Specificity and Sensitivity of the Streptozyme Test for the Detection of Streptococcal Antibodies

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Received for publication 21 August 1973

A comparison between the results of the streptozyme hemagglutination test and serological titers for anti-streptolysin O (ASO), anti-hyaluronidase (AH), anti-deoxyribonuclease B (ADN-B), and anti-nicotinamide adenine dinucleotidase (ANAD) was made in two groups of human sera. In one group, serological titers for all the four antibodies were lower than the threshold of sensitization reported by the producing firm. In the second group, the titer of at least one of the four antibodies was equal to or higher than the threshold. False-positive and false-negative reactions occur with those sera when one or more antibody titer is at or near the threshold of the test as described by the manufacturer. The test was positive for all sera where either the ASO was greater than 166 or the ANAD was greater than 270, and for 98% of the sera with ADN-B greater than 960. It is, therefore, concluded that the streptozyme test can be used as an adjunct to the clinical diagnosis of streptococcal infections and their nonsuppurative sequelae. It is less useful to assess the levels of antibodies in sera from general population surveys. For such sera, the relative specificity and sensitivity of the test might yield misleading results. Until more experience is gained with the test, caution should be used in its application to infant and older adult age groups, where significant streptococcal antibody titers are frequently near the threshold of the test.

The revised Jones criteria developed in 1965 by a subcommittee of the American Heart Association stressed the importance of documenting a prior streptococcal infection in establishing the diagnosis of acute rheumatic fever (15). Serological tests to detect antibodies to one or more of the streptococcal extracellular antigens are employed for this purpose. Although the anti-streptolysin O (ASO) test is most widely employed, it is not elevated in all cases of rheumatic fever or nephritis (10, 14). Ayoub and Wannamaker (2) pointed out the advantage of determining the antibodies to three different streptococcal extracellular products. With such a battery of tests, evidence for a prior streptococcal infection can be obtained in almost all patients with acute rheumatic fever and acute nephritis. However, the use of one or more of these tests taxes the available resources, supplies, and trained personnel for many clinical laboratories. There is a need, therefore, for alternative antibody assays that are reliable and simple to perform. The streptozyme test (Wampole Laboratories, Div. Denver Chemical Mfg. Co., Stamford, Conn.) has recently been developed for this purpose (6). It is a rapid passive hemagglutination test that detects at least five streptococcal antibodies: ASO, anti-deoxyribonuclease B (ADN-B), anti-hyaluronidase (AH), anti-nicotinamide adenine dinucleotidase (ANAD), and anti-streptokinase (ASK).

Others have compared the results obtained with the streptozyme test in human sera to those obtained on the same sera with the serological tests for detecting ASO, AH, ASK, and ADN-B (3, 8, 12). Although each of these studies revealed a good correlation between the results with the streptozyme test and the conventional serological tests, there has been inadequate assessment of the possible frequency of false-positive and false-negative results with the streptozyme test. This current study has been designed to answer this question. The results of the test have been determined on a group of antisera in which at least one antibody titer was elevated and a group of negative sera in which none of the four antibody titers was elevated.

MATERIALS AND METHODS

The streptozyme test employs a reagent that is a standard suspension of formaldehyde-treated sheep
erythrocytes sensitized with group A streptococcal extracellular products (6). The information supplied by the producing firm and data obtained by others who have used the test (3, 8, 12) indicate that a positive streptozyme test will usually be observed with sera when one or more of the streptococcal antibody titers are: ASO, ≥166 Todd units; ADN-B, ≥170; ANAD, ≥270; AH, ≥256; and ASK, ≥200.

The streptozyme test was performed according to the instructions of the producing firm and as reported in previous publications (6). In brief, inactivated sera were diluted 1:100 by adding 5 ml of isotonic saline to 0.05 ml of serum specimen by using a calibrated Kimax serological pipette. The test was performed by adding 1 drop of 1:100 diluted serum to 1 drop of streptozyme reagent on a glass slide, mixing the two with a disposable stirrer provided with the kit, and slowly tilting the slide for no more than 2 min. Positive sera showed a visible agglutination reaction, whereas negative sera did not. A test serum was scored as streptozyme positive when agglutination was comparable to the reaction obtained with the positive control serum provided by the firm. A negative control serum was used that had antibody titers below the threshold of the test. The streptozyme reagent was supplied through the courtesy of the producing firm.

Serological tests were performed for the streptococcal antibodies known to be detected by the streptozyme test with the exception of antibodies to streptokinase. ASO was determined by the Rantz and Randall dilution system (13). The ADN-B was performed by methyl-green-microtechnique of Nelson et al. (7, 11), and the ANAD test was done by the technique of Ayoub and Ferreti (1) by using sodium bismuthate. The AH test was done on heat-inactivated sera by using a 50% end point similar to that of Harris and Harris (5). All titrations were made in duplicate, and results were comparable.

The human sera were from the epidemiological studies on streptococcal diseases conducted in the Cairo area (4).

The double-blind procedure was employed throughout the study for the performance of the streptozyme test and the other antibody tests.

**RESULTS**

In Table 1 is shown the comparison between the results with the streptozyme test and the titers of ASO, ADN-B, ANAD, and AH on 177 human sera. For this comparison, as well as that in Tables 2 and 3, sera were classified as positive if one or more antibody titers were elevated as defined in Table 1 and negative if all four antibody titers were less than those given in Table 1. The streptozyme test was positive for 63% of the positive sera and negative for 83% of the negative sera. Further analysis was performed to determine the reasons for the occurrence of false-negative streptozyme test reactions in 37% of the positive sera and the occurrence of false-positive results in 17% of the negative sera.

| Sera | Streptozyme results |
|------|---------------------|
|      | Positive | Negative |
| Positive* | 90 (63)* | 52 (37) |
| Negative* | 6 (17) | 29 (83) |

* At least one antibody titer elevated: ASO > 166, ANAD > 270, ADN-B > 170, and AH > 256.
* Numbers in parentheses are percentages.
* All four antibody titers are: ASO < 166, ANAD < 270, ADN-B < 170, AH < 256.

In Table 2 are tabulated the median and geometric mean values for the antibody titers for the positive sera that gave a positive streptozyme reaction and for the positive sera that gave a negative streptozyme reaction. The range of antibody values are given in the footnotes. It is clear from this analysis that many of the positive sera with a negative streptozyme reaction had antibody titers near the sensitivity threshold of the streptozyme test.

In Table 3 are tabulated the median and geometric mean values for the antibody titers for the negative sera that gave a negative streptozyme reaction and for the negative sera that gave a positive streptozyme reaction. Included in the footnotes are the range of antibody titers. The median and geometric mean values for ASO and ADN-B, especially, are greater for the negative sera with a positive streptozyme reaction than the comparable values for the negative sera with a negative streptozyme reaction. Thus, the streptozyme test may be positive for negative sera with antibody titers that approach the sensitivity threshold of the test.

In a number of clinical and epidemiological circumstances, only a modest elevation occurs in one or more of the streptococcal antibodies. For this reason, an additional analysis of the data on the 177 sera was performed to determine the frequency of a positive streptozyme test reaction for those sera in which at least one of the four antibody titers was at or near the threshold of the sensitivity of the test. For this purpose, the sera were sorted into two categories for each one of the four antibodies. In one category the titer was near the threshold of sensitivity of the test; in the other category the titer was above the threshold; sera with titers below the threshold of sensitivity were excluded from the analysis. Thus, for example, there were 14 sera with an ASO of 166 and 35 sera in which the ASO was greater than 166. One hundred twenty-eight sera were excluded from this ASO analysis. The status of the titers for the other
antibody tests for the sera in the two categories was not considered for the purpose of this analysis of the comparison of the streptozyme test results and the ASO titers. In a similar way the 177 sera were sorted into two categories for each of the other three antibodies, and again the sera that had titers below the threshold of sensitivity were excluded from the analysis. The data are tabulated in Table 4. A negative streptozyme test was observed for 4 out of 14 sera (28%) with an ASO titer of 166 and 34 out of 63 (54%) sera with an ADN-B titer of 200 to 360. Similar percentages are seen for the other two antibodies.

The data also indicate that the streptozyme test was usually positive for those sera with at least one of the four antibody titers greater than the threshold of sensitivity of the streptozyme test. All sera with an ASO greater than 166 and all sera with an ADN greater than 270 gave a positive streptozyme reaction. Only 1 out of 50 sera with an ADN-B titer greater than 360 was missed by the test.

The test was positive for only 51 of 62 sera with an AH titer greater than 500, but this probably is a reflection of the well-known capriciousness of the AH test.

**DISCUSSION**

The results of this investigation show that the streptozyme test may give false-positive or false-negative reactions when an antiserum has a level of one or more streptococcal antibodies at or near the level of sensitivity of the streptozyme reagent. For example, 28% of the sera with an ASO of 166, 32% of the sera with an ANAD between 90 to <270, and 54% of the sera with an ADN-B between 200 and 360 gave a

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**Table 2. Comparison between streptozyme reaction and antibody titers for the antibody-positive sera**

| Streptozyme reaction | No. tested | Median and geometric mean values of antibody titers of positive sera* |
|----------------------|------------|------------------------------------------------------------------|
| Positive             | 90         | ASO: 125* (149) ANAD: 227* (182) ADN-B: 480* (407) AH: 576* (501) |
| Negative             | 52         | ASO: 50* (50) ANAD: 90* (99) ADN-B: 200* (196) AH: 217* (253) |

* As defined in Table 1.
* Figure represents median and between brackets the geometric mean.
* Range, 12 to 625.
* Range, 90 to 540.
* Range, 100 to 2,560.
* Range, 200 to 2,560.
* Range, 12 to 166 (four sera, 166).
* Range, 85 to 180.
* Range, 60 to 320.
* Range, 80 to 1,280.

**Table 3. Comparison between streptozyme reaction and antibody titers for the antibody-negative sera**

| Streptozyme reaction | No. tested | Median and geometric mean values of antibody titers of negative sera* |
|----------------------|------------|------------------------------------------------------------------|
| Negative             | 29         | ASO: 50* (33) ANAD: 90* (97) ADN-B: 60* (80) AH: 128* (135) |
| Positive             | 6          | ASO: 125* (92) ANAD: 90* (113) ADN-B: 120* (121) AH: 160* (170) |

* As defined in Table 1.
* Figure represents median and between brackets the geometric mean.
* Range, 12 to 125.
* Range, 85 to 180.
* Range, 40 to 160.
* Range, 80 to 240.
* Range, 50 to 125.
* Range, 90 to 180.
* Range, 80 to 160.
* Range, 92 to 204.
negative streptozyme test. Thus, in certain circumstances, the streptozyme test may have limited usefulness for detecting a significantly elevated antibody titer. For example, a serum of ASO 166 may be indicative of a streptococcal infection in an infant or older adult. This is due to the fact that streptococcal infections are uncommon in these two age groups. For this reason 95% of children under the age of 2 years have an ASO below 100 (10), and the response that occurs in such individuals after an infection may be below or near the level of sensitivity of the streptozyme test.

There was a good correlation with the results of the streptozyme test and the antibody tests for those sera in which there were distinctly elevated levels of ASO, ADN-B, and ANAD. Significantly high titer of these are usually encountered in streptococcal disease and their sequelae (10). A negative streptozyme reaction is unlikely to occur for sera with clinically elevated antibody titer to any one of these three tests.

Because 95% of the patients with rheumatic fever usually have a significantly elevated titer to one or more of the streptococcal antigens tested here, the streptozyme test can probably be used to support the clinical diagnosis of rheumatic fever. With such patients the instances of false negatives will be few, and the streptozyme test may be as useful as a single antibody value determined by conventional serological methods. It must be recognized, however, that in instances where the differential diagnosis is in question, a false-positive streptozyme test may be misleading. In such cases, there is no substitute for thorough serological investigation.

ACKNOWLEDGMENTS

The support, advice, and assistance of Robert Zimmerman, Contract Officer for this PL 480 Project, is gratefully acknowledged.

This investigation was supported by Public Health Service grant PL-480 (05-25-2).

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