Estimation of Antistreptococcal Esterase in Rheumatic Fever and Rheumatic Heart Disease

NOBUO WATANABE, M.D. AND YUKIYOSHI NAKAMURA, M.D.

In hemolytic streptococcal infection, a high ASE value in the absence of a rise in ASO has shown to occasionally occur. The relationship between ASO and ASE titers in rheumatic fever and rheumatic heart disease and the significance of these values was therefore studied. ASE values were measured by ASE Kit "Marupi" in 8 patients during the acute stage of rheumatic fever and in 9 patients with rheumatic heart disease, and then compared with the corresponding ASO values. Among the patients with rheumatic fever, a rise of ASO only was seen in 1 patient and a rise of ASE only was seen in another. In the remaining 6 patients, both ASE and ASO were elevated. Only in 1 patient with rheumatic heart disease was ASE found to be elevated.

As the method for serological diagnosis of hemolytic streptococcal infection, the measurement of ASO and ASK titers is used extensively. Stock et al.¹ originally discovered the occurrence of antibody against hemolytic streptococcus esterase (ASE) in the serum of patients with infection by hemolytic streptococcus.

The method for measurement of this antibody was subsequently developed by Hayano.² With infection by hemolytic streptococcus, high values of ASE in the absence of a rise in ASO was demonstrated. In the present study in an effort to clarify the significance of ASE measurement, we have measured ASE values in patients with rheumatic fever and rheumatic heart disease in order to evaluate the correlation with ASO.

MATERIALS AND METHODS

ASE was measured by using ASE Kit "Marupi", and ASO was measured by the Ranz-Landal method, in 8 patients in the acute stage

of rheumatic fever and in 9 patients with rheumatic heart disease. Figure 1 shows the principle of ASE measurement. S-acetylthiophenol was used as the substrate for streptoesterase (SE) and 5,5'-dithiobis-2-nitrobenzoic acid as the coloring agent. The optical density of thionitrobenzoic acid

Fig.1. Principle of ASE assay.
SpA = Protein A; SE = Streptococcal esterase; ASE = antistreptococcal esterase Antibody

Key Words:

Antistreptococcal esterase (ASE)
ASO
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Department of Pediatrics, Kyorin University, Tokyo, Japan

Mailing address: Nobuo Watanabe, M.D., Department of Pediatrics, Kyorin University, 6-20-2 Shinkawa, Mitaka, Tokyo 181, Japan

TABLE I PROCEDURE OF THE MEASUREMENT OF ASE VALUES

1. Test serum is diluted 51 times or 101 times with physiological saline.		
2. Diluted sample (100 μ l)	Hemolytic streptococcal esterase solution (100 μ l)	Solution of antibody absorbent* (100 μ l)
After mixing, incubated at $37 \pm 1^\circ\text{C}$ for 30 minutes.		
3. Washed with physiological saline twice. (100 – 1500 $\times$ g, 10 minutes)		
4. To the precipitate (immune complex bound to protein A) coloring solution (DNTB) is added and subjected to preliminary heating at $37 \pm 1^\circ\text{C}$.		
5. The substrate solution (SATP) is added and the mixture is incubated at $37 \pm 1^\circ\text{C}$ for 30 minutes.		
6. The solution for stopping the reaction is added.		
7. The mixture is immediately centrifuged and the optical density of the supernatant is measured at 412 nm.		
8. From the ΔOD of standard serum containing 300 ASE unit, the ASE content of the sample is calculated.		
(1) $\Delta\text{OD} = \text{OD of the sample} - \text{OD of the blank}$		
(2) $\text{ASE unit} = \frac{\Delta\text{OD of the sample}}{\Delta\text{OD of the standard serum} \times 300}$		

*Cell wall suspension of Protein A-bound staphylococcus aureus.

acid thus produced was measured at 412 nm. As the ASE reference standard, ASE-A from the SS379 strain (Type 40 Group A) and ASE-B from the H36B strain (Type Ib, Group B) were used. ASE-B was used only in selected patients.

The procedure is summarized in Table I.

RESULTS

Table II summarized ASO and ASE-A values in 8 patients with rheumatic fever. A rise of ASO only was seen in 1 patient and a rise of both ASO and ASE-A was noted in the remaining 6 patients. In Case R-8, ASO was 640 units, ASE-A 356 units, and ASE-B was 755 units, which indicated the presence of antibody against Type B hemolytic streptococcus esterase as well. In addition, in a particular patient who exhibited erythema marginatum during treatment with oral

TABLE II ASO, ASE IV RHEUMATIC FEVER

No.	Age	Sex	ASO	ASE
R-1	10	M	1250	527
2	7	M	625	306
3	14	F	500	328
4	13	M	1250	601
5	11	M	500	265
6	14	M	240	322
7	8	F	640	510
8	10	F	640	356

TABLE III ASO, ASE IN RHEUMATIC HEART DISEASE

No.	Age	Sex	ASO	ASE
C-1	19	M	50	91
2	8	F	250	124
3	17	F	50	44
4	6	M	100	100
5	14	F	50	70
6	8	M	< 12	35
7	14	M	240	174
8	9	F	320	397
9	12	F	166	114

medication for the prevention of post-streptococcal disease, the ASO rose from 250 to 320 units, and ASE-A from 165 to 346 units.

Table III summarizes ASO and ASE-A values in patients with rheumatic heart disease. In only 1 patient did ASE-A showed a high value while ASO stayed within normal range in all patients.

DISCUSSION

ASE said to change in a parallel fashion with ASO showing a high degree of correlation. According to our studies on scarlet fever, Type A β hemolytic streptococcus was recovered in all patients by throat culture. Despite the clinical manifestations of scarlet fever, no rise in ASO was seen at all, but a rise ASE-A found in 2 out of 19 patients is noteworthy. This would indicate that ASO and ASE are entirely different types of antibody.

As was pointed out by Hayano et al.³, ASE-B frequently rises along with ASE-A. Whether this is due to the simultaneous infection by B group hemolytic streptococcus or represents an ac-

companying phenomenon due to common antigenic property remains to be decided.

In rheumatic fever and rheumatic heart disease, the measurement of both ASO and ASK cannot demonstrate the antibody against hemolytic streptococcus in 100% of cases, creating difficulty in diagnosis in some cases. The addition of the measurement of ASE was shown to improve the accuracy of serological diagnosis.

According Kawakita,⁴ as the result of measurements in chronological sequence in inflammation of the upper respiratory tract, ASE returns to the normal range more slowly than does ASO. ASE may therefore be more sensitive than ASO as a method of exploring the etiology of acquired heart disease. According to Kawakita et al., the coefficient of diurnal and daily variations of serum ASE values stayed within 5%, and this appears particularly suitable for follow-up studies on the clinical course by using pair serum.

ASE measurement therefore appears to be of significance in patients with rheumatic fever and rheumatic heart disease.

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