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Isolation of *Legionella pneumophila* from Blood with the BACTEC System: a Prospective Study Yielding Positive Results

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In a prospective study of 16 patients with Legionnaires disease confirmed by cultural isolation of *Legionella pneumophila* from the respiratory tract, 38% (6 of 16) had positive blood cultures. Daily subcultures were made onto buffered charcoal-yeast extract plates from 6B aerobic and 7C anaerobic BACTEC blood culture bottles (Johnston Laboratories, Inc., Towson, Md.). Isolation of *L. pneumophila* was achieved from both aerobic and anaerobic bottles. *L. pneumophila* growth indices failed to exceed the BACTEC threshold limits; thus, the organism would have been overlooked despite its presence in the blood culture bottles. Bacteremic patients had statistically significant higher quantities of *L. pneumophila* isolated from sputum and visualized on direct fluorescent antibody stains. Thus, the potential exists for improved diagnosis of *Legionella* infection by a relatively noninvasive procedure (blood culture) with an instrument already in use in many hospital laboratories.

In 1947, Jackson reported the isolation of a "rickettsia-like" agent from blood of a febrile patient with respiratory insufficiency; guinea pig and embryonated eggs were used for isolation (8). This organism was classified 32 years later as *Legionella pneumophila* when it also was shown to be the causative agent of Legionnaires disease. Thus, the first clinical isolation of *L. pneumophila* was from blood. Since the American Legion Convention outbreak, there have been a few anecdotal reports of isolation from blood (1, 4, 5, 7, 9). In two of the reports, blood culture bottles supplemented with L-cysteine and ferric pyrophosphate were used (4, 7).

Chester et al. (1) isolated the organism with the BACTEC 460 automated blood culture system (Johnston Laboratories, Inc., Towson, Md.). We thus instituted a prospective study of known cases of Legionnaires disease to confirm whether bacteremia could be detected by the BACTEC system and to assess the clinical implications if bacteremia was found to be present.

MATERIALS AND METHODS

Patient selection. Sixteen patients with culture-confirmed Legionnaires disease seen in an 18-month period in our hospital constituted the study group (see Table 1). Respiratory secretions from all 16 patients grew *L. pneumophila*, serogroup 1. Fourteen of the cases were diagnosed from sputum specimens, and two were diagnosed from transtracheal aspirates.

Respiratory culture technique. All clinical samples were processed directly onto three plates: buffered charcoal-yeast extract (BYCE); selective BCYE containing polymyxin B (80 U/ml), cefamandole (4.0 μg/ml), and anisomycin (40 μg/ml); and selective differential BCYE containing polymyxin B (80 U/ml), anisomycin (40 μg/ml), vancomycin (1.0 μg/ml), bromcresol purple (0.001% [vol/vol]), and bromthymol blue (0.001% [vol/vol]). These three plates are modifications of previously described media (2, 3, 11). The plates were incubated at 35°C in room air in 70 to 80% humidity and held for 7 days. The cultures were examined daily with a stereomicroscope with a light source held at an angle slightly greater than 10° to the horizontal. The identity of all isolates was confirmed by slide agglutination with monospecific antisera.

Plate quantitation was based on a four-quadrant isolation streak. Designation was as follows: fewer than 10 colonies, very rare; between 10 and 100 colonies, rare; greater than 100 colonies in the first, second, and third streaks were light, moderate, and heavy, respectively.

Blood culture inoculation, incubation, and testing. Blood (5 to 10 ml) was drawn by the medical staff for blood culture. The blood was then transferred to a yellow-stoppered VACUTAINER tube (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) for transport to the laboratory. In the laboratory, one half of the sample was used to inoculate the 7C anaerobic bottle, and the remaining half was used to inoculate the 6B aerobic bottle. Both 7C and 6B bottles contained a 30-ml volume with enriched tryptic soy broth, anticoagulant, and ^{14}C-labeled substrates. All aerobic 6B blood culture bottles were incubated at 35°C on a reciprocal shaker for the first 48 h and tested on the BACTEC 460 three times on days 1 and 2. Thereafter, the bottles were tested once on days 3, 4, 5, 6, and 7. The anaerobic 7C bottles were incubated at 35°C without agitation and tested once a day for 7 days.

Subculture protocol. All available blood cultures from the 16 patients were subcultured to BCYE once the respiratory tract culture yielded *L. pneumophila*; subcultures were performed until the bottles were 7 days old. A tuberculin syringe was used to transfer 1.0 ml of the blood-broth mixture from both the 6B and 7C bottles. Because of the large volume being subcultured (1.0 ml), the entire surface of two BCYE plates was needed for each bottle. The inoculum was spread evenly with a plastic loop. The plates were allowed to dry and then incubated in humidified room air at 35°C. After 5 days, the colonies were counted and recorded. For the first two patients entering the protocol, only 0.1 ml from the aerobic 6B bottles was subcultured (see Table 2).

RESULTS

Table 1 summarizes the clinical and laboratory findings for the 16 study patients. Of the 16 patients with culture-

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TABLE 1. Timing of positive blood cultures, respiratory tract cultures, DFA positivity, erythromycin initiation, and outcome with respect to onset of pneumonia[a]

Patients	Underlying disease[b]	First positive blood culture	First positive sputum culture	First positive DFA	Start of erythromycin	Death
Bacteremic						
1	Congestive heart failure	0	0	0	0	4
2	Metastatic Ca of spine	0	0	0	2	6
3	Chronic lymphocytic leukemia	3	2	2	2	4
4	Ca of lung, leukemia, COPD	2	3	3	3	4
5	Ca of liver and gall bladder	5	5	Negative	5	7
6	Multiple myeloma, renal failure	4	4	4	None	6
Nonbacteremic						
7	Ca of lung, COPD	Negative	5[c]	Negative	6	Survived
8	Bowel obstruction, aspiration pneumonia	Negative	4	4	4	4
9	Ca of lung	Negative	3[c]	10	4	Survived
10	Lymphoma with spinal metastases	Negative	7	Negative	5	14
11	Ca of lung, COPD	Negative	2	Negative	2	Survived
12	Diabetes	Negative	1	Negative	1	Survived
13	Sézary syndrome, chronic lymphocytic leukemia	Negative	9	Negative	10[d]	12
14	Trauma	Negative	6	6	7	26
15	Ca of lung	Negative	4	4	6	12
16	Mesothelioma, COPD	Negative	2	Negative	7	8

[a] Pneumonia onset was defined by clinical criteria and X ray. Data indicate the number of days after onset of pneumonia, where 0 = day of pneumonia onset, 1 = day after onset, etc.
[b] Abbreviations: Ca, carcinoma; COPD, chronic obstructive pulmonary disease.
[c] Transtracheal aspirate specimen.
[d] Trimethoprim-sulfamethoxazole was used.

confirmed Legionnaires disease, 38% (6 of 16) yielded *L. pneumophila* from the BACTEC culture bottles. The sputum cultures for the six bacteremic cases showed moderate to heavy growth; in contrast, the sputum cultures of the 10 nonbacteremic cases showed very rare to light growth. The association of bacteremia with sputum culture quantitation was statistically significant ($P < 0.01$, Fisher exact test). All organisms isolated from both blood and respiratory tract belonged to serogroup 1.

The sensitivity of direct fluorescent-antibody (DFA) stain in this study was 56% (9 of 16). The DFA stain was positive in five of six bacteremic patients. However, the association for DFA positivity and presence of bacteremia was not statistically significant, given the limited sample size. On the other hand, higher quantification of organisms visualized by DFA (≥17 organisms) was significantly associated with bacteremia when compared with negative or lower quantification of DFA (≤16 organisms) ($P < 0.05$, Fisher exact test).

All (100%) bacteremic patients died during the hospital course as compared with six of ten (60%) patients without bacteremia; this relationship was not statistically significant.

Table 2 presents the daily quantitation of *L. pneumophila* in a BACTEC bottle for three patients. As was the case in all six positive patients, the growth indices did not exceed the BACTEC threshold limits. *L. pneumophila* was isolated from both bottles ranging from 1 to 107 CFU/ml and decreased slightly over the 7 days (Table 2). This low-level positivity was recognized when the blood culture for patient 2 was observed. As a result, the volume used for subculture was increased from 0.1 to 1.0 ml, and subculturing from the 7C anaerobic bottle was initiated as well.

DISCUSSION

Chester et al. isolated *L. pneumophila* postmortem from unsupplemented BACTEC 6B aerobic blood culture bottles from a patient with autopsy-proven Legionnaires disease (1).

TABLE 2. Growth indices and *L. pneumophila* quantitation from BACTEC blood culture bottles in three bacteremic patients

Day	Growth index value for patient[a]:									
	2		4				6			
	6B growth index	CFU/ml[b]	6B growth index	CFU/ml	7C growth index	CFU/ml	6B growth index	CFU/ml	7C growth index	CFU/ml
1	12	—[c]	8	—	3	—	8	—	6	—
2	13	20	11	—	9	—	11	—	8	—
3	11	10	9	23	10	21	8	—	10	—
4	8	10	6	—	10	—	11	—	10	—
5	10	10	9	10	9	23	6	107	9	18
6	8	0	2	—	8	18	10	40	10	18
7	7	0	6	2	8	16	7	13	7	13

[a] Growth index values did not attain the threshold necessary for radiometric positivity. Positivity for 6B is generally set at growth index ≥30; positivity for 7C is generally set at growth index ≥20.
[b] CFU/ml for patient 2 was increased by a factor of 10 for purposes of comparison.
[c] —, No data available.

Low-level radiometric readings were present in the aerobic bottles at 48 h and were maintained throughout the 7 days of incubation. Gram stains and subcultures of the bottles failed to demonstrate the presence of an organism. At day 7 of the incubation, both aerobic and anaerobic bottles were subcultured to BCYE agar; two of the three aerobic bottles yielded *L. pneumophila*. As a result, Chester et al. recommended that aerobic blood culture bottles be subcultured to *Legionella* media when BACTEC growth indices rise to the 30 to 60 range (1).

We instituted a prospective search for *L. pneumophila* in BACTEC blood culture bottles taken from pneumonia patients in whom respiratory tract cultures yielded *L. pneumophila*. Despite the fact that isolation of *L. pneumophila* from blood is presumed to be a rare event, we found that 38% (6 of 16) of the patients with culture-confirmed Legionnaires disease were bacteremic. Bacteremic patients had significantly higher quantities of *L. pneumophila* isolated from sputum ($P < 0.05$, Fisher exact test) and visualized on DFA stain ($P < 0.05$, Fisher exact test) when compared with nonbacteremic patients. All of the bacteremic patients succumbed to their disease within 2 to 6 days after pneumonia was defined (Table 1).

Unlike the experience of Chester, none of our *L. pneumophila*-positive blood cultures attained the BACTEC instrument threshold; thus, the organism would have escaped detection had subculturing not been performed as part of this protocol. This surprisingly high rate of bacteremia is consistent with the severe and protean clinical manifestations of the disease, including gastrointestinal symptoms, encephalopathy, and hepatic and renal dysfunction (6). To further underscore our finding, two cases of *L. pneumophila* endocarditis have recently been reported (10).

Currently, the most sensitive method of diagnosis of *L. pneumophila* is isolation of the organism from transtracheal aspirates (12). Serology is useful but generally requires obtaining a convalescent serum 4 to 6 weeks after onset of pneumonia, relegating its usefulness primarily for epidemiologic purposes. With the use of selective media, sputum culture is now feasible (12). (In our laboratory, the sensitivity of sputum culture now exceeds 70%.) The DFA stain is the most rapid means of diagnosis, but unfortunately, this test is relatively insensitive (range of 33 to 56%).

Since *Legionella* spp. are now recognized as relatively common causes of infection, there may be value in performing blind subcultures from blood culture bottles onto BCYE media. The efficiency from such an approach could presumably be increased if blind subculturing was restricted to high-risk patients; for example, patients in hospitals with *Legionella*-contaminated water supplies or patients with pneumonia who fail to respond to broad-spectrum antimicrobic therapy.

Adaptation of the BACTEC system, including addition of growth stimulators of *L. pneumophila* to blood culture bottles and radioactive labeling of *Legionella* metabolites, should result in a promising modality for rapid diagnosis of *Legionella* infection. In addition, blood may be particularly useful for isolation of *Legionella* in the absence of competing microorganisms from this source. Preliminary supplementation experiments in our laboratory with trace metals, amino acids, and other growth factors added to BACTEC 6B bottles resulted in luxuriant growth of *L. pneumophila* (10^4 CFU/ml) at 72 h (J. Rihs; unpublished data).

Thus, the potential exists for improved diagnosis of *Legionella* infections by a relatively noninvasive procedure (venipuncture) with an instrument already available in many hospital laboratories.

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