Diagnostic Value of Microscopic Examination of Gram-Stained Sputum and Sputum Cultures in Patients with Bacteremic Pneumococcal Pneumonia

Daniel M. Musher, Roberto Montoya, and Anna Wanahita

Infectious Disease Section, Medical Service, Veterans Affairs Medical Center, and Departments of Medicine and Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas

Clinicians continue to question the usefulness of microscopic examination of Gram-stained sputum specimens (“Gram staining”) and sputum culture for diagnosis of pneumonia. We analyzed the sensitivity of these techniques in 105 patients with pneumococcal pneumonia proven by blood culture. Gram staining revealed gram-positive cocci in pairs and chains, and culture yielded pneumococci in only 31% and 44% of all cases, respectively. However, sputum specimens were never submitted for examination in 31 cases; in 16 others, the specimen was inadequate and a culture was not done. Excluding these cases, the sensitivities of Gram staining and culture were 57% and 79%, respectively. If patients receiving antibiotics for >24 h had been excluded, Gram staining would have suggested pneumococci in 63%, and culture results would have been positive in 86%. Sensitivity increased in inverse proportion to the duration of antibiotic therapy (P < .05). Microscopic examination of sputum samples before antibiotics were administered and performance of culture within 24 h of receipt of such treatment yielded the correct diagnosis in >80% of cases of pneumococcal pneumonia.

Attempts to determine the relative roles of common bacterial pathogens as etiological agents of community-acquired pneumonia (CAP) have yielded widely divergent results [1, 2]. In the preantibiotic era, Streptococcus pneumoniae was known to cause >95% of cases of lobar pneumonia [3]. Patients who did not provide an adequate sputum specimen at admission might do so later, without the diagnosis being affected by administration of an effective antimicrobial drug. The definition of “lobar” was somewhat circular, because the word described, in part, a syndrome but was also related to the culture result [3, 4]. Nevertheless, pneumococci caused ≥80% of all cases of pneumonia that led to hospitalization [4]. By contrast, modern studies implicate pneumococcus in 11%–70% of cases of CAP [1, 2]. These lower numbers may reflect the failure to obtain sputum specimens for microbiological analysis, the failure to process specimens properly, the dampening effect of antibiotic therapy, and/or a change in the natural history of pneumonia.

In the present investigation, we attempted to determine the likelihood that the microbiological study of sputum would identify the causative agent in bacterial pneumonia, specifically by studying the frequency with which microscopic examination of Gram-stained sputum specimens (“Gram staining”) and bacterial culture yielded the correct diagnosis in a large number of cases in which the etiology was confirmed by positive blood culture results. Our hypotheses were that (1) microbiological studies of sputum specimens are reliable when valid specimens are used, (2) a substantial proportion of patients who are admitted to the hospital for pneumonia are unable to provide valid specimens,
and (3) an admixture of nonvalid data (or nondata) with valid data may lead to the erroneous impression that microbiological techniques are themselves poorly reliable for diagnosis of pneumococcal pneumonia.

METHODS

With use of an automated list of all positive blood culture results at the Michael E. DeBakey Veterans Affairs Medical Center (Houston, TX), we identified patients whose blood culture(s) yielded *S. pneumoniae* during the period of January 1997 through December 2002. During these years, all medical records were computerized, and all clinical, laboratory, radiological, and pharmacy data were fully available. We selected for inclusion all patients with bacteremic pneumococcal pneumonia, which was defined by ≥1 blood culture positive for *S. pneumoniae*, a clinical picture consistent with pneumonia, and a chest radiograph that demonstrated a pulmonary infiltrate. This study was approved by the Institutional Review Board at Baylor College of Medicine (Houston, TX).

We determined whether sputum samples were submitted for microbiological analysis, and we recorded the results of Gram staining and culture from the first such sample submitted for each patient. Specific attention was paid to the laboratory assessment of the adequacy of the sample, as determined microscopically. Our laboratory defines as adequate any specimen that has areas that contain ≥10 WBCs for each 1 epithelial cell at magnification of 400×. For the purposes of this study, a positive Gram staining result indicates predominant gram-positive cocci in pairs and chains at magnification of 1000×. A positive sputum culture result is one that yields *S. pneumoniae* on 5% sheep blood agar or 5% sheep blood agar containing colymycin and nalidixic acid after overnight incubation at 37°C under 5% CO₂. In our automated medical records, an accession number cannot be assigned to a sputum sample unless the time at which it was obtained is recorded. The timing of antibiotic administration is also documented electronically.

Statistics. Fisher’s exact test was used to determine differences between groups of subjects, and χ² analysis was applied to determine the significance of the trend. Statistical analysis was performed using Sigma Stat statistical software (SPSS). Significance was defined as *P* < .05.

RESULTS

**Sputum Gram staining and culture.** In the 6 years of the study, 105 cases met our criteria for bacteremic pneumococcal pneumonia. Overall, microbiological study appeared to be poorly sensitive. Gram-positive cocci were seen in pairs and chains in 33 cases (31%; defined as a “positive Gram staining result”), and culture yielded *S. pneumoniae* in 46 cases (44%; defined as a “positive culture” result). In 31 cases, however, no sputum sample was ever submitted; when these 31 cases are subtracted from the denominator, the sensitivity of Gram staining increases to 45%, and the sensitivity of culture increases to 62% (figure 1). In an additional 16 cases, the sample received by the laboratory was judged to be inadequate. If these 16 cases are excluded from analysis, the sensitivity of Gram staining increases to 57%, and that of culture increases to 79%.

**Effect of antibiotics.** Antibiotic therapy may be initiated at varying time intervals before sputum samples are obtained for microbiological study, and such treatment might adversely affect the ability to identify the causative agent. We stratified the 58 patients who provided a valid sputum sample on the basis of the time that elapsed between initiation of antibiotic therapy and submission of the sample: ≥24 h, <24 but ≥6 h, <6 h, and no antibiotics received before the sputum specimen was obtained (figure 2). Among 7 patients who had received antibiotics for ≥24 h before a sputum sample was submitted, Gram staining results were positive for only 1 patient (14%), and culture results were positive for 2 (29%). With exclusion of these 7 patients, the overall sensitivity of Gram staining increased to 63%, and the sensitivity of culture increased to 86%. Of 18 patients who had been treated for <24 h but ≥6 h, 9 (50%) had a positive Gram staining result, and 16 (89%) had a positive culture result. Among an additional 18 patients who were treated for <6 h, 11 (61%) had a positive Gram
staining result, and 14 (78%) had a positive culture result. Finally, for the 15 patients who had received no antibiotics, the sensitivity of Gram staining was 80% and that of the culture was 93%. The trends toward increasing sensitivity with decreasing duration of antibiotic treatment were significant ($P < .005$ and $P < .05$ for results of Gram staining and culture, respectively; determined by $\chi^2$ analysis for significance of the trend).

Thus, in patients with bacteremic pneumococcal pneumonia who provided an adequate sputum specimen and who had received no prior antibiotics, Gram staining detected pneumococci in 80% of cases; the sensitivity was significantly reduced for samples obtained $\geq 6$ h after antibiotic therapy was initiated ($P < .05$, by Fisher’s exact). In contrast, cultures of samples obtained within $24$ h after antibiotic therapy was started yielded pneumococci in 88% of cases, with the likelihood of obtaining a positive culture result only decreasing $>24$ h after treatment was started; after $6$ h but within $24$ h of antibiotic use, culture was more sensitive than Gram staining ($P = .03$). There was no case in which the Gram staining result was positive and the culture result was negative, suggesting that the problem does not arise because of error in selecting or identifying pneumococcal colonies on blood agar plates.

**DISCUSSION**

The results of the present study show that the apparent sensitivity of microscopic examination of Gram-stained sputum specimens and sputum culture for diagnosis of pneumococcal pneumonia is highly dependent on which cases are included in the denominator. We studied patients in whom the etiological diagnosis could not be questioned—namely, those with a clinical syndrome of pneumonia and isolation of *S. pneumoniae* from the blood. On the basis of data for the entire population of patients with bacteremic pneumococcal pneumonia, the sensitivity was low: 31% and 44% for Gram staining and culture, respectively. Exclusion of the nearly one-third of patients who failed to provide a sputum specimen (a proportion similar to that in a recently reported, large prospective study [5]) substantially improved the apparent outcome. As increasingly stringent conditions relating to the duration of antibiotic administration limited the denominator, the apparent sensitivity of both techniques improved. Our results show that (1) Gram staining documents pneumococci in 80% of sputum specimens obtained before administration of antibiotics, and (2) culture yields *S. pneumoniae* in a similar proportion in samples obtained up to $24$ h after antibiotic therapy has begun. In those of our patients who provided an adequate sputum sample and who had not been treated with antibiotics, the sensitivities of Gram staining and culture were 80% and 93%, respectively.

These results help to explain discrepancies among earlier articles that reported that sensitivity of Gram staining in proven cases of pneumococcal pneumonia ranged from 20% to 69% and that the sensitivity of culture ranged from 29% to 94% (summarized in table 1). A confounding effect of prior antibiotic therapy has been cited in older studies [12, 18, 19], some of which excluded patients who received these agents, but these findings were generally based on smaller numbers of cases, and the precise time relationships were not examined. Some investigators dismiss microbiological studies altogether [20]. Two recent studies of all-cause CAP [20, 21] identified pneumococci in 5%–10% of cases; one of these studies [20] stated that Gram staining suggested pneumococci as a cause in 0%. Clearly, the technique itself cannot be held to be faulty if it is not utilized, if it is applied to inadequate specimens, or if it is applied after confounding factors have been introduced.

Our study has certain limitations. First, to have a homogeneous series of cases with proven etiology, we confined ourselves to patients with pneumococcal pneumonia. Studies on which we have worked [22–24] and other studies [15, 25], however, have reported that Gram staining and culture of sputum samples obtained from patients with bacterial pneumonia caused by *Haemophilus influenzae* [22], *Moraxella catarrhalis* [23], or *Staphylococcus aureus* [24] are even more reliable, which also seems to be the case with pneumonia due to gram-negative bacilli (D.M.M., unpublished data). Second, we studied only patients with bacteremic disease. Although one might postulate that such patients have larger numbers of organisms in their sputum than do nonbacteremic patients, we are unaware of studies—or even of anecdotal data—to support that concept. Third, our laboratory’s criterion that a valid sample contain $\geq 10$ inflammatory cells for each epithelial cell is more stringent than that of Murray and Washington [26], who required a ratio

---

**Figure 2.** Relationship between the duration of antibiotic treatment and results of Gram staining and culture. The yield of sputum Gram staining (open bars) and culture (shaded bars) for detection of *Streptococcus pneumoniae* in patients with proven pneumococcal pneumonia, shown on the vertical axis, is inversely proportional to the duration of antibiotic treatment, shown on the horizontal axis.
Table 1. Findings of bacteriological evaluations of sputum samples obtained from patients with bacteremic pneumococcal pneumonia.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>No. of cases</th>
<th>Microscopy criteria</th>
<th>Positive results, %</th>
<th>Patients with prior antibiotic therapy excluded</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rathbun et al. [6]</td>
<td>1967</td>
<td>69</td>
<td>NR</td>
<td>NR</td>
<td>55</td>
<td>No &gt;83% of cases determined by mouse inoculation</td>
</tr>
<tr>
<td>Fiala [7]</td>
<td>1969</td>
<td>25</td>
<td>NR</td>
<td>NR</td>
<td>56</td>
<td>Yes Extensively cited article; collection and processing was uncontrolled; Gram staining was not performed</td>
</tr>
<tr>
<td>Barrett-Connor [8]</td>
<td>1971</td>
<td>33</td>
<td>NR</td>
<td>NR</td>
<td>49</td>
<td>No Some cases involving lung aspirates in the denominator; 95% yield by mouse inoculation</td>
</tr>
<tr>
<td>Tempest et al. [9]</td>
<td>1974</td>
<td>56</td>
<td>NR</td>
<td>NR</td>
<td>75</td>
<td>No Denominator was cases involving lung aspirates</td>
</tr>
<tr>
<td>Davidson et al. [10]</td>
<td>1974</td>
<td>17</td>
<td>NR</td>
<td>NR</td>
<td>82</td>
<td>No Denominator was cases involving lung aspirates</td>
</tr>
<tr>
<td>Drew [11]</td>
<td>1977</td>
<td>31</td>
<td>&gt;5 PMNs and ≤1 SEC per OIF</td>
<td>~50</td>
<td>94</td>
<td>Yes 81% of all sputum samples were judged to be adequate</td>
</tr>
<tr>
<td>Guzzetta et al. [13]</td>
<td>1983</td>
<td>14</td>
<td>&gt;25 PMNs and ≤10 SECs per LPF</td>
<td>43</td>
<td>36</td>
<td>Yes</td>
</tr>
<tr>
<td>Perlino [14]</td>
<td>1984</td>
<td>19</td>
<td>More SECs than PMNs</td>
<td>NA</td>
<td>81</td>
<td>Yes Cultures performed only if microscopy criteria were met</td>
</tr>
<tr>
<td>Gleckman et al. [15]</td>
<td>1988</td>
<td>36</td>
<td>&gt;25 PMNs and ≤10 SECs per LPF</td>
<td>69</td>
<td>78</td>
<td>Yes Study was prospective</td>
</tr>
<tr>
<td>Watanakunakorn et al. [16]a</td>
<td>1997</td>
<td>59</td>
<td>≤10 SECs per HPF</td>
<td>20</td>
<td>29</td>
<td>No Only 55% of patients with pneumonia provided a valid sample</td>
</tr>
<tr>
<td>Torres et al. [17]a</td>
<td>1998</td>
<td>43</td>
<td>NR</td>
<td>26</td>
<td>49</td>
<td>No Only 61% of patients with pneumonia provided a sputum sample</td>
</tr>
</tbody>
</table>

NOTE. HPF, high-powered field; LPF, low-powered field; NA, not available; NR, not recorded; OIF, oil-immersion field; PMN, polymorphonuclear leukocyte; SEC, squamous epithelial cell.

* Only these studies provide the reader with a true denominator—namely, the total number of patients with pneumococcal pneumonia, proven by a positive blood culture result.

of 25 polymorphonuclear cells for 10 epithelial cells. Nevertheless, only 16 sputum samples were excluded by this requirement, some or most of which might have also been excluded by the less stringent criteria. It is also interesting to note that the illustrative figures in the article by Murray and Washington [26] fulfill our criteria. Fourth, although many of the patients in this series were included in a prospective study of pneumococcal disease [27], we did not originally plan to examine various techniques of processing sputum [28], and our analysis of the microbiological findings was retrospective. An advantage of this approach, however, is that we succeeded in documenting the potential usefulness of Gram staining and culture of sputum samples under routine laboratory conditions, with no special protocol in place.

What light does our study shed on the frequency with which *S. pneumoniae* causes CAP? Of 100 patients hospitalized with pneumococcal pneumonia, ~20 are expected to have a positive blood culture result [3, 29]. If, as our data show, sputum culture yields an organism in 44% of the remaining cases, then, overall, pneumoccus will be identified in 55 of the 100 patients. Thus, at our medical center, *S. pneumoniae* actually causes CAP nearly twice as frequently as we recognize. At other hospitals, pneumococci have only been identified in sputum samples obtained from 15%–30% of patients with proven pneumococcal pneumonia [16, 17], raising the possibility that other systematic problems contribute to underreporting of pneumococci as a cause of pneumonia. Thus, the actual incidence of pneumococcal infection in CAP may be substantially greater than suggested in some recent studies [3, 20, 21]. Our data provide further evidence to support the hypothesis that many patients with CAP of undetermined etiology may actually have pneumococcal pneumonia [30, 31].

As bacteria become increasingly resistant to available antibiotics, it seems appropriate to turn away from empiricism toward increased diagnostic precision. Such precision would be enhanced by prompt examination of a high-quality sputum specimen. Even when therapy for CAP is started empirically, the knowledge that pneumococcus is responsible may help the clinician to simplify therapy at the time of change from parenteral to oral treatment. Many patients, however, cannot provide sputum samples at the time of admission to the hospital, and delays in treatment may lead to a worse outcome [32, 33].
In light of these considerations, it seems reasonable to maximize efforts to obtain sputum for microbiological study during the first few hours that a patient is in the hospital. If that is not possible, microscopic evaluation of a Gram-stained sputum sample is likely to be useful within the first 6–12 h of therapy, and a culture may provide useful data using a sputum sample obtained up to 24 h after antimicrobial therapy has been begun.

Acknowledgments

We thank Edward Graviss for performing statistical analysis.

Financial support. Department of Veterans Affairs Merit Review Program.

Conflict of interest. All authors: No conflict.

References