Original Article

Bacteriological investigation of infected pressure ulcers in spinal cord-injured patients and impact on antibiotic therapy

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Study design: Retrospective.

Objectives: To improve the use of bacteriological results for treating spinal cord-injured patients with infected pressure ulcers.

Setting: Microbiology and Orthopaedics Department, Ambroise Paré University Hospital, Boulogne-Billancourt, France.

Methods: Tissue specimens, sampled at the end of the surgical intervention from unbridled and cleaned ulcers were analysed. Drainage liquids were cultured at day 1 (D1) and day 5 (D5) postsurgery. For part of the patients, a presurgery superficial sample was analysed and compared with the surgical and postsurgical samples.

Results: In all, 168 surgical samples from 101 patients, 183 D1 and 104 D5 wound drainage liquids were included in this study. Out of the 168 surgical samples 17 (10%) had a negative culture, whereas 151 (90%) had a positive culture. For drainage liquids, the culture was negative in 48% and 56% of the samples at D1 and D5, respectively. The most frequently isolated species were enterobacteria, followed by staphylococci and streptococci.

Conclusion: Culturing deep tissue specimens sampled from the surgically cleaned and unbridled ulcers allows for the isolation of the bacterial species that are really involved in the ulcer infection. As the identification of these bacteria and their antibiotic susceptibility are available, when the culture results of the D1 postsurgical drainage liquid is also available, it is easier to choose targeted antibiotic treatment.

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Keywords: infected pressure ulcers; surgical sampling protocol; targeted antibiotic therapy

Introduction

Infection of pressure ulcers constitutes, besides urinary tract infections, the most frequent complication in spinal cord-injured (SCI) patients in spite of preventive measures and adapted technical equipment.^{1,2} More than 85% of SCI patients develop an infected pressure ulcer at least once in their life, 70% of them having multiple ones.^{3–5} Several factors are involved in the development of pressure ulcers such as diminished activity and mobility of the patients leading to frailty of the skin, and the immunological change induced by the section of the spinal cord.^{4,6} The most fragile anatomical sites where pressure ulcers may occur are the ischiosacral tuberosity, the trochanter and the sacrum. Infections of pressure ulcers at these sites are

frequent and may lead to cellulitis and abscesses, in addition to osteomyelitis or arthritis of the proximal joints, with bacteria from the direct environment that are mostly the urogenital and digestive tracts.⁷ As infections of these sites probably start by colonisation, it is often difficult to decide if the isolated species are colonising saprophytes or are at the origin of deep tissue infection.⁸

The surgical treatment of infected pressure ulcers by musculocutaneous flaps, which is necessary in most of the cases, has to be accompanied by an adapted antibiotic treatment, but an empirical antibiotic treatment is difficult to initiate due to the large variety of bacterial species involved in such infections.⁹ It has been proposed to use third-generation cephalosporins and glycopeptides for the first-line treatment of extended pressure ulcers.^{1,7} Such a blind treatment should not be continued longer than 3–5 days and must be adapted in relation to the results of the bacteriological

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investigation.^{1,7} Once the antibiotic treatment can be targeted, it should be maintained for 3 weeks if no general symptoms (fever, osteomyelitis, arthritis, bacteraemia) are present. Otherwise, the treatment must be continued for up to 6 weeks.^{5,10}

In order to target the antibiotic treatment in SCI patients undergoing surgical treatment by musculocutaneous flaps, to bacterial species really involved in the infectious process, we established a presurgery protocol and a sampling procedure of deep tissues whose results are presented here.

Materials and methods

Patients and patient data

In all, 101 patients, hospitalised in the septic unit of the orthopaedic surgery department during the year 1999, were enrolled in this study, 33 of them being women and 68 men. The mean age was 44 years for the male patients and 57 years for the female patients. The patients underwent 1.5 surgical interventions (extremes between 1 and 6) during the same sojourn at the hospital. The data concerning each patients' antibiotic treatment were collected by the bacteriologist when the bacteriological results became available.

Sampling protocol

The central surface of the pressure ulcer was sampled with a simple humid swab at the admission for 77 of the patients, whereas deep tissue specimens were sampled for all patients ($N=101$) at the end of the surgical intervention after extensive cleansing and debridement of the ulcer. Drainage liquids were investigated at day 1 (D1) and if possible at day 5 (D5) postsurgery.

Bacterial identification and antibiotic susceptibility

The bacterial culture was carried out in a semiquantitative way in order to estimate the bacterial load. The media used comprised, in general, Columbia sheep blood agar plates, incubated in aerobic and anaerobic atmospheres, a chocolate agar plate to permit growth of fastidious bacteria and a Drigalski plate for the growth of Gram-negative rods (bioMérieux, Marcy l'Etoile, France). Species identification and antibiotic susceptibility testing were carried out for each colony type. Bacterial identification of enterobacteria, nonfermenting

bacteria, streptococci and coagulase-negative staphylococci was carried out with the Api system (bioMérieux, Marcy l'Etoile, France). *Staphylococcus aureus* was identified by the positive result of the coagulase test.

Antibiotic susceptibility of the isolates was determined using the agar disc diffusion method (Biorad, Marnes la Coquette, France), and interpreted according to the recommendations of the French Committee of Antibiogram.¹¹

Results

During the 1-year study period, we analysed 168 surgical samples from 101 patients, 183 wound drainage samples at D1 postsurgery and 104 drainage samples at D5. For nine of the 101 patients, no drainage liquid was obtained, and 58 drainage liquids that were expected to be sampled at D5 following the sampling protocol were not received at the microbiology laboratory, because the absence of drainage liquid had caused removal of the draining system prior to D5.

The results of the bacterial culture of surgical samples and wound drainage liquids are presented in Table 1. From the surgical samples 17 (10%) did not yield bacterial growth, whereas 116 (69%) were positive with one or two bacterial species and 35 (21%) with at least three bacterial species. From 8% of the samples obtained during surgery, four or more bacterial species were isolated.

For about one-half of the D1 (48%) and D5 (56%) drainage liquids, no bacteria could be isolated. One or two species were found in 44% of D1 and 40% of D5 drainage liquids, and only rarely three (4%) or more species (0–2%) were cultivated from drainage liquids.

The comparative results of species isolated from surgical samples and subsequent drainage liquids are presented in Table 2. In case of a negative culture of the surgical samples, the corresponding drainage liquids were also negative in 78%, but positive with one or two bacterial species in 22%. When the surgical samples of deep tissue were positive with one bacterial species, the corresponding drainage liquids were negative in 54%, and positive with the same bacterial species, sometimes accompanied by one or more different bacterial species, in 29% of the cases. In 17% of the cases, different species were isolated from drainage liquids and surgical samples. When two or three bacterial species were isolated from the surgical samples, roughly one-third of

Table 1 Culture results of surgical samples and drainage liquids

Specimen	Negative culture	Positive culture				
		No. (%) of samples with <i>n</i> different bacterial species				
		1	2	3	4	≥5
Surgical samples ($N=168$)	17 (10)	67 (40)	49 (29)	21 (13)	9 (5)	5 (3)
Drainage liquids on D1 ($N=183$)	88 (48)	45 (25)	35 (19)	8 (4)	4 (2)	3 (2)
Drainage liquids on D5 ($N=104$)	58 (56)	27 (26)	15 (14)	4 (4)	0	0

Table 2 Results of the bacteriological culture of wound drainage liquids (D1 and D5, $N = 287$) in relation to the cultural results of the surgical samples ($N = 168$)

<i>Culture result of surgical samples</i>	<i>N</i>	<i>Culture result of the corresponding wound drainage liquids</i>	<i>N</i>	<i>%</i>
Negative	17	Negative	31	78
		1 species	8	20
		2 species	1	2
1 Species	67	Negative	62	54
		1 Identical species	17	15
		1 Identical species + 1 different species	13	11
		1 Identical species + 2 different species	1	1
		1 Identical species + 3 different species	1	1
		1 Identical species + 5 different species	1	1
		1 Different species	16	14
		2 Different species	4	3
2 Species	49	Negative	18	26
		2 Identical species	7	10
		2 Identical species + 1 different species	2	3
		1 Identical species	14	20
		1 Identical species + 1 different species	15	22
		1 Identical species + 2 different species	2	3
		1 Different species	8	12
		2 Different species	1	1
		3 Different species	2	3
3 Species	21	Negative	11	37
		3 Identical species	3	10
		2 Identical species	4	13
		2 Identical species + 1 different species	2	7
		2 Identical species + 3 different species	1	3
		1 Identical species	6	20
		1 Identical species + 1 different species	3	10
4 Species	9	Negative	18	72
		4 Identical species	3	12
		4 Identical species + 2 different species	1	4
		1 Different species	3	12
≥ 5 Species	5	Negative	6	75
		2 Species	2	25

the corresponding D1 and D5 drainage liquids (26% and 37%, respectively) were negative, and in two-thirds the same bacterial species were identified (58% and 63%, respectively), accompanied or not by a bacterial species different from those isolated from the surgical samples. In case of isolation of four or more bacterial species from the surgical samples, two-thirds of the subsequent drainage liquids yielded a negative culture.

The bacterial species isolated are presented in Table 3. As multiple isolates from the same sample are possible, there were 289 isolates from surgical samples and 239 isolates from drainage liquids at D1 and D5. Enterobacteria (29%) and staphylococci (28%) were nearly equally isolated from surgical samples, followed by enterococci (16%). *Escherichia coli* and *Proteus mirabilis* were the dominant species among enterobacteria, but the single species that was the most frequently isolated from surgical samples was *Enterococcus faecalis* ($N = 45$, 16%). Among *S. aureus* isolates, the methicillin-resistant

phenotype ($N = 38$, 13%) was more frequent than the methicillin-susceptible one ($N = 28$, 10%).

In drainage liquids, enterobacteria (44%) dominated as in surgical samples, followed by staphylococci (28%), again with the predominance of methicillin-resistant *S. aureus* ($N = 35$, 15%) over methicillin-susceptible *S. aureus* ($N = 19$, 8%). Enterococci and streptococci (14%) were much less often isolated from drainage liquids than from surgical samples (26%).

Table 4 shows the categorised results of the bacteriological culture of specimen sampled at the surface of the pressure ulcer at the hospital admission of the patient, compared with the bacterial culture results from surgical samples in the depth of the ulcer and drainage liquids. This analysis was carried out for 77 of the 101 patients. In 36% of the cases, the results of the presurgery samples were in good agreement with the results of the surgical samples and the drainage liquids, as in 4% both were culture negative, in 9% both grew

only one bacterial species and in 23% both were polymicrobial. In 64% the two types of samples, presurgery and surgical samples or drainage liquids, yielded discordant results, as in 11% the surgical samples or drainage liquids were culture positive, whereas the presurgery sample has been culture negative. In 44% the surgical samples or drainage liquids were polymicrobial compared with a monomicrobial

Table 3 Bacterial species isolated from surgical samples and wound drainage liquids

Species	Number (%) of positive samples with the bacterial species	
	Surgical sample (N = 289)	Drainage liquid (N = 239)
Enterobacteria	83 (29)	104 (44)
<i>Escherichia coli</i>	33	48
<i>Proteus mirabilis</i>	28	22
<i>Morganella morganii</i>	6	11
<i>P. vulgaris</i>	2	2
<i>Providencia stuartii</i>	0	1
<i>Klebsiella</i> spp.	6	10
<i>Citrobacter</i> spp.	4	4
<i>Enterobacter</i> spp.	4	5
<i>Serratia marcescens</i>	0	1
Other Gram-negative rods	29 (10)	32 (13)
<i>Pseudomonas aeruginosa</i>	20	26
<i>Acinetobacter baumannii</i>	8	6
<i>Flavobacterium</i> spp.	1	0
Staphylococci	80 (28)	68 (28)
<i>Staphylococcus aureus</i> MR	38	35
<i>S. aureus</i> MS	28	19
<i>Coagulase-negative staphylococci</i> ^a	14	14
Streptococci and enterococci	76 (26)	33 (14)
<i>Enterococcus faecalis</i>	45	25
Streptococci of groups A, B, G, F	16	6
Other streptococci ^b	15	2
Other ^c	21 (7)	2 (1)

MR: methicillin resistant; MS: methicillin susceptible.

^a*Staphylococcus epidermidis*, *S. capitis*, *S. lugdunensis*, *S. hominis*. ^b*Streptococcus intermedius*, *S. mitis*, *S. equi*. ^cAnaerobes, coryneforms, *Candida*

presurgery sample, and in 9% the surgical or post-surgical samples were culture negative or monomicrobial compared with polymicrobial presurgery samples.

In most of the cases, targeted antibiotic therapy was started on the basis of the results of the surgical samples. If the surgical samples as well as the drainage liquids at D1 and D5 were culture negative, no antibiotic treatment was given, except when the patient's general condition made it necessary. In case of a positive surgical sample, the isolated species were treated by the narrowest spectrum antibiotic therapy possible. The same strategy was chosen in case of negative surgical but positive drainage liquid sample, which was the case for seven patients, for whom the results of the drainage samples had to be awaited to target antibiotic treatment.

The antibiotics mostly used to treat the patients were third-generation cephalosporins, ie cefotaxime for *E. coli* and *P. mirabilis* infection, cefepime for enterobacterial species producing cephalosporinase at normal or high levels such as *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella morganii* or *Providencia stuartii*. Sometimes a fluoroquinolone molecule, in most cases ciprofloxacin, was associated. When enterobacteria were associated to enterococci, an ureidopenicillin (eg piperacillin) or a combination of an ureidopenicillin with a beta-lactamase-inhibitor (eg piperacillin/tazobactam) was given.

In case of *Pseudomonas aeruginosa* infection, ceftazidime was used in most of the cases always associated with another active molecule like ciprofloxacin or fosfomycin.

Glycopeptides were used in the case of methicillin-resistant *S. aureus* infection, and replaced by penicillin M when the antibiogram was available and showed that the *S. aureus* isolate was methicillin susceptible. Streptococci and enterococci were treated by aminopenicillins when their susceptibility was confirmed. No vancomycin-resistant enterococci were isolated.

Discussion

During a 1-year period, we analysed 168 surgical samples from 101 SCI patients, as well as 183 wound drainage samples at D1 and 104 drainage samples at D5, following a strict sampling protocol to improve the interpretation of bacteriological results of the often

Table 4 Comparison of the culture results of presurgery samples, and surgical samples and drainage liquids for 77 patients (100%)

Presurgery sample with	Surgical sample and/or drainage liquid with		
	Negative culture (N = 5)	Monomicrobial culture (N = 18)	Polymicrobial culture (N = 54)
Negative culture (N = 11)	3 (4%)	6 (8%)	2 (3%)
Monomicrobial culture (N = 41)	0	7 (9%)	34 (44%)
Polymicrobial culture (N = 25)	2 (3%)	5 (6%)	18 (23%)

polymicrobial samples and the appropriate antibiotic therapy.

It is clear that the profitability of specimen sampled in pressure ulcers varies in relation to the site and depth of the sample.^{1,7} Moreover, it has been shown that high-pressure cleansing of the infected site permits the reduction of the contaminating cutaneous flora by 90%.¹ From the surgical samples of our study, only 10% yielded a negative culture, which is not surprising as patients were selected for infection of their pressure ulcer. Such culture-negative surgical samples were mostly obtained from secondary interventions during the same hospital sojourn, which were necessary when the extent of the primary ulcer was very large or the initial bacterial load was very heavy. Nevertheless, from the majority of culture-positive surgical samples (69%), we isolated only one or two bacterial species, indicating correct cleansing prior to sampling. This finding was confirmed by the higher part of culture negative results in drainage liquids (48% at D1 and 56% at D5) than in surgical samples (10%), indicating again exhaustive surgical cleansing. Thus, by sampling at the end of the surgical intervention after cleansing and unbridling of the pressure ulcer, it is possible to isolate the bacterial species that are responsible for infection of the pressure ulcer and to avoid isolation of contaminating species.

In about two-thirds of the cases, one or more of the bacterial species isolated from surgical samples were also isolated from the drainage liquids. This finding supports the hypothesis that culturing of drainage liquids is a powerful means to survey the postsurgical evolution that can, in case of persistent infection, lead to a new surgical intervention as has been shown previously.¹²

Most of the Gram-negative rods implicated in infection of the pressure ulcers were *E.coli* and *P. mirabilis* as was found in other studies.^{10,13} This may be explained by the fact that the infection starts probably by colonisation of frail skin with bacteria from the urogenital and digestive tracts.⁷ In both surgical samples and drainage liquids, bacterial species that are naturally multidrug-resistant to different antibiotics, such as *P. aeruginosa*, *Stenotrophomonas maltophilia* or *Acinetobacter baumannii*, were quite rarely isolated in our study (10% of the surgical samples and 13% of drainage liquids, respectively), although these species have been reported to be frequently isolated in SCI patients.^{10,14,15} On the contrary, among *S.aureus* isolates, the methicillin-resistant phenotype dominated over the methicillin-susceptible one.

As the identification of species isolated from the surgical samples becomes available 48 h after surgery, we wanted to know if sampling the surface of the pressure ulcer, which can be carried out at hospital admission of the patient, could accelerate the knowledge process of the infectious agent and thus accelerate targeting of the antibiotic therapy. For only 28 (36%) of the 77 patients who had such a presurgery sample, similar culture results were found in both the presurgery sample and the surgical sample/liquid drainage. More-

over, when the culture results of the presurgery samples were discrepant from the surgery samples or drainage liquids, the latter were much more frequently informative (55%) – in terms of the number of isolated bacterial species – than the presurgery samples (9%). In these cases, if the treatment had been chosen on the basis of the results of the presurgery samples, the choice of treatment would have missed a certain number of bacterial species responsible for the deep tissue infection, bearing the risk for the patient of not being treated correctly and for the hospital ecology of selecting antibiotic-resistant mutants.

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