

# Immunoblotting in the diagnosis of culture negative endocarditis caused by streptococci and enterococci

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## Abstract

**Aim**—To improve the diagnosis of culture negative endocarditis by diagnosing cases due to streptococci and enterococci.

**Method**—Serum samples were immunoblotted against extracts of the commonest streptococci and enterococci. They were selected from patients with a cardiac murmur, persistent pyrexia and at least three negative blood cultures. The presence of patterns of endocarditis species specific antigenic bands was measured and correlated with clinical outcome.

**Results**—Negative serology was found in 28 patients where the diagnosis of endocarditis was rejected or, if proved, staphylococcal, yeast, Gram negative, systemic lupus erythematosus, due to Q fever or *Chlamydia psittaci* or non-bacterial thrombotic. Positive serology was found in 27 of the 34 patients where the response to antibiotics suggested streptococcal or enterococcal infection. In 22 of these there was objective evidence of endocarditis. Positive serology was also found in three of four further patients with vegetations at necropsy.

**Conclusion**—The identification of patterns of antibody response on immunoblotting can be used to make a specific diagnosis of streptococcal or enterococcal endocarditis in the absence of positive blood cultures.

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Keywords: Immunoblotting, culture negative endocarditis, serodiagnosis.

Prior to the development of antibiotics, infective endocarditis was universally fatal with patients dying within a few months of diagnosis.<sup>1</sup> The advent of antibiotics and valve replacement surgery along with our ability to isolate the specific micro-organism by blood culture has led to a reduction in mortality to between 14 and 46%.<sup>2</sup> However, there remain patients with active endocarditis where repeated blood cultures are negative. The incidence ranges from 2.5 to 31%.<sup>3</sup> One important cause is the prior administration of antibiotics. This reduced the incidence of positive blood cultures in patients with documented streptococcal endocarditis<sup>4</sup> and was more likely to have occurred in patients with culture negative rather than culture positive endocarditis.<sup>5,6</sup> Exclusion of these cases and the

handling of blood cultures in an experienced laboratory has led to a revised incidence of culture negative endocarditis of approximately 5%.<sup>7</sup>

The diagnosis of culture positive endocarditis has been confirmed by a variety of serological techniques measuring antibody. These have either demonstrated a raised total antibody as with immunofluorescence,<sup>8,9</sup> indirect enzyme linked immunosorbent assay (ELISA)<sup>10,11</sup> and counterimmunoelectrophoresis<sup>12</sup> or characterised individual patterns of antigenic bands by immunoblotting.<sup>13-15</sup> The latter is species specific for *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus mutans*, *Streptococcus bovis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus sanguis*, and *Streptococcus lactis*.<sup>14,15</sup> This presents the possibility of detecting the same patterns of antibody reactivity in the serum of patients with culture negative endocarditis (CNE). This would lead to a more precise laboratory diagnosis and the initiation of appropriate antibiotic therapy or the continued search for another cause of the pyrexia.

The definitive diagnosis of endocarditis is dependent on the demonstration of vegetations on a heart valve at surgery or necropsy or a peripheral embolus.<sup>7</sup> Recently, a secondary set of clinical criteria has been developed.<sup>16</sup> This consists of major criteria (positive echocardiogram and typical blood culture) and minor criteria (predisposition, fever, vascular phenomena, immunological phenomena, suggestive echocardiogram, and suggestive microbiology). There are also patients with a fever, cardiac murmur and negative blood cultures in whom the diagnosis of CNE is queried by clinicians. This can occur in the absence of objective clinical signs of endocarditis and a negative echocardiogram.

We examined serum samples from such patients as well as those who have objective evidence of vegetations, demonstrated by a peripheral embolus, a positive echocardiogram, surgical excision, or necropsy. They were immunoblotted against extracts of streptococci and enterococci to test for the species specific, endocarditis specific antigenic bands listed in table 1. These were derived from previous work.<sup>14,15,17</sup> The entry requirements for the present study were persistent pyrexia of greater than 38°C for at least five days, a cardiac murmur and a minimum of at least three negative blood cultures. The patients were then subdivided according to their final clinical diagnosis. This took into account evidence of vegetations such as positive echocardiograms,

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Table 1 Species specific antigenic bands to which the presence of antibody was considered to be diagnostic of endocarditis

Bacteria	Antibody class	Apparent molecular weight (kDa) (no. of bands)
<i>E faecalis</i>	NCTC 755	IgM/IgG 88-90, 45-47
<i>E faecium</i>	NCTC 7171	IgM/IgG 82-90 (3 bands)
<i>S bovis</i>	NCTC 8183	IgM/IgG 70-140 (≥5 bands)
<i>S sobrinus</i>	MUCOB 263	IgM/IgG 220, 200, 190
<i>S agalactiae</i>	MUCOB 204	IgM 82, 71, 66-67
		IgG 34-36, 31-33, 30
<i>S pneumoniae</i>	Clinical isolate	IgM/IgG 150-190 (≥4 bands)
		IgM 123, 100, 90
<i>S gordonii</i>	F90A/NCTC 7868	IgM/IgG 180, 80-82, 45
<i>S oralis</i>	NCTC 7864	IgM/IgG 180, 80-82

vegetations seen at necropsy or surgery, response to empirical antibiotic therapy or a final diagnosis such as lupus non-bacterial verrucous endocarditis or one of the conditions associated with non-bacterial thrombotic endocarditis.<sup>18</sup> This study thus assessed the use of immunoblotting (where this was consistent with the clinical progress of the patient) in the diagnosis of streptococcal or enterococcal endocarditis in the absence of positive blood cultures.

**Methods**

**CLINICAL SAMPLES**

Serum samples were collected from patients referred with possible CNE. They all had a history of at least three negative blood cultures, a persistent murmur and a pyrexia of 38°C or greater for at least five days.

The patients were subdivided as follows: group 1, no objective evidence of vegetations (table 2). Group 1A patients were treated empirically and successfully for streptococcal or enterococcal endocarditis by two weeks of intravenous penicillin or amoxycillin plus an aminoglycoside followed by four weeks of oral chemotherapy. In group 1B patients the diagnosis of endocarditis was subsequently rejected. Group 2, evidence of vegetations as shown by a positive echocardiogram. Some cases had suggestive immunological and vascular phe-

nomena and in others the vegetations were demonstrated at surgery. The patients were treated successfully for either streptococcal or enterococcal endocarditis (group 2A; table 3), staphylococcal (group 2B), yeast (group 2C), Gram negative (group 2D) or Q fever, or *Chlamydia psittaci* (group 2E) endocarditis (table 4). Group 3, vegetations were confirmed at necropsy (table 5). Group 4, diagnosed as having lupus non-bacterial verrucous endocarditis (table 5). All patients had a positive echocardiogram and systemic lupus erythematosus (SLE). Group 5, the patients had one of the conditions associated with non-bacterial thrombotic endocarditis (table 5). There was no evidence of infective endocarditis as judged by a response to empirical antibiotic therapy where this was initiated. Some patients had a positive echocardiogram.

**IMMUNOBLOTTING**

*Isolates*

Extracts were prepared from *Enterococcus faecalis* NCTC 775, *Enterococcus faecium* NCTC 7171, *Streptococcus bovis*, type 1, NCTC 8183, *Streptococcus sobrinus* (Manchester University Collection of Bacteria, MUCOB 263), *Streptococcus oralis* NCTC 7864, *Streptococcus gordonii* NCTC 7868, *S gordonii* F90A, *Streptococcus agalactiae* (MUCOB 204), and *Streptococcus pneumoniae* (isolated from a patient with endocarditis<sup>15</sup>). *S sobrinus* (MUCOB 263) was previously referred to as *S mutans* (MUCOB 263) but is closer in phenotype to *S sobrinus*.<sup>19</sup> *S gordonii* F90A was previously referred to as *S sanguis* F90A, but has been reclassified.<sup>20</sup> Isolates were cultured in brain heart infusion broth for 48 hours at 37°C in a rotary shaker. Cultures were pelleted by centrifugation at 2500 × g for 15 minutes, resuspended in 10mm Tris/HCl (pH 7.5) and repelleted. Fragmentation was performed at -20°C in an X-press. The disintegrated cells were centrifuged at 10000 × g for 10 minutes.

Table 2 Clinical details of patients in groups 1A and 1B

Case no.	Antibiotics prior to blood culture	Positive serology	Additional clinical details
<b>Group 1A</b>			
1		<i>E faecalis</i>	
2		<i>E faecalis</i>	
3		<i>S gordonii</i>	Myocardial infarct
4		<i>S gordonii</i>	
5	Amoxycillin	<i>S gordonii</i>	Pneumonia, atrio-ventricular defect
6		<i>S gordonii</i>	Splinter haemorrhages
7	Amoxycillin	<i>S mutans</i>	<i>S epidermidis</i> (single blood culture bottle)
8	Amoxycillin	<i>S pneumoniae</i>	Pneumonia, aortic stenosis
9		<i>S oralis</i>	Arthralgia
10		<i>S agalactiae</i>	Splinter haemorrhages
11			Prosthetic aortic valve, congestive cardiac failure, rectal bleeding caused by polyp removed at sigmoidoscopy
12			Dental extraction, splinter haemorrhages, mitral valve disease
<b>Group 1B</b>			
13		<i>S sobrinus</i> IgG only	Polymyalgia rheumatica. Previous <i>S mutans</i> endocarditis in mitral valve 4 months previously
14			Rheumatic mitral valve disease. No vegetations at surgery
15			Rheumatoid arthritis
16			Congestive cardiac failure. No vegetations at necropsy
17			<i>C albicans</i> osteomyelitis
18			Crohn's disease
19	Oral amoxycillin		Urinary tract infection <i>Escherichia coli</i> , prosthetic aortic valve
20	Oral trimethoprim		<i>Yersinia fredericksonii</i> from blood culture, mitral valve disease, parathyroid adenoma, raised IgM to hepatitis A

Table 3 Clinical details of patients in group 2A

Case no.	Antibiotics prior to blood culture	Positive serology	Additional clinical details
21		<i>E faecalis</i>	Cerebrovascular accident, Roth spots, clubbing
22	Penicillin and gentimicin	<i>E faecium</i>	Vegetations at surgery
23	Penicillin and gentimicin	<i>S bovis</i>	Carcinoma of the sigmoid colon
24		<i>S bovis</i>	Left ventricular failure, associated aortic and mitral valve incompetence
25		<i>S gordonii</i>	Atrial fibrillation
26		<i>S gordonii</i>	Vegetations at surgery sterile on culture
27	Penicillin	<i>S gordonii</i>	Dental abscess, ventricular septal defect, cerebrovascular accident
28		<i>S oralis</i>	Cerebrovascular accident
29		<i>S gordonii</i>	
30		<i>S oralis</i>	Splenomegaly, vegetations at surgery
31		<i>S sobrinus</i>	<i>S mutans</i> endocarditis 18 months previously
32	Amoxycillin	<i>S pneumoniae</i>	Pneumonia
33	Benzylpenicillin	<i>S pneumoniae</i>	Prior pneumococcal meningitis
34	Amoxycillin	<i>S pneumoniae</i>	Chronic obstructive airways disease
35	Clindamycin	<i>S pneumoniae</i>	Pneumococcal osteomyelitis, vegetations at surgery
36	Penicillin and gentamicin	<i>S oralis/S gordonii</i>	Mitral valve prolapse
37	Vancomycin	<i>S oralis/S gordonii</i>	<i>S epidermidis</i> (single bottle blood culture)
38	Penicillin		Prosthetic aortic valve
39			Splenomegaly
40			Prosthetic mitral valve
41			Aortic stenosis, night sweats
42			Vegetations at surgery sterile on culture

Table 4 Clinical details of patients in groups 2B to 2E

Case no.	Antibiotics prior to blood culture	Positive serology	Additional clinical details
Group 2B			
43	Penicillin		Intravenous line <i>S epidermidis</i> , responded vancomycin and gentamicin
44	Vancomycin		Intravenous line <i>S epidermidis</i> , responded vancomycin and gentamicin
45			Intravenous line <i>S epidermidis</i> responded flucloxacillin and gentamicin
Group 2C			
46			Yeast seen on Gram stain of aortic prosthetic valve obtained at surgery. Responded to amphotericin B
Group 2D			
47			<i>Haemophilus parainfluenzae</i> sputum, endocarditis 18 years previously, valve culture negative
Group 2E			
48			Q fever, complement fixation test positive (1 in 1024)
49			<i>C psittaci</i> , complement fixation test positive (1 in 512)

Table 5 Clinical details of patients in groups 3, 4 and 5

Case no.	Antibiotics prior to blood culture	Positive serology	Additional clinical details
Group 3			
50	Vancomycin	<i>E faecalis</i>	<i>E faecalis</i> isolated from vegetations at necropsy
51	Flucloxacillin and fusidic acid		Septic arthritis, <i>S aureus</i> isolated from vegetations at necropsy
52	Benzylpenicillin	<i>S pneumoniae</i>	Prior <i>S pneumoniae</i> meningitis, necropsy confirmed sterile vegetations
53		<i>S oralis/S gordonii</i>	Necropsy confirmed sterile vegetations
54			Necropsy confirmed sterile vegetations
Group 4			
55*			SLE
56*			SLE
57*			SLE, mitral incompetence
58*			SLE
Group 5			
59			Chronic myeloid leukaemia
60			Hodgkin's lymphoma
61			Non-Hodgkin's lymphoma
62			Lung cancer
63*			Thrombocytopenia, Henoch Schonlein purpura
64*			Rheumatic heart disease, Goodpasture's syndrome
65	Cefotaxime and gentamicin		Adenocarcinoma of the prostate
66	Vancomycin and gentamicin		Acute myeloid leukaemia, invasive aspergillosis

\* positive echocardiogram.

The supernatant was used in further experiments and stored at  $-20^{\circ}\text{C}$ .

*Sodium dodecyl polyacrylamide gel electrophoresis*  
The supernatants were solubilised in 2.6% sodium dodecyl sulphate and 2-mercapto-

ethanol at  $100^{\circ}\text{C}$  for five minutes. Electrophoresis of these preparations was carried out on a 10% polyacrylamide gel in a discontinuous buffer system. Protein ( $10\mu\text{g}$ ) was applied to each well. Molecular weight standards were the Amersham Rainbow markers. These were myosin 200000, phos-

phorylase B92500, bovine serum albumin 69 000, ovalbumin 46 000, carbonic anhydrase 30 000, trypsin inhibitor 21 500, and lysozyme 14 300.

#### Immunoblots

Proteins were transferred onto nitrocellulose paper in a buffer containing 25 mM TRIS, 192 mM glycine and 20% methanol (pH 8.3). Transfer was performed at 25°C with a current of 500 milliamps for 30 minutes in a transphor cell (LKB Laboratories). Free protein sites were saturated by incubation in 3% bovine serum albumin (Sigma, Poole, Dorset, UK) in buffered saline (0.9% NaCl and 10 mM TRIS (pH 7.4)) at 4°C overnight. The nitrocellulose membrane was then incubated at 25°C for two hours with each patient's serum diluted 1 in 10 in 3% bovine serum albumin and 0.5% Tween 20 in buffered saline. After washing five times for 30 minutes in 0.9% saline and 0.5% Tween 20, the nitrocellulose was incubated for one hour at 25°C with alkaline phosphatase conjugated anti-human IgM or IgG (Sigma). The conjugate was diluted 1 in 1000 in 3% bovine serum albumin in buffered saline. After washing as described above, the nitrocellulose was incubated for 15 minutes at 25°C with buffer (100 mM TRIS/HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl<sub>2</sub>) containing a mixture of 66 µl/10 ml of nitroblue tetrazolium (NBT 50 mg/ml in N, N-dimethylformamide 70%) and 33 µl/10 ml of 5-bromo-4-chloro-3 indolyl phosphate (BC1P 50 mg/ml in N, N-dimethylformamide 70%). The reaction was stopped by washing in water. Immunoblots were semi-quantified by reflectance densitometry (Chromoscan 3; Joyce Loebel) for the bands defined in table 1. A scan height greater than 40mm was counted as positive.

#### Results

For the 20 patients in group 1, where objective evidence of endocarditis was lacking, serum samples from 10 were positive on immunoblotting against *E faecalis* or a specific streptococcal species. All of these patients responded to antibiotic therapy as expected for streptococcal or enterococcal endocarditis. This was also the case in patients 11 and 12 who did not produce antibody. In the eight cases where the diagnosis of endocarditis was subsequently rejected only patient 13 produced an IgG response against *S mutans*. This was due to a successfully treated episode of *S mutans* endocarditis four months previously. All patients in group 2 had confirmed endocarditis and were subdivided according to the spectrum of the antimicrobials used in therapy (groups 2A to 2D) or positive atypical serology (group 2E). A positive antibody response was detected in cases 21–37 who were managed as either streptococcal or enterococcal endocarditis. In case 23 the high antibody response (fig 1) against *S bovis* prompted a search for a large bowel neoplasm which was subsequently found in the sigmoid colon. In case 31 immunoblotting suggested a partially treated *S mutans* endo-

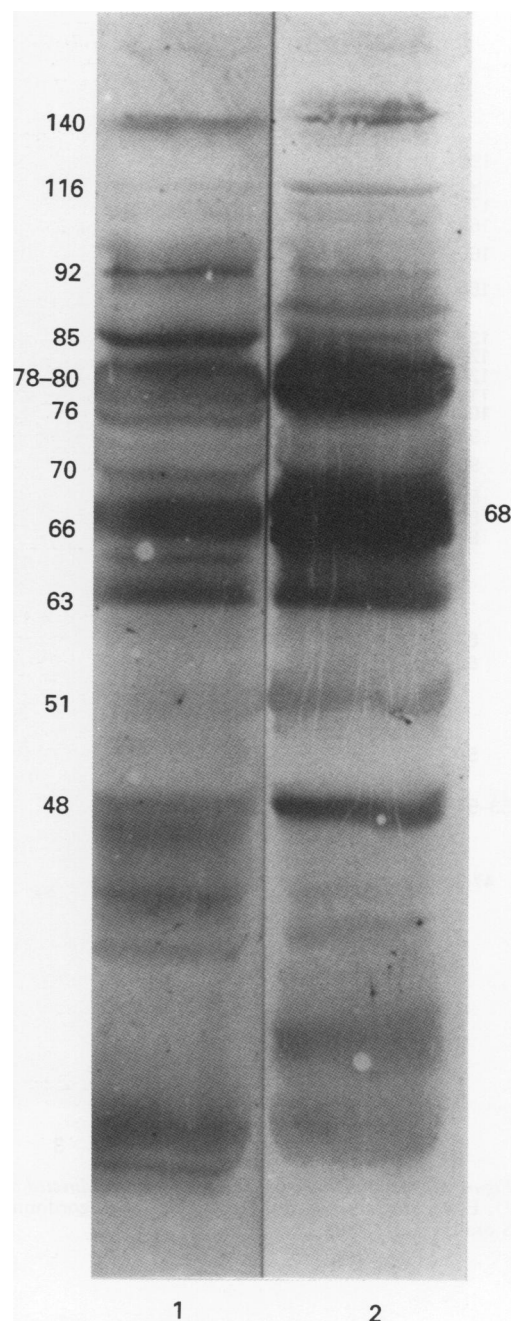


Figure 1 Serum from case 23 assayed for IgM (lane 1) and IgG (lane 2) directed against *S bovis*.

carditis as there was IgM directed against bands at 220, 200 and 190. In cases 32–35 the previous history suggested pneumococcal infection and the serology active *S pneumoniae* endocarditis. Case 33 had a persistently high IgM antibody titre (fig 2). In cases 36 and 37 the immunoblots for both *S oralis* and *S gordonii* were positive, suggesting that some of the immunodominant antigens were shared. In cases 38–42 the negative results did not correlate with clinical outcome. In contrast, the negative serology in cases 43–49 was consistent with staphylococcal (group 2B), yeast (group 2C), Gram negative (group 2D), or atypical (group 2E) endocarditis.

In group 3 patients vegetations were seen at necropsy and their culture in case 50 confirmed *E faecalis* endocarditis. In case 51 a negative

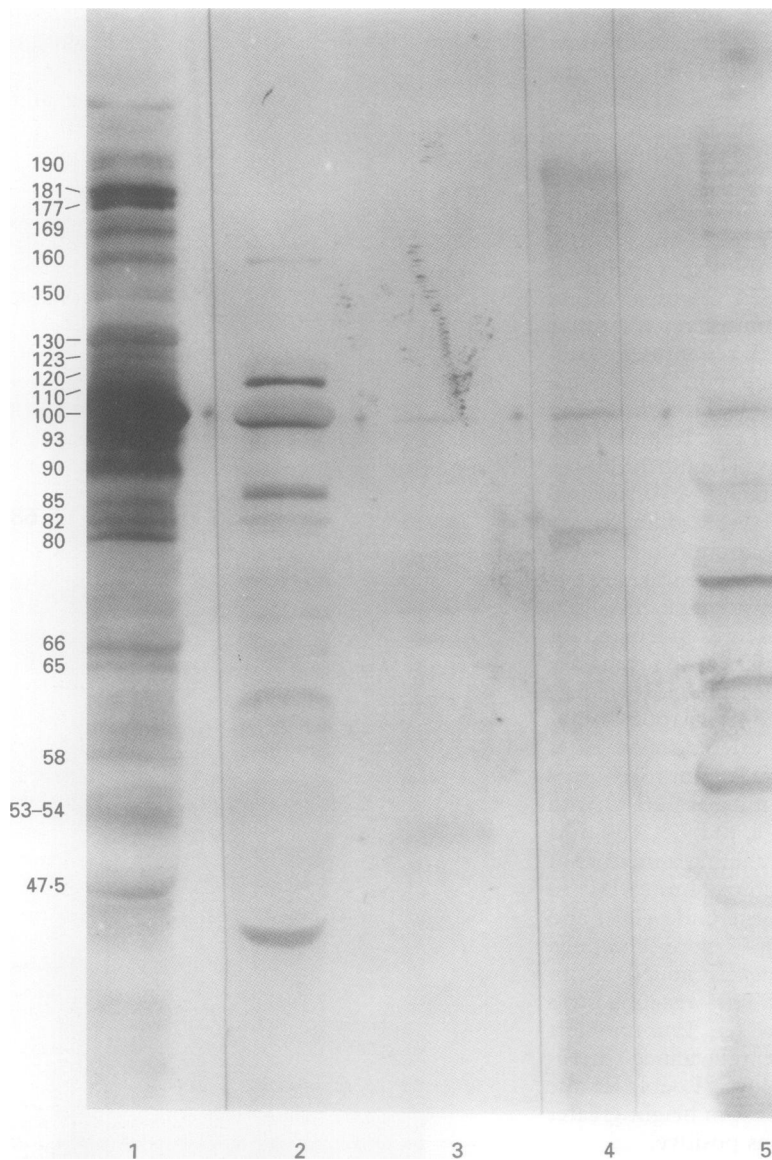


Figure 2 Serum from case 33 assayed for IgM directed against *S pneumoniae* (lane 1), *E faecalis* (lane 2), *E faecium* (lane 3), *S gordonii* NCTC 7868 (lane 4), and *S oralis* NCTC 7864 (lane 5).

antibody response was reported and the vegetations grew *S aureus*. In cases 52–54 the vegetations were sterile. In case 52 the history of *S pneumoniae* meningitis, in conjunction with high titres of specific antibody, suggested partially treated *S pneumoniae* endocarditis.

In cases 55–66 the diagnosis of streptococcal or enterococcal endocarditis was rejected and a diagnosis of lupus non-bacterial verrucous (group 4) or non-bacterial thrombotic (group 5) endocarditis was reached. None of these patients demonstrated a positive antibody response.

For the purpose of calculating the sensitivity, specificity and efficiency of the test for diagnosing streptococcal and enterococcal endocarditis it was assumed that groups 1B, 2B, 2C, 2D, 2E, 4, 5, and case 51 from group 3 were true negatives. They had a negative antibody response and a final alternative diagnosis. True positives, as defined by a positive antibody response and response to appropriate antimicrobial therapy, included cases 21–37

from group 2A, and by a positive antibody response and isolation of the bacteria at necropsy or causing meningitis prior to the development of endocarditis, cases 50 and 52 from group 3. Case 53 (group 3) has been included as a true positive on the basis of the antibody response. Cases 38–42 from group 2A were antibody negative but responded to treatment for streptococcal or enterococcal endocarditis. These cases have been defined as false negatives with the caveat other bacteria such as those in the HACEK (*Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, *Kin-gella*) group would have responded to this regimen.<sup>21</sup>

The patients in group 1A failed to fulfil the necessary criteria for a diagnosis of endocarditis<sup>7,16</sup> and have been excluded from this analysis. Cases 54 (group 3) has also been omitted. This is due to the lack of response to antimicrobials, the absence of antibody and the sterile culture of vegetations at necropsy making it impossible to define the case as a true or false negative. There were no false positives. Having made the above assumptions, the sensitivity of the test was 85%, the specificity 100% and efficiency 91%.

## Discussion

Research on the serology of culture positive endocarditis caused by streptococci and enterococci has repeatedly confirmed the high levels of antibody. An immunofluorescence titre equal or greater than 400 when the patient's serum was examined against the corresponding isolate was virtually diagnostic of endocarditis.<sup>9</sup> An indirect ELISA test for antibodies against the serotype I antigen from nutritional variant streptococci (NVS) was positive in 23 of 31 serum samples from patients with NVS endocarditis. In contrast, only 10 of 30 serum samples from patients with non-NVS streptococcal endocarditis contained antibody.<sup>10</sup>

Crossed immunoelectrophoresis demonstrated a significant association ( $p < 0.002$ ) between the finding of viridans streptococci in the blood and the development of specific precipitins. This did not apply to *S aureus* endocarditis, but did permit the identification of five of 23 cases of CNE as caused by a viridans streptococcus.<sup>12</sup>

The dissection of the antibody response in endocarditis by immunoblotting was originally applied to cases due to *E faecalis*.<sup>13,14</sup> This not only confirmed the high level of species specific antibody but introduced the concept that the presence of antibody against specific antigens could distinguish between a bacteraemia and a bacteraemia complicating endocarditis. Individual *E faecalis* antigens have been cloned, sequenced, expressed, and used as the basis of an indirect ELISA for measuring this specific antibody.<sup>22,23</sup>

The concept was then applied to other streptococci.<sup>15</sup> In the case of *S mutans* one of the antigens has been identified as antigen I/II and its immunodominance confirmed by ELISA in five patients with *S mutans* endocarditis.<sup>11</sup> A clone obtained by screening a  $\lambda$  ZAP DNA

library derived from *S. sobrinus* MUCOB 263 with serum from a patient with *S. mutans* endocarditis had, on sequencing, a 99.2% homology to the Spa A antigen of *S. sobrinus* and 68.4% homology to the antigen I/II of *S. mutans*.<sup>19</sup> In the case of *S. pneumoniae* an immunodominant cell surface protein of approximately 90 kilodaltons, pneumococcal surface protein, has been described. This may be the band presented in table 1.<sup>24</sup>

The identification of the viridans streptococci in a routine clinical laboratory is difficult. For example, only three of 16 identical viridans streptococcal endocarditis isolates sent to five institutions were identified as the same species.<sup>25</sup> In our original work immunoblotting was carried out against *S. sanguis* F90A.<sup>14</sup> This was subsequently reclassified as *S. gordonii* F90A.<sup>20</sup> For the purpose of this study, antibody was measured against this isolate, *S. gordonii* NCTC 7868 and *S. oralis* NCTC 7864. Antibody against a conserved band at 80–82 kilodaltons was present in all of the isolates when reacted with serum from culture positive cases, regardless of whether the originating laboratory had reported the causative bacteria as *S. oralis*, *S. gordonii*, *S. sanguis*, *S. mitis*, or *S. mitior*. This band reacted with a monoclonal antibody specific to the conserved epitope LKVIRKNIV from candidal heat shock protein (HSP) 90 (Burnie JP, personnel communication).<sup>26</sup> This suggested that this molecule is a member of the ubiquitous HSP90 group of proteins. The presence of shared antigens explains the lack of species specificity in the antibody response of cases 36, 37 and 53. Antibody was measured against *S. oralis* NCTC 7864 because immunoblotting serum from cases where the causative streptococcus was known to be definitely either *S. gordonii* or *S. oralis* had shown a slightly different pattern.<sup>17</sup>

Burnie *et al*<sup>14</sup> examined serum samples from seven possible cases of CNE and showed that only the two cases where the diagnosis of streptococcal endocarditis was likely produced an antibody response on immunoblotting. The present study extended this to a further 66 patients. Negative serology was observed in the 28 patients where the diagnosis of streptococcal or enterococcal endocarditis was no longer considered valid. Positive serology was a predictor of response to appropriate antimicrobial therapy in cases 1–10 (group 1A) and cases 21–37 (group 2A). The negative serology, despite a response to antibiotic therapy in cases 11 and 12 (group 1A) and cases 38–42 (group 2A), might have been due to either infection by micro-organisms in the HACEK group or NVS where the examination of serum samples by immunoblotting from culture positive patients has been disappointing.<sup>15</sup> This paper has also delineated a group of patients (cases 1–10, group 1A) with pyrexia, cardiac murmur, negative blood cultures, a positive antibody test, a response to appropriate antimicrobials, and a negative echocardiogram. This should act as a cautionary note against basing the diagnosis of endocarditis solely on echocardiography.<sup>16</sup>

The series also reinforced the significance of antibiotic therapy before taking blood cultures. In the 30 cases where serology suggested either streptococcal or enterococcal endocarditis, prior antibiotic therapy had been given in 14. This agrees with the findings of other authors.<sup>3–6</sup> It revealed a series of six patients (cases 8, 32–35 and 52) where the causative agent was thought to be *S. pneumoniae*. This would indicate that infection of the heart valve was present during the original illness. Its subacute nature and the administration of sublethal concentrations of antibiotics meant that it had slowly progressed to present as CNE several months later. In case 23 the association between *S. bovis* endocarditis and a large bowel neoplasm was seen<sup>27</sup> with the original diagnostic clue being the positive antibody titre against *S. bovis* on immunoblotting.

The current management of CNE is based on the empiric use of either penicillin or ampicillin in combination with gentamicin.<sup>21</sup> This regimen is effective against streptococci, some enterococci and most organisms in the HACEK group. Outcome has been correlated with response to this therapy. In 52 patients with CNE 92% of the patients who became afebrile after one week of antimicrobial therapy survived, compared with 50% of the patients who remained febrile after one week ( $p < 0.02$ ).<sup>6</sup> Immunoblotting serum samples against extracts from the commonest streptococci and enterococci provides a way of making a positive diagnosis in a proportion of these cases.

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