A comparative study of the use of bioactive glass S53P4 and antibiotic-loaded calcium-based bone substitutes in the treatment of chronic osteomyelitis

A RETROSPECTIVE COMPARATIVE STUDY

The treatment of chronic osteomyelitis often includes surgical debridement and filling the resultant void with antibiotic-loaded polymethylmethacrylate cement, bone grafts or bone substitutes. Recently, the use of bioactive glass to treat bone defects in infections has been reported in a limited series of patients. However, no direct comparison between this biomaterial and antibiotic-loaded bone substitute has been performed. In this retrospective study, we compared the safety and efficacy of surgical debridement and local application of the bioactive glass S53P4 in a series of 27 patients affected by chronic osteomyelitis of the long bones (Group A) with two other series, treated respectively with an antibiotic-loaded hydroxyapatite and calcium sulphate compound (Group B; n = 27) or a mixture of tricalcium phosphate and an antibiotic-loaded demineralised bone matrix (Group C; n = 22). Systemic antibiotics were also used in all groups.

After comparable periods of follow-up, the control of infection was similar in the three groups. In particular, 25 out of 27 (92.6%) patients of Group A, 24 out of 27 (88.9%) in Group B and 19 out of 22 (86.3%) in Group C showed no infection recurrence at means of 21.8 (12 to 36), 22.1 (12 to 36) and 21.5 (12 to 36) months follow-up respectively, while Group A showed a reduced wound complication rate.

Our results show that patients treated with a bioactive glass without local antibiotics achieved similar eradication of infection and less drainage than those treated with two different antibiotic-loaded calcium-based bone substitutes.

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Chronic osteomyelitis may follow haematogenous, post-traumatic or post-operative bacterial colonisation. Staphylococcus aureus (S. aureus), and other Gram-positive pathogens, are the most commonly involved. Complete eradication of infection remains challenging. Given the limited ability of antibiotics to penetrate poorly vascularised or devitalised infected bone and surrounding tissues, treatment often requires surgical intervention, including removal of all implants, infected or necrotic bone, and any severely affected soft tissues.

The management of any bone defect resulting from the septic process itself or surgical debridement has been addressed in various ways. These include local or free soft-tissue flaps, bone transport, or often the local antibiotic treatment delivered via loaded polymethylmethacrylate (PMMA), bone graft or bone substitutes.

The main drawbacks of antibiotic-loaded PMMA include possible thermal damage to the antibiotic as the cement cures, the need for a second intervention to remove the PMMA and the facilitation of small colony variant multiresistant strains through inadequate concentrations of the slowly released antibiotic, including possible biofilm formation on the bone cement itself.

To overcome these problems, antibiotic-loaded bone grafts or bone substitutes have been proposed. Unlike bone grafts, bone substitutes do not require harvesting and processing, have unlimited availability, have no risk of transmitting viral disease, and provide predictable antibiotic release. Local antibiotic delivery has been shown to provide high local antibiotic levels in the presence of low serum concentrations, thereby reducing the risk of systemic toxicity and side effects. The local antibiotics in bone substitutes currently supplement systemic antibiotic therapy and, eventually, may even replace it.

Bioactive glass (BAG) has been shown to have antibacterial, osteoconductive and angiogenic properties, which could make it suit-
able to treat bone defects in infections. In particular, BAG-S53P4 (composition SiO$_2$, Na$_2$O, CaO, P$_2$O$_5$) has been reported to facilitate tissue growth by chemically binding to bone matrix, thereby promoting new bone formation,\textsuperscript{19} while antibacterial properties are related to an increase in the local pH and osmotic pressure through the release of sodium and calcium ions and phosphorus salts, which renders the environment hostile for bacterial adhesion and the subsequent proliferation of bacterial pathogens. A remarkably large spectrum of bactericidal activity has been attributed to this biomaterial,\textsuperscript{15,20,21} and its antibiofilm properties have recently been described.\textsuperscript{22} No induction of bacterial resistance to BAG-S53P4 has been reported to date.

Despite the increasing amount of \textit{in vitro} evidence of the antibacterial properties of BAG, and its long-term clinical use in treating chronically infected bone in craniomaxillofacial surgery,\textsuperscript{2,3,22} to our knowledge only two clinical studies, with a limited series of 11 and 3 patients respectively, have explored the potential use of BAG for the treatment of chronic osteomyelitis in the orthopaedic setting.\textsuperscript{18,26}

Given the recent approval of BAG S53P4 (BonAlive, BonAlive Biomaterials Ltd, Biolinja, Finland) as a medical device in Europe for the treatment of osteomyelitis, we undertook a study to evaluate its efficacy and safety in a large continuous series of patients treated for chronic osteomyelitis involving the long bones. This was a retrospective, non-randomised comparative study between a consecutive series of patients treated with two different calcium-based antibiotic-loaded bone substitutes or with bioactive glass S53P4. The study was performed in two centres that specialise in the treatment of bone and joint infections. The main outcomes of interest were rate of recurrent infection and local or systemic side effects.

**Patients and Methods**

This study consisted of three consecutive series of patients, age > 18 years, with a clinical, laboratory and radiologically established diagnosis of chronic osteomyelitis of the long bones (duration > six months) who required surgical debridement and bone void filling. Exclusion criteria were segmental bone defects, associated septic arthritis, or the need for concomitant local plastic surgical procedures.

Surgical debridement was combined with systemic antibiotic administration and the local application of bioactive glass S53P4 (BonAlive) (Group A; n = 27), antibiotic-loaded hydroxyapatite and calcium sulphate (PerOssal, aap Biomaterials GmbH, Dieburg, Germany) (Group B; n = 27), or a combination (230:1 w/w) of tricalcium phosphate (Calcibon granules, Biomet Deutschland GmbH, Berlin, Germany) and teicoplanin-loaded demineralised bone matrix (Targobone, Ossacur AG, Oberstenfeld, Germany) (Group C; n = 22).

Primary endpoints of the study were an absence of recurrent or persistent infection at follow-up based on an absence of a draining sinus or local clinical signs of acute inflammation, with a normal CRP and ESR and no need for further surgery at any stage during follow-up. The secondary endpoint was the absence of local or systemic side effects from the local application of the bone substitute. Data were collected by two observers (NL, DR).

All patients gave informed consent to participate in the study, which was approved by the ethics committee of our institution and conducted in accordance with institutional standards. The study was partially funded by the Italian Ministry of Health (grant no. 4060/10).

**Patient characteristics.** All patients underwent pre-operative clinical examination, laboratory investigation and radiological evaluation, which included CT, MRI and isotopic bone scans, according to their history, duration of symptoms, previous surgical procedures and clinical presentation. Demographic information, time of surgical intervention from first diagnosis of infection, number of previous operations, pathogenesis, site of infection, clinical presentation, anatomo-pathological classification according to Cierny and Mader,\textsuperscript{2} and whether the patient had impaired immunity, according to the McPherson classification,\textsuperscript{3} are reported in Table I.

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Table I. Pre-operative data of the three groups of patients

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Total number of patients</td>
<td>27</td>
<td>27</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>19/8</td>
<td>16/11</td>
<td>14/8</td>
<td>0.69</td>
</tr>
<tr>
<td>Mean age (yrs), SD (range)</td>
<td>45.2, SD 13.6 (19 to 80)</td>
<td>47.0, SD 13.1 (24 to 74)</td>
<td>44.9, SD 14.2 (23 to 77)</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean ESR (mm/h), SD (range)</td>
<td>60.8, SD 44.3 (15 to 122)</td>
<td>55.3, SD 38.7 (18 to 108)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Site (tibia/femur/humerus/other)</td>
<td>8/8/9/10</td>
<td>8/8/11</td>
<td>4/8/10</td>
<td>0.90</td>
</tr>
<tr>
<td>Stage (Cierny–Mader classification) (1/2/3/4)</td>
<td>8/1/6/2</td>
<td>8/2/15/2</td>
<td>5/1/15/1</td>
<td>0.98</td>
</tr>
<tr>
<td>Host type (McPherson classification) (A/B/C)</td>
<td>9/1/7/1</td>
<td>10/1/15/1</td>
<td>10/1/12/0</td>
<td>0.80</td>
</tr>
<tr>
<td>Clinical presentation at the time of surgery (acute/subacute/chronic)</td>
<td>8/7/12</td>
<td>8/8/11</td>
<td>6/6/10</td>
<td>1.00</td>
</tr>
<tr>
<td>Patients with draining sinus at the time of surgery</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean ESR (mm/h), SD (range)</td>
<td>58.5, SD 41.7 (18 to 115)</td>
<td>60.8, SD 44.3 (15 to 122)</td>
<td>55.3, SD 38.7 (18 to 108)</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean serum C-reactive protein (mg/l), SD (range)</td>
<td>98.9, SD 73.3 (8 to 180)</td>
<td>98.9, SD 73.3 (8 to 180)</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>
**Surgical procedure.** Surgery was performed according to the same protocol in all patients and by the same surgical team. In brief, with the patient lying supine, a surgical incision was made at the focus of the osteomyelitis, using the previous scar when present. After accurate dissection of the soft tissues, bone was exposed. In some cases intra-operative fluoroscopy was used to localise the lesion. Removal of all foreign materials, when present, including all metallic materials and bone substitutes and macroscopically infected/necrotic tissues, was then performed. Opening of the osteomyelitic focus was usually performed with a surgical oscillating saw and osteotomes, in order to make a bone window approximately 1 cm to 2 cm wide and 2 cm to 8 cm long, depending on the infected site. Debridement of the medullary canal was then performed with curettes, osteotomes and motorised burrs. The medullary canal proximal and distal to the lesion was opened. Repeated lavage with saline and haemostasis were then performed. The use of a tourniquet was avoided or kept to a minimum.

After debridement and re-gowning and re-gloving, the bone defect was filled with the following bone substitutes:

- **Group A:** BAG S53P4 granules and no local antibiotic;
- **Group B:** PerOssal pellets, intra-operatively loaded with antibiotics targeted to the infecting pathogen, whenever a pre-operative antibiogram was available (n = 21). In the remaining 6 patients, a combination of vancomycin and meropenem was used;
- **Group C:** Calcibon granules mixed with Targobone. All 22 patients in this group had a pre-operative positive culture examination for Gram-positive pathogen(s) sensitive to teicoplanin.

Patients of Group A were operated from 2010 to 2012, while patients of Group B and C were operated from 2009 to 2012.

The defects were packed and the granules gently impacted until no more biomaterial could be accommodated. No steps were taken to prevent the biomaterial spreading within the intramedullary canal or being distributed outside the bone, other than by suturing the fascia over the bone defect. The size of the bone defect was measured during surgery, based on the amount of bone substitute required.

One patient in Group A underwent intramedullary nailing and one had an Ilizarov external frame applied. One patient in Group B and two in Group C received an axial external fixator. In all cases, the need for supplementary stabilisation resulted from mechanical instability at the septic focus, owing to previous trauma, surgery or local debridement. All operations were performed as one-stage procedures, including bone stabilisation when needed. Suction drains were not used in any group.

Post-operatively, patients from all groups received four to 12 weeks of systemic antibiotic therapy, often determined by prior consultation with an infectious disease specialist and/or our microbiology department. A combination of two antibiotics was usually administered, targeted to the isolated microorganism(s) when known, or based on local policies when the cultures were negative (usually intravenous vancomycin or teicoplanin and meropenem for 14 days, followed by a combination of oral levofloxacin and rifampicin after discharge home). Thromboprophylaxis with low molecular weight heparin was provided four to six weeks post-operatively in all cases.

**Microbiological analysis.** Microbiological analysis was conducted on any removed foreign material, swabs and tissue samples (n = 5 to 8). Samples were collected aseptically at operation and delivered to the laboratory for analysis within 30 minutes. Tissue samples and foreign materials were processed by sonication as previously described.27

Swabs and 100 μl of sonicated samples were seeded onto chocolate agar (CA), mannitol salt agar (MSA), MacConkey agar (MC) Schaedler blood agar (SBA), Sабouraud agar (SA,) brain–heart infusion broth (BHI) and thioglycolate broth (TH). CA and MC plates were incubated at 37°C for 24 hours both in a 10% CO₂-enriched atmosphere and an aerobicosis (‘normal’ atmosphere). Incubation of MSA and SA lasted 48 hours in the aerobicosis, whereas SBA was incubated anaerobically at 37°C for 48 hours. After incubation, growth and colony counts were recorded for both aerobes and anaerobes. BHI and TH were incubated for 15 days at 37°C and checked daily for microbial growth.

Identification was performed at both biochemical (Vitek2 Compact, Biomérieux, Marcy l’Etoile, France) and genotypic level. Genotypic identification was performed by DNA sequencing of about 80 base pairs of variable regions V1 and V3 of the 16S rRNA gene by pyrosequencing (PSQ96RA, Diatech, Jesi, Italy). Obtained sequences were inserted in the Basic Local Alignment Search Tool (BLAST)28 to achieve accurate identification.

**Post-operative follow-up.** All patients underwent clinical and laboratory evaluation, including haemochromocytometric analysis with leukocyte formula (study of leukocyte morphotypes) and determination of ALT, AST, creatinine, CRP and ESR, at 15 and 30 days and at three, six, nine, 12, 18, 24 and 36 months after surgery.

Wound healing after the debridement was assessed by an independent observer (LM), according to the following clinical score:

- **Excellent:** complete wound healing achieved uneventfully in 15 days, the surgical wound remaining dry, without any serum leakage at all times;
- **Good:** wound healing achieved within 15 days, with some drainage or leakage;
- **Fair:** a wound showing prolonged sterile drainage or serum leakage, with time to healing >15 days and less than six weeks, eventually requiring additional sutures;
- **Poor:** no wound healing for more than six weeks, or a wound requiring additional surgical procedures.

Radiographs were obtained at three, six and 12 months post-operatively, and then yearly thereafter. Radiographic examination was performed by an experienced radiologist in order to discover the bone substitute incorporation in
the host bone, measured as the amount of bone substitute still visible on the radiograph, presence of osteolysis, periosteal reaction or fracture lines at the site of surgical debridement at follow-up.

Any early and late side effects that might relate to the local application of bone substitutes, were recorded at each visit.

**Statistical analysis.** Statistical evaluation of the data was performed using SPSS v12.1 (SPSS Inc., Chicago, Illinois). After the verification of normal distribution and the homogeneity of the variance, quantitative data were examined by analysis of variance (ANOVA) using Bonferroni correction or the non-parametric Kruskal–Wallis test for those variables not normally distributed. The Mann–Whitney U test was used to detect significant differences between groups by adopting Bonferroni correction.

Qualitative data are reported as frequencies and percentages and compared using the two-tailed Fisher’s exact test, and p < 0.05 was considered statistically significant.

**Results**

Pre-operative data did not differ significantly among groups (Table I). Isolated microorganisms are shown in Figure 1. The most common pathogen in all groups was *S. aureus*, with a predominance of methicillin-resistant strains.

The estimated sizes of the bone defects are reported in Table II. Primary closure of the wound was obtained in all patients.

Compared with Group A, the mean hospital stay was 2.5 (31%) and 3.2 (40%) days longer for Groups B and C, although this difference was not found to be statistically significant (Table II). Systemic antibiotic therapy was generally continued with a combination of two antibiotics for approximately six weeks in all groups. Intravenous administration was usually performed for the first two weeks, followed by oral administration whenever possible, according to the sensitivity of the isolated pathogen(s).

In Group A, wound healing was considered excellent in 18 patients, good in six, fair in one owing to prolonged

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**Table II. Post-operative data**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean follow-up (mths), SD (range)</td>
<td>21.8, SD 7.2 (12 to 36)</td>
<td>22.1, SD 8.0 (12 to 36)</td>
<td>21.5, SD 7.9 (12 to 36)</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean hospital stay (days), SD (range)</td>
<td>8.0, SD 6.9 (4 to 18)</td>
<td>10.5, SD 8.1 (6 to 24)</td>
<td>11.2, SD 8.3 (7 to 23)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean systemic antibiotic treatment duration (weeks), SD (range)</td>
<td>6.6, SD 2.1 (4 to 12)</td>
<td>6.5, SD 2.2 (4 to 12)</td>
<td>6.6, SD 1.9 (5 to 12)</td>
<td>0.99</td>
</tr>
<tr>
<td>Wound healing (excellent/good/fair/poor)</td>
<td>18/6/1/1</td>
<td>11/9/7/1</td>
<td>6/10/6/0</td>
<td>0.02</td>
</tr>
<tr>
<td>Prolonged serum wound leakage (&gt; 2 weeks)</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean bone defect (ml), SD (range)</td>
<td>29.3, SD 13.0 (10 to 60)</td>
<td>33.8, SD 24.4 (6 to 76)</td>
<td>31.8, SD 18.4 (10 to 0)</td>
<td>0.66</td>
</tr>
<tr>
<td>No infection recurrence (%)</td>
<td>25 (92.6)</td>
<td>24 (88.9)</td>
<td>19 (86.3)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

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**Fig. 1**

Graph of microorganisms isolated from the different patient groups.
serum leakage (which finally required further closure) and poor in one. This last patient developed skin necrosis and delayed bone exposure, which required a local muscular flap eight months after surgery. Neither patient showed any infection at the latest follow-up.

Overall, prolonged serum leakage (more than two weeks from surgery), was observed in only one patient (3.7%) in Group A, compared with eight patients (29.6%) in Group B (p = 0.02) and six (27.2%) in Group C (p = 0.03, Mann–Whitney U test).

Other complications included one deep vein thrombosis each in Group A and Group B, a late fracture at the surgical site in one patient in Group A, and the development of renal insufficiency in one patient in Group C.

One patient in Group A (tetraplegic and tracheostomised) died 13 months after surgery from the sequelae of a pre-existing recurrent pneumonia, and one in Group B died in a car accident 27 months after surgery. No recurrence of infection at the osteomyelitic site was evident clinically or radiologically in either patient at their last follow-up.

At a mean follow-up of 21.8 months (12 to 36), two patients in Group A showed a recurrence of infection at six months from debridement; one of them also suffered a spontaneous fracture at the site of infection, requiring external fixation. Overall, 27 patients (92.6%) in Group A showed no sign of recurrence/persistence of infection at final follow-up.

After a similar period of follow-up, 24 patients (88.9%) in Group B showed no clinical or laboratory signs of recurrent infection (one required a delayed plastic procedure one month after surgery, due to poor soft-tissue healing) and 19 patients (86.4%) in Group C were free of infection. The difference was not statistically significant (Table II). Considering only patients with at least two years’ follow-up, we observed two recurrent infections out of 20 patients (10%) in Group A, two out of 21 patients in Group B (9.5%) and two out of 16 in Group C (12.5%).

At latest follow-up, the radiographs showed partial incorporation of all the three bone substitutes under study; the biomaterial could still be seen on the plain radiographs, although there were no signs of osteolysis or periosteal reactions.

Discussion
We found that BAG-S53P4 is as effective as two different calcium-based antibiotic-loaded bone substitutes, with a significant reduction in prolonged wound serum leakage and a trend towards reduction in hospital stay. Persistent serum wound leakage has previously been reported with calcium-based bone substitutes, and is a fairly common occurrence, in contrast to our experience with the use of BAG-S53P4.

Our results, obtained in chronic bone infections caused by a number of different pathogens, including methicillin- and multi-resistant Gram-positive and Gram-negative strains, and even in mixed flora, reflect and extend the range of antibacterial activity of BAG-S53P4, previously reported in in vitro studies and in other clinical applications. We are unaware of any reported bacterial resistance to this compound. Our results on a larger population confirm the observations of previous studies, showing approximately a 90% eradication rate at a mean of 21.8 months’ follow-up (12 to 36), irrespective of the isolated pathogen and the host type.

Of the two patients showing recurrent infection, one had a polymicrobial aetiology (Pseudomonas aeruginosa, S. aureus and Enterococcus spp.), and in the other MRSA was isolated at surgery. Further analysis of the failed cases suggests, for one patient, an insufficient filling of the bone defect, as he had a long infected nail crossing both the tibia and the femur, after a knee arthrodesis; this was removed at the time of debridement and bioglass application. Filling such a large defect was not feasible in this particular case. In a previous study on 11 patients, Lindfors et al proposed that outcome after BAG-S53P4 treatment might be related to proper filling of the cavities. The other failed patient in Group A had a reactivation of osteomyelitis of the tibia after two years, at a site which had been infected for 22 years prior to surgery. This patient had a concomitant soft-tissue defect which, with hindsight, should probably have received simultaneous soft tissue flap coverage at the time of debridement, but this was not performed because direct closure was achieved.

We have shown that BAG-S53P4 can be safely and effectively used to fill bone defects after debridement in bone infections without local antibiotics. Even though both in vitro and in vivo studies have reported on a possible combination of bioactive glass with antibiotics to treat osteomyelitis, to our knowledge there is no evidence that this association provides better clinical results than BAG-S53P4 alone.

We acknowledge the limitations of this study. It was retrospective in nature, but the homogeneous inclusion criteria followed in our department for over a decade, and the remarkable similarities between the continuous series of patients included in this study, may reduce the influence of this potential bias. In addition, the clinical evaluation of wound healing used in this study was based on a simple descriptive scoring and has not been validated. In this regard there is no available score of wound healing specifically validated for bone infection surgery, and all reported scores showed bias and questionable inter-observer reproducibility, often including variables not directly related to the wound, such as the length of hospital stay. We have reported on only one BAG. Since the 1960s when this biomaterial was first described for clinical use, various BAG compositions have been shown to have bone binding and antibacterial properties. However, the respective capacity of bone bonding and resorption rates are highly affected by the chemical composition. Therefore, evaluation of the clinical impact of each bioglass is needed in order to assess
their respective clinical safety and efficacy profiles. With respect to bone graft substitutes, only one study on 19 patients affected by chronic osteomyelitis and treated with PerOssal has so far been reported. In general, the use of all available bone substitutes for the treatment of osteomyelitis has only limited supporting evidence.

In conclusion, notwithstanding the limitations described, this study confirms and reinforces previous limited reports on the safety and efficacy of BAG-S53P4 in the treatment of chronic osteomyelitis of the long bones, in the presence of multiresistant strains, in immunocompromised hosts, and without the need for supplementary local antibiotics.

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References