



Two-stage hip revision arthroplasty for periprosthetic joint infection without the use of spacer or cemented implants

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Abstract

Introduction There is no gold standard for performing a two-stage exchange arthroplasty to treat periprosthetic joint infection (PJI). The use of spacers and the anchorage principles of the revision prosthesis remain controversial. Herein, we report the success rate of a two-stage total hip replacement procedure without using a spacer and only pressfit cementless implants.

Methods Between 2009 and 2015, 57 patients with chronic late-onset PJI were treated using a two-stage prostheses exchange without spacer. The average age was 66.7 years (47–83 years). The mean follow-up was 53.9 ± 25 months. Treatment included microbiologic diagnostics and a high-efficiency antimicrobial therapy in between the operations for six weeks and a two week antibiotic-free interval before reimplantation of the cementless prostheses. After implantation, antibiotics were stopped. This study was approved by the institutional review board.

Results Ninety-six percent of the patients had prior unsuccessful PJI treatment in other hospitals. The most common microorganism was *Staphylococcus epidermidis* (50.9%), followed by *Propionibacterium acnes* (17.5%) and *Staphylococcus aureus* (14%). In 42.1% cases, mixed infections were found. All patients could be treated using a cementless implant. In 91.2%, PJI remission was achieved, while 8.6% had chronic PJI with implant retention. Overall, nine prostheses (15.8%) were replaced owing to ongoing PJI or fractures. Mean modified Harris Hip Score was 60.85 (range: 22–88). None of the patients died.

Conclusion We demonstrated a high success rate for two-stage exchange of infected total hip arthroplasty. Spacer-free treatment does not negatively affect success rate or function. Implantation of an uncemented pressfit prosthesis was possible in all patients.

Keywords PJI · Periprosthetic joint infection · Two-stage exchange · Cementless · Pressfit · Spacer

Introduction

The number of endoprosthetic procedures is increasing annually, and concomitantly, the number of revision surgeries is on the rise. The incidence of periprosthetic joint infection (PJI) following total hip arthroplasty (THA) procedures increased from 1.99 to 2.18% in the period from 2001 to 2009 in the USA [1].

Till date, there is no international gold standard for the treatment of PJI. The outcome of two-stage revision surgery for implant replacement versus single-stage procedures is being investigated. In addition, no definitive guidelines are provided for the different procedures. The two-stage exchange is considered most appropriate for chronic difficult-to-treat cases. Complex interdisciplinary treatment strategies with modular implants and treatment-resistant pathogens represent not only a professional but also a financial challenge. Therapy regimens with one or two-stage implant replacements are on average approximately 3.4–6 times more cost-intensive than

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the primary implantation itself [2]. However, in difficult-to-treat and difficult late-onset cases, the two-stage exchange remains the Benchmark, owing to the published high remission rates [3]. Essentially, no standard operating procedure for the two-stage exchange could be agreed upon [4], and there are a lot of controversial points like the duration of prostheses-free intervals or the right time to carry out the replantation [4]. It yet remains unclear if a spacer should be used, and whether the new prostheses should be cemented. Antimicrobial impregnated static or articulating spacers are often used to manage dead space and deliver local antimicrobial therapy until a permanent prosthesis can be implanted. The reported overall complication rate of spacers is very high (22–58%) [5]. In detail, there are about 17% luxations, 14% fractures of the bone, 10% damages to the spacer, and damage to the acetabular bonestock [5]. Fifty-three to 68% of all polymethyl methacrylate (PMMA) spacers show bacterial contamination after removal [6]. Used since the 1970s, PMMA is today the most widely used carrier system for local antibiotics and the base of spacers. However, there are some disadvantages that limit the use of PMMA: First is the need for its surgical removal in a subsequent procedure. Used in bone defects, the non-resorbable PMMA prevents osteointegration in the desired bone. Despite the initial antibiotic potency, the surface of the cement acts as an adhesive for bacteria and can itself lead to bacterial colonization, as well as the development of bacterial resistance and biofilm formation [7]. Furthermore, its unfavourable release and an incomplete elution limit regular deployment. In addition to the active substance, the size of the surface involved in the diffusion is decisive. The rate of release of the PMMA bone cement decreases by 6–12% [8]. Today, favorable antibiotic carriers like degradable calcium-sulfate show superior elution kinetics and less biofilm development [9–11]. The time from resection arthroplasty to reimplantation varies significantly from two weeks to several months, and the use of antibiotic-impregnated cement and spacers has not been evaluated in randomized controlled trials [4].

In case of replantation of the new prostheses, the type of fixation, i.e., cemented versus non-cemented, remains controversial. Although the optimal component fixation method in primary THA is still debated, several studies have shown increased use of the cementless technique in most parts of the world [12, 13]. Worldwide use of cementless fixation for primary total hip arthroplasty (THA) is on the rise despite some evidence from the world's registries suggesting inferior survivorship compared with cemented techniques [12]. However, data for septic revision cases are lacking.

Advantages of cementation are lower perioperative fracture rate in osteoporosis and osteopenia and a better post-operative pain control, whereas, in up to 45% of the revisions, severe peri-operative incidents occur [14]. On the other hand, there are some specific aspects for revision surgery. There is an over

80% reduction in cement-bone interface shear strength between primary and revision arthroplasty [15], which explains the higher loosening and revision rates of cemented implants reported in these cases. In the case of a new revision, there are clearly more difficult prerequisites for cemented prostheses.

Despite all previous findings, there is yet no standard concerning details of the two-stage exchange including the use of spacers and the anchorage principles of the revision implant [14].

Aim

The aim of the current study was therefore to evaluate the success rate of the two-stage total hip replacement procedure without using any spacer and no cemented implants. Success rates and clinical outcomes are shown.

Material and methods

Patient characteristics

Patients with chronic and late-onset periprosthetic infection of the hip joint were included. The infected implants were total hip arthroplasties, no hemiarthroplasties. None of the patients fulfilled the criteria for a single-step exchange according to the guidelines of the *Infectious Diseases Society of America* (IDSA Guidelines) [4]. All patients fulfilled the criteria of a late-onset chronic infection. Patients with early infections or the possibility of a one-stage replacement or debridement, antibiotics, and implant retention (DAIR) procedure were excluded. The study was approved by the institutional review board (IRB). Between 2009 and 2015, 57 patients were included with a minimum follow up of 14 months. The mean follow-up period was 53.9 ± 25 months, while the suggested minimum follow-up in the study protocol was 14 months.

Diagnostics

- PJI was diagnosed in all patients according to the current published diagnostic criteria [16–18], and all patients were retrospectively proofed to fit the IDSA guidelines [4] and the latest consensus meeting [17, 18] regarding the presence of a periprosthetic infection. Effective antibiotic therapy requires reliable bacterial detection; we therefore performed a sterile joint puncture (sent as blood culture) pre-operatively and obtained three to five tissue samples and a joint puncture intra-operatively. In addition, blood and synovial diagnostics were performed according to the mentioned guidelines [17].

Surgical procedure

- A two-stage exchange was performed as previously published [19], recently redescribed [20] and modified as follows:

This strategy involves at least two procedures. In the first step, cultures were obtained, all infected tissue was debrided, and the components and PMMA were removed [21]. A calculated systemic antibiotic therapy was initiated after intra-operatively collecting microbiological samples during the first intervention. The calculated systemic therapy was performed using a broad-range antibiotic that provided good penetration of the soft tissue. The antibiotics were changed three days after the procedure in accordance with the results of the actual antibiogram. Antibiotics were selected according to the principles of antibiotic stewardship, taking into account sensitivity and tolerability. Six weeks of systemic antibiotic therapy was administered, an initial intravenous therapy of two weeks followed four weeks of pathogen-specific highly bioavailable oral antimicrobial treatment [22, 23]. No spacers were used. A Girdlestone Situation (Resection Arthroplasty) was performed. In the prostheses-free periods, no biofilm-targeting antibiotics were used (like Rifampin). Clinical and laboratory monitoring for efficacy and toxicity was performed. Monitoring of outpatient antimicrobial therapy followed published guidelines [24]. After six weeks of high-efficient antimicrobial therapy, an antimicrobial-free interval of two weeks was observed, during which the patient was evaluated for any signs of ongoing infection, by using inflammatory markers and clinical assessment [4, 20, 21]. If there was evidence of ongoing infection, a repeat radical debridement procedure was performed including tissue sample-testing and followed by further antimicrobial therapy before attempted reimplantation.

If the “PJI Assessment” showed no evidence of ongoing PJI, a new prosthesis was implanted, without any cement. We used different types of prostheses according to the existing

bone stock at the femoral bone or the acetabulum. Table 1 provides details of all implants used in the study. The antimicrobial treatment with intravenous application was carried out until the reimplantation cultures were finalized as negative. In case of absence of PJI signs and/or negative tissue samples, antibiotic therapy was discontinued. If reimplantation cultures were positive, a DAIR procedure was carried out as previously published [19] and recently redescribed [25] including the use of Rifampin if the bacteria were gram-positive and in the absence of contraindications. If no remission occurred after one DAIR procedure, the prostheses was removed, and the two-stage exchange procedure was ready to be started. Figure 1 summarizes this workflow and Figs. 2 and 3 are showing two characteristic case-reports.

Patient monitoring

Monitoring of the patients was carried out according to published guidelines [4, 24]. Evaluation of the patient included a physical examination and examination of wound healing, current clinical symptoms, drug allergies, and intolerances, comorbid conditions, prior and current microbiology results from aspirations and surgeries, and antimicrobial therapy for the PJ. A test for C-reactive protein (CRP) as well as complete blood count and electrophoresis was performed. A plain radiograph was performed in all patients prior to and after surgery. Blood cultures for aerobic and anaerobic organisms as well as procalcitonin were obtained in cases of patients presenting with fever.

Success rate, outcome measures, and statistics

Clinical outcome was assessed after the minimum follow-up of 14 months (mean follow-up: 53.9 ± 25 months). “Success” and “remission” of PJI were defined as the absence of clinical, radiological, and biological (i.e., inflammatory markers) signs of infection [4] after the individual follow-up. In addition, we described the success of the treatment directly after the prostheses implantation during hospital stay (Table 2(a, b)), and after the whole follow-up (Table 2(c, d)). If the cementless revision implant had to be exchanged for any reason, it was defined as “removed” or “exchanged” and reasons are given (Table 2(c)).

The modified Harris Hip Score (mHHS), which has high validity and reliability [26], was used to measure the functional outcome. The HHS is a functional outcome score to assess pain, function, absence of deformity, and range of movement. When the patient-reported portion of the score is completed and the physical examination is omitted, it is referred to as the mHHS, and shows a maximum of 91 instead of 100 (HHS). There are no clinically meaningful differences in outcomes between the mHHS and the HHS [27]. The statistical analysis was performed using IBM SPSS statistics version 21 (IBM Germany GmbH, Ehningen, Germany).

Table 1 Used cementless Pressfit implants as revision implant after two-stage exchange

Acetabular component		Number	Percent
Allofit	(Zimmer-Biomet, CH)	48	84.2
MRS-Titan	(Peter Brehm, Germany)	7	12.3
Trabecular Metal	(Zimmer-Biomet, CH)	2	3.5
Femural component		<i>n</i>	<i>%</i>
MRP-Titan	(Peter Brehm, Germany)	41	71.9
Hyperion	(Zimmer-Biomet, CH)	7	12.3
Metabloc	(Zimmer-Biomet, CH)	3	5.3
Revitan	(Zimmer-Biomet, CH)	3	5.3
Mutars	(Implantcast, Germany)	3	5.3

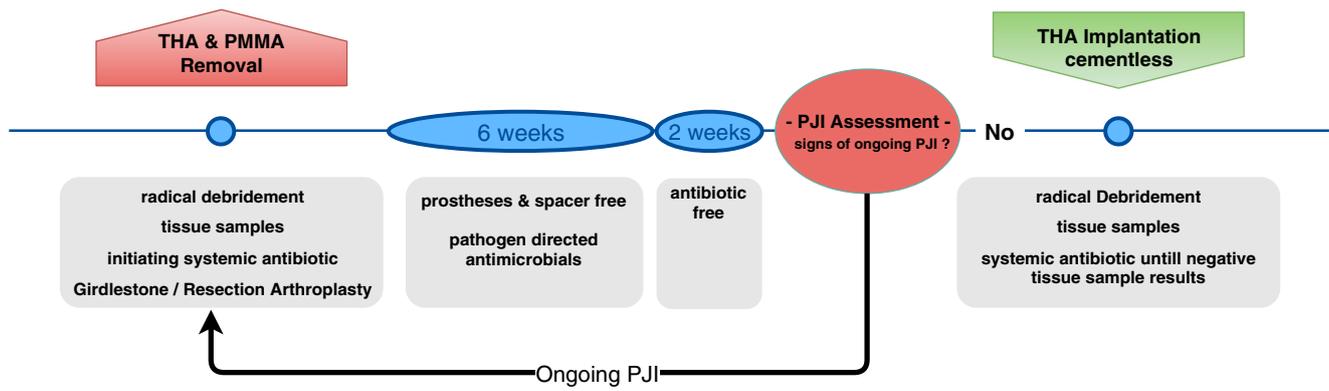


Fig. 1 Workflow of two-stage cementless and spacer-free exchange of infected total hip arthroplasty

Fig. 2 Radiograph of a 70-year-old patient with late-onset PJI suffering from persistent pain and reduced mobility (a). Removal of the endoprosthesis was carried out, as well as a second revision (b). After third look surgery showed negative intraoperative cultures, cementless reimplantation was performed (c). The patient showed a mHHS of 86 points and no clinical signs of PJI in the final follow-up (d). Implants (Cup/Stem): Allofit S (Zimmer, Switzerland)/MRP-Titan (Peter Brehm, Germany)

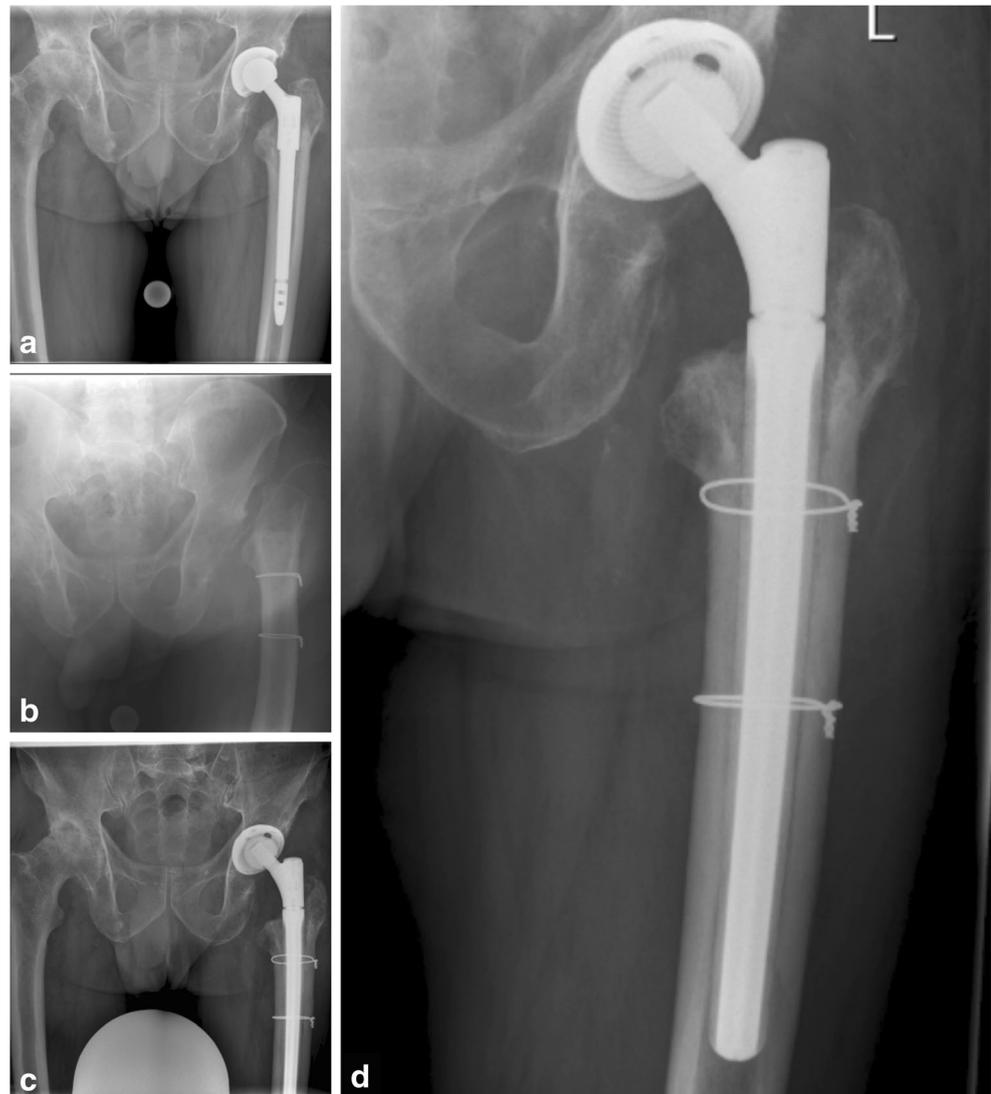


Fig. 3 Fifty-five-year-old patient suffered a periprosthetic fracture of the acetabulum and in the aftermath an implantation of a revision cup was performed; later on, she presented with a chronic fistula and PJI of the THA (a). The THA was removed (b). After two revision stages, reimplantation was carried out by using a revision cup in case of an acetabular defect type PAPROSKY IIIB (c). After 2.5 years, the patient moves mostly without crutches, showing no signs of a PJI and reaches a mHHS of 74 points (d). Implants (Cup/Stem): MRS-/MRP-Titan (Peter Brehm, Germany)



Results

Patient characteristics

Between January 2009 and December 2015, 57 patients were treated using the aforementioned modified two-stage prostheses exchange without using a spacer or cemented implants and could be followed-up.

The average age was 66.7 years (range: 47–83 years, standard error of the mean [SEM] 1.22); 47.4% of the patients were male ($n = 27$) and 52.6% were female ($n = 30$). The mean follow-up period was 53.9 ± 25 months, while the minimum follow-up in study protocol was 14 months. All patients had American Society of Anesthesiologists (ASA) criteria 3 or 4, 14% had diabetes, and 73.7% had hypertension.

Ninety-six percent of the patients had undergone prior unsuccessful PJI treatment in other hospitals. Two patients (4%) started PJI therapy in our hospital, while 68% of patients ($n =$

39) had one and 28% ($n = 16$) had two surgical revisions in other hospitals before. All patients only qualified for a two-stage procedure. Three patients (5.3%) had an acute PJI with massive local signs of infection (rubor, calor, dolor) and two (3.5%) had a chronic fistula. All other patients presented with a late-onset and low-grade infection without clinical signs of infection.

Microbiologic testing

In 57.1% patients, a microorganism could be isolated prior to prostheses removal in synovial culture and in 59.6% during the removal, or in 19.3% while the PJI assessment during the antibiotic-free period. In 12.3% of the cases, a microorganism could be isolated while implanting the new cementless implant. In sum, bacteria could be isolated ($n = 57$) in all of the cases. The most common bacterium was *Staphylococcus epidermidis* (50.9%), followed by *Propionibacterium acnes*

Table 2 A and B are related to the time during hospital stay. C and D show outcome after the complete follow-up

		Number	Percent
A	Status of PJI during hospital stay after reimplantation of the implant		
	Remission	50	87.7
	Ongoing PJI	7	12.3
B	In-hospital complications and further surgery after implantation of the implant	<i>n</i>	%
	None	32	56%
	Closed reduction after prosthesis luxation	7	12%
	Hematoma evacuation	5	9%
	Exchange of head/ball/neck component due to instability	5	9%
	DAIR Procedure	5	9%
	Prosthesis removal	2	4%
	Material failure/fracture of component	1	2%
C	Status of implant after whole follow-up	<i>n</i>	%
	Preserved	48	84.2
	Removed/exchanged	9	15.8
	Ongoing PJI	4	
	Periprosthetic fracture of femur	2	
	Periprosthetic fracture of pelvis	2	
	Fracture of modular stem/neck	1	
D	Status of infection after whole follow-up	<i>n</i>	%
	Remission	52	91.2
	Chronic PJI	5	8.8

(17.5%), *S. aureus* (14%), and *Enterococcus faecalis* (10.5%). In 42.1% of the cases, mixed infections with different types of bacteria were found (Fig. 4).

Systemic antimicrobial therapy administration was performed in accordance with the antibiograms, most frequently using ampicillin/sulbactam or clindamycin, and in about one third of cases using a combination therapy with rifampin (Table 3).

In the prostheses-free interval, no spacer was used. After PJI assessment and mentioned remission of PJI, only cementless implants were used. According to the bone stock, cementless implants of different companies were used (Table 1); in particular, a combination of Allofit acetabular component (Zimmer-Biomet, Winterthur, Switzerland) and MRP-Titan modular femoral Stem (Peter Brehm, Weisendorf, Germany).

After performing the cementless implantation, we observed signs of ongoing PJI in 12.3% patients and performed, in this case, either a DAIR procedure or another prostheses removal. Overall in 44% of the cases, after implantation, another surgery had to be performed. Table 2 shows the surgical outcome and complications. After the completed follow-up, 84.5% ($n = 48$) of the implants could be retained and remission of PJI could be detected in 91.2% ($n = 52$). Overall, nine prostheses had to be exchanged during the follow up, owing to four ongoing PJI and two periprosthetic fractures of the femur,

two fractures of the pelvis, and one fracture of the modular stem/neck component. In total, five patients (8.8%) showed persistent PJI of the hip, which was treated with persistent drainage ($n = 2$) and three underwent antimicrobial suppression therapy. In between the follow-ups, no patient died.

Patients were evaluated after a minimum follow-up of 14 months, while the mean follow-up period was 53.9 ± 25 months. The mHHS was used. The maximum possible points are 91 and the minimum is 0.

Mean mHHS was 60.85 (min–max 22–88, SEM 2.05). Detailed functional outcome is shown in Table 4.

Discussion

- Although PJI is a rising problem, and overarching treatment concepts and guidelines are published, details and outcomes as well as gold standards remain unclear. In a difficult-to-treat collective with over 90% patients unsuccessfully handled in other hospitals before, we hereby present our outcomes. In summary, we performed a two-stage or three-stage procedure without using any type of spacers or cemented implants. In 57.1% patients, a microorganism could be isolated prior to prostheses removal in synovial culture and in 59.6% during the removal; in sum, bacteria could be isolated in all of the cases. Comparable

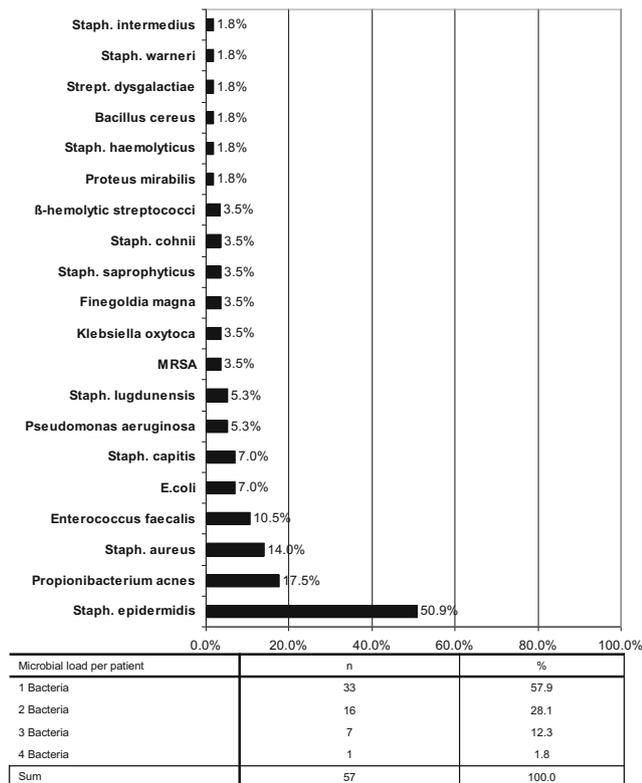


Fig. 4 Bacterial spectrum. Multiple nomination possible

outcome of culture-negative and culture-positive periprosthetic hip joint infection for patients undergoing two-stage revision was just reported [28]. Thus, in this concept, there are implant and spacer-free intervals in which biofilm-effective antimicrobials are not used. After successful implantation of the cementless revision implant and negative tissue samples antimicrobial therapy is stopped. Antimicrobial therapy is therefore highly effective limited on the necessary durations. In contrast, other researchers recommend an additional biofilm-active therapy after implantation of the revision implant for a further 6 weeks in all patients [19, 29, 30]. We tried to avoid this because of the severe side effects of these antimicrobials; it is shown that adverse effects are present in over 30% of the cases and leads to a drop of 18% in the cure rate [31–33]. After performing the cementless implantation, we observed signs of ongoing PJI in 12.3% patients and performed, in this case, either a DAIR procedure or another prostheses removal. In this study, we had to use rifampin in only 35% of the cases and try to limit the usage in cases in which a DAIR procedure was carried out after implant replantation or positive tissue samples after replantation. Antimicrobial impregnated static or articulating spacers are often used to manage dead space and deliver local antimicrobial therapy until a permanent prosthesis is placed [34, 35]. However, the complication rate of

Table 3 Used antibiotics. Multiple nomination and combinations possible. Rifampin is only used in combination

Antibiotic	Number	Percent
Ampicillin + Sulbactam	46	80.7%
Rifampin	20	35.1%
Clindamycin	19	33.3%
Levofloxacin	18	31.6%
Vancomycin	12	21.1%
Ciprofloxacin	8	14.0%
Piperacillin/tazobactam	5	8.8%
Fosfomycin	5	8.8%
Doxycyclin	4	7.0%
Tigacyclin	3	5.3%
Linezolid	3	5.3%
Gentamycin	2	3.5%
Meropenem	1	1.8%
Ceftazidim	1	1.8%
Imipenem	1	1.8%
Tetracyclin	1	1.8%

placing spacers is reported to be 22–58% and hence, in our opinion, too high for a method with suspected benefit [5]. In all, there were about 17% luxations, 14% bony fractures, 10% spacer-related damages and damage to the acetabular bonestock [5]. Fifty-three to 68% of all PMMA-spacers show bacterial contamination after removal [6]. Therefore, we avoided the use of any type of spacer. In addition, if a local antibiotic needs to be used, favorable antibiotic carriers such as degradable calcium-sulfate show superior elution kinetics and less biofilm development [10, 11, 36–38]. We currently reported their use [11, 25, 39]. The concerns of spacer-free therapy include muscle shrinking that leads to impaired function after replantation. Marczak et al. [40] compared a two-stage exchange with using a spacer and without. In 99 hips, they could detect that the re-infection rate in spacer group was quite twice as much as in the non-spacer group (13.3 vs. 6.4%). One spacer luxation was seen, but the functional outcome was better in the spacer vs. the non-spacer group according to the HHS (66 vs 58), while this working group does “believe that the difference in the HHS is not based on the use of the spacer” [40]. Tikhilov et al. could identify blood and culture-specific values as risk factors for a recovery of PJI but not the kind of used spacer articulating or not, whereas there was no non-spacer group [41]. We report a mean mHHS of 60.85 (equals an HHS of about 67%) in this collective. In particular, > 80% patients did not require pain relievers or required only aspirin and > 80% could use the stairs and achieve a walking distance of at least 500 m. Thus, in this difficult-to-treat collective, an appropriate outcome was achieved. There is a lack of

Table 4 Functional outcome

Clinical outcome and modified Harris hip score		Number	Percent
Pain	Bedridden, pain in bed	0	0.0%
	Serious, limited activity	2	3.5%
	Moderate, tolerable pain, more than aspirin	8	14.0%
	Mild, may take aspirin	12	21.1%
	Slight, no compromise in activity	16	28.1%
	None	19	33.3%
Limp	Severe or unable to walk	4	7.0%
	Moderate	31	54.4%
	Slight	21	36.8%
	None	1	1.8%
Support	Not able to walk or two crutches	3	5.3%
	Two canes	25	43.9%
	One crutch	4	7.0%
	Cane most of the time	6	10.5%
	Cane for long walks	11	19.3%
	None	8	14.0%
	Distance walked	Bed/chair only	2
	Indoor only	7	12.3%
	500 m	19	33.3%
	1000 m	17	29.8%
	unlimited	12	21.1%
Stairs	Not able to walk or two crutches	4	7.0%
	In any manner	5	8.8%
	Normally using a railing	39	68.4%
	Normal	9	15.8%
Shoes/socks	Not able	12	21.1%
	With difficulty	22	38.6%
	With ease	23	40.4%
Sitting	Unable to sit comfortably	3	5.3%
	On high chair >30 min	7	12.3%
	Comfortably	47	82.5%
Public transportation	Unable to use without help	20	35.1%
	Able to use without help	37	64.9%
mHHS (0–91)	Min 22 max 88 mean 60.85 SEM 2.05 SD 15.48		

comparable studies, due to different definitions of PJI and treatment protocols. Cabarita et al. [42] showed comparable results of a mean HHS of 69 (spacer-free) to 74 (with spacer); 15% of the study patients showed spacer-related complications. Du et al. [43] reported an HHS of 47.56 ± 14.23 in a collective of 266 hips with articulating spacers. Spacer-related complications were seen in 14.3%.

- The success rates of two-stage exchange have been published in the *Lancet* [3] ranging from 65 to 100%, but the reasons for this range and the particular factors that affect outcomes are unknown [3]. Gomez et al. [44] report sobering results of two-stage revision with spacer placement for PJI. Nearly 20% of patients in their study who had a

spacer placed never did not require a new prosthesis, and nearly 20% of those who did get a new prosthesis ultimately failed treatment. The authors reported a 7.5% mortality rate in the intermediate period after resection arthroplasty [44, 45]. By contrast, we report a PJI remission rate of 91.2%, while 8.8% ($n = 5$) had chronic PJI with implant retention. Overall, nine prostheses (15.8%) were replaced owing to ongoing PJI or fractures. We used modular implants in most cases; accordingly, we observed in one case, a prosthesis-related failure of the neck. In between the follow-ups, no one had died. We only used uncemented, pressfit implants given the specific background of revision surgery. The bearing surface which

was used was ceramic-on-crosslink-polyethylene (CoP). It was shown that ceramic-on-ceramic or CoP has advantages in the risk of PJI in primary THA [46]. There is an over 80% reduction in cement-bone interface shear strength between primary and revision arthroplasty [15] and thus higher loosening and revision rates of cemented implants in these situations [47]. In case of a new revision, there are clearly more difficult conditions with cemented prostheses. Other studies showed that treatment of infected THA with two-stage revision using uncemented components at the second stage yields results similar to revision with antibiotic-loaded cemented components, while a 10.3% infection recurrence rate was found in this series at the minimum two year follow-up [48].

Limitations

- Our study has several limitations. First, our sample size is relatively small because of the strict inclusion criteria. Second, the current study has no control group, and a prospective randomized trial would be valuable. A long-term follow-up could identify late-onset recurrences.

Conclusion

- Two-stage exchange of infected THA can lead to high success rates in treating late-onset PJI. The spacer-free procedure does not negatively affect the outcome concerning success rate or function, as per latest literature. In every case, the implantation of an uncemented pressfit prostheses was possible. The success rate of treatment was shown in 91.2% cases, while in 84.2%, the uncemented revision implant was preserved even at the final follow-up.

Compliance with ethical standards

Study is based on institutional review board (IRB) approval.

Conflict of interest The authors declare that they have no conflict of interest.

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