Streptococcal Antibody Tests in Rheumatic Fever*

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ABSTRACT

Although the incidence of rheumatic fever has declined significantly over the last decade, testing for antibodies to streptococcal extracellular enzymes maintains an important role in differentiating this disease from others with similar characteristics. Detection of antibodies to streptolysin O and DNase-B remain the more popular single antibody tests while the streptozyme test, which detects antibodies to five distinct streptococcal extracellular products, has been increasingly used in recent years as a screening test. Several new procedures detecting antibodies to different somatic antigens have been developed, the most promising of which seem to be anti-Group A carbohydrate tests. Because antibodies to the group A carbohydrate remain for several years in patients with persistent rheumatic valvular disease, this test should aid in the differentiation of rheumatic from non-rheumatic heart disease.

Introduction

Streptococcus pyogenes or group A streptococcus causes several kinds of infections that can be followed by nonsuppurative inflammatory sequelae. Glomerulonephritis can follow practically any kind of group A streptococcal infection, while rheumatic fever follows essentially infections of the upper respiratory tract. The major clinical importance of these sequelae stems from their potential to cause severe acute or chronic damage to the heart (rheumatic heart disease) or the kidneys.

In recent years, both the incidence and the severity of rheumatic fever has declined in industrialized countries, parallel to a decrease in the severity of streptococcal pharyngitis. The number of reported cases of acute rheumatic fever per 100,000 population in the United States was 1.79 in 1974 and decreased to 0.06 in 1983.12 However,
rheumatic fever is still frequently found in many underdeveloped countries.

While post-streptococcal glomerulonephritis may be regarded as an immune complex disease, the pathogenesis of rheumatic fever is less clear. As in glomerulonephritis, acute and chronic damage is not associated with the presence of the microorganism in the lesions. Thus, tissue damage is likely to be caused by streptococcal antigens and/or host substances elaborated in the course of an exaggerated response to group A streptococci. However, neither the nature of the bacterial products nor the mechanisms leading to inflammatory reactions have been unequivocally clarified. Host factors seem to be important as evidenced by the fact that only a small percentage of individuals infected with group A streptococci develop rheumatic fever. Moreover, some streptococcal strains seem to be more apt than others to induce the disease. Some aspects concerning the pathogenesis of rheumatic fever have been recently reviewed.

Acute rheumatic fever is usually diagnosed using Jones Criteria which were originally proposed by Jones in 1944 and revised several times thereafter (table I). According to this scheme, “the presence of two major criteria or one major and two minor criteria indicates a high probability of the presence of acute rheumatic fever if supported by evidence of a preceding group A streptococcal infection.” Thus, with few exceptions, evidence of a recent streptococcal infection is mandatory for the diagnosis of acute rheumatic fever.

For several decades the determination of antibodies directed against extracellular products of group A streptococci, and especially anti-streptolysin O (ASO) antibodies, has been extremely helpful in establishing the diagnosis of acute poststreptococcal disease. Since acute rheumatic fever follows infection in a time period averaging three weeks, culture methods are far less satisfactory than serological methods. Of course, in addition to their clinical value, serological tests have proven invaluable for studies concerning the epidemiology and pathogenesis of streptococcal infections. For example, from an historic point of view, the observation that antibodies directed against the group A streptococcus are uniformly elevated in the course of acute rheumatic fever has been essential in establishing its streptococcal etiology. This paper will be a review of the important characteristics of selected tests with special references to the diagnosis of rheumatic heart disease.

Group A streptococci produce several antigens, some of which are shown in table II. Levels of antibodies to certain extracellular antigens, such as streptolysin O (SLO) and deoxyribonuclease B (DNase B), have been extensively investigated in terms of their relationship to preceding streptococcal infections. The most widely used methods for the detection of anti-streptococcal antibodies in clinical laboratories are probably the anti-streptolysin-O (ASO), the anti-deoxyribonuclease B (ADN-B), the anti-hyal-

### TABLE I

<table>
<thead>
<tr>
<th>Major Manifestations</th>
<th>Minor Manifestations</th>
</tr>
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<tbody>
<tr>
<td>Carditis</td>
<td>A. Clinical</td>
</tr>
<tr>
<td>Polyarthritus</td>
<td>Previous rheumatic</td>
</tr>
<tr>
<td>Chorea</td>
<td>fever</td>
</tr>
<tr>
<td>Erythema marginatum</td>
<td>Arthralgia</td>
</tr>
<tr>
<td>Subcutaneous nodules</td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>B. Laboratory</td>
</tr>
<tr>
<td></td>
<td>Acute phase reactants</td>
</tr>
<tr>
<td></td>
<td>Prolonged P-R interval</td>
</tr>
</tbody>
</table>

**Supporting Evidence of Streptococcal Infection**

- Increased titer of anti-group A streptococcal antibodies
- Positive throat culture for group A streptococcus
- Recent scarlet fever

The presence of two major criteria, or of one major and two minor criteria, indicates a high probability of acute rheumatic fever if supported by evidence of a preceding group A streptococcal infection.
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TABLE II

Group A Streptococcal Antigens

<table>
<thead>
<tr>
<th>Cellular</th>
<th>Extracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-specific polysaccharide</td>
<td>Streptolysin O</td>
</tr>
<tr>
<td>M protein</td>
<td>DNase A, B, C, D</td>
</tr>
<tr>
<td>M-associated protein (MAP)</td>
<td>Hyaluronidase</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>NADase</td>
</tr>
<tr>
<td>R &amp; T proteins</td>
<td>Streptokinase</td>
</tr>
<tr>
<td>Protoplast membrane</td>
<td>Pyrogenic toxins</td>
</tr>
</tbody>
</table>
| Teichoic acids | Ururonidase (AH), and the streptozyme (SZ) tests. These are effective methods, but there are also several new tests that have been developed which detect antibodies to somatic antigens. Antibodies to extracellular streptococcal antigens rise during the first month after infection and plateau for three to six months before declining.4,5 Normal levels are generally found six to 12 months after the infection.15 Thus, patients with chorea, an often delayed manifestation of rheumatic fever, might have normal antibody titers.1 A positive anti-streptococcal antibody test is generally considered one in which there is a two dilution increase in titer between acute and convalescent serum specimens.1 Serum samples should be obtained at two to four week intervals and tested simultaneously. However, in most instances, only one serum sample is available and efforts have been made to determine “normal” values for anti-streptococcal antibodies.5,18,23,27 These values vary with the age of the population under study, geographical location, and the season of the year. Generally, a value is considered abnormally elevated if it exceeds the upper limit of normal with normalcy being defined as that titer exceeded by no more than 15 to 20 percent of the normal population.

Anti-streptolysin-O Test

This is the most commonly used test for the detection of streptococcal anti-

bodies. Streptolysin-O (SLO) is an oxygen labile toxin capable of lysing a wide range of mammalian cells but not bacterial protoplasts or L forms.8 This differential effect can be explained by the ability of streptolysin O to bind only to membranes containing cholesterol. Free cholesterol (i.e., non-esterified or protein bound cholesterol) also interacts with SLO and inhibits lytic activity.45 Although serum cholesterol is not in a state allowing for inhibition of SLO, the beta lipoprotein fraction of normal human sera shows a slight inhibition.48 Streptolysin-O is produced by group A, group G, and human group C streptococcal strains but not by other streptococci.26,43 Other Gram positive bacteria, however, such as the pneumococci, Clostridium perfringens, and Listeria monocytogenes, can produce antigenically related oxygen and cholesterol inhibitable cytolysins. Although cross reactions with SLO are found in the sera of horses immunized with these cytolysins, cross reactions are rare with human sera.43

The ASO tests predominantly used are the hemolytic tests, but an ASO latex screening test is also available. The hemolytic test is a neutralization test which works on the premise that reduced SLO has the ability to lyse erythrocytes of many animals species. The ASO level is measured in Todd units by recording the highest dilution of serum inhibiting hemolysis.41 The main advantage of the test is that it has good reproducibility and is well standardized. An additional advantage is that it is widely commercially available and its value in the diagnosis of post-streptococcal sequelae has been carefully assessed.

However, high levels of serum beta lipoprotein in liver disease and serum contamination with products of the growth of bacteria such as Bacillus cereus and Pseudomonas spp. can result in falsely elevated ASO titers.26,44 Since
only reduced streptolysin O has hemolytic properties, oxidation of streptolysin O can also result in false positive titers.\textsuperscript{26,43} Another advantage of the ASO test is a relative lack in sensitivity. Only about 85 percent of patients with acute rheumatic fever have increased ASO levels.\textsuperscript{4,5} The remaining 15 percent have, however, elevated antibody levels to other extracellular antigens, such as DNase B\textsuperscript{5} and hyaluronidase.\textsuperscript{19} Moreover, the ASO test is not a reliable indicator of skin infections. This probably results from the combination of SLO with free cholesterol present in epidermal tissues and a consequent decrease in its antigenic properties.\textsuperscript{43} Thus, although skin infections do not give rise to rheumatic fever, it is generally advisable to perform another test in addition to the ASO test to rule out a recent streptococcal infection when rheumatic fever is suspect. In the absence of information about normal values in a given geographic area and as a general guide, ASO titers equal or superior to 250 Todd units in adults and equal or superior to 333 in children should be considered increased.\textsuperscript{1} Another type of ASO test is the latex screening test.\textsuperscript{28} This test requires only a single dilution of the patient’s serum. It is a rapid slide test in which enough SLO coupled to latex particles or erythrocytes is added to the serum to bind 200 international units of ASO per ml. The presence of more than 200 international units of ASO results in macroscopically visible agglutination. This test is useful as a screening test, although its high cost often prevents screening of a large number of sera in most situations.

**Anti-deoxyribonuclease B Test**

Another test frequently used to detect group A streptococcal infections is the ADN-B test. This is a neutralization test which detects antibodies to the enzyme DNase-B produced by group A and by a few strains of group C and G streptococci.\textsuperscript{5,25} Other enzymes, such as DNase A, C, and G, are produced not only by group A but also by other streptococci.\textsuperscript{26} This test involves incubation of DNase-B with several dilutions of the patient’s serum followed by the addition of deoxyribonucleic acid (DNA). As the DNase-B combines with the antibodies in the serum, the DNase-B is neutralized so that it is no longer capable of depolymerizing the DNA substrates. Polymerization of DNA can be assessed either by the addition of agents, such as ethanol, capable of coagulating polymerized DNA, or by dyes such as methyl green which retains its color when combining with polymerized, but not depolymerized, DNA.

The ADN-B test offers some advantages over the ASO test. First, patients with streptococcal skin infections or their sequela (notably glomerulonephritis) often have high ADN-B, but not ASO, titers.\textsuperscript{5} Second, the ADN-B test is not associated with false positive results owing to bacterial growth in serum samples, liver disease, or oxidation of the antigen. However, false positive results can occur in patients with elevated DNase serum levels, such as in hemorrhagic pancreatitis.\textsuperscript{26}

The ADN-B test is probably the best single test for the serologic detection of streptococcal infections. However, some patients with rheumatic fever may have low ADN-B titers and increased sensitivity is obtained by performing a second streptococcal antibody test.\textsuperscript{4,25} In fact, if two different tests are performed on the same sample, abnormally elevated streptococcal antibody levels can be detected in 90 percent of patients.

As with ASO, ADN-B titers are likely to vary seasonally and geographically. “Upper normal levels” of ADN-B are generally lower than ASO before 13 years of age, but they are similar to ASO in older age groups.\textsuperscript{27}
Other Antibody Tests to Extracellular Antigens

The anti-hyaluronidase (AH) test is also a neutralization test which is commercially available. Its principle is very similar to that of the ADN-B test. Potassium hyaluronate is used instead of DNA as a substrate to detect enzymatic inhibition. The AH test has the same advantages over ASO as ADN-B. It is associated with less false positive reactions than ASO and it is of value in detecting skin infections. It is, however, less reproducible and less well studied than ADN-B. Other antibody tests based on neutralization of biological activities of streptococcal exoenzymes are the anti-streptokinase test and the anti-nicotinamide adenine dinucleotide glycohydrolase test. These tests, however, have been less accurately standardized and evaluated than the ASO and ADN-B tests.

Streptozyme

A test that has been commonly used in recent years for the detection of post-streptococcal sequelae is the Streptozyme SZ test. In contrast to the neutralization tests described previously, the SZ test is a quick, simple procedure which has the potential of simultaneously detecting antibodies to several different extracellular antigens. The SZ test is a two minute hemagglutination test whose reagents consist of sheep red blood cells sensitized with the supernatant of group A streptococcal cultures. The SZ test is believed to detect antibodies to at least five different exoenzymes produced by group A streptococci, namely SLO, DNase-B, hyaluronidase, streptokinase, and nicotinamide adenine dinucleotide glycohydrolase. The SZ test is a qualitative test that can be used also as a quantitative test. Sera producing agglutination at a 1:100 dilution can be further tested using serial dilutions to determine an end point.

Several attempts have been made to correlate the sensitivity and specificity of the SZ test in detecting streptococcal infections. Streptozyme SZ has been shown to detect rising antibody titers in human and experimental animal infections before ASO or ADN-B. Although the sensitivity of the SZ may be considered satisfactory, more studies appear to be necessary to assess better its specificity. In a study of 162 sera from a stratified random sample of school children, a good correlation was found between SZ and ASO and ADN-B titers in young school children. However, there appeared to be a steady increase in the percentage of false positive SZ specimens with increasing age. Thirty-six out of 81 (44.4 percent) young adults had positive SZ titers in the presence of low ASO and ADN-B titers. That the titer of 1:100 suggested by the manufacturer might be too low is also indicated by another study. These observations suggest that the upper limits of normal for the SZ test should probably be further assessed.

Other disadvantages of the SZ test are that some variability in strength of different lots of SZ reagents can occur and that it employs an as yet uncharacterized antigen preparation. Accordingly, the possibility that the SZ test measures antibodies to non-group A streptococcal products should be more thoroughly investigated.

Newer Methods

There are several other methods that have recently been developed which have the potential of giving the clinician other options besides conventional methods for the detection of post strep-
toccocal complications. In the course of streptococcal infections, an immune response develops not only to extracellular but also to cellular components such as M protein,\textsuperscript{14,35,39} M-associated protein (MAP),\textsuperscript{46,47} group A carbohydrate,\textsuperscript{11,22,30} peptidoglycan,\textsuperscript{21,36} teichoic acids,\textsuperscript{29} and protoplast membrane.\textsuperscript{6} Only tests detecting antibodies to A carbohydrate, MAP, and streptococcal protoplast membrane (SPM) will be reviewed here, since they appear more promising than other tests in the detection of post-streptococcal sequelae.

Methods by which anti-group A carbohydrate (ACHO) antibodies can be measured include bacterial agglutination,\textsuperscript{13} passive hemagglutination,\textsuperscript{37} and radioimmunoassay.\textsuperscript{15} The ACHO tests are still experimental, but they are very attractive methods for the detection of streptococcal infections and rheumatic valvular disease. In a study of children with group A streptococcal respiratory infection and age and season-matched controls, there was less overlap between the two groups in ACHO titers (measured by passive hemagglutination) than in ASO and ADN-B titers.\textsuperscript{17} Elevation of ACHO titers occurred more frequently than ASO or ADN-B titers among infected children.\textsuperscript{17} Levels of raised ACHO antibodies returned to normal in a period of five months in almost all subjects.

There appears to be a persistence of elevated levels of ACHO in patients with chronic rheumatic valvular disease.\textsuperscript{15} The level of ACHO declines to normal values six to 12 months after the infection in rheumatic fever patients without rheumatic carditis.\textsuperscript{4,15} However, in patients with persistent valvular disease, high levels of ACHO antibodies are maintained for several years. Patients with congenital valvular heart disease or with mitral prolapse do not have elevated levels of ACHO antibodies.\textsuperscript{2} It has been suggested that elevated ACHO in the presence of normal ASO titers may be helpful in differentiating rheumatic from non-rheumatic mitral insufficiency.\textsuperscript{2,4} Accordingly, persistently elevated levels of ACHO with normal ASO levels over a period of two years or more suggests rheumatic heart disease. Of course, the combination of elevated ACHO and ASO levels would have no predictive value since these can result from a recent uncomplicated infection.

The reason for the persistence of ACHO antibodies in patients with rheumatic valvulitis has not been clarified. It has been suggested that this could result from the continuous release from damaged valves of glycoproteins cross-reactive with streptococcal group A polysaccharides.\textsuperscript{15} However, a valvular damage \textit{per se} is not likely to be involved since ACHO levels are normal in patients with non-rheumatic valvular disease. Alternatively, persistent ACHO levels could result from a state of specific hypersensitivity to this antigen in patients with rheumatic valve disease.\textsuperscript{3,4}

Another method that can be of interest as a procedure for detecting post-streptococcal sequelae is the determination of serum antibodies to MAP. Anti-MAP antibodies were the only antibodies found to be higher in patients with acute rheumatic fever than in patients with acute glomerulonephritis.\textsuperscript{47} It is interesting that in another study total serum immunoglobulin A (IgA) levels were also found to be higher in patients with acute rheumatic fever than in patients with acute glomerulonephritis, although serum immunoglobulin G (IgG) was increased in both groups of patients.\textsuperscript{34} However, IgG but not IgA seems to represent the major class of antibodies reacting with MAP, as determined by an enzyme-linked immunosorbent assay (ELISA) test.\textsuperscript{34} These data indicate that the determination of anti-MAP antibodies and of total serum IgA concentration may be useful adjunctive tests in the diagnosis of rheumatic fever.
An ELISA test for detection of anti-streptococcal proplast membrane (SPM) antibodies has been recently described. It is believed that group A SPM are involved in the pathogenesis of both rheumatic fever and post-streptococcal glomerulonephritis and there is a well documented cross reactivity between SPM antigens and human tissues. Anti-SPM levels do not appear to be comparable to ASO levels, probably because the anti-SPM tests preferentially detect antibodies to infecting strains belonging to the same M-type used for antigen preparation. In several serum samples from one patient with uncomplicated streptococcal sore throat, however, there was a correlation between anti-SPM antibodies and ASO titers.5 In conclusion, over the past 10 years the development of new detection methods for post-streptococcal sequelae has given laboratories more procedures from which to choose. These methods should continue to improve as new discoveries are made in the actual progression of streptococcal infection and post-infection complications.

References


