% serum methods for the blood LTT; patients with CBD (●) and beryllium-exposed control subjects (○).

We reviewed these data on our 16 patients with CBD with elevated blood LTTs in the context of this streamlined assay. Patients had an average of 3.5 abnormal values (range, 2 to 6) among six beryllium-stimulated cell groups (BeSO_4 concentrations of 1×10^{-4} , 1×10^{-5} , and 1×10^{-6} mol/L on 2 harvest days). We required an abnormal blood test to include two or more abnormal responses from the six groups tested. If test results include an isolated abnormal value (presence of a single elevated value of the six cell groups), reporting the peak SI may be misleading. We suggest using the median of the three highest SIs to avoid basing interpretation on an isolated outlying value.

The rarity of CBD may require blood and BAL samples to be shipped to laboratories with experience in doing the test. We have not found delays of 24 hours to affect quantitative or qualitative results of the blood test. Although we have not studied the effects of transport or delays in performing the LTT for BAL samples, we have had no difficulty with samples sent to us by overnight courier. Blood and BAL samples split between our research laboratory and sent by overnight courier to a laboratory on the East Coast resulted in qualitative agreement for all four BAL samples and four of five blood samples. The discrepant blood result was positive in our laboratory and negative in the other laboratory with a different method (data not presented).

In conclusion, our results suggest that the blood LTT can be used to diagnose CBD, in most cases eliminating the need for BAL. In patients with non-caseating granulomas and/or MNC infiltrates, an abnormal blood LTT differentiates CBD from other granulomatous diseases. The sensitivity of the blood re-

sponse is reproducible between laboratories with the same method. Our recommendations for conducting the assay make the blood LTT less resource intensive and more acceptable for use in surveillance applications, as well as in clinical diagnosis.

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REFERENCES

1. Epstein PE, Dauber JH, Rossman MD, Daniele RP. Bronchoalveolar lavage of a patient with chronic berylliosis: evidence for hypersensitivity pneumonitis. *Ann Intern Med* 1982;97:213-6.
2. Cullen MR, Kominsky JR, Rossman MD, et al. Chronic beryllium disease in a precious-metal refinery: clinical epidemiologic and immunologic evidence for continuing risk from exposure to low level beryllium fume. *Am Rev Respir Dis* 1987;135:201-8.
3. Rossman MD, Kern JA, Elias JA, et al. Proliferative response of bronchoalveolar lymphocytes to beryllium. *Ann Intern Med* 1988;108:687-93.
4. Newman LS, Kreiss K, King TE, Seay S, Campbell PA. Pathologic and immunologic alterations in early stages of beryllium disease: reexamination of disease definition and natural history. *Am Rev Respir Dis* 1989;139:1479-86.
5. Saltini C, Winestock K, Kirby M, Pinkston P, Crystal RG. Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. *N Engl J Med* 1989;320:1103-9.
6. Kreiss K, Newman LS, Mroz MM, Campbell PA. Screening blood test identifies subclinical beryllium disease. *J Occup Med* 1989;31:603-8.
7. Williams WR, Jones Williams W. Development of beryllium lymphocyte transformation tests in chronic beryllium disease. *Int Arch Allergy Appl Immunol* 1982;67:175-80.
8. Jones Williams W, Williams WR. Value of beryllium lymphocyte transformation tests in chronic beryllium disease and in potentially exposed workers. *Thorax* 1983;38:41-4.
9. Bargon J, Kronenberger H, Bergmann L, Buhl R, Meier-Sydow J, Mitrou P. Lymphocyte transformation test in a group of foundry workers exposed to beryllium and nonexposed control. *Eur J Respir Dis* 1986;69(suppl 146):211-5.
10. Rom WN, Lockey JE, Bang KM, Dewitt C, Johns RE. Reversible beryllium sensitization in a prospective study of beryllium workers. *Arch Environ Health* 1983;38:302-7.
11. Preuss OP, Deodhar SD, Van Ordstrand HS. Lymphoblast transformation in beryllium workers. In: Jones Williams W, Davies BH, eds. *Proceedings of the Eighth International Conference on Sarcoidosis and other Granulomatous Diseases*. Cardiff, Wales: Alpha Omega Publishing, 1980:711-4.
12. Deodhar SD, Barna B, Van Ordstrand HS. A study of the immunologic aspects of chronic berylliosis. *Chest* 1973; 63:309-13.
13. Stokes RF, Rossman MD. Blood cell proliferation response to beryllium: analysis by receiver-operating characteristics. *J Occup Med* 1991;33:23-8.
14. Freiman DG, Hardy HL. Beryllium disease: the relation of pulmonary pathology to clinical course and prognosis based on a study of 130 cases from the U.S. Beryllium Case Registry. *Hum Pathol* 1970;1:25-44.
15. Oppenheim JJ, Schecter B. Lymphocyte transformation. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. Washington, D.C.: American Society for Microbiology, 1980: 223-45.
16. Watters LC, Schwartz MI, Cherniak RM, et al. Idiopathic pulmonary fibrosis: pretreatment bronchoalveolar lavage cellular constituents and their relationship with lung histopathology and clinical response to therapy. *Am Rev Respir Dis* 1987;135:696-704.
17. Rosner B. *Fundamentals of biostatistics*. Boston: Duxbury, 1982.

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