OCCURRENCE OF ANTIBODIES TO TEICHOIC ACID IN PATIENTS WITH DISEASES OTHER THAN STAPHYLOCOCCAL INFECTION

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SUMMARY. To determine the usefulness of the teichoic acid antibody (TAA) test in conditions where unspecific viral and bacterial antibodies are often encountered, we measured TAA by the gel-diffusion method in 475 patients without known staphylococcal disease; they included 213 patients with arthritis, 108 with liver diseases, 100 with gastro-intestinal disorders and 54 with acute pharyngitis. Positive controls were 104 patients with *Staphylococcus aureus* bacteraemia and 203 healthy adults were negative controls. Thirteen (6%) of the healthy adults had positive TAA titres (≥ 4), and the highest titre was 8 in two people (1%). Positive titres were found in 38% of patients with *S. aureus* bacteraemia and high titres (≥ 8) were seen in 24%. Among the patients with arthritis, positive TAA titres were found significantly more often than in healthy controls in patients with *Yersinia* arthritis ($p < 0.01$) and systemic lupus erythematosus (SLE; $p < 0.02$). In other patient groups, the percentage of positive TAA titres did not differ significantly from that in healthy adults. Eight (2%) of the 475 patients without known staphylococcal infection had TAA titres ≥ 8 but these high titres were not associated with any particular disease group. Only two of these eight patients had slightly raised antibody to staphylococcal α -haemolysin. We conclude that the TAA test cannot be used as a reliable indicator of septic staphylococcal disease in patients with *Yersinia* arthritis or SLE, but that in general, TAA titres ≥ 8 point strongly to *S. aureus* infection even in patients with autoimmune or liver diseases.

INTRODUCTION

The teichoic acid antibody (TAA) test has proved valuable in the diagnosis of bacteraemic infections caused by *Staphylococcus aureus* (Larinkari *et al.*, 1977; Tuazon *et al.*, 1978; Wheat, Kohler and White, 1978; Bayer *et al.*, 1980; Tenenbaum and Archer, 1980). Especially high TAA titres have been found in staphylococcal endocarditis and in other complicated staphylococcal infections with metastatic abscess formation (Crowder and White, 1972; Nagel *et al.*, 1975; Tuazon and

Sheagren, 1976; Tuazon *et al.*, 1978; Wheat *et al.*, 1978; Bayer *et al.*, 1980; Tenenbaum and Archer, 1980). Patients with atopic dermatitis often have high TAA titres without evidence of clinical infection (Larinkari, 1982a).

Teichoic acids are the major group antigen of *S. aureus*, but they are present, as a rule, in all gram-positive bacteria either as cell wall or cell membrane components (Knox and Wicken, 1973). The wall teichoic acid of *S. aureus* is a ribitol-phosphate polymer substituted with α - and β -linked N-acetylglucosamine residues which are the main antigenic determinants (Sanderson, Strominger and Nathenson, 1962; Torii, Kabat and Bezer, 1964). Cross-reactions can be expected in TAA assay, because many gram-positive and also some gram-negative bacteria have structures that are antigenically similar to those of the ribitol teichoic acids of *S. aureus* (Juergens, Sanderson and Strominger, 1963; Brundish and Baddiley, 1968; Kane and Karakawa, 1969; Aasen and Oeding, 1971; Argaman, Liu and Robbins, 1974; Gmeiner *et al.*, 1977). In practice, however, false positive TAA test results have been fairly uncommon (Crowder and White, 1972; Larinkari *et al.*, 1977, Leffell, Folds and Wasilauskas, 1978; Martin, Greenberg and Wallace, 1979). They have been found usually in low titres in some patients with streptococcal and diphtheroid endocarditis, (Hoppes and White, 1975; Nagel *et al.*, 1975; Tuazon *et al.*, 1978; Bayer *et al.*, 1980). Relatively high TAA titres have also been reported in a small fraction of healthy controls as well as patients with non-infectious diseases (Leffell *et al.*, 1978; Tenenbaum and Archer, 1980; Larinkari, 1982a).

High levels of unspecific bacterial and viral antibodies have been reported to occur especially in patients with systemic lupus erythematosus and in patients with different liver diseases (Vesikari and Laitinen, 1974; Triger, 1976) but the occurrence of TAA in these patients has not been studied. To further define the value of the TAA test in the diagnosis of staphylococcal infections we have determined the TAA titres in various patients, without a history of staphylococcal infection, and compared the findings with those in healthy controls and in a group of patients with *S. aureus* bacteraemia.

MATERIALS AND METHODS

Controls

Healthy controls. This group consisted of 99 healthy blood donors and 104 other healthy adults; 116 were males and 87 were females, with mean age 32 (range 16–62) years. One serum sample was examined from each person. This material was used as the control group in two earlier papers (Larinkari, 1982a and b) but it is now analysed more accurately by age.

*Patients with *S. aureus* bacteraemia.* This positive control group comprised 104 patients with blood-culture-proven bacteraemia caused by *S. aureus*; 61 were males and 43 were females, with mean age 47 (range 2–87) years. Included were 14 patients with either liver disease or connective tissue disease as their basic illness: four had rheumatoid arthritis (RA), three had alcoholic liver disease, three cirrhosis of unknown etiology and one each had Goodpasture's syndrome, cholangitis and chronic aggressive hepatitis; one patient had uric arthritis and gallstones. The serum samples were taken 5 days to 4 weeks after the first positive blood culture. All patients had samples taken during the second week of their illness. Sixteen patients have been included in a previous report. (Larinkari, 1982b).

Patients with nonstaphylococcal disease

A total of 475 patients in four main disease groups—arthritis, liver disease, gastro-intestinal disease and pharyngitis—were studied. The age and sex distribution of these patients is shown

TABLE I

Age and sex distribution of 475 patients without known staphylococcal disease

Disease group	Patients			Age	
	number tested	males	females	mean	range
Arthritis	213	99	114	36	13-75
Liver disease	108	52	56	49	19-79
Gastro-intestinal disease	100	44	56	42	15-82
Pharyngitis	54	25	29	27	3-71

in Table I. The diagnosis in the patients was based on generally accepted clinical, histological, microbiological and immunological criteria. None of the patients had a culture-proven staphylococcal bacteraemia in their history. Serum samples were taken from patients with acute conditions, at weekly intervals for 3-6 weeks. Follow-up sera taken during different stages of the disease were available also from several patients with chronic diseases. Only the highest TAA titre is given from each patient.

Arthritis patients. This group included 213 patients divided into the following subgroups: reactive arthritis, 56 patients (28 after *Chlamydia trachomatis* infection, 22 after *Yersinia enterocolitica* infection, four after *Salmonella* infection and two after *Shigella* infection); connective tissue disease, 56 (39 with definite or classical rheumatoid arthritis, nine with systemic lupus erythematosus, and eight with other connective tissue disease); Reiter's disease, 32; rheumatic fever, seven; other known polyarthritis, five; polyarthritis of unknown aetiology, 57 patients.

Liver diseases. This group consisted of 108 patients with the following diagnoses: alcoholic liver disease, 32; primary biliary cirrhosis, 21; liver metastases, 13; chronic active hepatitis, five; other liver diseases, (cirrhosis of unknown aetiology, infectious hepatitis, fatty degeneration, fibrosis of liver, hepatic sarcoidosis, cholangitis, hepatomegaly, icterus) 37.

Gastro-intestinal diseases. 100 patients with different gastro-intestinal diseases had the following diagnoses: malabsorption syndrome, 60; acute gastroenteritis, 32 (10 with *Salmonella* infections, two with acute appendicitis and 20 with gastroenteritis of unknown aetiology); and eight patients had ulcerative colitis.

Pharyngitis. Throat cultures were taken from 54 patients with signs and symptoms of acute pharyngitis. β -haemolytic streptococci were isolated from 35 specimens (group A, 12; group C, nine; group G, three; not group A, B, C, D or G, 11).

Methods

TAA assay. TAA was measured by the gel diffusion method described previously (Larinkari *et al.*, 1977). Twofold dilutions of serum were tested against the antigen, a sonicate of *S. aureus* strain Copenhagen diluted 200-fold. This strain contains both α - and β -linked N-acetylglucosamine, predominantly the latter (Sanderson *et al.*, 1962). Teichoic acid precipitation lines were identified by comparison with purified teichoic acid from *S. aureus* Copenhagen as reference. Titres ≥ 4 were considered to be positive results.

*Assay for antibody to the α -haemolysin of *S. aureus* (AStA).* AStA determinations were done by inhibition by the patient's serum of haemolysis of rabbit red blood cells by α -haemolysin using the previously described method with minor modifications (Widholm, 1951). Values (IU/ml) were measured by comparing dilutions of patients' sera with the standard serum (Behringwerke®, Marburg, West Germany); this had an AStA content of 1 IU/ml that gave a 50% haemolysis end point. Values ≥ 2 IU/ml were considered to be positive results. AStA was measured only in patients with TAA titres ≥ 8 .

Statistical methods. The Chi square test with Yates's correction was used for statistical comparisons.

RESULTS

The occurrence of positive TAA titres in healthy controls and in patients with bacteraemia caused by *S. aureus* is shown in table II. Thirteen (6%) of the 203 healthy controls had TAA titres ≥ 4 . The highest titre was 8, seen in two persons. The positive titres were evenly distributed amongst all age groups. In the positive control group of 104 patients with *S. aureus* bacteraemia, the frequency of TAA titres ≥ 4 was 38%, 24% had titres ≥ 8 and 9% had titres ≥ 16 . The highest titre, 32, was seen in one patient. High TAA titres were especially common in patients with *S. aureus* bacteraemia and liver disease or connective tissue disease as the basic illness (TAA ≥ 8 in 50% and 33% respectively).

The TAA titres of 213 patients with arthritis are summarized in table III; 26

TABLE II

Occurrence of positive teichoic acid antibody titres in 203 healthy controls and in 104 patients with bacteraemia caused by S. aureus

Subject group	Number of subjects tested	Number (percent) of patients with TAA titre		
		≥ 4	≥ 8	≥ 16
Healthy controls	203	13(6)	2(1)	0
age group 16-25 years	40	3(8)	0	0
26-35 years	89	6(7)	1(1)	0
36-45 years	51	2(4)	0	0
46-62 years	23	2(9)	1(4)	0
<i>S. aureus</i> bacteraemia	104	40(38)	25(24)	9(9)
patients with liver disease	8	6(75)	4(50)	2(25)
patients with connective tissue disease	6	2(33)	2(33)	2(33)

TAA = teichoic acid antibody.

TABLE III

Occurrence of positive teichoic acid antibody titres in 213 patients with arthritis

Diagnosis	Number of patients tested	Number (percent) of patients with TAA titre	
		≥ 4	≥ 8
Reactive arthritis	56	10(18)	1(2)
<i>Chlamydia trachomatis</i> infection	28	4(14)	1(4)
<i>Yersinia enterocolitica</i> infection	22	6*(27)	0
other known triggering infection	6	0	0
Connective tissue disease	56	8(14)	2(4)
rheumatoid arthritis	39	5(13)	1(3)
systemic lupus erythematosus	9	3†(33)	1(11)
other connective tissue disease	8	0	0
Reiter's disease	32	3(9)	2(6)
Rheumatic fever	7	0	0
Other polyarthritis	5	0	0
Polyarthrititis of unknown etiology	57	5(9)	0

TAA = teichoic acid antibody.

* $p < 0.01$; † $p < 0.02$ compared with healthy controls.

patients (12%) had TAA titres ≥ 4 . Patients with *Yersinia* arthritis and SLE had TAA titres ≥ 4 significantly more often than healthy controls ($p < 0.01$ and $p < 0.02$ respectively). Single high titres (≥ 8) occurred also in some other disease groups: one patient with reactive arthritis after *Chlamydia* infection, one with rheumatoid arthritis and two patients with Reiter's disease.

Amongst the 208 patients with various gastro-intestinal and liver diseases, (table IV) TAA titres ≥ 4 were most often seen in patients with ulcerative colitis (12%) and primary biliary cirrhosis (10%). Titres ≥ 8 occurred only sporadically amongst patients with malabsorption syndrome, primary biliary cirrhosis and infective hepatitis.

The highest TAA titre in the 54 pharyngitis patients was 4, seen in six patients (table V); all harboured β -haemolytic streptococci in the throat, and three of these strains belonged to group A.

TAA titres ≥ 8 were found in eight patients; their clinical details are given in table VI. Only two of these patients had ASTa values above normal; both were 2.5 IU/ml. One patient with positive ASTa and TAA tests also had atopic dermatitis and her spleen

TABLE IV

Occurrence of positive teichoic acid antibody titres in 108 patients with liver diseases and 100 patients with gastro-intestinal diseases

Diagnosis	Number of patients tested	Number (percent) of patients with TAA titre	
		≥ 4	≥ 8
Liver diseases	108	6(6)	2(2)
alcoholic liver disease	32	2(6)	0
primary biliary cirrhosis	21	2(10)	1(5)
liver metastases	13	0	0
chronic active hepatitis	5	0	0
other liver disease	37	2(5)	1(3)
Gastro-intestinal diseases	100	4(4)	1(1)
malabsorption syndrome	60	2(3)	1(2)
acute gastroenteritis	32	1(3)	0
ulcerative colitis	8	1(12)	0

TAA = teichoic acid antibody.

TABLE V

Occurrence of positive teichoic acid antibody titres in 54 patients with pharyngitis

Throat culture result	Number of patients tested	Number (percent) of patients with TAA titre	
		≥ 4	≥ 8
Positive for β -haemolytic streptococci	35	6(17)	0
group A	12	3(25)	0
not group A	23	3(13)	0
Negative for β -haemolytic streptococci	19	0	0

TAA = teichoic acid antibody.

TABLE VI

*Clinical details of eight patients with teichoic acid antibody titres ≥ 8 , without evidence of *S. aureus* infection*

Underlying disease	Age	Sex	TAA titre	ASta* value (IU/ml)	Additional diagnosis
Reiter's disease	34	M	8	<0.64	none
Reiter's disease	42	M	8	0.9	none
reactive arthritis due to					
<i>Chlamydia trachomatis</i> infection	26	M	8	1.25	none
sero-negative rheumatoid arthritis	50	F	8	0.64	none
systemic lupus erythematosus	20	F	8	0.64	none
malabsorption syndrome	32	F	16	2.5	atopic dermatitis, splenectomy
primary biliary cirrhosis	31	F	16	0.64	none
acute viral hepatitis	37	F	8	2.5	psoriatic arthritis

TAA = teichoic acid antibody.

*ASta = antibody to staphylococcal α -haemolysin.

had been removed. The other patient had viral hepatitis and chronic arthritis, probably associated with psoriasis.

DISCUSSION

In this study, relatively high TAA titres (≥ 4) sometimes occurred in obviously healthy persons and in patients with different diseases but without known *S. aureus* infection. A TAA titre ≥ 4 was twice as common in arthritis patients as in healthy controls. Patients with *Yersinia* arthritis and SLE were most likely to have positive TAA tests. This result resembles the previous findings of unspecific viral antibodies in SLE (Vesikari and Laitinen, 1974). Patients with Group A streptococcal pharyngitis quite often had positive TAA tests, but this group was too small for the findings to reach statistical significance. The distribution of TAA titres in patients with various gastro-intestinal and liver diseases was not different from that in healthy controls. High TAA titres (≥ 8) occurred in a few healthy persons (1%) as well as patients with non-staphylococcal diseases (2%) and these were not concentrated in any particular patient group. These figures are very much smaller than the 24% found among patients with *S. aureus* bacteraemia.

The positive TAA findings in persons without active staphylococcal disease could be due to infection with cross-reactive bacteria. β -linked N-acetylglucosamine is the main antigenic determinant both in the ribitol teichoic acid of *S. aureus* and group A streptococcal polysaccharide (Juergens *et al.*, 1963), which may explain the moderately high TAA titres in patients with streptococcal pharyngitis; β -linked N-acetylglucosamine is a prominent antigenic determinant also in *Streptococcus bovis* (Kane and Karakawa, 1969). Cross-reactions may be due also to ribitol phosphate compounds; the pneumococcal C substance is a ribitol teichoic acid (Brundish and Baddiley, 1968) and a minority of *S. epidermidis* strains have ribitol teichoic acids (Aasen and Oeding, 1971). Antibodies to *Haemophilus influenzae* type b capsular polysaccharide have been shown to cross-react with several gram-positive bacteria that contain ribitol teichoic acids in their cell walls, including *S. aureus*, *Bacillus subtilis*, *B. pumilus* and *Lactobacillus plantarum*, as well as a few pneumococcal and *Escherichia coli* serotypes that contain similar polysaccharides in their cell envelopes (Argaman *et al.*, 1974). Most of these cross-reactions are, however, weak. Ribitol phosphate has been

identified as one of the main constituents of lipopolysaccharide of three *Proteus mirabilis* strains (Gmeiner *et al.*, 1977).

In patients without *S. aureus* infection, TAA has most often been reported in patients with endocarditis or bacteraemia caused by streptococci or pneumococci, *S. epidermidis*, diphtheroids and *E. coli* (Crowder and White, 1972; Hoppes and White, 1975; Nagel *et al.*, 1975; Larinkari *et al.*, 1977; Leffell *et al.*, 1978; Tuazon *et al.*, 1978; Tenenbaum and Archer, 1980).

Another possibility is that the high TAA titres in healthy persons and in patients without known *S. aureus* infection may be due to silent staphylococcal infections. This is less likely because the AS_ta values were normal in eight of the 10 persons with TAA titres ≥ 8 . On the other hand, it is known that in *S. aureus* bacteraemia positive TAA and AS_ta results often occur in different patients (Larinkari *et al.*, 1977). One of the two patients with a slightly elevated AS_ta and a high TAA titre had atopic dermatitis, a condition in which elevated AS_ta values and TAA titres are likely to occur (Mustakallio, 1966; Larinkari, 1982a). The other patient had viral hepatitis, arthritis and psoriasis, conditions in which AS_ta and TAA usually remain in the normal range (Mustakallio, 1966; Larinkari, 1982a).

From the data presented we conclude that the TAA test cannot be used as a reliable indicator of septic staphylococcal infection in patients with *Yersinia* arthritis or SLE, but that, in general, a high TAA titre (≥ 8) points strongly to significant infection caused by *S. aureus* even in patients with autoimmune diseases or liver diseases. At the lower level of positivity (TAA titre 4) the specificity of the TAA test for *S. aureus* infection is less good, as these low titres are seen in 6% of normal adults.

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