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INFECTION

A sophisticated antibiotic-loading protocol in articulating cement spacers for the treatment of prosthetic joint infection

A RETROSPECTIVE COHORT STUDY

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Objectives

The optimal protocol for antibiotic loading in the articulating cement spacers for the treatment of prosthetic joint infection (PJI) remains controversial. The objective of the present study was to investigate the effectiveness of articulating cement spacers loaded with a new combination of antibiotics.

Methods

A retrospective cohort study involving 114 PJI cases treated with implantation of an articulating cement spacer between 2005 and 2016 was performed. The treatment outcomes of the conventional protocol (i.e. gentamicin and vancomycin (GV protocol)) were compared with those reported using the sophisticated antibiotic-loading protocol (i.e. vancomycin, meropenem, and amphotericin (VMA protocol)).

Results

There were 62 and 52 PJI cases treated with the GV and VMA protocols, respectively. Antimicrobial susceptibility testing revealed that 22/78 of all isolates (28.2%) in this series were resistant to gentamicin, whereas there were no vancomycin-, meropenem-, or amphotericin-resistant strains. The overall infection recurrence rates were 17.7% (11/62) and 1.9% (1/52), respectively ($p = 0.006$). In patients with a negative preoperative culture, there was no infection recurrence reported in the VMA cohort (0/45 (0%) *vs* 10/54 (18.5%) in the GV cohort; $p = 0.002$). Multivariate analysis indicated that the VMA protocol correlated with a decreased risk of infection recurrence compared with the GV protocol ($p = 0.025$).

Conclusion

The sophisticated VMA protocol for the loading of antibiotics in articulating cement spacers, as part of a two-stage exchange, was associated with a reduced rate of infection recurrence. This proposed protocol appears to be safe and effective, especially in patients with negative culture results prior to the first-stage operation.

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Keywords: Two-stage revision, Articulating cement spacers, Prosthetic joint infection, Antibiotics

Article focus

- Protocol and outcomes of antibiotic loading in articulating cement spacers of two-stage revision.

Key messages

- The vancomycin, meropenem, and amphotericin antibiotic-loading protocol for articulating cement spacers yields better infection control than the gentamicin/vancomycin protocol.

Strengths and limitations

- A new antibiotic-loading protocol was proposed and comprehensively tested in comparison with the conventional protocol.
- This was a retrospective comparative study and therefore bias may exist.

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Introduction

Prosthetic joint infection (PJI) is one of the most catastrophic complications following total joint arthroplasty.[1] Two-stage revision remains the optimal procedure for eradicating infection, with reported success rates ranging from 80% to 95%.[2,3] An antibiotic-loaded bone cement (ALBC) articulating spacer is commonly used for the local delivery of antibiotics and maintenance of joint function in the interim period.

Various antibiotics with different elution characteristics have been used in the antibiotic cement. The most commonly used antibiotics are gentamicin, tobramycin, and vancomycin.[4] Currently, there are established guidelines available regarding the selection of antimicrobials to be used in ALBC for the management of patients who are culture-positive prior to the first-stage operation. However, approximately 5% to 12% of PJIs are culture-negative, and a larger proportion of cases are not culture-positive prior to the first-stage procedures. Although it plays an important role in the successful treatment of PJI, the empirical selection of antibiotic to be loaded in articulating cement spacers for the treatment of patients infected with undetermined organisms prior to the first-stage operation remains unclear.[5] Furthermore, the percentage of PJIs caused by Gram-negative bacilli or multidrug-resistant bacteria, as well as that of polymicrobial and fungal PJIs, is increasing. Therefore, improved combinations of antibiotics loaded into articulating cement spacers are required to cover a wider range of pathogens encountered in PJIs and reduce the risk of reinfection.[6-8]

In our institution, both the GV protocol (i.e. articulating cement spacers containing gentamicin and vancomycin) and VMA protocol (i.e. articulating cement spacers loaded with vancomycin, meropenem, and amphotericin if necessary) have been used in an effort to improve the success rate of the two-stage exchange for the treatment of PJI.

The purpose of the current study was to investigate the clinical outcomes associated with the two aforementioned protocols in the treatment of PJI, especially in cases with negative culture prior to the first-stage operation.

Patients and Methods

Patient enrolment. A retrospective review of our institutional PJI database between 1 January 2005 and 31 June 2016 was performed to include all patients who underwent implantation of an antibiotic-loaded articulating cement spacer as part of an intended two-stage exchange for the treatment of prosthetic hip or knee joint infection. The diagnosis of PJI was finally determined by our PJI panel after evaluation of all available perioperative information based on the Musculoskeletal Infection Society (MSIS) criteria.[9] The exclusion criteria included the following: surgical treatment for PJIs in multiple joints; less than two years of follow-up after implantation of the articulating cement spacer unless an endpoint event (e.g. reinfection) occurred; two-stage revision for non-prosthetic joint infection; and two-stage revision with only a superficial infection.

Identification of pathogenic organisms. Joint aspiration was performed in every patient suspected of PJI. In the occurrence of a 'dry tap' during aspiration, the preoperative culture was considered to be negative. Microorganisms isolated from the sinus tract and draining wound were not thought to be the definite pathogens of PJI. Only microorganisms isolated from joint aspiration were considered to be the definite preoperative pathogens of PJI. Intraoperatively, synovial fluid samples were collected and analyzed microbiologically (Gram stain and culture). Prosthetic soft tissue with inflammatory changes was collected for microbiological and histopathological assays. Since August 2011, sonication of explanted prostheses, followed by incubation of the resulting sonicate fluid, was also carried out for the detection of pathogens.[10] Samples harvested preoperatively and intraoperatively were inoculated in both BD Bactec Aerobic and Anaerobic blood bottles, and analyzed using a BD Bactec 9240 automated blood culture system (Becton, Dickinson and Company, Franklin Lakes, New Jersey) for five days. Detection of microorganisms warranted further investigation using the VITEK automated microbial identification system (bioMérieux, Marcy-l'Étoile, France). The antimicrobial susceptibility of Gram-positive and Gram-negative bacteria was tested using the minimum inhibitory concentration (MIC) method, while the antimicrobial susceptibility of fungus was tested using the microtitration method.

The antimicrobial-loading protocols in ALBC for the treatment of PJI. All patients were initially treated with resection arthroplasty, including radical debridement, removal of a prosthesis, implantation of an articulating ALBC, and administration of systemic antimicrobial agents for the control of joint infection. At the first stage, in some cases, Orthosonics System for Cemented Arthroplasty Revision (OSCAR; Orthosonics, Maidenhead, United Kingdom) was used to remove the firmly fixed bone cement in deep marrow cavity.

Prior to 1 June 2012, the GV antimicrobial-loading protocol for articulating cement spacers included gentamicin at 0.5 g per 40 g of cement and vancomycin at 3 g per 40 g of cement.[11] The treatment strategy was gradually changed after 1 January 2012 to the new VMA antimicrobial protocol using articulating cement spacers impregnated with vancomycin, meropenem, and amphotericin. Between 1 January 2012 and 1 June 2012, both antimicrobial protocols were used in our institution according to surgeon choice. The VMA antimicrobial protocol was used for all patients from 1 June 2012. The detailed antibiotic-loading method of the sophisticated VMA protocol is shown in Table I.

Table I. Detailed information of the vancomycin, meropenem, and amphotericin protocol (antibiotics added per 40 g cement)

Infection	Vancomycin, g	Meropenem, g	Amphotericin, g
G+ without sinus	2.5	0.5	0
G+ with sinus	3	1	0
G- without sinus	0.5	2.5	0
G- with sinus	1	3	0
Fungus without sinus	0.5	0.5	0.15
Fungus with sinus	1	1	0.1
Culture-negative	2	2	0
Polymicrobial (G+ and G-)	2	2	0
Fungus and G+	1.5	0.5	0.1
Fungus and G-	0.5	1.5	0.1

G+, Gram-positive bacteria; G-, Gram-negative bacteria

The administration of intravenous antibiotics was continued and adjusted postoperatively according to the intraoperative culture and antimicrobial susceptibility testing for two weeks during hospitalization. Discharged patients continued to receive oral antibiotics for at least four weeks. After completion of the antibiotic treatment, the levels of ESR and CRP were measured at least three times. A gradual decrease in the levels of inflammatory markers compared with those observed at the time of implant removal – in combination with absence of symptoms related to infection – was a prerequisite for proceeding with reimplantation.[12]

Data collection and outcome measures. Basic information, such as age, sex, infection type, antibiotic-resistant microorganisms, prior irrigation and debridement (I&D), host grade,[13] and sinus was extracted from our institutional medical records. The preoperative and intraoperative culture outcomes and associated antimicrobial susceptibility test outcomes were extracted from the database of our institutional microbiology laboratory. In addition, treatment outcomes, the latest follow-up date, complications, and time of their occurrence were recorded by chart review and/or targeted telephone interview.

The assessment of the treatment outcome was initiated at the time of the first operation for PJIs, which was the first stage of two-stage exchange in our study.[14] To balance the different follow-up timespans between the two protocols, the follow-up results at two years were utilized to compare the outcomes. In addition, infection control was defined as not meeting the MSIS criteria for PJIs and not requiring further surgery. The latter excluded the planned reimplantation of a two-stage exchange, a procedure for a complication related to the antibiotic spacer, or a planned operation to address soft-tissue problems between the two stages.[15]

Statistical analysis. A chi-squared test or Fisher's exact test was used as appropriate for the analysis of categorical data. The Shapiro–Wilk test was used to test the normality of the data. An independent Student's *t*-test or the non-parametric Mann–Whitney U test was used for between-group comparisons of numerical data. A Kaplan–Meier survival analysis was used to compare the survival rates between different protocols. Logistic regression was performed to identify risk factors for reinfection. Model fitting for the logistic regression included all risk factors that were significant in the univariate analysis. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed using SPSS 21 (IBM, Armonk, New York) and Prism 5 (GraphPad Software, La Jolla, California).

Results

A total of 130 patients with PJI treated with implantation of articulating cement spacers as part of intended two-stage exchange were identified. Of those, 114 patients were eligible for analysis.

The GV protocol cohort included 62 PJI cases (31 knees and 31 hips), while the VMA protocol cohort included 52 PJI cases (26 knees and 26 hips). The two cohorts were comparable in terms of age, sex, infection type, antibiotic-resistant microorganisms, prior I&D, host grade,[13] and sinus (Table II).

Microbiological profiles. In the GV and VMA protocol cohorts, there were 20 (32.3%) and 9 (17.3%) culture-negative cases, respectively ($p = 0.085$, chi-squared test). The causative microorganism was identified preoperatively in only 12.9% (8/62) and 13.5% (7/52) of patients in the GV and VMA protocol cohorts, respectively ($p = 1.000$, chi-squared test). *Staphylococcus aureus* was the most commonly isolated bacterium in both cohorts ($p = 0.132$, chi-squared test). Overall, the distribution of microbiological species between the GV and VMA protocols was not significantly different (Table III).

Overall, in this series, 22/78 of the tested isolates (28.2%) were resistant to gentamicin, whereas there were no vancomycin-, meropenem-, or amphotericin-resistant strains identified. For *S. aureus*, the rates of resistance against gentamicin and vancomycin were 19.2% and 0.0%, respectively ($p = 0.019$, Fisher's exact test). For coagulase-negative staphylococci (CoNS), the rates of resistance against gentamicin and vancomycin were 33.3% and 0.0%, respectively ($p < 0.001$, Fisher's exact test). For Gram-negative strains, the rates of resistance against gentamicin and meropenem were 31.3% and 0.0%, respectively ($p = 0.015$, Fisher's exact test). The detailed rates of antibiotic resistance for the GV and VMA protocols are shown in Table IV.

Treatment outcomes. Among all patients, 28/114 patients (24.6%) retained the spacer as a definitive treatment method because of general health conditions, psychological factors, or acceptable function of the articulating cement spacer *in situ*. For the 86/114 patients (75.4%) who proceeded with the intended reimplantation, the mean interval between stages was 5.5 months (3 to 32) (Fig. 1, Table II).

The overall rates of infection recurrence in the GV and VMA protocol cohorts were 17.7% (11/62 patients) and 1.9% (1/52 patients), respectively ($p = 0.006$, chi-squared

Table II. Demographic characteristics of overall patients

Demographic	GV protocol (n = 62)	VMA protocol (n = 52)	p-value
Mean age, yrs (range)	67.5 (36 to 86)	64.9 (42 to 84)	0.189*
Female, n	34	28	1.000†
Sinus, n	31	22	0.454†
Host grade, n			0.279†
A	28	23	
B	30	21	
C	4	8	
Multiple infections, n	11	6	0.434†
Multiple-resistant microorganisms, n	19	19	0.553†
Prior irrigation and debridement, n	14	13	0.827†
Reimplantation, n	47	39	1.000†
Dislocated or fractured spacer, n	7	1	0.069†
Spacer exchange, n	10	0	0.002‡§
Mean interval time, mths (SD; range)	5.5 (4.9; 3 to 32)	5.4 (3.5; 3 to 21)	0.844*
Mean follow-up, mths (range)	112.1 (24 to 174)	43.6 (24 to 74)	< 0.001*§
Mean ASA score (range)	2.0 (1 to 3)	1.8 (1 to 3)	0.203*
Preoperative negative culture, n	54	45	1.000†

*Independent Student's *t*-test
†Chi-squared test
‡Fisher's exact test
§Statistically significant
GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin; ASA, American Society of Anesthesiologists

Table III. Microbiological profiles of overall patients

Causative microorganism	GV protocol (n = 62)	VMA protocol (n = 52)	p-value
Staphylococcus aureus, n	11	10	1.000*
MRSA, n	5	3	0.726*
CoNS, n	11	16	0.124*
MRCoNS, n	6	10	0.422*
Streptococcus species, n	0	2	0.206†
Enterococcus species, n	3	0	0.249†
Gram-negative bacillus, n	3	5	0.466*
Fungus, n	4	2	0.687*
Mycobacterium tuberculosis, n	0	2	0.206†
Polymicrobial, n	10	6	0.592*
Negative culture, n	20	9	0.085*

*Chi-squared test
†Fisher's exact test
GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin; MRSA, methicillin-resistant *Staphylococcus aureus*; CoNS, coagulase-negative staphylococci; MRCoNS, methicillin-resistant coagulase-negative staphylococci

test). For patients without reimplantation, these rates were 53.3% (8/15 patients) and 7.7% (1/13 patients), respectively (p = 0.010, chi-squared test). For patients with reimplantation, these rates were 6.4% (3/47 patients) and 0.0% (0/39 patients), respectively (p = 0.108, Fisher's exact test).

Preoperatively, 54 and 45 patients in the GV and VMA protocol cohorts were culture-negative and the rates of infection recurrence were 18.5% (10/54 patients) and 0.0% (0/45 patients), respectively (p = 0.002, Fisher's exact test). Among preoperative culture-negative patients without reimplantation, the rates of infection recurrence were 53.8% (7/13 patients) and 0.0% (0/12 patients), respectively (p = 0.005, Fisher's exact test). Among preoperative culture-negative patients with reimplantation, the rates of infection recurrence were 7.3% (3/41 patients) and 0.0% (0/33 patients), respectively (p = 0.249, Fisher's exact test).

Of note, there were no antibiotic-related complications observed in either of the groups.

In the Kaplan–Meier survival analysis, the overall survival rates at two years using infection recurrence as the endpoint were 81.7% and 97.5% in the GV and VMA protocol cohorts, respectively (p = 0.007, log-rank test) (Fig. 2a). In patients without reimplantation, these rates were 45.8% and 91.7%, respectively (p = 0.014, log-rank test) (Fig. 2b). In patients with reimplantation, these rates were 93.1% and 100.0%, respectively (p = 0.111, log-rank test) (Fig. 2c).

In patients with negative preoperative culture, the two-year survival rates in the GV and VMA protocol cohorts were 81.0% and 100.0%, respectively (p = 0.003, log-rank test) (Fig. 3a). Among preoperative culture-negative patients without reimplantation, these rates were 45.5% and 100.0%, respectively (p = 0.003, log-rank test) (Fig. 3b). Among preoperative culture-negative patients with reimplantation, these rates were 92.2% and 100.0%, respectively (p = 0.119, log-rank test) (Fig. 3c).

In summary, lower rates of infection recurrence and higher rates of survival were observed in the VMA protocol cohort. These results further confirmed the better performance of the proposed VMA protocol over the GV protocol in the interim period.

Analysis of risk factors for treatment failure. There were no significant differences in the rates of reinfection between the GV and VMA protocol cohorts in terms of age, sex, host grade, multiple infections, MRSA or MRCoNS, negative culture, or preoperative negative culture. However, patients with sinus exhibited a significantly increased rate

Table IV. Antibiotic susceptibility of infected microorganisms (resistant isolates/total isolates). Microorganisms isolated from polymicrobial infection were also included in the analysis

Antibiotic	GV protocol				VMA protocol			
	S	C	G-	F	S	C	G-	F
Gentamicin	2/14	6/17	1/10	NT	3/12	6/19	4/6	NT
Meropenem	NT	NT	0/10	NT	NT	NT	0/6	NT
Vancomycin	0/14	0/17	NT	NT	0/12	0/19	NT	NT
Amphotericin	NT	NT	NT	0/7	NT	NT	NT	0/2

GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin; S, *Staphylococcus aureus*; C, coagulase-negative staphylococci; G-, Gram-negative bacteria; F, fungus; NT, not tested

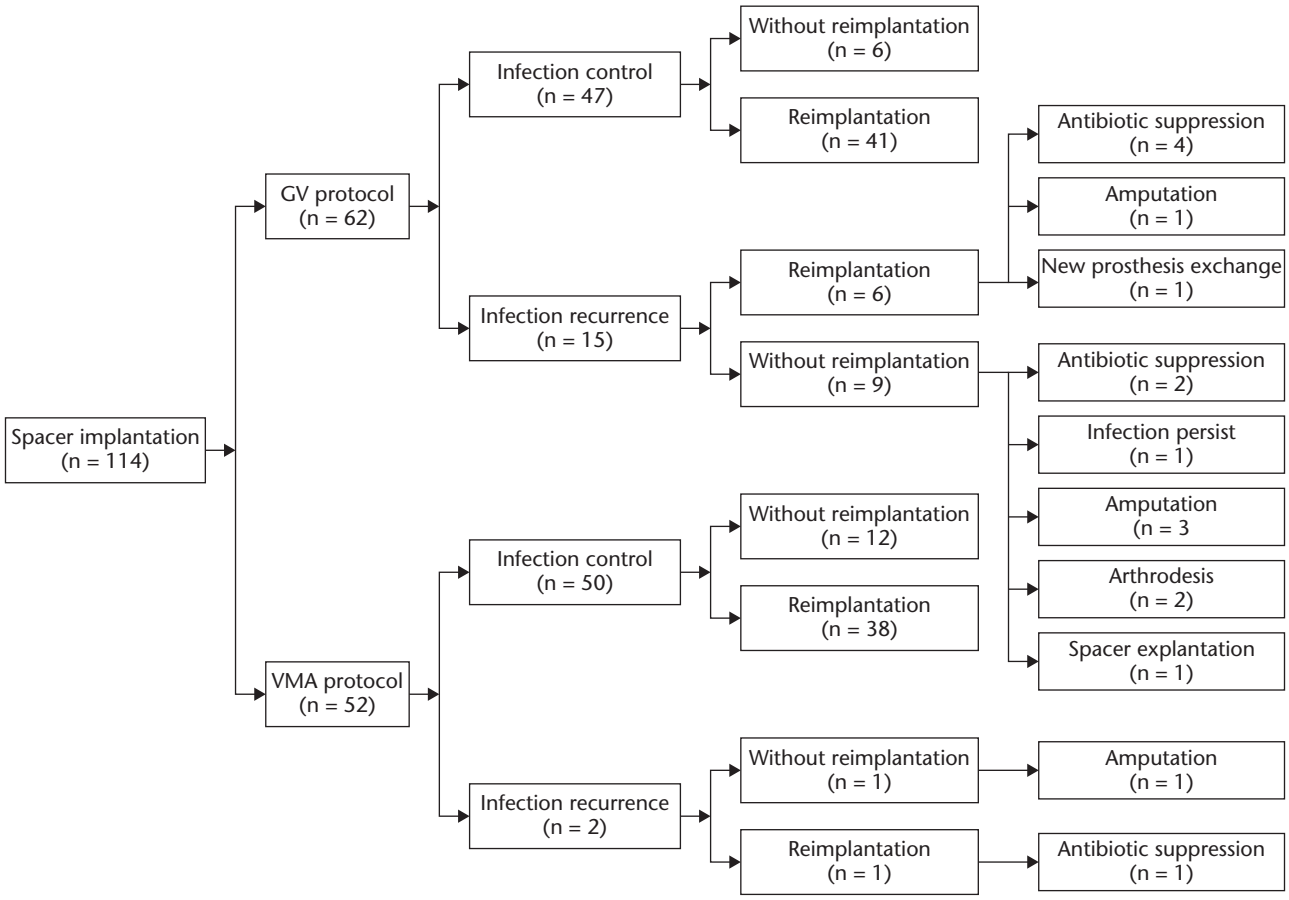

Fig. 1

The outcome flowchart of 114 prosthetic joint infection (PJI) cases treated with different antibiotic-loading protocols. The results shown in this flowchart were obtained at the last follow-up. GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin.

of recurrence ($p < 0.001$, chi-squared test). Moreover, it was found that use of the VMA protocol was associated with a reduced risk of reinfection compared with use of the GV protocol ($p = 0.006$, chi-squared test) (Table V).

A multivariate regression model indicated that although sinus was associated with an increased trend, it was not shown to be a significant predictor of failure ($p = 0.997$). The VMA protocol was associated with a reduced risk of reinfection compared with the GV protocol (odds ratio (OR) = 0.087; 95% confidence interval 0.010 to 0.733; $p = 0.025$) in the multivariate analysis.

Discussion

Two-staged revision with the implantation of an articulating antibiotic spacer for PJI remains one of the most reliable methods for the eradication of infection.[16] However, the most appropriate antibiotics to be added to a cement spacer remains a matter of debate.[17] Currently, guidelines are available for cases in which the causative pathogen is confirmed preoperatively. Although new techniques for the detection of causative pathogens have emerged (e.g. sonication of an explanted prosthesis, 16s ribosomal DNA (rDNA) test, and next-generation

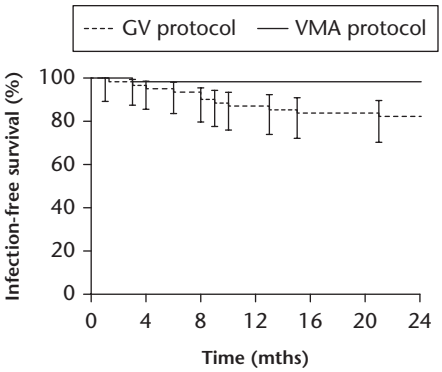

Fig. 2a

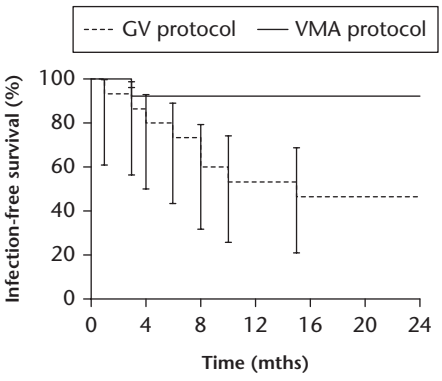

Fig. 2b

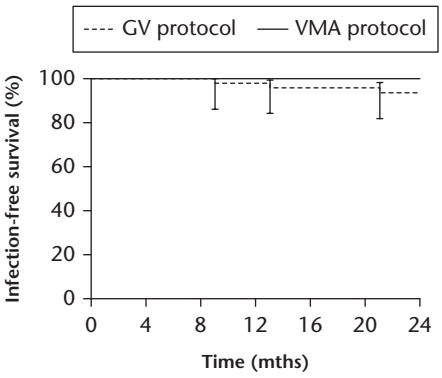

Fig. 2c

Kaplan–Meier infection-free survival analysis of overall patients. a) Survival rates of patients with and without reimplantation (p = 0.007, log-rank test). b) Survival rates of patients without reimplantation (p = 0.014, log-rank test). c) Survival rates of patients with reimplantation (p = 0.111, log-rank test). GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin.

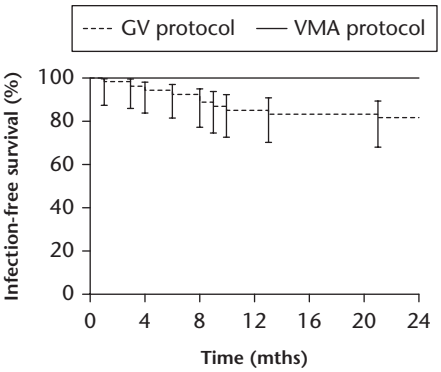

Fig. 3a

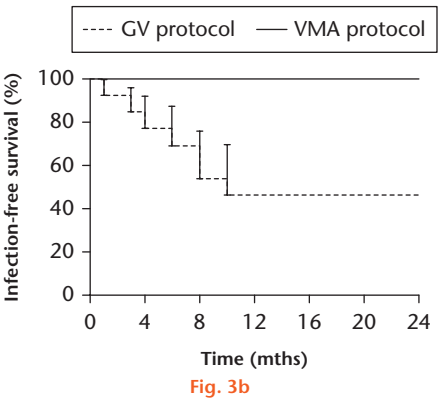

Fig. 3b

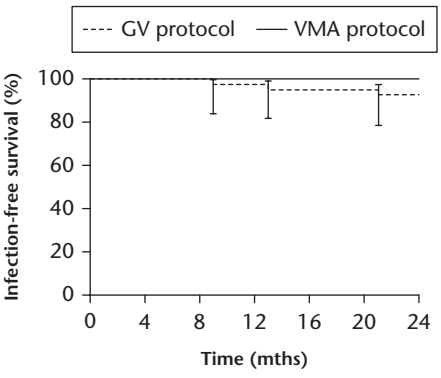

Fig. 3c

Kaplan–Meier infection-free survival analysis of patients with negative preoperative culture. a) Survival rates of patients with and without reimplantation (p = 0.003, log-rank test). b) Survival rates of patients without reimplantation (p = 0.003, log-rank test). c) Survival rates of patients with reimplantation (p = 0.119, log-rank test). GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin.

sequencing),[18,19] microbiological information can only be obtained a few days after the first-stage operation. In our institution, the causative pathogen can only be confirmed in < 20% of patients prior to the first-stage operation of the two-stage revision. The relatively low preoperative culture rate may be partially influenced by the strict microbiological process implemented in our institution. First, lavage was not performed in the case of 'dry tap' during joint aspiration. This may influence the accuracy of the culture, and cause infection in an otherwise aseptic arthroplasty.[20,21] Second, microorganisms isolated from the sinus tract and draining wound were considered to be potentially contaminated pathogens from the sinus tract and draining wound closure, rather than the pathogens that caused PJI.[22,23] Third, preoperative aspiration was inoculated in BD Bactec blood bottles and cultured for only five days, which was recommended by most studies in order to avoid the increase of false-positive results with prolonged culture.[10,24,25] Therefore, establishing an effective protocol to guide the selection of antibiotics for articulating cement spacers is of great importance.

Table V. Risk factors for infection recurrence in univariate analysis

Variable	Total	Treatment failure (n = 12)	Treatment success (n = 102)	p-value
Mean age, yrs (range)	66.3 (36 to 86)	68.6 (36 to 86)	66.0 (38 to 85)	0.430*
Female, n	62	6	56	0.769†
Sinus, n	53	12	41	< 0.001‡†
Host grade, n				0.895†
A	52	5	47	
B	50	6	44	
C	12	1	11	
Antibiotic protocol, n				0.006‡†
GV	62	11	51	
VMA	52	1	51	
Preoperative negative culture, n	99	10	89	0.658†
Non-MRSA, MRCoNS (1), n	37	3	34	(1) *vs* (2): 0.694; (1) *vs* (3): 0.189; (1) *vs* (4): 0.625†
MRSA, MRCoNS (2), n	31	4	27	(2) *vs* (3): 0.428; (2) *vs* (4): 0.355†
Multiple infections (3), n	17	4	13	(3) *vs* (4): 0.055†
Negative culture (4), n	29	1	28	All: 0.171†

*Independent Student's *t*-test
†Chi-squared test
‡Statistically significant
N/A, not applicable; GV, gentamicin and vancomycin; VMA, vancomycin, meropenem, and amphotericin; MRSA, methicillin-resistant *Staphylococcus aureus*; MRCoNS, methicillin-resistant coagulase-negative staphylococci

The combination of vancomycin and gentamicin in a cement spacer is commonly used to treat PJI with a potential synergistic effect against *Staphylococcus* biofilms.[26] However, there is increasing evidence of bacterial resistance in infected hip and knee prosthetic joints against gentamicin. It has been reported that approximately 50% of staphylococci responsible for prosthetic infections were resistant to gentamicin, while none were resistant to vancomycin.[27] It was also observed that a third of all Gram-negative bacteria were resistant to gentamicin.[28] In another study, the frequency of gentamicin resistance was 56.4% among isolated bacteria from PJI cases. Moreover, the emergence of small-colony forms of *S. aureus* in gentamicin monotherapy and its inactivity against enterococcal isolates renders this antibiotic agent an unsuitable choice when used alone, rather than in combination with another antibiotic.[29] Furthermore, among CoNS from the revision total hip and knee arthroplasties performed for infection, 77% were resistant to gentamicin. During a ten-year period (between 2001 and 2010), the rate of resistance of CoNS to gentamicin increased from 32% to 47%.[30]

Meropenem has a broad spectrum of *in vitro* activity against Gram-positive and Gram-negative pathogens, including extended-spectrum β-lactamase (ESBL) and AmpC beta-lactamase producing Enterobacteriaceae.[31] Meropenem retains antimicrobial properties following exposure to high temperatures, which occur during poly(methyl methacrylate) (PMMA) curing,[32] and thermal stability is important for the loading of antibiotics in cement spacers.[33] In our study, the resistance rate of Gram-negative bacteria to meropenem was lower compared with that observed for gentamicin. This finding is consistent with those of previous reports.[34] In addition, the combination of meropenem and vancomycin was more effective in treating polymicrobial infection and preoperative culture-negative infections. Meropenem is consistently associated with low resistance rates in Enterobacteriaceae and staphylococci isolates and did not exhibit a widespread change in resistance rates between 1999 and 2008. In contrast, *Escherichia coli* strains and all Enterobacteriaceae strains exhibited a consistently increasing rate of resistance against aminoglycosides.[35]

In vitro antibiotic elution, antibacterial activity, and mechanical performance of vancomycin and carbapenem for broad-spectrum coverage have been studied in several studies.[27,28,36] Notably, the mechanical properties of the bone cement were negatively affected following the addition of 0.5 g of vancomycin to a bone cement containing 0.5 g of meropenem.[27] Additionally, a combination of 1.25% vancomycin and 1.25% meropenem may be an interesting compromise between the introduction of antibacterial properties and preservation of mechanical properties.[36] Another *in vitro* study showed that the combination of vancomycin and meropenem in a cement spacer was effective in inhibiting the growth of *Staphylococcus, Enterococcus, Pseudomonas*, and *E. coli*.[28] To the best of our knowledge, a more comprehensive *in vivo* evaluation of carbapenem used in spacers has not previously been reported. The successful addition of ertapenem to the cement spacers for the treatment of polymicrobial PJIs in the two-stage revision has been described; however, that study did not include a control group, and the efficacy and safety of ertapenem-containing spacers in the treatment of monomicrobial PJIs were not evaluated.[37]

The addition of meropenem increases the elution of the vancomycin from the cement spacer and broadens its antibacterial spectrum.[27] Therefore, the VMA protocol

contains a relatively low dose of antibiotics of each type to balance the antimicrobial activity and reduce systemic toxicity in patients in whom vancomycin was administered intravenously after the first-stage operation.[38] Furthermore, a synergetic antibacterial effect was observed against *S. aureus* when subinhibitory vancomycin and meropenem were combined *in vitro*.[39] Although the elution of amphotericin B from the cement remains controversial, the implantation of amphotericin B-loaded cement spacers has been reported to eradicate fungal PJIs successfully.[40] Moreover, the addition of antibiotics to cement spacers for the treatment of fungal PJI may lead to a reduction in the incidence of secondary bacterial joint infections.[8] Our protocol against fungal PJI, which has not been previously studied, consists of vancomycin, meropenem, and amphotericin. It was efficacious in eradicating fungus PJI without noticeable nephrotoxicity.

A previous study suggested that the number of patients who failed to undergo reimplantation was consequential, and almost 20% of patients who underwent resection arthroplasty and spacer insertion did not undergo a subsequent reimplantation.[14] In our study, in patients who did not undergo reimplantation, the rate of infection control in the VMA protocol cohort was markedly higher than that reported in the GV protocol cohort. However, in patients with reimplantation, the outcome was not significantly different between the two protocols. This evidence suggests that the favourable overall results observed in patients treated with the VMA protocol are derived from better infection control in the interim period.

The limitations of this study should be recognized. First, this was a single-centre retrospective study with potential uncontrolled selection biases among subgroups. Secondly, the duration of the follow-up for each group was different; however, we compared the rate of infection control at two years after implantation of an articulating cement spacer to balance the bias. Thirdly, the *in vitro* antibiotic elution, antibacterial activity, and mechanical performance of the VMA antibiotic-loading method were not tested in the present study, which needs further research. Finally, the retrospective period was relatively long. The method of microbiological culture advanced during the examined period. For example, due to the application of the culture of sonicated explanted prostheses, the intraoperative culture-negative rate decreased in the VMA protocol. In addition, the experience of surgeons increased during the study period, which may bring potential bias. However, the methods to fabricate spacers were the same in VMA and GV protocols, and the same surgeons were involved in both protocols. In this way, the potential confounding factor was mitigated.

Despite the aforementioned limitations, the VMA antibiotic-loading protocol was associated with a lower rate of infection recurrence and a better rate of survival compared with the conventional GV protocol. Moreover, multivariate analysis revealed that the VMA protocol correlated with a lower risk of infection recurrence, and the protocol was especially applicable to patients with preoperative negative culture.

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