Antistreptolysin O titer profile in acute rheumatic fever diagnosis

Claudia Saad Magalhães Machado, Katya Ortiz, Alessandra de Lourenço Budin Martins, Roberto Salvador Martins, Nilton Carlos Machado

Abstract

Objective: to determine ASO titer profile by establishing ARF differential diagnoses of other diseases with high levels of ASO antibodies.

Methods: we investigated 78 patients with ARF at onset and follow-up, 22 with isolated chorea at onset, 45 with recurrent oropharyngeal tonsillitis, and 23 with recent flare of juvenile idiopathic arthritis. We tested ASO with automated particle-enhanced immunonephelometric assay (Behring®-Germany). The ASO (IU/ml) titers were assessed at the following time intervals: 0-7 days, 1-2 weeks, 2-4 weeks, 1-2 months, 2-4 months, 4-6 months, 6-12 months, 1-2 years, 2-3 years, 3-4 years, and 4-5 years after onset of ARF.

Results: ASO titers in patients diagnosed with ARF had a significant increase up to the 2-4-month time interval ($P<0.0001$). Baseline levels were observed afterwards in patients under regular penicillin prophylaxis. The levels of ASO in ARF were also significantly higher than in patients with isolated chorea, recurrent oropharyngeal infections or juvenile idiopathic arthritis ($P=0.0025$), when age-matched samples of these groups were compared. The test’s sensitivity was 73.3% and the specificity was 57.6%, and it was calculated taking into account the upper limit of normality at 320 IU/ml, as well as the established diagnosis of ARF. The test’s specificity and positive predictive value increased with rising or higher titers, being higher with titers above 960 UI/ml.

Conclusion: this reappraisal of ASO profile in ARF patients indicates a remarkable response during the acute phase, and that points to the extent to which ASO levels may differentiate ARF from other diseases with high levels of ASO antibodies, as coincidental but unrelated streptococcal infection or chronic arthritis flareup.


Introduction

Recent studies in pediatric rheumatology have dealt with the clinical characteristics of the acute phase of rheumatic fever, emphasizing its high prevalence as well as growing diagnostic difficulties. These difficulties result from the nonexistence of pathognomonic signs and specific lab exams. In the last 50 years, the diagnosis of Acute Rheumatic Fever (ARF) has been guided by Jones criteria through the observation of a variable combination of signs and symptoms that occur after streptococcal oropharyngeal infection; however, in daily medical practice, the interpretation of some clinical situations becomes a diagnostic challenge. Carditis is the most frequent clinical...
Among the cases that accomplished the follow-up protocol, 78 were related to acute rheumatic fever, which manifested itself through polyarthritis and/or carditis, and 22 presented Sydenham’s chorea as isolated manifestation. ARF diagnosis was made according to reviewed Jones criteria\(^{11}\) and the diagnosis of isolated chorea was based on clinical manifestations and absence of acute carditis signs (heart murmur, pericarditis, heart insufficiency) or manifestation of arthritis. All patients were submitted to secondary prophylaxis with benzathine penicillin every three weeks in supervised, regular administrations.

45 patients with recurrent oropharyngeal infections, with at least 4 annual episodes of pharyngitis and tonsillitis and prescription of antibiotic therapy for all cases were recruited from the General Pediatric Outpatient Clinic for follow-up and investigation during the same period.

23 patients with juvenile idiopathic arthritis, according to the recent definition proposed by the ILAR- International League of Associations for Rheumatology,\(^{12}\) were evaluated to presentation or recent reactivation during the same recruitment period of ARF cases; the onset of JIA was categorized as oligoarticular (14 cases), polyarticular (4 cases), and systemic (5 cases).

The study was approved by the Ethics Committee on Research of the Botucatu School of Medicine (Universidade Estadual de São Paulo); patients were recruited after the consent of parents or legal guardians.

**ASO measurement**

The measurement of ASO levels was performed through latex agglutination nephelometric assay,\(^{10}\) using the NALatex-ASL reagents (Behring-Behringwerke AG, Marburg-Germany). The method consists of immunochemical agglutination of polystereine particles, covered in antistreptolysin-O with antibodies present in human serum. This agglutination determines an increase in the turbidity of reagents, which is measured through nephelometry. The quantitative measurement of ASO concentration is carried out by measuring the turbidity of reagents, considering the peak agglutination rate and using known concentration patterns for comparison. The results are automatically calculated through logarithmic function and expressed in international units - IU/ml. The results obtained through the latex agglutination test are comparable to those presented in neutralization tests with hemolysis inhibition, i.e. 1IU=1.04 U Todai.\(^{13,14}\) The serum ASO levels were assessed at predetermined periods, starting in the first week and extending to 5 years after diagnosis.

**Statistical analysis**

The statistical comparison of ages in different groups of patients was carried out through analysis of variance with posttest: Tukey test. The statistical comparison of ASO
values at different intervals, and ASO values at presentation, according to diagnosis, was obtained through nonparametric tests - Kruskal-Wallis test and Multiple Comparison test (Dunn). The significance level and graphic representation of results were provided by a specific software (Graph-Pad Prism Software Inc. version 2.0,1995).

Results

Demographic characteristics and ages of patients are shown in Table 1. The statistical comparison of ages in the four groups of patients revealed a significant difference (P<0.05), presenting low values for the group with recurrent oropharyngeal infections in comparison with patients affected by ARF, isolated chorea, and juvenile idiopathic arthritis, which showed no significant difference.

The sequential analyses of ASO in patients diagnosed with ARF (with the exception of isolated chorea cases) initiated in the first week and carried out at intervals of 0-7 days, 7-15 days, 15-30 days, 1-2 months, 2-4 months, 4-6 months, 6-12 months, 1-2 years, 2-3 years, 3-4 years and 4-5 years are shown in Figure 1 together with the representation of median values, interquartile range, and a confidence interval of 95%. The number of patients evaluated in each period is shown in Table 2, together with their respective ASO geometric mean values. By considering an upper normal limit of 320 IU/ml for the study population, we observed a striking serum ASO level response in the first two weeks of diagnosis, with a gradual titer reduction until the 2-4-month interval; after an interval of 4-6 months, these levels reached base values. An individual significant variation, defined by a confidence interval of 95% (Figure 1) and adjusted by the geometric mean in Table 2, was observed. These values present lower variation after the interval of 1-2 years.

The statistical comparison of ASO values at each of the intervals revealed a significant difference (P< 0.0001), and the multiple comparison test at all intervals showed significant differences in the values from the first week to

![Figure 1 - Serum ASO levels (median, interquartile range, and 95%CI) in patients with ARF, from diagnosis up to 5 years of follow-up](image.png)

Table 1 - Demographic characteristics and age-related comparison of patients with Rheumatic Fever, Isolated Chorea, Recurrent Oropharyngeal Infections, and Juvenile Idiopathic Arthritis

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Gender</th>
<th></th>
<th>Age (months)</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Acute Rheumatic Fever</td>
<td>78</td>
<td>37</td>
<td>41</td>
<td>116</td>
<td>111±24</td>
<td></td>
</tr>
<tr>
<td>(B) Isolated Chorea</td>
<td>22</td>
<td>10</td>
<td>12</td>
<td>127</td>
<td>15±33</td>
<td></td>
</tr>
<tr>
<td>(C) Recurrent Oropharyngeal Infections*</td>
<td>45</td>
<td>24</td>
<td>21</td>
<td>75</td>
<td>77±30*</td>
<td></td>
</tr>
<tr>
<td>(D) Juvenile Idiopathic Arthritis</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td>110</td>
<td>113±26</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P<0.05, Post-test (Tukey): (A=B=D) ≠ C
Table 2 - ASO in Rheumatic Fever (Polyarthritis and/or Carditis) from diagnosis up to 5 years of follow-up

<table>
<thead>
<tr>
<th>ASO IU/ml</th>
<th>0-7d</th>
<th>8-15d</th>
<th>16-30d</th>
<th>1-2m</th>
<th>2-4m</th>
<th>4-6m</th>
<th>6-12m</th>
<th>1-2a</th>
<th>2-3a</th>
<th>3-4a</th>
<th>4-5a</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>13</td>
<td>26</td>
<td>29</td>
<td>22</td>
<td>21</td>
<td>39</td>
<td>33</td>
<td>29</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>G</td>
<td>567</td>
<td>603</td>
<td>448</td>
<td>413</td>
<td>362</td>
<td>252</td>
<td>217</td>
<td>210</td>
<td>176</td>
<td>124</td>
<td>147</td>
</tr>
</tbody>
</table>

* P<0.0001

n number of patients
G Geometric Mean

* Kruskal-Wallis test and Dunn Multiple Comparisons
Upper normal limit of 320 IU/ml

Figure 2 - Comparison of ASO levels among patients with recurrent oropharyngeal infections, according to age.

Discussion

The variable clinical manifestation of ARF and the absence of pathognomonic signs allowed the establishment of diagnostic criteria. It is crucial that previous streptococcal infection be confirmed in order to establish the diagnosis. However, when the most typical manifestations of the disease are not clearly defined, we have a problem with identifying the first bout of infection and establishing a
secondary prophylaxis with penicillin, which is a determinant factor for the prevention of relapses.

Oropharyngeal cultures are essential (regarded as gold standard exams) for the definitive diagnosis of oropharyngeal infections; however, their low positivity rate in the diagnosis of ARF restricts their use. The determination of antibodies to fight extracellular products of streptococci has been of great value in clinical practice and for the epidemiological investigation of streptococcal infections and their sequels. The determination of antistreptolysin-O (ASO) may substantially contribute to diagnosis as its titers reach high values in the presence of major clinical manifestations, declining regardless of the subsequent course of the disease.

The sequential analysis of ASO was conducted on 78 consecutive ARF cases and the distribution of titers at different stages of the disease produced a remarkable response in its acute phase. The decline in ASO levels was also assessed in this population protected from streptococcal infections through secondary prophylaxis with benzathine penicillin every 3 weeks, showing that these levels remain stable 4 months after the initial bout of infection. The result of the geometric mean for ASO values, at the diagnosis of ARF in our patients (534 IU/ml), was comparable to the results described by Ayoub & Wannamaker (446 U/ml) in the 1960s in the United States, and by Berrios et al. (460 U/ml) in the 1980s in Chile, showing that recent sociodemographic changes did not influence this profile. In practice, an increase or decline in ASO levels, with a difference of two titers in relation to the upper normal limit, is usually interpreted as a sign of previous streptococcal infection. This is not applicable, however, to isolated chorea, where ASO levels may be normal in the clinically active phase of the disease.

To correctly interpret the test, we have to consider that high ASO titers are simply signs of previous oropharyngeal infection, supporting the diagnosis of ARF, but, isolatedly, they neither prove nor measure the activity of the disease, with possible manifestation in other diseases. A considerable number of patients are referred to us due to recurrent oropharyngeal infections and high ASO levels, with no manifestation of major clinical signs of rheumatic fever. The effects of repeated exposure to streptococci were evaluated in these patients, considering their ages for comparison with ARF patients.

Normal limits should be previously established for the target population as these vary according to age, time of the year, geographical situation, and prevalence of streptococcal infections. The reference values for children have been reported in the literature (see Table 5) as occurring in different geographical regions. The recent study was used as a reference for the establishment of an upper normal limit of 320 IU/ml, using the latex agglutination test, and a confidence interval of 95%.

The characterization of immunoresponses to streptococci, as far as age is concerned, showed that infants

---

### Table 3 - Comparison between ASO values at onset of Acute Rheumatic Fever (ARF), Isolated Chorea, Recurrent Oropharyngeal Infections, and Juvenile Idiopathic Arthritis

<table>
<thead>
<tr>
<th></th>
<th>ARF*</th>
<th>Isolated Chorea</th>
<th>Recurrent Oropharyngeal Infections</th>
<th>Juvenile Idiopathic Arthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>26</td>
<td>12</td>
<td>36</td>
<td>23</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>549</td>
<td>296</td>
<td>216</td>
<td>287</td>
</tr>
</tbody>
</table>

*P=0.0025

**n** number of patients

G Geometric Mean

* Kruskal-Wallis test and Dunn Multiple Comparisons

Upper normal limit of 320 IU/ml

---

### Table 4 - ASO values during the first week of ARF manifestation, according to Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Accuracy

<table>
<thead>
<tr>
<th>ASO IU/ml</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥320 IU/ml</td>
<td>73.3%</td>
<td>57.6%</td>
<td>46.8%</td>
<td>80.9%</td>
<td>62.9%</td>
</tr>
<tr>
<td>≥640 IU/ml</td>
<td>53.3%</td>
<td>81.3%</td>
<td>59.2%</td>
<td>77.4%</td>
<td>71.9%</td>
</tr>
<tr>
<td>≥960 IU/ml</td>
<td>23.3%</td>
<td>93.2%</td>
<td>63.6%</td>
<td>70.5%</td>
<td>69.6%</td>
</tr>
</tbody>
</table>

---

### Figure 3 - Comparison of ASO levels (median, interquartile range, and 95% CI) at the onset of acute rheumatic fever (ARF), chorea, recurrent oropharyngeal infections (ROI), and juvenile idiopathic arthritis (JIA).
Table 5 - Reference values (upper normal limit) for ASO in infants and children, according to the literature

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Location</th>
<th>ASO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klein GC, Baker CN, Jones WL</td>
<td>1971</td>
<td>USA</td>
<td>170 U</td>
</tr>
<tr>
<td>Renneberg J, Soderström M, Prellner K et al.</td>
<td>1989</td>
<td>Sweden</td>
<td>200 U</td>
</tr>
<tr>
<td>Mhalu FS, Matre R</td>
<td>1995</td>
<td>Tanzania</td>
<td>200 U</td>
</tr>
<tr>
<td>Francescantonio PLC, Silva NA</td>
<td>1996</td>
<td>Goiânia</td>
<td>320 U</td>
</tr>
<tr>
<td>Quaresma MR, Leser PG, Ferraz MB</td>
<td>1997</td>
<td>São Paulo</td>
<td>320 U</td>
</tr>
<tr>
<td>Kaplan EL, Rothermel CD, Johnson DR</td>
<td>1998</td>
<td>USA</td>
<td>240 U</td>
</tr>
</tbody>
</table>

and preschool children present lower ASO levels. In school-age children, repeated exposure to streptococcal antigens allows the characterization of secondary anamnestic response, which begins some days after infection and reaches its peak within 3-6 weeks. Actually, secondary response was found in patients older than 4 years who presented more than 4 annual episodes of pharyngitis and tonsillitis.

The low specificity of the ASO test is a diagnostic limitation since patients whose arthritis is caused by diseases other than ARF may have high ASO levels as a result of a recent streptococcal infection that is coincident but unrelated. We should bear this possibility in mind as chronically high ASO levels may occur in up to one third of patients with chronic polyarthritis as a manifestation associated with inflammatory process, even though the relationship between the inflammatory process and exposure to streptococci remains still unknown.

Patients with isolated chorea, recurrent oropharyngeal infections, and juvenile idiopathic arthritis also presented high ASO levels at diagnosis; however, these values had lower significant differences in relation to those presented by patients with ARF, whose manifestation occurred through polyarthritis and/or carditis.

Individually, there was a great variation in ASO levels at all stages of the disease. An isolated ASO result may have low sensitivity, imposing a limitation as to the use of ASO measurement, once not all patients respond by increasing the production of this antibody. Such difficulty may be overcome through the combined determination of other antistreptococcal antibodies such as antideoxyribonuclease B, antihyaluronidase, antistreptokinase and antistreptodornase or through the serial determination of ASO. Nevertheless, this may have a high cost-benefit ratio in such a way that an isolated test could produce a higher value for diagnosis when ASO levels exceed 640 IU/ml.

The epidemiology of rheumatic fever and other poststreptococcal manifestations have been a recent concern even in industrialized countries and their prevalence in our environment draws attention not only to the necessity of preventive measures but also to measures that could facilitate precise diagnoses and identification of new outbreaks of infection. We conclude that it is necessary to consider the sensitivity and specificity of the test in relation to other diseases that present high ASO levels, in addition to the interpretation of upper normal limits, so that sequential or isolated ASO levels shown by ARF patients can be interpreted with more accuracy.

References

Antistreptolysin O titer profile... - Machado CSM et alii


Correspondence:
Dra. Claudia S.M. Machado
Depto. de Pediatria – Fac. Medicina de Botucatu, UNESP
CEP 18618-970 – Botucatu, SP, Brazil
Phone/fax: +55 14 6802.6274 / 6802.6083
E-mail: cmachado@fmb.unesp.br