The Diagnosis of Streptococcal Pharyngitis by the Antibody-Coated Bacteria Test

DAVID L. SEWELL, PH.D.,*† RICHARD E. BRYANT, M.D.,† ABDEL L. RASHAD, M.D., PH.D.,* and MICHAEL J. MILLER, M.D.†

*Department of Clinical Pathology and †Infectious Diseases Division, ‡University of Oregon Health Sciences Center and Clinical Pathology Service, Veterans Administration Medical Center, Portland, OR 97201

ABSTRACT

Fluorescein isothiocyanate-labelled goat antihuman immunoglobulin (anti-IgG, anti-IgA, anti-IgM) and anticomplement conjugates were used to detect antibody or complement-coated bacteria from the oropharynx of patients with pharyngitis. Throat smears prepared from patients with a positive culture for group A beta-hemolytic streptococci had significantly more bacteria that stained with labelled antihuman IgG than smears prepared from patients with a negative culture. When compared to the results of a throat culture, the sensitivity, specificity, and predictive value of a positive and negative antihuman IgG stained smear was 91 percent, 94 percent, 85 percent, and 96 percent, respectively. The results of smears stained with antihuman IgA, IgM or complement did not correlate statistically with the isolation of group A beta-hemolytic streptococci.

Introduction

The early diagnosis of Group A streptococcal pharyngitis makes it possible to select appropriate antimicrobial therapy to prevent suppurative and non-suppurative complications. Although there are problems associated with the differentiation of the streptococcal carrier state, the subclinical infection, and the acute infection, the throat culture is the accepted method of diagnosis.9 Recently, the Gram-stained smear of pharyngeal secretions has been used for early diagnosis of streptococcal pharyngitis.4 However, clinical signs and symptoms, bacteriologic data and even antistreptococcal antibody titers are not totally reliable indicators of streptococcal pharyngitis.4,8,11 This study evaluated the usefulness of the antibody-coated bacteria (ACB) test7 for diagnosing streptococcal pharyngitis from throat smears.

Materials and Methods

Throat swabs from 88 patients presenting with pharyngitis of unknown etiology and 24 healthy controls were examined. Seven patients had one additional culture following antibiotic treatment. The swabs were placed in sterile phosphate buffered saline (pH 7.0), vortexed, and centrifuged at 1000 × g for one minute. The supernatant was centrifuged at 5000 × g for 10 minutes and the sediment was resus-
pended in 0.1 ml phosphate buffered saline (PBS). Aerobic and anaerobic sheep blood agar plates were inoculated with 0.001 ml of the sediment and incubated 18 to 24 hours. Acetone fixed smears were prepared for microscopic examination.

The smears were treated with fluorescein isothiocyanate (FITC) labeled goat antihuman immunoglobulin (anti-IgG, anti-IgA, and anti-IgM) and antihuman complement (anti-C₃) conjugates.* All smears were incubated at room temperature for 30 minutes, washed twice with PBS and examined by fluorescent microscopy. The fluorescent intensity of the stained cells was graded from one to four plus. Ten random fields from each smear were examined. Bacteria exhibiting three to four plus fluorescence were counted. Preincubation of a few positive smears with unlabelled antihuman immunoglobulin blocked the subsequent binding of FITC-labeled antihuman immunoglobulin and demonstrated the specificity of the reaction. Smears were grouped according to the total number of positive organisms seen in ten fields. The breakpoint of 25 positive bacteria per 10 fields was determined from the healthy control group. The average count and range for the control group was 7 and 0 to 23 positive bacteria per 10 fields. All beta-hemolytic streptococci (BHS) isolated were identified by Lancefield grouping using commercial antisera.† Significance levels were determined by the Chi-square method. The sensitivity, specificity and predictive value of both positive and negative results were determined according to Feinstein.⁶

Results

Fifty-four of 119 throat swabs were positive for BHS. Thirty-two of the positive cultures were group A BHS. Patients with positive cultures for group A BHS had significantly (p < 0.001) more antibody coated bacteria on throat smears than did the control groups (table I). Only 18 percent of the smears prepared from specimens with positive cultures for nongroup A BHS stained with FITC-labeled antihuman IgG. Excluding the specimens from the post-treatment patients, the sensitivity [true positive (TP)/TP + false negative (FN)], specificity [true negative (TN)/TN + false positive (FP)], predictive value of a positive result (TP/TP+FP), and predictive value of a negative result (TN/TN+FN) of the ACB test was 91 percent, 94 percent, 85 percent, and 96 percent, respectively. If the results from the post-treatment group are included in the analysis, the specificity and predictive value of a positive test decline to 91 percent and 78 percent, respectively. Although cultures were negative for BHS following antibiotic treatment, 43 percent of the throat smears stained with FITC-labelled antihuman IgG.

The throat smears were also stained with FITC-labelled antihuman IgA, IgM, and complement. None of the smears contained significant numbers of bacteria coated with IgM or complement when stained with the appropriate antiserum (data not shown). As shown in table II, approximately 30 percent (16/54) of the throat smears prepared from patients with cultures positive for BHS (groups A,B,C,G) were stained with FITC labelled antihuman IgA (p < 0.01). The difference between antihuman IgA stained smears of patients with positive cultures for group A BHS and those of the other study groups was not statistically significant.

Discussion

Group A BHS in tonsillar exudates was coated with immunoglobulins that blocked the subsequent binding of antibody directed against streptococcal sur-

---

* Hyland Laboratories, Costa Mesa, CA.
† Burroughs-Wellcome, Research, Triangle Park, NC.
face antigens. This observation suggested that the antibody coated bacteria should react with antibody directed against human immunoglobulin. In fact, bacteria in throat smears prepared from patients with positive cultures for group A BHS (or in a few cases of nongroup A BHS) stained intensely with FITC-labelled antihuman IgG. This probably represents a specific immune response directed against the bacterium causing the tissue inflammation and destruction. The bacteria stained with FITC-labelled antihuman IgG following antibiotic treatment may be nonviable group A BHS or secondary pathogens. The latter seems unlikely since the immunoglobulin coating of normal oropharyngeal flora was not increased in patients with symptomatic pharyngitis of unknown etiology. The results with the nongroup A BHS are consistent with the observations that these BHS may cause a clinically and immunologically significant infection but more often are associated with asymptomatic cases.

Crawford et al reported that the Gram-stained smear of pharyngeal secretions was a useful technique for the prompt diagnosis of streptococcal pharyngitis. The Gram-stained smear (interpreted by five observers) had a mean sensitivity, specificity and predictive value of a positive result of 73 percent, 96 percent, and 71 percent. The Gram-stained smear technique was criticised because of the low sensitivity (27 percent false negative tests) and the considerable expertise needed to evaluate the smears. In comparison, the ACB test had fewer false negative tests (9 percent) and a similar specificity (94 percent). Since the ACB test is a fluorescent procedure, the interpretation of the smear also requires some expertise. Additional studies are needed to determine the value of the ACB test in differentiating the streptococcal carrier state from true infection.

Acknowledgments

These studies were supported by a grant from the Medical Research Foundation of Oregon and the Tartar Trust. The authors also wish to acknowledge the technical assistance of Mrs. Virginia Morthland.

References


---

### Table I

<table>
<thead>
<tr>
<th>Culture Results</th>
<th>&lt; 25 Positive Bacteria/10 Fields</th>
<th>≥ 25 Positive Bacteria/10 Fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-hemolytic streptococci (group A)</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci (not group A)</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Normal flora (symptomatic patients)</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Normal flora (post-treatment patients)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Normal flora (healthy controls)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Fluorescein isothiocyanate
+Significant difference at p < 0.001

### Table II

<table>
<thead>
<tr>
<th>Culture Results</th>
<th>&lt; 25 Positive Bacteria/10 Fields</th>
<th>≥ 25 Positive Bacteria/10 Fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-hemolytic streptococci (group A)</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci (not group A)</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Normal flora (symptomatic patients)</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Normal flora (post-treatment patients)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Normal flora (healthy controls)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Fluorescein isothiocyanate