Gram Stain and Quantitative Sputum Culture in Bacteriological Assessment of Lower Respiratory Tract Infection

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Abstract: We evaluated the clinical role of Gram-stained smears and quantitative culture in examining sputum specimens from patients with respiratory infections. Fresh sputum specimens (75 prospectively and 86 retrospectively) from elderly patients with predominantly chronic pulmonary disorders were submitted to this study. The respiratory pathogenic organisms were isolated more frequently from high quality sputum defined by a modified sputum screening system on Gram-stained sputum smears. In this study, 34.8% of specimens examined were qualified for rejection. A significantly positive correlation between the number of dominant bacteria on Gram-stained smears and the subsequently isolated pathogens was demonstrated. The modified sputum screening system used in the present study is recommended for routine laboratory use to save technical and financial resources, and to enhance the diagnostic value of sputum specimens.

Key words: Gram stain, Quantitative culture method, Sputum cytology, Lower respiratory tract infection

INTRODUCTION

The clinical usefulness of Gram-stained sputum smears and sputum culture has remained a matter of considerable debate due to the confounding effect of oropharyngeal contamination upon expectorated specimens (Martin et al., 1978). Some authorities have even suggested that little is gained by examining sputum specimens (Lentino and Lucks, 1987). In the less developed parts of the world where the subject of cost-effectiveness and the burden of respiratory infections remain issues of critical concern, the potential available alternative means of enhancing the diagnostic value of sputum samples should be explored.

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The use of initial microscopic examination have been suggested as a significant method of saving technical time and financial resources besides providing useful clinical information for clinicians (Matsumoto *et al.*, 1978; Martin *et al.*, 1978). Several microscopic screening systems for sputum have been devised (Wong *et al.*, 1982). Some of these systems, however, appear rather unrealistic for routine use by service-oriented clinical laboratories especially those handling large numbers of specimens against limited technical and financial resources. In this regard, the present study was designed to evaluate a modified, simple screening system using Gram-stained sputum smears and quantitative sputum culture in the bacteriological assessment of lower respiratory tract infections.

**MATERIALS AND METHODS**

*Sputum samples*: Fresh sputum specimens submitted to the Department of Internal Medicine (Institute of Tropical Medicine, Nagasaki University) from adult patients with potential lower respiratory infections were investigated. The expectorated sputum specimens were prospectively (February to July, 1989) and retrospectively (January to June, 1988) assessed.

*Gram-stained smear and sputum quality*: The method for Gram staining of sputum specimens was based on Hucker modification (Sonnenwirth, 1980). The sputum quality was defined by the combined numbers of squamous epithelial cells (SEC) and polymorphonuclear cells (PMNC) observed at lower magnification ($\times$ 100) on Gram-stained smears. At least one third of each smear was observed. The quality of sputum specimens were defined as below; Good quality: PMNC$>$25 and SEC$>$10, Borderline quality: PMNC$>$25 and SEC$>$10, Poor quality: PMNC$>$25 and SEC$>$10. The morphologic characteristics of bacteria on Gram-stained smears were observed under $\times$ 1000 oil immersion objective, and recorded. The presence of various bacteria was recorded semi-quantitively based on a four-category scale: 0 = none, + = rare, ++ = moderate, +++ = numerous. Moderate or numerous bacteria observed on Gram-stained smears were evaluated to be dominant.

*Sputum quantitative culture*: To each ml of sputum specimen, 0.2ml of N-acetyl-L-cysteine (176.2mg/ml, Senju Pharmaceutical Company, Japan) was added and vortexed. Serial ten-fold dilution of sputum sample was made in sterile saline. Using a flame sterilized calibrated (0.01 ml) wire loop, one loopful of each dilution was inoculated on the corresponding portions of trypticase soy agar (Becton Deckinson Microbiology Systems, USA) containing 7% rabbit blood. Plates were incubated for 18-24 hours at 35°C under 5% CO$_2$, and colony count were done. For specific identification, appropriate tests were done using standard procedures.

*Clinical informations*: Clinical data corresponding to specific date of sputum collection was obtained from patient case records at Nagasaki University Hospital, Nomozaki Municipal Hospital and Tagami Hospital.
RESULTS AND DISCUSSION

A total of 161 sputum specimens were examined; 75 prospectively and 86 retrospectively. The patients involved in this study were generally elderly with a mean age exceeding 60 years and an approximately balanced sex ratio. Over 80% of these patients were associated with chronic respiratory disorders; chronic bronchitis, bronchiectasis, diffuse panbronchiolitis and chronic pulmonary emphysema. The distribution of sputum specimens in this study in terms of their quality, as defined by a criteria previously described, is shown in Table 1. Over 60% of the total specimens satisfied the criteria for good quality. However, 34.8% of the total sputum specimens belonged to the criteria for poor quality. The isolated pathogens based on the quantitative culture method in the present study consisted of Pseudomonas aeruginosa (21.7%), Streptococcus pneumoniae (20.7%), Haemophilus influenzae (18.5%), Branhamella catarrhalis (16.3%), Staphylococcus aureus (14.1%) and others (8.7%). These distribution of respiratory causative organisms corresponded to a previous report (Matsumoto, 1988). We evaluated the frequency of isolation of specific pathogens from good quality or poor quality sputum in the prospective and retrospective study. It was recognized that the frequency of isolation of specific pathogens is generally higher from good quality sputum specimens than from poor ones, with exceptions of Branhamella catarrhalis and Staphylococcus aureus in the retrospective study (Figure 1). In the prospective study, only 6 specific pathogens including 4 Pseudomonas aeruginosa strains were isolated from poor quality sputums. We also examined the relationship between the numbers of dominant bacteria on Gram-stained smears and subsequently isolated specific pathogens. A significantly positive correlation between the number of dominant bacteria on Gram-stained smear and the subsequently isolated pathogens was demonstrated in Figure 2 (p<0.001, r=0.90). The relationship between clinical and laboratory-based parameters (body temperature, C-reactive protein level and white blood cell counts) did not achieve significant levels of correlation.

The management of lower respiratory tract infections is significantly simplified if a specific pathogen is accurately determined. Routine sputum cultures are noteworthy for providing misleading results due to oropharyngeal contamination and irregular distribution

Table 1. Distribution of sputum quality defined by a modified criteria on Gram-stained smears in prospective and retrospective study

<table>
<thead>
<tr>
<th>Sputum quality</th>
<th>Number of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prospective</td>
</tr>
<tr>
<td>Poor</td>
<td>23</td>
</tr>
<tr>
<td>Borderline</td>
<td>1</td>
</tr>
<tr>
<td>Good</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
</tr>
</tbody>
</table>
Fig. 1. Comparison of the frequency of isolated specific organisms from good or poor quality sputum specimens as defined in the prospective and the retrospective study.
Good quality sputum; Poor quality sputum;
PA: Pseudomonas aeruginosa, HI: Haemophilus influenzae,
SP: Streptococcus pneumoniae, BC: Branhamella catarrhalis,
SA: Staphylococcus aureus.
of bacteria within the specimen (Bartlett, 1981). This makes correct recognition of true pathogens difficult. The present study demonstrated that the pathogenic bacteria were more frequently originated from good quality sputum. Some authorities suggested that a cost-effective sputum screening system should reject at least 22% of the specimens received (Heineman and Radano, 1979). On the other hand, 34.8% of specimens examined in this study qualified for rejection. This study further demonstrates that a morphological and semiquantitative assessment of bacteria on Gram-stained smears has a definite role in predicting presence of significant pathogens. Therefore, Gram-stained sputum smears can greatly aid clinicians to make presumptive etiological diagnosis, and permit the laboratory to enhance the isolation of pathogenic bacteria by selective culture methods. A recent report similarly indicated that laboratory-based interpretation of microbiologic results could improve physicians to make decisions (Mizrachi and Valensteine, 1987). It should, however, be noted that isolation from culture of two or more morphologically similar bacterial species could not be distinguished by a Gram-stained sputum smear.

In conclusion, this study shows that Gram-stained smears and quantitative sputum culture together have a significant role in enhancing the diagnostic value of expectorated sputum specimens. The criteria used in this study would appear to be more adaptable for routine laboratory use.
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REFERENCES


