Serological detection of Gram-positive bacterial infection around prostheses


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Coagulase-negative staphylococci produce an exocellular glycolipid antigen which has potential as a serological marker of infection in bone. The value of this newly detected antigen was investigated by enzyme-linked immunosorbent assay (ELISA) in 15 patients with culture-proven infection of prostheses caused by Gram-positive bacteria. The antigen was purified by gel-permeation chromatography from the culture supernatants of coagulase-negative staphylococci grown in a chemically defined medium.

There were significant differences (p < 0.0001) between the serum IgG and IgM levels in patients with infection due to Gram-positive staphylococci and those of a control group of 32 patients with no infection. The ELISA test, which has potential for the diagnosis of infection, may be valuable in distinguishing between staphylococcal infection around prostheses and aseptic loosening.

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Staphylococcus aureus and coagulase-negative staphylococci are the most common causes of infection around prostheses. Early and accurate diagnosis of infection is a major problem, and it is particularly important to distinguish between infection and mechanical (aseptic) loosening. False-positive cultures arising from skin contaminants make the interpretation of microbiological culture from joint aspirates difficult. A major prospective evaluation of criteria for microbiological diagnosis of infection around a prosthesis recommended that the cut-off point for a definite diagnosis of infection should be three or more operative specimens which yield an indistinguishable organism. Current tests available to aid the diagnosis of infection around a prosthesis include the ESR, the level of C-reactive protein (CRP), plain radiography, radio-isotope scans, aspiration, biopsy and histology. Serological methods based on the detection of elevated levels of antibody to microbial antigens offer rapid, non-invasive detection of infection. However, the identification of a suitable antigen, for the accurate diagnosis of staphylococcal sepsis remains unresolved. This is compounded by the likelihood that many individuals may have a significant level of antibody against the common cellular antigens of staphylococci, such as the wall peptidoglycan and teichoic acids, derived from exposure to their own commensal skin flora.

A characteristic feature of infections around prostheses is the biofilm in which the organisms adhering to the components of the device are embedded. The biofilm layer consists of polysaccharide slime which obscures the microorganisms from detection by host defences. It follows that exocellular antigens produced by the organisms and released from the biofilm may stimulate an antibody response, which can be detected and distinguished from the basal level of coagulase-negative staphylococcal antibody. On this basis, previous attempts to devise serodiagnostic tests have utilised exocellular proteins of Staph. aureus. The serum IgG response to these antigens in patients with osteomyelitis associated with artificial joints was significantly higher than in patients with uninfected joints or in healthy normal control subjects. In contrast to Staph. aureus, coagulase-negative staphylococci produce few exocellular proteins, but a number of exocellular carbohydrate and teichoic acid antigens have been described. In one study, partially purified exocellular antigen produced by a single strain of coagulase-negative staphylococci grown in brain-heart infusion broth was shown to be useful in the serodiagnosis of orthopaedic infections. This material was presumed to be either a polysaccharide or teichoic acid but could not be identified further because of the presence of components derived from the complex medium. We have since investigated the chemical nature and the antigenic...
properties of exocellular material produced by a wide range of coagulase-negative staphylococci in a chemically-defined growth medium, and have identified a novel short-chain-length form of the cellular lipoteichoic acid with potential for the serodiagnosis of Gram-positive bacterial infections.\textsuperscript{19} We now describe the results of the use of this antigen in the serological diagnosis of infection around a prosthesis.

**Patients and Methods**

Between July 1997 and July 1998, 47 patients were entered into the study. We used strict inclusion criteria and excluded any patient with a history within the last six months of possible infection such as of the upper respiratory tract or the urinary tract, abscess, dental sepsis or procedures, invasive urological procedures, intra-abdominal sepsis, osteomyelitis, subacute bacterial endocarditis, and skin disorders (dermatitis, psoriasis, eczema).

Fifteen patients had proven infections with loosening of the prosthesis caused by Gram-positive bacteria, giving a positive culture. There were seven men and eight women with a mean age of 68.5 years (34 to 82).

In a control group of 32 patients, 21 had a recent closed fracture; there were 15 men and six women with a mean age of 33 years (16 to 57). The remaining 11 patients had primary osteoarthritis of the hip; there were five men and six women with a mean age of 70.2 years (62 to 84).

The preoperative clinical evaluation assessed pain, function and range of movement according to the scale of Merle D’Aubigné and Postel (0 to 6).

Anteroposterior and true lateral radiographs of the pelvis were obtained before surgery. A full blood-cell count, ESR, measurement of CRP, lateral radiographs of the pelvis were obtained before surgery. A full blood-cell count, ESR, measurement of CRP, and culture of mid-stream urine and aspirate from the hip were routinely obtained. Blood (10 ml) was taken from each patient and was analysed by ELISA.

Revision surgery was performed in an operating suite with horizontal laminar flow by a senior surgeon (RBCT). The void volume of the column (determined by elution of blue dextran) was 8 ml. The material in fractions 10 to 15 was pooled and used directly to coat the ELISA plates after 100-fold dilution in carbonate buffer. Selection of these fractions effectively removed any bacterial products of low molecular weight and components of the culture medium from the culture supernatant and was found to be essential for an effective assay.

Microtitration plates (Immulon 2, Dynex Technologies, Chantilly, Vermont) were coated with either crude culture supernatant or pooled fractions from the FPLC purification.

Antigen preparations were diluted with 100 volumes of sodium carbonate/bicarbonate buffer (0.05 M, pH 9.6) before coating wells with 100 µl at 4°C for 18 hours. After removal of excess antigen, wells were washed with TBS-Tween. Unbound sites were blocked by incubation in the same buffer (1 hour, 4°C). For measurement of IgG titres, patient sera (200 µl), diluted 1:400 in TBS-Tween unless indicated, were added to the first well in each row of the microtitre plate. The samples were then double-diluted across the microtitre plate by serial transfer of 100 µl from the first well into the adjacent well in each row containing 100 µl of TBS-Tween. For measurement of IgM titres the serum was preincubated with Gullsorb (Gull Laboratories Inc, Salt Lake City, Utah) for 30 minutes at 37°C to absorb competing IgG molecules. The Gullsorb adsorbent was removed by allowing it to sediment out and the serum remaining in the supernatant was diluted in TBS-Tween as before. After application of the diluted sera, the plates were incubated for 18 hours at 4°C. After removal of excess serum and washing with TBS-Tween, bound IgG was detected by addition of 100 µl of Protein A-horseradish peroxidase conjugate (0.5 µg/ml in TBS-Tween; Sigma, Poole, UK) and incubation for two hours at 4°C. After removal of conjugate and washing with TBS-Tween, 100 µl of chromogenic substrate were added to each well. The substrate contained 10 µg of 3,3’,5,5’-tetramethylbenzidine (Sigma) dissolved in 1 ml of dimethyl sulfoxide and diluted into 100 ml of sodium acetate/citrate buffer (0.1 M, pH 6.0) containing 10 µl of H₂O₂ (20% v/v). When the colour had developed sufficiently (5 minutes at 20°C), the reaction was stopped by addition of 100 µl of sulphuric acid (1 M) to each well and the absorbance measured at 450 nm with an Anthos 2001 plate reader (Labtec Instru-
ments, Ringmer, UK). Assays were also performed using goat anti-human IgM-peroxidase conjugate (Sigma) in place of protein A-peroxidase to measure the level of IgM in sera, which had been adsorbed with Gullsorb. Control ELISA assays with uncoated wells (blocked with Tween 20), or wells coated with material prepared from brain-heart infusion medium alone (processed by FPLC in the same way as the culture supernatant) and patient sera and conjugates, produced no colour.

**Statistical analysis.** The Mann-Whitney U test was used to determine the significance of differences between the test and the control groups. A result was considered significant if \( p < 0.05 \).

## Results

### Laboratory tests.

In the infected group the ESR, CRP and white cell count (WBC) were measured in ten patients with loosening of prostheses caused by Gram-positive bacteria. The ESR was elevated above 30 mm/hour in all patients. The CRP was >6 ng/l in seven patients (70%), and normal in the remainder. In all ten patients the WBC was within the normal range.

In the control group, the ESR, CRP and WBC were normal.

### Serology.

In the infected group, serology was defined as positive if IgG titres were greater than 1:20 000 (Fig. 1a).
Fourteen patients with septic loosening of prostheses due to Gram-positive bacteria had positive serology. Only one patient in this group had negative serology. In 13 patients in this group the IgM titres were determined (Fig. 1b).

In the control group one patient had raised IgG titres (22 000) but in the remainder they were all under 20 000 (Fig. 2a). This represents a false-positive rate of 3%. Serum IgM titres were determined in 21 patients in this group (Fig. 2b).

The serum IgM levels to this novel antigen were not significantly elevated (mean IgM titre 3992 ± 4094 sd, n = 13) compared with the control group (mean IgM titre, 3533 ± 3136, n = 21) (p = 0.7903). By contrast, the serum IgG levels were significantly elevated (mean IgG titre 64 000 ± 47 451, n = 15) compared with the control group (mean IgM titre, 8469 ± 5136, n = 32) (p < 0.0001). The sensitivity of this test was 93.3%, with a false-positive rate of 3%. The specificity of the test was 96.9%.

Discussion

It is vitally important to distinguish between loosening of a prosthesis caused by low-grade sepsis and aseptic loosening since the diagnosis of infection alters the management plan for the revision procedure.\textsuperscript{21,22} If the infecting organism can be identified appropriate antibiotic therapy can be
administered in the perioperative period and additional antibiotic with the correct sensitivity may be added to the bone cement. Unfortunately, at present, there is no single method which can distinguish, with confidence, between septic and aseptic loosening.

A careful history may provide the first indication of indolent sepsis. Serial laboratory tests are important in the evaluation of any patient undergoing revision surgery. In one study, it was reported that the ESR was greater than 30 mm/hour in 75% of 65 patients with infected hips. By contrast, another report noted that in 46% of 52 patients with active sepsis the ESR was less than 30 mm/hr, and the relevance of the ESR in helping to detect occult sepsis was questioned. In aseptic cases the CRP level should return to normal within three weeks of surgery. An elevated CRP may be more indicative of infection than a rise in the ESR, but is still non-specific. The measurement of both CRP levels and ESR is marginally more accurate.

Radiological features suggestive of septic loosening of a prosthesis such as peristeal elevation, endosteal scalloping, or areas of osteolysis, may also be seen in aseptic loosening when there is an extensive macrophage reaction. Radio-isotope bone scanning using 99mTc-MDP is also non-specific, identifying only areas of increased uptake due to new bone formation, which may occur in the presence of loosening with or without infection. Although white cell scans with radio-isotope labelling are more sensitive than conventional bone scanning, they have a high incidence of false-positive results. Aspiration arthrography is used as a routine test by some surgeons before revision procedures, whereas others use it more selectively in patients in whom they consider infection to be more likely. The significance of the results after aspiration of the hip remains controversial. Some authors have reported a positive diagnosis of infection in up to 90% of infected hips, whereas others have found positive results in only 10% of cases. Most authors agree that the sensitivity for this procedure is of the order of 70%.

In our study the serum IgG and IgM levels, specific to a novel antigen in a group of patients with infection around the prosthesis caused by Gram-positive bacteria and a positive culture, were determined before operation. The serum IgM levels to this novel antigen were not significantly elevated in comparison with those of the control group (p = 0.7903). This is probably because an early rise in the serum IgM titre at the onset of the infection had been missed at the time that the measurements were taken. The serum IgG levels to this novel antigen, however, were significantly elevated in comparison with those of the control group (p < 0.0001). A threshold of 20,000 was used. Previous work using ELISA has shown that it can differentiate between patients with catheter-related infections and control subjects. The sensitivity and specificity of this test (93.3% and 96.9%, respectively) suggested that it could be useful in detecting occult infection.

In our study the relevance of the organisms isolated from the tissue samples was determined using antibiogram patterns, which have low discrimination. To confirm that the isolates were identical, however, would require molecular genotyping using pulsed-field gel electrophoresis. By comparison the ELISA described in our study provided a rapid, sensitive and relatively inexpensive serodiagnostic method for the detection of Gram-positive bacterial infection of prostheses which could be easily adapted into the routine microbiology laboratory. The cost was £6 per test.

We suggest that the ELISA IgG assay to the novel antigen may provide accurate preoperative information to evaluate a painful arthroplasty. Identification of the causative organism before operation by the ELISA for this novel antigen would help the surgeon to decide whether a one- or two-stage revision procedure was appropriate.

The test may also assist in the management of patients in whom the microbiological results are either negative (e.g. because of the use of antibiotics) or based on a single isolate of an organism which may be either a contaminant or a possible pathogen. The test needs further evaluation, however, to confirm its value in the clinical setting.

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