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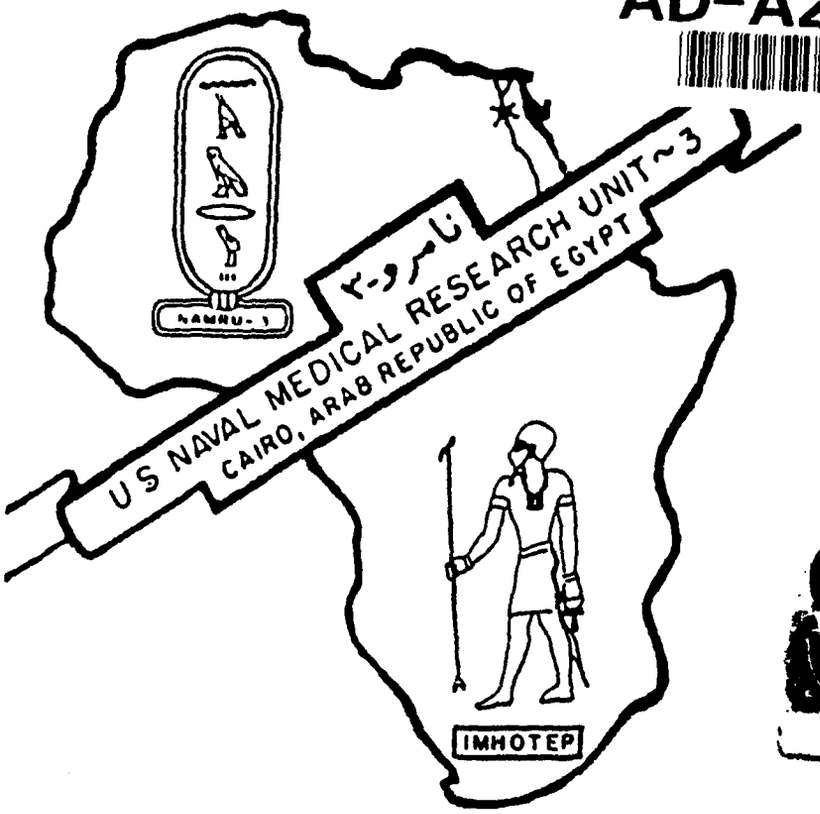

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INTERPRETING A SINGLE ANTISTREPTOLYSIN O TEST:
A COMPARISON OF THE "UPPER LIMIT OF NORMAL" AND
LIKELIHOOD RATIO METHODS

BY

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Single serologic tests may occasionally influence clinicians in making diagnoses. The antistreptolysin O (ASO) test is a frequently used tool for detecting recent Streptococcus pyogenes infection and is helpful in the diagnosis of diseases like rheumatic fever. Using data from a 1989 prospective study of 600 healthy male military recruits, in which 43% experienced S. pyogenes upper respiratory tract infection (2-dilution rise in ASO), this report compared two methods of interpreting a single ASO titer. Using the "upper limit of normal" (80 percentile) method, recruits with an ASO titer of greater than 400 showed evidence of recent S. pyogenes infection. This method had a sensitivity and specificity of only 65.9 and 81.9% respectively. In contrast to the "yes-no" dichotomy of the "upper limit of normal" method, the likelihood ratio method statistics were ASO value specific, more consistent with clinical judgement, and better emphasized the caution clinicians must use in interpreting a single ASO test.

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INTERPRETING A SINGLE ANTISTREPTOLYSIN O TEST: A COMPARISON OF THE "UPPER LIMIT OF NORMAL" AND LIKELIHOOD RATIO METHODS

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Abstract—Single serologic tests may occasionally influence clinicians in making diagnoses. The antistreptolysin O (ASO) test is a frequently used tool for detecting recent *Streptococcus pyogenes* infection and is helpful in the diagnosis of diseases like rheumatic fever. Using data from a 1989 prospective study of 600 healthy male military recruits, in which 43% experienced *S. pyogenes* upper respiratory tract infection (2-dilution rise in ASO), this report compared two methods of interpreting a single ASO titer. Using the "upper limit of normal" (80 percentile) method, recruits with an ASO titer of greater than 400 showed evidence of recent *S. pyogenes* infection. This method had a sensitivity and specificity of only 65.9 and 81.9% respectively. In contrast to the "yes–no" dichotomy of the "upper limit of normal" method, the likelihood ratio method statistics were ASO value specific, more consistent with clinical judgment, and better emphasized the caution clinicians must use in interpreting a single ASO test.

Antistreptolysin O *Streptococcus pyogenes* Likelihood ratio Upper limit of normal Rheumatic fever Infection

INTRODUCTION

The reemergence of more virulent strains of *Streptococcus pyogenes*, accompanied with their numerous clinical manifestations, has led clinicians to more frequently consider the probability of *S. pyogenes* infection. In the absence of positive cultures, serologic tests are often used. At present, there is no perfect serologic technique to confirm recent *S. pyogenes* infection. The most commonly available and easiest assay to perform is the antistreptolysin O titer (ASO). This test is particularly effective at detecting upper respiratory tract *S. pyogenes* infection [2, 7]. A rise in ASO titer occurs in the second week after infection and reaches its maximum value at 4–6 weeks [13]. When using an appropriate dilution scheme, a 2-dilution incremental rise or greater in ASO titer is usually accepted as serologic confirmation of recent infection [2]. When acute and convalescent sera are not available, a single ASO value above the "upper limits of normal" (80 percentile) is considered evidence of recent infection [8, 9, 12]. This "upper limit of normal" (ULN)

Informed consent was obtained from all patients. Data and specimen collection procedures were approved by the Naval Medical Research Institute's Committee for the Protection of Human Subjects.

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ASO titer varies with population, age, and individual laboratory [1, 2, 7, 8]. The dichotomous ULN method fails to quantify the likelihood that individual ASO test results reflect recent infection. No matter their magnitude, all ASO titers above the ULN are classified in the same fashion. The purpose of this study was to compare the ULN method with a likelihood ratio method of interpreting a single ASO test. The likelihood ratio method offers a continuum of risk estimation, based upon the magnitude of the test result. Data from a previous study of healthy military recruits was available for this comparison.

METHODS

Study group

Data for this investigation were collected during a previously reported epidemiologic study of 873 male U.S. Marine Corps recruits who entered recruit training camp in January 1989 [4]. Serum and throat cultures were obtained from all recruits within 48 h of entering camp (pre-training sample) and again 11 weeks later (post-training sample). Questionnaire data were obtained from recruits at the time of the pre-training blood sample. Six hundred recruits met the enrollment criteria: denial of a sore throat during the 2-week period before entering camp and donation of both pre-training and post-training sera samples.

Laboratory studies

ASO titers [10] were determined using 0.10 log dilution increments: 1:100, 1:120, 1:160, 1:200, 1:240, 1:320, 1:400, 1:480, 1:640, 1:800, 1:960, 1:1280, 1:1600, 1:1920, and 1:2560. ASO and throat culture methodology has been reported previously [4]. Reagents for ASO testing were purchased from DIFCO (Detroit, MI). Logarithm (base 10) conversion of ASO titers was performed prior to analysis. Because exact titers were not determined for ASO values < 100, these sera were arbitrarily assigned the ASO value of 50 before geometric mean titers were calculated.

Statistical analyses

A chief aim of this work was to examine two methods of interpreting a single ASO titer as a predictor of recent *S. pyogenes* infection. In this study a post-training ASO titer was used as the single test and a 2-dilution rise or greater in ASO titer (pre-training to post-training) was defined as serologic confirmation of infection. Using various post-training ASO cutoff points, calculations of sensitivity and specificity were made. Sensitivity was defined as the proportion of recruits with a 2-dilution or greater rise (pre-training to post-training) in ASO titer who had a post-training ASO titer above a determined cutoff point. Specificity was defined as the proportion of recruits without a 2-dilution or more rise in ASO titer who had a post-training ASO titer less than or equal to a cutoff point. Likelihood ratio statistics were determined for post-training throat culture results and each post-training ASO value by defining infection as a 2-dilution rise in ASO titer (pre-training to post-training) and using the formulae [3, 5]:

$$\text{LR} = \frac{\text{(Probability of results among people with infection)}}{\text{(Probability of results among people without infection)}}. \quad (1)$$

Pre-test odds of infection were calculated from pre-test probability of infection (prevalence) using the equation:

$$\text{Pre-test odds} = \frac{\text{(Pre-test probability)}}{(1 - \text{Pre-test probability})}. \quad (2)$$

Post-test odds of infection were calculated by using the formula:

$$\text{Post-test odds} = \text{Pre-test odds} * \text{likelihood ratio}. \quad (3)$$

Post-test probability of infection was calculated by applying the following equation:

$$\text{Post-test probability} = \frac{\text{(Post-test odds)}}{(1 + \text{Post-test odds})}. \quad (4)$$

Overall post-test odds of infection considering 2 independent tests were calculated using the following formula:

$$\text{Overall post-test odds} = \text{Pre-test odds} * \text{likelihood ratio1} * \text{likelihood ratio2}. \quad (5)$$

The Kruskal–Wallis test was used to compare non-parametric distributions.

RESULTS

The distribution of recruit ASO titers is shown in Table 1. The geometric mean pre-training and post-training ASO titers were 200 and 295 respectively. The ULN (80-percentile)

Table 1. Distribution of pre-training and post-training serum ASO titers among 600 healthy U.S. Marine Corps recruits, 1989, San Diego, California

ASO Titer	Pre-training (number of recruits)	Post-training (number of recruits)	Sensitivity	Specificity
<100	97	80	—	23.4
100	39	26	—	31
120	54	32	100	40.4
160	53	45	96.9	51.2
200	59	46	90.3	59.6
240	106	61	81.4	70.8
320	35	33	75.6	76
400	51	45	65.9	81.9
480	48	67	53.1	91.8
640	25	37	44.2	95.9
800	18	38	32.9	98.5
960	9	34	21.3	99.7
1280	3	11	17.4	100
1600	1	19	10.1	100
1920	0	9	6.6	100
2560	2	17	0	100

Sensitivity and specificity calculations were made for post-training ASO titers referenced at various cutoff points and compared to a 2-dilution rise (over 11 weeks) in ASO standard.

titer for pre-training ASO was 400. Two hundred fifty-eight (43%) of the 600 recruits had a 2-dilution or greater rise in ASO (pre-training to post-training).

Twenty-six (4.3%) of the 600 recruits had throat cultures positive for *S. pyogenes* upon enrolling in the study. Recruits with a positive pre-training throat culture had higher pre-training and post-training ASO titers, but were at no greater risk of infection (2-dilution rise in ASO titer), as compared with recruits with a negative pre-training throat culture for *S. pyogenes*. Geometric mean pre-training titers were 380 (throat culture positive for *S. pyogenes*) and 192 (throat culture negative) (Kruskal–Wallis test, $p < 0.001$). Geometric mean post-training titers were 463 (pre-training throat culture positive for *S. pyogenes*) and 289 (pre-training throat culture negative), respectively (Kruskal–Wallis test, $p = 0.025$).

Sixty-six (11%) of the 600 recruits had post-training throat cultures positive for *S. pyogenes*. The likelihood ratios of recruit infection (2-dilution rise in ASO titer) with negative and positive throat cultures were 0.85 and 3.82, respectively.

The sensitivity and specificity of the single post-training ASO titer (examined at various cutoff points) predicting a 2-dilution rise in ASO (pre-training to post-training) are recorded in Table 1. Similarly, likelihood ratios for individual post-training ASO titers predicting a 2-dilution rise in ASO are recorded in Table 2. Likelihood ratios were multiplied by an array of

Table 2. The probability of recent *S. pyogenes* infection determined from a single ASO test in a population of 600 U.S. Marine Corps Recruits, 1989, San Diego, California

Pre-test probability of infection p	Pre-test odds of infection $p/(1-p)$	Post-test probability of infection or predictive value after a single ASO test for specific ASO titers (likelihood ratios)							
		240 (0.8)	320 (1.1)	400 (1.7)	480 (1.3)	640 (2.2)	800 (4.3)	960 (9.9)	1280 (13.3)
0.05	0.05	0.04	0.06	0.08	0.07	0.11	0.19	0.34	0.41
0.10	0.11	0.08	0.11	0.16	0.12	0.19	0.32	0.52	0.60
0.15	0.18	0.12	0.16	0.23	0.19	0.28	0.43	0.64	0.70
0.20	0.25	0.17	0.22	0.30	0.25	0.35	0.52	0.71	0.77
0.25	0.33	0.21	0.27	0.36	0.30	0.42	0.59	0.77	0.82
0.30	0.43	0.25	0.32	0.42	0.36	0.48	0.65	0.81	0.85
0.35	0.54	0.30	0.37	0.48	0.41	0.54	0.70	0.84	0.88
0.40	0.67	0.35	0.42	0.53	0.47	0.60	0.74	0.87	0.90
0.45	0.82	0.39	0.47	0.58	0.51	0.64	0.78	0.89	0.92
0.50	1.00	0.44	0.52	0.63	0.57	0.69	0.81	0.91	0.93

Likelihood ratios shown here should only be applied to similar populations. The likelihood ratios for ASO titres greater than 1:1280 could not be calculated due to division by zero. Final probablities were calculated using the product of the odds of disease before the test $[p/(1-p)]$ and the likelihood ratio for the ASO titer. These odds of disease after the ASO $(x:y)$ were then converted to probabilities by using the formula: $x/(x+y)$.

potential pre-test odds of infection yielding post-test odds of infection (formula 3). These post-test odds were then converted to probabilities or positive predictive values of infection (formula 4).

DISCUSSION

Diagnosis of *S. pyogenes* upper respiratory tract infection is not easy. The condition is confounded by a lack of consistently reliable "diagnostic" symptoms or clinical signs [4, 8, 11], multiple serologic markers with quantitatively unpredictable responses [8], and by asymptomatic *S. pyogenes* carriers [6]. Clinicians generally do not rely upon serologic data for diagnoses of acute *S. pyogenes* infections such as uncomplicated pharyngitis, because often patients will not have had time to develop an immune response. Rather, serologic tests are of greater value for confirming a complication of *S. pyogenes* infection such as rheumatic fever.

There are a number of difficulties interpreting an ASO test. Streptococci other than *S. pyogenes* may elevate the ASO titer. ASO response to *S. pyogenes* infection is dependent upon the site; throat infections are more likely to cause ASO elevations than are skin infections [2, 4, 7]. Not all acute infections result in ASO elevations, especially if treated early with antibiotics. Finally, the elevation in ASO occurs several weeks after infection. Thus a single ASO test has poor sensitivity.

Even considering these limitations, when both acute and convalescent sera are unavailable, laboratories often use an ASO titer above an "upper limit of normal" or some other cutoff point as evidence of recent *S. pyogenes* infection. The package insert for the ASO reagents used in this study describes values >100 as elevated in adults. This cutoff point is clearly too low for the present study population as 77.3% of recruits would have been classified as having elevated ASO titers before they were exposed to the *S. pyogenes* epidemic, which is unlikely. Using the ULN (80-percentile pre-training ASO) titer as a cutoff point, recruits with post-training ASOs greater than 400 had evidence of recent *S. pyogenes* infection. This method of detecting infection had a sensitivity and a specificity of 65.9 and 81.9%, respectively (Table 1), and missed 34.1% of true infections [8]. The dichotomous ULN method values an ASO titer of 480 the same as an ASO titer of >2560 because both are greater than the 80 percentile value for this population. Similarly, an ASO titer of 40 is valued the same as an ASO titer of 400 because both are at or below the 80 percentile value. This logic is not consistent with clinical reasoning, yet such are the limitations of the ULN methodology.

In contrast, the likelihood ratio values (Table 2) calculated in this study gave more clinically useful information. They provided a ratio of the odds of infection to the odds of no infection for individual ASO titers. These statistics were more intuitive because the higher the ASO titer, the more confident the clinician could be in estimating recent *S. pyogenes* infection. Additionally, if the probability of infection was known prior to the ASO test, a post-ASO test probability of disease could be calculated [5] (Table 2). Likelihood ratios offer an additional value in that they may be used multiplicatively (formula 5) to evaluate overall probability for a series of independent tests [3]. The post-test odds of infection for a preceding test may be used as the pre-test odds of infection for the next test in the series (formula 3).

As an example, assume that a clinician frequently sees members of a population similar to this study population. He or she knows from previous serologic testing that on average 15% of patients with sore throats and fevers develop serologic evidence for *S. pyogenes* infection. The clinician evaluates a 19-year-old male with recent trauma to his right ankle and subsequent ankle tenderness and swelling. The patient reports a history of an untreated sore throat and fever 3 weeks ago. The physician obtains a throat culture and a serum sample from this patient. The next day the laboratory reports an ASO titer of 640. Using Table 2, the patient now has a 2.2:1 relative odds of recent *S. pyogenes* infection compared to no infection. Using the pre-test probability of 0.15 (pre-test odds $= 0.18$), the clinician calculates a 28% probability that the patient recently suffered a *S. pyogenes* infection. The following day the laboratory reports that the throat culture was positive for *S. pyogenes*. The clinician now uses the previous probability of 28% to calculate a new pre-test odds of 0.39. These odds are then multiplied by the likelihood ratio for a positive throat culture (3.82) which results in an overall post-test odds of disease of 1.49 which translates to an overall probability of recent infection of 0.60 (alternately the clinician could have used formula 5 for this calculation). These results may encourage him to consider acute rheumatic

fever in his differential diagnosis. In contrast, by the ULN method, the clinician would only have a dichotomous ASO result which favored recent *S. pyogenes* infection. The result would be the same whether the ASO titer was 480 or 2560. The clinician would have had no objective way to combine the results of the single ASO test and the results of the throat culture.

Our study has several limitations. Although it is recognized that most rheumatic fever patients experience an elevation in ASO titer, no subject in this study developed rheumatic fever. However, the 2-dilution rise in ASO titer using a 0.10 log scale is a common standard for epidemiologic studies of *S. pyogenes* and rheumatic fever infections. Upper limits of normal and likelihood ratios for ASO tests should be calculated at individuai laboratories for specific populations. We recognize that this may not be practical in all settings. Additionally, due to the poor sensitivity of the ASO test, the likelihood ratios should be considered as conservative. When using these likelihood ratios, a high likelihood ratio for *S. pyogenes* infection should be valued more than a low likelihood ratio.

In summary, interpreting a single ASO test is difficult. Clinicians should not rely upon package insert guidelines but should compare a titer value with values in similar populations run at the same laboratory. A single test has the most diagnostic value when it results in a high titer. The magnitude of this diagnostic value is best interpreted by statistics such as the likelihood ratio. In contrast to the "yes–no" dichotomy of using the ULN method to predict recent *S. pyogenes* infection, likelihood ratio statistics, although not always practical, offer a continuum of risk estimation which is more consistent with clinical judgment.

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