THE JOURNAL OF BONE & JOINT SURGERY (BRITISH)

Improved detection of infection in hip replacements

A CURRENTLY UNDERESTIMATED PROBLEM

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Our aim was to determine if the detection rate of infection of total hip replacements could be improved by examining the removed prostheses. Immediate transfer of prostheses to an anaerobic atmosphere, followed by mild ultrasonication to dislodge adherent bacteria, resulted in the culture of quantifiable numbers of bacteria, from 26 of the 120 implants examined. The same bacterial species were cultured by routine microbiological techniques from only five corresponding tissue samples. Tissue removed from 18 of the culture-positive implants was suitable for quantitative tissue pathology and inflammatory cells were present in all samples. Furthermore, inflammatory cells were present in 87% of tissue samples taken from patients whose implants were culture-negative. This suggests that these implants may have been infected by bacteria which were not isolated by the techniques of culture used.

The increased detection of bacteria from prostheses by culture has improved postoperative antibiotic therapy and should reduce the need for further revision.

J Bone Joint Surg [Br] 1998;80-B:568-72.
Received 17 October 1997; Accepted after revision 9 January 1998

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0301-620X/98/48473 $2.00

Despite the use of ultra-clean-air operating theatres and antibiotic-impregnated bone cement,[1] between 2%[2] and 15%[3] of all revision hip operations are because of infection of the implant.[4] Unfortunately, after revision the rate of infection is higher than after primary procedures, with as many as 40% of revised hips becoming reinfected.[5] This has been attributed to the presence of unrecognised infection at the time of the first revision,[5] possibly because bacteria colonising the surface of implants grow predominantly in adherent biofilms[6] and may not be detected by aspiration or by standard culture techniques.[7] In addition, the well-proven incidence of anaerobic bacteria in joint infection[8] emphasises the need for special methods of culture.

We aimed to improve the isolation of bacteria from hip prostheses removed at revision operations by using mild ultrasonication to dislodge bacteria adhering to the surface of the implant, and strict anaerobic techniques.

Patients and Methods

From March 1996 to May 1997 we retrieved 120 hip prostheses from patients at revision operations. There were 70 women and 50 men with a mean age of 69 years (27 to 92). The mean interval from the previous joint replacement to the revision operation was 8.8 years (4 months to 24 years). All had standard preoperative care.

The skin was painted with Betadine (Seton Healthcare, Oldham, UK) and the incision area covered with an adhesive plastic drape. All operations were carried out with vertical laminar air flow and the operating team wore disposable impervious drapes. Routine antibiotic prophylaxis consisted of 2 g of cefamandole (Kefadol; Dista Products Ltd, Basingstoke, UK) given intravenously at the time of the induction of anaesthesia. Further doses of 1 g of cefamandole were given at eight and 16 hours after surgery. Cefamandole is recommended by the British National Formulary for surgical prophylaxis since it is a broad-spectrum antibiotic for both Gram-positive and Gram-negative bacteria. The removed femoral and acetabular components were placed aseptically in separate sterile bags. Tissue in contact with the implants was also removed and placed in sterile bottles. All specimens were immediately placed in an anaerobic jar for transport to an anaerobic cabinet.

Bacterial isolation and identification. Bacteria attached to the implant surfaces were dislodged into Ringer's solution (25% v/v, 100 ml) by immersion for five minutes in a 150 W ultrasonic bath operating at a nominal frequency of 50 Hz, which has been shown to have no effect on the viability of the infecting micro-organisms.[9]

Total viable bacterial counts were performed as follows: five 0.5 ml volumes of sonicate were plated on to each of blood agar (BA) and anaerobic blood agar (ABA) plates. A known weight of tissue was homogenised for three minutes in Ringer's solution (25% v/v, 5 ml). Three 0.5 ml samples of homogenised tissue were plated on to BA and ABA plates. The remaining homogenised tissue was added in equal amounts to tryptone soya broth (TSB) and cooked meat broth (CMB) for enrichment. The BA and ABA plates were incubated at 37°C aerobically and anaerobically, respectively, and examined after 1, 2, 4 and 7 days. The TSB and CMB cultures were incubated at 37°C aerobically and anaerobically, respectively. Both broths were subcultured on days 7 and 14, on BA for aerobic incubation and on ABA for anaerobic incubation. All plates were incubated at 37°C for 48 hours.

To determine the likelihood of contaminating bacteria being introduced during sampling, 20 retrieved orthopaedic implants were sterilised in a hot-air oven and processed as described above.

All the organisms isolated were Gram stained and identified using commercially available biochemical test galleries (API20A, APIStaph; Biomurieux, France).

Histological examination. Tissue specimens were fixed in unbuffered formal saline. Representative samples were processed and embedded in paraffin wax and 5 μm sections were cut and stained using haematoxylin and eosin.

All slides were subsequently assessed blindly and independently by two histopathologists. The stained sections were scanned at low power and the areas of the slide containing the heaviest inflammatory infiltrate were selected for further examination at higher magnification (×400). If they showed mostly blood clot or were otherwise unsuitable, there was no further assessment, but if the sample was satisfactory, the number of inflammatory cells per high-power field was assessed in five randomly selected fields within the most inflamed area of tissue. The three groups of inflammatory cells assessed were polymorphonuclear leucocytes, lymphocytes and tissue macrophages. For these types of inflammatory cell, the slide was given a score per

Table I. Results of bacterial isolation and tissue pathology in 26 revision hip arthroplasties (see text)

		Implant sampled by sonication		Tissue sampled by homogenisation		Inflammatory cell score*		
Case	Prosthetic component	Isolate(s)	Total viable count (CFU/implant)	Isolate(s)	Total viable count (CFU/g of tissue)†	PMN	LYM	MAC‡
1	Femoral	*S. epidermidis*	$>10^6$	*S. epidermidis*	2.5×10^4	1	1	2
2	Acetabular	*S. epidermidis*	3×10^4	-	-	0	1	2
3	Acetabular	*S. epidermidis*	5×10^2	-	-	1	1	3
4	Femoral	*S. epidermidis*	1.6×10^4	*S. epidermidis*	1×10^3	2	1	1
	Acetabular	*S. epidermidis*	4.1×10^3					
5	Acetabular	*S. capitis*	5×10^2	-	-	0	0	1
6	Acetabular	*S. hominis*	$>10^6$	-	-	0	1	2
7	Femoral	*S. aureus*	1×10^4	*S. aureus*	Isolated by enrichment	0	1	0
	Acetabular	*S. aureus*	$>10^6$					
8	Acetabular	*S. epidermidis/S. capitis*	7.5×10^3	-	-	2	2	2
9	Acetabular	*S. epidermidis/S. hominis*	2×10^3	-	-	0	2	3
10	Femoral	*S. epidermidis/S. hominis*	3×10^4			1	2	3
	Acetabular	*S. epidermidis*	4×10^4					
11	Femoral	*P. acnes*	5×10^2	-	-	NS§	NS	NS
12	Femoral	*P. acnes*	1×10^5	-	-	0	2	3
13	Femoral	*P. acnes*	2×10^3	-	-	NS	NS	NS
14	Femoral	*P. acnes*	1×10^4	*P. acnes*	1.3×10^3	0	1	2
15	Femoral	*P. acnes*	2×10^2	*P. acnes*	1×10^2	2	2	3
	Acetabular	*P. acnes*	4×10^2					
16	Femoral	*P. acnes*	2×10^2	-	-	2	2	3
17	Femoral	*P. acnes*	2×10^2	-	-	1	1	1
18	Acetabular	*P. acnes*	1.6×10^3	-	-			
19	Femoral	*P. acnes*	2.2×10^3	-	-	0	1	2
20	Femoral	*P. acnes*	5×10^4	-	-	NS	NS	NS
21	Femoral	*P. acnes*	6×10^2	-	-	NS	NS	NS
	Acetabular	*P. acnes*	5×10^2					
22	Acetabular	*P. acnes*	8×10^2	-	-	NS	NS	NS
23	Femoral	*S. sciuiri/P. acnes*	1.5×10^3	-	-	NS	NS	NS
24	Femoral	*S. epidermidis/P. acnes*	2×10^4	-	-	NS	NS	NS
25	Femoral	*Micrococcus* sp./*P. acnes*	1×10^3	-	-	1	2	3
26	Femoral	*S. haemolyticus/P. acnes*	1×10^3	-	-	1	1	1
	Acetabular	*P. acnes*	5×10^2					

* mean of five replicates
† mean of three replicates
‡ PMN, polymorphonuclear leucocyte; LYM, lymphocyte; MAC, tissue macrophage
§ not suitable for histological examination

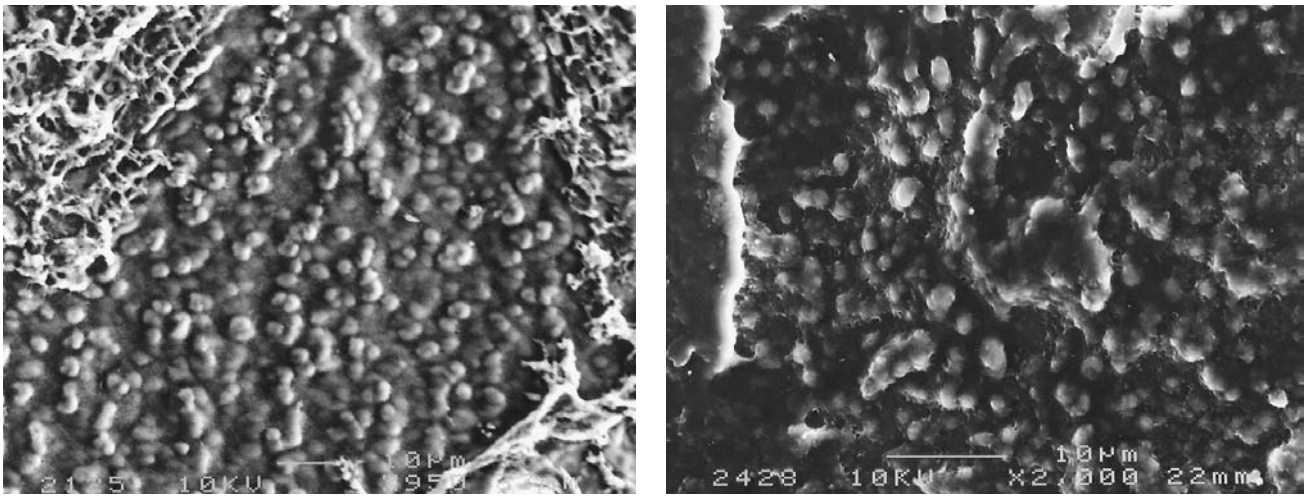

Fig. 1

SEM showing the bacterial biofilm on a retrieved orthopaedic implant viewed at a magnification of (a) ×700 and (b) ×1500.

high-power field: 0, absent; 1, 1 to 10 cells; 2, 10 to 20 cells; or 3, 20 or more cells.

Results

Quantifiable numbers, from 2×10^6 to $>10^6$, of bacteria were cultured from 26 of the 120 samples examined (22%). The infection in seven of the samples was caused by a single *Staphylococcus* species. Three other samples were infected by a combination of two *Staphylococcus* species. The anaerobic bacterium, *Propionibacterium acnes,* was isolated as the single infecting organism in 12 samples and a further four samples were infected by a combination of *P. acnes* and an aerobic Gram-positive coccus (Table I); 16 of the 26 samples (62%) therefore involved *P. acnes*. SEM confirmed that bacteria were able to grow within a confluent biofilm on the surface of the prosthesis (Fig. 1). Organisms similar to those isolated from the implants were cultured from only five of the 26 corresponding tissue samples. All the other tissue samples were culture-negative. No bacteria were isolated from the 20 control prostheses.

Tissue samples from 18 of the 26 culture-positive implants were suitable for histological examination and inflammatory cells were present in all 18 samples (Table I). Lymphocyte and macrophage infiltration was more extensive than polymorphonuclear leucocyte infiltration with 17 samples having lymphocyte and macrophage scores of 1 to 3 compared with only ten with polymorphonuclear leucocyte scores of 1 to 3 (Table II).

Tissue samples from 63 of the 94 culture-negative implants were also suitable for histological examination; inflammatory cells were present in 55 (87%). Lymphocyte and macrophage infiltration was again more extensive than polymorphonuclear leucocyte infiltration. Of the 55 tissue samples, 52 and 54 had lymphocyte and macrophage scores of between 1 and 3, respectively, whereas only 31 samples had a similar level of polymorphonuclear leucocyte infiltration (Table II).

We examined the hospital records of 18 of the 26 patients with culture-positive implants and of 52 of the 94 patients with culture-negative implants. Of the 18 culture-positive patients, 12 had a preoperative diagnosis of either aseptic loosening or dislocation, and the other six patients had been suspected of having infection. In four of these six patients, however, no bacteria had been cultured from either joint fluid aspirated preoperatively or from tissue samples removed at the time of surgery. Preoperative aspiration of joint fluid in the other two patients resulted in the isolation of *Staphylococcus epidermidis* (case 4) and *S. aureus* (case 7). Similar organisms to those isolated preoperatively were cultured from the implants after sonication and from the surrounding tissue after homogenisation. Of the 52 patients with culture-negative implants, 50 had a preoperative diagnosis of either aseptic loosening or dis-

Table II. The relationship between inflammatory cell presence and bacterial isolation by culture

	Inflammatory cell score											
	PMN*				**LYM†**				**MAC‡**			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Number of culture-positive implants	8	6	4	0	1	10	7	0	1	4	6	7
Number of culture-negative implants	32	24	5	2	11	17	30	5	9	8	15	32

* polymorphonuclear leucocyte
† lymphocyte
‡ tissue macrophage

location and two a preoperative diagnosis of suspected septic loosening. No bacteria were cultured from aspirated joint fluid removed from these two patients.

Discussion

We used mild ultrasonication to disrupt adherent microbial biofilms formed on the surface of retrieved orthopaedic implants to improve isolation and allow direct quantitation of the infecting bacteria. We have previously shown the efficacy of ultrasound in dislodging adherent bacteria growing within microbial biofilms on the surface of peritoneal catheters[10] and ureteral stents.[11] Of the 120 orthopaedic implants retrieved and examined, we cultured viable bacteria in quantifiable numbers from 26 (22%). Our review of records from 18 of the 26 patients with culture-positive implants showed that infection had been suspected in only six and that in only two had this suspicion been confirmed by the culture of bacteria from aspirated joint fluid. Previous studies examining only tissue samples have been reported to show an incidence of infection of from 2% to 15%.[2,3] We isolated similar types of viable bacteria from only five corresponding tissue samples, possibly because of the bacteriostatic or bactericidal effects of the cefamandole administered at the time of operation or because of the host's defences. The bacteria growing within a biofilm on the surface of the device would have a degree of protection from both. Our results therefore support the limited study of Gristina and Costerton[7] who found that sonication of tissue, bone and methylmethacrylate cement removed with 25 implants recovered larger numbers of bacteria than from joint fluid, or swabs of excised tissue and prosthetic surfaces. If we had not used ultrasonication most of the samples diagnosed as showing infection would have been misdiagnosed as non-infected, and would not have received appropriate postoperative therapy.

The organisms which we isolated are similar to those reported by others,[5,12,13] but the proportion from which the obligate anaerobe, *Propionibacterium acnes*, was isolated (62%) was much higher than the 0% to 27% which has previously been reported for anaerobes.[2,12] The increase in detectable viable bacteria is probably due to the mild ultrasonication, which dislodges bacteria from within a biofilm, together with immediate transfer of the retrieved implants to an anaerobic atmosphere. It is unlikely that the bacteria represent contamination: no bacteria were isolated from 20 sterilised implants which provided control samples. We also found a good correlation between the organisms isolated and the inflammatory response in the adjacent tissue. It has previously been shown that the presence of acute or chronic inflammation in tissue samples correlates well with intraoperative cultures and therefore can be used to aid the diagnosis of infection.[14-16] In our study, tissue from 18 infected samples was suitable for histological examination; inflammatory cells were present in all 18. Lymphocyte and macrophage infiltration predominated, which suggests low-grade indolent infection rather than the acute inflammatory reactions which would be characterised by more extensive polymorphonuclear leucocyte infiltration. This was not unexpected; 16 of the 18 implants had been in situ for more than two years.

In some patients (cases 15 and 16, Table I) we isolated low numbers of viable bacteria despite high inflammatory scores. This suggests that we are still underestimating the total viable bacterial numbers present on the prostheses. Gristina and Costerton[7] examined the surfaces of retrieved implants by EM and noted that sometimes the morphological forms of the isolated bacteria were different, concluding that some bacteria within the biofilm were not cultured even after ultrasonication.

The two possible sources of infecting bacteria are operative contamination and haematogenous spread from a distant source of infection. In only one of our samples, was infection by *Staphylococcus aureus* alone (case 7), and this implant had been in situ for less than one year. Previous reports suggest that virulent organisms such as *Staphylococcus aureus* are usually responsible for early infections.[13,17] Our other positive samples were all infected by either coagulase-negative staphylococci or *Propionibacterium acnes*. These low-virulence organisms, part of normal skin and dental microbiota, often cause delayed clinical infections with a long interval between the inoculation of the bacteria and the onset of symptoms. This may often mimic the natural 'aseptic' loosening of a prosthesis.[12] In our study, 23 of the 25 implants infected by these bacteria had been in place for over two years and would have been classified as delayed infections.

Some delayed infections are caused by bacterial contamination at the time of operation. Kamme and Lindberg[13] demonstrated a relationship between wound contamination and postoperative infection by *Staphylococcus epidermidis* and *Propionibacterium acnes*. Delay in the diagnosis may be due to the low virulence and slow growth of the organism, the small size of the inoculum and the suppression of infection by prophylactic antibiotics. Late infections are also due to the haematogenous spread of bacteria from a distant source of infection. Bartzokas et al[18] showed that *Streptococcus sanguis* isolates responsible for implant infections could be isolated from the mouths of the corresponding patients, and concluded that the route was possibly haematogenous. More recently, Stoll et al[19] reported the culture of *Propionibacterium acnes* and *Peptostreptococcus micros* from the synovial fluid of a patient with an infected total knee prosthesis. The patient also had an abscess at the root of a wisdom tooth suggesting a dental origin of the infection. Similarly, Debelian, Olsen and Tronstad[20] showed that *Propionibacterium acnes* strains isolated from the root canal of patients during endodontic therapy were similar to those isolated from blood, suggesting that this organism can spread haematogenously. Its antigen has also been detected in the synovial fluid of patients suffering from rheumatoid arthritis.[21] The bacteria which we isolated

could therefore have resulted from intraoperative seeding or haematogenous spread.

We found high inflammatory scores in 87% of the tissue samples from patients whose implants were culture-negative. Infiltration with lymphocytes and macrophages was predominant, which suggests that the implants removed from these patients may have also been infected by low-virulence organisms which were not isolated by the techniques used. Similar results were obtained by Fehring and McAlister[14] in a smaller study of nine patients in whom the histological evaluation was positive and the cultures negative; in six there was a high clinical suspicion of infection.

We believe that infection of retrieved hip prostheses is currently underestimated. We recommend that the removed implants are sonicated to dislodge adherent bacteria and then sampled directly to improve bacterial isolation under anaerobic conditions. The examination of tissue alone does not always result in the isolation of the causative organism. The improved detection and isolation allow the use of suitable antibiotics for a longer postoperative period, which should improve the clinical outcome. We are now using polyclonal antisera and monoclonal antibodies specific for the infecting bacteria and 16S rRNA oligonucleotide probes to increase the detection of non-culturable bacteria.

We wish to thank Martin Clulow and James McKenna for technical assistance and the Electron Microscopy Unit, the Queen's University of Belfast and the Department of Bacteriology, Belfast City Hospital.

Dr M. Tunney and the work were funded by the Arthritis and Rheumatism Council for Research, UK (Project grant number P0522); Gordon Ramage was funded by a Department of Education for Northern Ireland Studentship; and Donna Hanna was funded by a European Social Fund Grant.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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