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Clinical evaluation of a fluorescent antibody test for the serological diagnosis of streptococcal endocarditis

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SUMMARY Serum fluorescent streptococcal antibody tests were carried out on 71 patients with clinically suspected infective endocarditis, and a final diagnosis of endocarditis was obtained in 46 patients.

A serological diagnosis of streptococcal endocarditis was obtained in 10 patients who had persistently negative blood cultures, as fluorescent streptococcal antibody titres equal to or greater than 400 were detected against at least one of four strains of streptococci used as heterologous antigens. There were no false positive fluorescent antibody results with heterologous antigens during tests on 29 patients who had either non-streptococcal endocarditis, a final diagnosis other than endocarditis, or streptococcal sepsis not associated with endocarditis. A negative result with the heterologous antibody test could not, however, exclude a diagnosis of streptococcal endocarditis as six of 11 patients with endocarditis due to *Streptococcus viridans* or *Str bovis* confirmed on blood culture had serum fluorescent antibody titres less than 400 against all the heterologous streptococcal antigens tested.

Homologous fluorescent streptococcal antibody titres equal to or greater than 400, using the patient's own blood culture isolate as the antigen, were found in the serum samples of 14 of 15 patients with endocarditis caused by viridans streptococci, three patients with enterococcal endocarditis, two patients with endocarditis caused by *Str pneumoniae,* and one patient with *Str bovis* endocarditis. In contrast, all five patients who had clinically insignificant streptococcal bacteraemias had serum fluorescent homologous antibody titres of only 100 or less. These results showed that the homologous serum fluorescent streptococcal antibody test could help to decide the clinical importance of a streptococcus which is initially isolated from only one or two of a number of inoculated blood culture bottles.

Some patients in whom a clinical diagnosis of infective endocarditis is strongly suspected have negative or equivocal blood culture results, and it has been suggested that these patients may often have undetected streptococcal endocarditis.[1] Occasionally, patients' blood cultures may give negative results during the first few days of incubation of the broths and then yield growth of a slow growing streptococcus on subcultures during subsequent days or weeks from one or more bottles. It would be useful to have serological tests which could help detect streptococcal infection more rapidly in patients with clinically suspected endocarditis, especially when the blood culture results are equivocal. A serological diagnosis would also be helpful when the blood cultures are completely negative. A preliminary report indicated that patients with confirmed streptococcal endocarditis usually have serum fluorescent antibody titres against homologous streptococcal antigen of between 400 and 1600, whereas antibody titres of 100 or less are found in healthy patients with asymptomatic streptococcal bacteraemia, without cardiac disease, who have undergone dental extraction.[2] This previous report also showed that patients with endocarditis due to viridans streptococci sometimes develop antibody titres equal to or greater than 400 against one or more indicator strains of viridans streptococci included in a heterologous antibody test. We have been asked by clinical microbiologists at many different hospitals in Britain to carry out the serum fluorescent streptococcal antibody tests in patients with a possible clinical diagnosis of

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endocarditis who initially had negative or equivocal blood culture results. We report here our experience together with a follow up of patients so that a clinical evaluation of the usefulness of the test may be assessed. The same set of heterologous antigens of viridans streptococci that was reported in the previous paper[2] was used throughout this period.

Patients and methods

PATIENTS AND SERUM SAMPLES

Patients investigated included those with a possible clinical diagnosis of endocarditis in whom blood cultures were initially negative, patients who had a viridans streptococcus of uncertain clinical importance isolated from blood cultures, and a few patients who were finally shown to have non-cardiac sepsis due to viridans streptococci. When the blood culture results were equivocal a strain of streptococcus was occasionally sent through the post together with the serum sample. All the serum fluorescent streptococcal antibody tests were carried out in the Department of Clinical Microbiology at St Stephen's Hospital. Results of this test are included only where there were reasonably complete clinical as well as laboratory details available during the follow up of the patients.

SERUM FLUORESCENT STREPTOCOCCAL ANTIBODY TEST

Four positive standard reference sera, obtained from patients with endocarditis, were included with every batch of tests. The first, second, third, and fourth reference sera had high fluorescent antibody titres (between 1 in 800 and 1 in 1600) against *Str mitior, Str sanguis II, Str mutans,* and *Str bovis I,* respectively. A pooled negative control serum was also tested against each antigen.

Overnight cultures on blood agar of *Str mitior* (isolated from the blood of a patient with endocarditis, patient N), *Str sanguis II* (from a patient with endocarditis, patient L), *Str mutans* (from a patient with endocarditis, patient P), and *Str bovis I* (from a patient with endocarditis, patient C) were used as the heterologous antigens; the patient's own blood culture isolates were used for homologous antibody tests. Each culture was emulsified in buffer to give a density of between 10^7 and 10^8 organisms per millilitre using Brownes' tubes. A loopful of each antigen suspension was spread on to each well of a Teflon coated slide, dried, and flamed.

Buffer containing 180 g of potassium chloride, 8·06 g of disodium hydrogen phosphate (Na_2HPO_4), 6·97 g of sodium dihydrogen phosphate (NaH_2PO_4), 2 g of sodium fluoride, 2 g of sodium azide, and 2 g of sodium arsenate in 2 l of distilled water, as stock solution, was freshly diluted 1/10 in distilled water for use at pH 7·2.

Each serum sample was initially diluted 1/50 and then in serial double dilutions up to 1/1600 in fresh buffer. Each heat fixed antigen smear was covered with diluted serum and incubated in a moist chamber at 37°C for 45 min. The serum was then washed off with buffer and the slide was immersed in fresh buffer for 5 min. Conjugate, fluorescein labelled sheep antihuman immunoglobulin (Burroughs Wellcome Ltd), diluted 1/32 in buffer, was added to cover the smear and the slide was incubated in the moist chamber for 45 min at 37°C. After incubation the slide was washed in buffer, dried, and mounted in 50% glycerol in buffer. All the slides were examined by incident light fluorescence using a Reichert-Fluovar microscope. Negative smears were checked by phase contrast microscopy to confirm the presence of streptococci in the fields examined. The titre was defined as the highest dilution of serum that gave definite fluorescence.

Results

Results of serum fluorescent antibody test, using heterologous streptococcal antigens, in patients with blood culture positive streptococcal endocarditis

Eleven patients had a diagnosis of endocarditis due to either *Str viridans* or *Str bovis* confirmed by repeated isolation of the causative organism from blood cultures (Table 1). Positive results with serum antibody titres equal to or greater than 400 were obtained in six patients using the set of heterologous antigens. With four of these patients a more rapid serological result was available compared with the isolation of the streptococcus from blood cultures. A detailed example of the timing of serological and blood culture results in one of these four cases is given in Table 2.

Results of serum fluorescent antibody test using heterologous antigens in patients with evidence of streptococcal endocarditis but in whom blood cultures were negative

Streptococcal endocarditis was finally detected in 10 patients by the serum fluorescent antibody test alone (Table 1, cases 12 to 21). With most of these patients there was a firm clinical diagnosis of infective endocarditis and appropriate antibiotic treatment for streptococcal endocarditis was always given, but in a few patients the diagnosis was less certain. An example where the results of the serum fluorescent streptococcal antibody test greatly influenced the management of the patient was in a case of cerebral haemorrhage (case 2, Table 2). A follow up test on this patient 18 months later

Table 1 *Patients with final microbiological diagnoses of endocarditis caused by Str viridans or Str bovis who had heterologous streptococcal antibody tests carried out*

Patient no	Results of serum fluorescent streptococcal antibody test with heterologous antigens				Final results of blood cultures and identity of isolates	Antibody titre to homologous antigens
	Str mitior	*Str sanguis II*	*Str mutans*	*Str bovis I*		
1	800(+)	100*	100	100	*Str mitior*	1600(+)
2	800(+)	100	100	100	*Str mitior*	1600(+)
3	1600(+)	100	200	100	*Str sanguis II*	1600(+)
4	400(+)	100	100	100	*Str viridans*	1600(+)
5	100	100	100	400(+)	*Str bovis I*	1600(+)
6	400(+)	100	100	100	*Str viridans*	1600(+)
7	100	100	100	100	*Str mitior*	1600(+)
8	100	100	100	100	*Str mitior*	100
9	100	100	100	100	*Str mutans*	1600(+)
10	100	100	100	100	*Str salivarius*	1600(+)
11	100	100	100	100	*Str milleri*	1600(+)
12	800(+)	100	100	100	Negative	NA
13	100	100	400(+)	1600(+)	Negative	NA
14	800(+)	100	100	1600(+)	Negative	NA
15	100	100	100	1600(+)	Negative	NA
16	800(+)	100	100	100	Negative	NA
17	1600(+)	100	400(+)	100	Negative	NA
18	400(+)	100	100	100	Negative	NA
19	800(+)	100	100	100	Negative	NA
20	100	400(+)	100	400(+)	Negative	NA
21	400(+)	100	100	100	Negative	NA

*100 stated for antibody titres equal to or less than 100.
(+) = positive result indicated by titres equal to or greater than 400.
NA = not applicable.
Str viridans in blood culture column indicates that viridans streptococci could not be further identified.

showed that the serum fluorescent streptococcal antibody titre to *Str mitior* had fallen from 800 to less than 100. Over a shorter period a fall in the IgM streptococcal antibody titre was also shown with this case using fluorescein labelled sheep antihuman IgM (Wellcome Diagnostics Ltd) diluted 1/32 as the class

Table 2 *Brief details of two cases where a serum fluorescent streptococcal antibody test provided a microbiological diagnosis at a time when blood cultures failed to isolate an organism*

Brief clinical details	Date	Blood cultures	Fluorescent antibody results
Case 1 (= patient 1 of Table 1)			
Young woman with unexplained febrile illness investigated elsewhere for preceding three months.		Negative	
Admitted to Westminster Hospital. Noted to have mitral murmur. Blood cultures collected over 48 h after admission.	13 July	4 sets collected before antibiotics	
Echocardiogram negative			
Penicillin and streptomycin started.	15 July	Negative so far	
Fluorescent antibody test reported positive	16 July	Negative	On 16 July:
	17 July	Negative	Titre of 800 to *Str mitior* reported with
	18 July	Probable Gram positive cocci on microscopy of one broth	comment that this provided serological evidence of *Str viridans* endocarditis
Blood culture positive	21–23 July	Slow growing *Str viridans* isolated from 3/4 sets. Subsequently identified as *Str mitior*	
Case 2 (= patient 12 of Table 1)			
Women aged 67 yr admitted to Westminster Hospital with drowsiness, headache, and fever. Systolic murmur of uncertain clinical importance noted. Antibiotics started in view of possible diagnosis of endocarditis. Treatment discontinued after only 1 week when computed tomogram showed cerebral haemorrhage. Antibiotics restarted when serological results confirmed endocarditis and a full 6 week course given.	10 December	6 sets blood cultures collected before antibiotic treatment Negative after 3 weeks incubation.	On 22 December: Antibody titre of 800 to *Str mitior* reported. Report stated that this result was "strongly suggestive of endocarditis due to a viridans streptococcus" IgM *Str mitior* antibody titre positive at 200.
After neurological recovery the patient disclosed a history of dental scaling 17 days before admission.			March: Follow up serum showed antibody titre of 400 to *Str mitior* and IgM *Str mitior* less than 50

Table 3 *Results of serum fluorescent streptococcal antibody test in patients who had endocarditis due to organisms other than Str viridans or Str bovis confirmed microbiologically*

Patient no	*Organism causing endocarditis*	*Results of serum fluorescent streptococcal antibody tests*	
		Heterologous antigens	*Homologous antigens*
22	*Str faecalis*	—	800
23	*Str faecalis*	—	1600
24	*Str faecium*	—	800
25	*Str pneumoniae*	—	1600
26	*Str pnuemoniae*	—	1600
27	Group B haemolytic streptococci	—	800
28	*Str pyogenes*	—	NT
29	*Staph aureus*	—	NA
30	*Staph aureus*	—	NA
31	*Staph epidermidis*	—	NA
32	*Neisseria sicca*	—	NA
33	*Coxiella burnetii*	—	NA

— = antibody titre equal to or less than 100 with the same indicator strains of viridans streptococci stated in Table 1.
NT = not tested.
NA = not applicable.

specific conjugate.

Results of serum fluorescent antibody test using heterologous streptococcal antigens in patients with endocarditis caused by organisms other than Str viridans or Str bovis

Twelve patients with endocarditis caused by organisms other than viridans streptococci (including enterococci, pneumococci, β-haemolytic streptococci, and staphylococci) gave negative results with the heterologous streptococcal fluorescent antibody test despite the presence of occasional high antibody titres against homologous antigen (Table 3). The serum samples referred to St Stephen's

Table 4 *Results of serum fluorescent streptococcal antibody test in 20 patients who initially were suspected of having possible endocarditis but who had a final alternative diagnosis*

Patient no	*Blood culture (final result)*	*Fluorescent streptococcal antibody test*				*Final diagnosis or other clinico-pathological comments*
		Str mitior	*Str sanguis II*	*Str mutans*	*Str bovis I*	
34	Kingella isolated	—	—	—	—	*Kingella kingae* septicaemia
35	Neg	—	—	—	—	Dissecting aneurysm of aorta (PM)
36	Neg	—	—	—	—	Collagen disease
37	Neg	—	—	—	—	No evidence of endocarditis (PM)
38	Neg	—	—	—	—	Infected arteriovenous shunt and nephritis
39	*Str mitior* isolated	—	—	—	—	Contaminated intravenous line without good evidence of endocarditis
40	Neg	—	—	—	—	Osteomyelitis
41	Neg	—	—	—	—	Haemolytic anaemia, not due to endocarditis
42	Neg	—	—	—	—	Systemic lupus erythematosus
43	Neg	200	—	—	—	Cardiac failure and chest infection not associated with endocarditis
44	Neg	—	—	—	—	Collagen disease
45	Neg	—	200	—	—	Cardiological problems from an attack of *Str viridans* endocarditis 1 year previously but did not have current new attack of endocarditis; patient improved without antibiotic treatment
46	Neg	—	—	—	—	Pyrexia of undetermined origin in patient with previous heart surgery but endocarditis not present
47	Neg	—	—	—	—	Disseminated gonococcal infection
48	Neg	—	—	—	—	Pelvic inflammatory disease in woman with a heart murmur but endocarditis not present
49	Neg	200	—	—	—	These patients were treated for endocarditis although the diagnosis was considered "doubtful"
50	Neg	—	—	—	—	
51	Neg	—	—	—	—	Cerebrovascular accident in patient with mitral stenosis but endocarditis not present
52	Neg	—	—	—	—	Hodgkin's disease
53	Neg	—	—	—	—	Patient with prosthetic heart valve had fever and recent erythromycin treatment; myeloma and no endocarditis

Neg = negative.
— = antibody titre ≤100.
(PM) = evidence at postmortem examination.

Table 5 *Results of serum fluorescent streptococcal antibody test using homologous antigen in patients who had equivocal blood culture findings initially*

Patient no	Initial blood culture findings and other comments	Result of serum fluorescent streptococcal antibody test using initial blood culture isolate as antigen	Final blood culture results	Final clinical and microbiological diagnosis
54	Only 1 of 12 blood culture bottles yielding growth of *Str mitior* after 7 days' incubation	1600	4 of 12 blood culture bottles yielding growth of *Str mitior* after 28 days' incubation	Infective endocarditis due to *Str mitior*
55	Only 1 of 4 blood culture bottles from this child yielded growth of *Str sanguis* after a few days' incubation	800	No change from initial blood culture result	Infective endocarditis due to *Str sanguis*
56	Only 1 of 6 blood culture bottles yielded growth of *Str viridans* after 3 days' incubation but Gram positive cocci seen in broths of 2 further bottles. Organism slow growing on blood agar	1600	*Str viridans* isolated from 4 of 6 blood culture bottles	Infective endocarditis due to *Str viridans*
57	Only 2 of 9 blood culture bottles showed Gram positive cocci in broths during microscopy after 5 days' incubation. Blood agar subculture plate from 1 broth had slow growing strain of viridans streptococcus which was directly used as homologous antigen (patient had recently received talampicillin from general practitioner)	400	6 of 9 blood culture bottles finally yielded growth of *Str viridans* during 3 weeks' incubation	Infective endocarditis due to *Str viridans*
58	Only 2 of 6 blood culture bottles yielded growth of viridans streptococcus after 2 days' incubation	400	4 of 6 blood culture bottles yielded growth of *Str viridans* after 5 days' incubation	Infective endocarditis due to *Str viridans*
59	*Str viridans* isolated from 2 blood culture bottles, belonging to different sets, of 4 sets collected after 3 days' incubation	100	No change from initial blood culture result	Patient died. Necropsy showed anaplastic squamous cell carcinoma of lung and an abdominal aortic aneurysm
60	*Str milleri* isolated from 1 of 9 blood culture bottles after 5 days' incubation from elderly woman with pyrexia of undetermined origin	50	No change from initial blood culture result	Undetected focus of infection, Patient better after 10 days oral antibiotic treatment. Probably not endocarditis
61	Child had a total of 9 sets (18 bottles) of blood cultures collected. From 1 set (both bottles) a mixture of *Str sanguis* and *Str salivarius* was isolated after a few days' incubation	Less than 100 against each blood culture isolate	No change from initial blood culture result	Unexplained febrile illness during months after heart surgery settled without antibiotics and was not due to endocarditis
62	*Str faecalis* isolated from 1 to 6 blood culture bottles during one week incubation *Str viridans* isolated from 1 of 6 blood culture bottles after one week incubation (child had recently received oral penicillin)	Less than 50	No change from initial blood culture result	Infective episode in a leukaemic patient probably not due to endocarditis. Responded to short course of antibiotics
63		Less than 50	No change from initial blood culture result	Child had a congenital heart defect, but unexplained transient febrile episode was thought unlikely to be due to endocarditis

Hospital for fluorescent antibody tests from case 32, a child, and case 33, an adult, were from patients who initially presented with blood culture negative endocarditis. Q fever endocarditis in case 33 was subsequently diagnosed serologically by another laboratory using *Coxiella burnetii* complement fixation tests.

Serum fluorescent streptococcal antibody test in other cases of blood culture negative endocarditis

Eight other adult patients had firm clinical diagnoses of endocarditis and negative serum fluorescent streptococcal antibody test results using heterologous antigens. The causative organism in each patient remained unknown.

Results of serum fluorescent streptococcal antibody test in patients who subsequently were given alternative diagnoses

Twenty patients in whom a clinical diagnosis of endocarditis was initially suspected were subsequently considered not to have endocarditis. Most of these patients had good evidence of alternative diagnoses (Table 4). As all 20 patients had serum fluorescent antibody titres below 400 there was no evidence of a false positive reaction in this group.

Results of serum fluorescent streptococcal antibody test in patients with non-cardiac sepis due to viridans streptococci

There were three patients with *Str milleri* liver abscess or empyema and one patient with *Str sanguis* spinal osteomyelitis, who had serum fluorescent streptococcal antibody titres between 200 and 800 against homologous antigens. The same serum samples from these patients had antibody titres equal to or less than 100 against the standard set of heterologous streptococcal antigens.

Results of serum fluorescent streptococcal antibody test with homologous antigens in patients who had equivocal blood culture results

Five patients who initially had equivocal blood culture results were rapidly shown to have high antibody titres equal to or greater than 400 against their own blood culture isolates (homologous antigens). All five patients were finally diagnosed as having streptococcal endocarditis (Table 5). A negative serum fluorescent antibody test against homologous antigens was obtained in five other patients with equivocal blood culture results who were subsequently considered not to have endocarditis; further clinical details are included in Table 5.

Discussion

The microbiological diagnosis of streptococcal endocarditis is not usually difficult to establish as the causative organisms are most often isolated from repeated blood culture sets within 48 h of their collection.[3] Factors that may cause the blood cultures to be negative or give delayed positive results include recent antibiotic treatment, infection due to fastidious strains of streptococci and the use of suboptimal blood culture techniques. The results of investigating patients with a possible clinical diagnosis of endocarditis using the serum fluorescent streptococcal antibody test with heterologous streptococcal antigens suggest that this serological test may be helpful when a positive result is obtained. It is noteworthy that a positive serological test with heterologous streptococcal antigens was obtained in 16 of 21 patients who finally had good clinical evidence of bacterial endocarditis. No false positive results were obtained with heterologous antigens in 12 patients with endocarditis caused by organisms other than *Str viridans* or *Str bovis* or in 20 patients initially suspected of having possible endocarditis but in whom there was a subsequent alternative diagnosis. Therefore a positive result with heterologous streptococcal antigens with an antibody titre greater than 400 was specific for serious streptococcal infections. Patients with viridans streptococcal infections other than endocarditis did not have sera reacting with the set of heterologous antigens and a positive result with these antigens strongly suggested the presence of endocarditis due to streptococci. When the results of blood culture subsequently became positive there was sometimes still an advantage for the use of the serum fluorescent streptococcal antibody test as the results were obtained more rapidly with the antibody test during investigations on four patients. In 10 patients the antibody test gave the only microbiological evidence of streptococcal endocarditis and occasionally this greatly influenced the clinical management of the patient (case 2, Table 2). A high titre of class specific IgM streptococcal antibodies was also detected against heterologous antigen in an early serum sample from case 2. This suggests that IgM specific streptococcal antibody tests may be useful to help confirm current active infection when early serum samples are available.

The results of the study also clearly show that a negative result obtained with the serum fluorescent streptococcal antibody test with heterologous antigens does not exclude endocarditis due to viridans streptococci as there were frequent false negative reactions. These false negative reactions could sometimes be explained by infection due to strains of viridans streptococci which were not included in the set of indicator strains such as *Str milleri* and *Str salivarius*. Furthermore, some patients with infective endocarditis caused by *Str mutans* and *Str mitior* developed high homologous antibody titres but failed to react serologically with the indicator strains of *Str mitior* or *Str mutans* used in the study. One other report of the use of a serum fluorescent streptococcal antibody test for the serological diagnosis of streptococcal endocarditis also found individual strain variability in reactions with sera from patients with *Str viridans* endocarditis, and no indicator strain of *Str mitior* was found particularly suitable as a heterologous antigen.[4] In our experience the one strain that reacted more than any other strain with sera from patients with endocarditis was the *Str mitior* strain from patient N. The *Str sanguis II* strain used in our study had poor reactivity and might usefully be replaced by another strain in the future. The *Str mutans* strain also showed poor reactivity not only with sera from patients with *Str mitior* endocarditis but also with the one case of *Str mutans* endocarditis investigated. *Str milleri* endocarditis has been reported more frequently in the past few years and it may be worthwhile to include a heterologous *Str milleri* antigen for future clinical tests. Although *Str bovis* is not strictly a viridans streptococcus but a Lancefield group D streptococcus, it was interesting that some patients in the blood

culture negative group gave strong serological reactions with *Str bovis* and either *Str mitior* or *Str mutans*. There are probably some antigenic cross reactions between some strains of viridans streptococci and *Str bovis*.

So far our clinical evaluation suggests that the serum fluorescent streptococcal antibody test using the current set of heterologous antigens is specific for detecting endocarditis due to *Str viridans* or *Str bovis* but that its sensitivity needs to be further improved. The indicator strains used in this study were mainly selected as heterologous antigens on an empirical basis since three of the four strains used had been studied previously.[2] Theoretical knowledge about which cell wall antigens react with antibodies in this serum fluorescent antibody test is lacking. Therefore, the optimal adjustment of the panel of heterologous antigens to improve the sensitivity of the test must depend largely on clinical trial and error assessments. Information about some other investigations such as circulating immune complexes was not usually available when serum samples for heterologous antibody tests were received from other hospitals. It is possible that certain patients may have had negative fluorescent antibody test results because of antigen-antibody complexes, rather than the use of inappropriate antigens. This possibility deserves investigation and tests to detect streptococcal antigen as well as antibody may also need to be developed.

The serum fluorescent streptococcal antibody test results using homologous antigens from patients with equivocal blood culture results has shown that this test is simple and useful diagnostically as positive results were nearly always associated with subsequent confirmation of endocarditis. Negative results also usually indicated a clinically insignificant streptococcal bacteraemia. In the few patients who were monitored over a long period after the treatment of streptococcal endocarditis it was apparent that the homologous antibody titre took at least 18 months to two years to fall from original titres of 800 to 1600 to subsequent final titres of less than 100 against homologous antigens. This test is therefore of little practical value for any monitoring of treatment

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