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ORIGINAL STUDY

Screening femoral heads from living donors : A prospective study comparing swab-agar versus bone fragment-broth culture

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Femoral heads are an important source of allograft bone used in reconstructive orthopaedic surgery. The sterility of donor material is of major importance for the recipient. Femoral heads intraoperatively retrieved during hip arthroplasty from medically screened living donors are routinely checked with a surface swab to exclude microbiological contamination. There is, however, evidence that swab cultures have limited sensitivity. We therefore prospectively compared two ways of screening femoral heads. Bacterial recovery from swabs in Amies transport medium taken intraoperatively, subsequently transported to the microbiology laboratory and inoculated on agar and in broth was compared with the recovery from a bone fragment also taken intraoperatively but immediately inoculated into Wilkins Chalgren broth. Forty femoral heads were tested with both methods. Bacteria were cultured neither from the femoral surface swabs nor from the femoral fragments. Consequently no distinct conclusions regarding the sensitivity of both techniques could be drawn. In addition the bacterial yield of two swabs in Amies transport medium streaked on a variety of culture media other than the conventional agar plates was also studied. Culturing of these swabs resulted in the detection of bacteria that are predominantly considered contaminants.

Keywords : femoral head ; bone banking ; culture ; sterility ; swab.

INTRODUCTION

Femoral heads from patients undergoing a total hip arthroplasty are saved intraoperatively, stored in bone banks according to stringent guidelines and used as allograft bone for filling and reconstructing bone defects (*2,3,4*). Patients giving informed consent to the donation of their femoral head are preoperatively carefully screened to rule out medical conditions that are contraindicated for tissue banking and to exclude transmittable bacterial and viral infections. The conventional procedure to verify the microbiological sterility of the intraoperatively retrieved femoral head includes a surface swab in

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Amies transport medium which is transported to the laboratory. The swab is then streaked onto blood agar and inoculated into a liquid thioglycolate broth. All media are incubated for two days at 36°C with 5% CO_2 when indicated.

It is, however, known that a swab is a rather insensitive technique to check sterility (*7,10*). Only manifestly contaminated products may be detected by means of the swab technique. Additional problems of the swab technique are its variable ability in removing organisms from the examined tissue on the one hand and in transferring the organisms, if present, from the swab to the culture medium on the other hand. To address this problem, one can take several swabs as an option (*11,12*). An alternative method is the use of a different culture technique, such as inoculation of a femoral fragment in a sterile nutrient broth. The aim of this study was to compare the sensitivity of these two culture methods : a femoral head fragment inoculated in Wilkins Chalgren broth versus the conventional swab method. In addition, we evaluated whether additional swabs inoculated on a larger variety of culture media enhances the yield of bacteria.

MATERIALS AND METHODS

Material for microbiological examination was prospectively collected from 40 femoral heads obtained during primary hip arthroplasty. Among the 40 donors, there were 28 men and 12 women. Donor ages ranged from 37 to 80 years (mean : 66 years). The right femoral head was banked 21 times and the left 18 times. The side of origin was unknown in one case. The reason for donating the femoral head was coxarthrosis in 39 donors and chondrolysis in one donor.

All operations were carried out in an operating room equipped with a vertical laminar airflow measuring three by four meters. When a suitable donor in accordance with the inclusion criteria according to Belgian Health Council standards (*2,3*) gave informed consent, two Copan swabs in Amies transport medium (Brescia, Italy) were taken from the surface of the retrieved femoral head. Additionally a fragment (+/- 1 cm^3) of the same femoral head was put into a rich nutrient broth (Wilkins Chalgren anaerobic broth, Oxoid Ltd, England). Both the swabs and the broth were sent to the microbiology laboratory. In the lab, one swab was streaked onto blood agar plate and inoculated into thioglycolate broth. Plate and thioglycolate broth were incubated for 48 hours at 36°C in 5% CO_2 when indicated (conventional method). In addition, the two swabs were cultured separately on aerobic blood and chocolate agar plates, anaerobic blood agar plates and finally the swabs were placed in Wilkins Chalgren broth. The plates and the broths inoculated with the swabs intended to filter the effect of the culture method used. By using a Wilkins Chalgren broth for both the swab and the bone fragment, we were able to determine the effect of the sample type (swab versus tissue fragment) on bacterial recovery. The disadvantage of this protocol was the increased risk of contamination by performing multiple manipulations with the two swabs.

For each operation, the following parameters were registered : time of incision, time of taking the femoral head swab, last cleaning time of the theatre floor and the number of persons present in the laminar flow chamber. At induction of anaesthesia, all donors received cefazoline 2 g intravenously as prophylactic antibiotic. After the operation, the patients' records were followed up to evaluate evidence of infectious complications.

The donated femoral head itself was placed in a sterile double sealed jar and stored in a freezer at -85°C.

RESULTS

Neither the conventional cultures (swab on agar and in thioglycolate broth), nor the bone fragments inoculated in Wilkins Chalgren broth revealed any positive result. Statistics for comparison of the two techniques could not be performed since there were no positive outcomes and hence there was no difference between the two groups.

The results with regard to the multiple swab cultures inoculated on various culture media are shown in Table I. Nine femoral head donors had either a positive swab and/or a positive broth culture. There were 4 donors with a positive agar culture, 3 with a positive broth culture and 2 with a positive culture both on agar and in broth. The positive cultures were nevertheless considered as caused by contaminating microorganisms for reasons explained hereunder.

In table II, data of the registered parameters are shown for all 40 donors, including those with multiple swab cultures inoculated on various culture media. There was no difference in time interval between starting the incision and taking the culture

Table I. — Results for the multiple swab cultures from 40 femoral head donors on various culture media

	Positive result on agar	Positive result in broth	Positive result on agar and in broth
N°. donors	4	3	2
N°. CNS	3	3	1
N°. *Staphylococcus aureus*	1	0	0
N°. *Peptostreptococcus* spp.	1	0	0
N°. *Propionibacterium* spp.	1	0	0
N°. *Corynebacterium* spp.	0	0	1

Table II. — Registered parameters for donors with positive cultures compared to donors with negative cultures

	Femoral head donors with multiple positive swab cultures			Observed parameters for all 40 femoral head donors[4]
	Positive result on agar[1]	Positive result in broth[2]	Positive result on agar and in broth[3]	
N°. donors	4	3	2	40
Mean time interval incision - culture (range)	58' (49' – 1h 5')	1h 15' (1h 10' - 1h 20')	1h 37' (1h 35' - 1h 40')	1h 17' (20' - 3h 30')
Mean time interval cleaning floor - incision (range)	21h 32' (53' - 2d 14h 50')	1d 7h 32' (20' - 2d 14h 45')	1d 13h 27' (12h 45' - 2d 14h 10')	20h 44' (20' - 4d 14h 10')
Mean N° of persons in Charnley chamber	4	3	4	4

[1]Parameters available from 3 donors
[2]Parameters available from 2 donors
[3]Parameters available from 2 donors
[4]Parameters available from 26 donors.

between donors with positive multiple swab cultures and the whole group of donors. Regarding the mean time interval between cleaning the theatre floor and the incision, there was a longer interval between donors with positive cultures and those with a negative culture, although the range was overlapping and the difference was not statistically significant. The number of persons in the clean air theatre did not differ between the two groups.

The mean period of patient follow-up was 82 (36-236) days. When verifying the medical records in search for complications, no problems were reported for 35 of the 40 patients. Two of the 40 donors suffered a minor non-infectious problem. The first patient had persisting thigh pain (145 days postoperative), the second patient suffered from a trochanteric bursitis (145 days postoperative). For the three remaining patients, no data were available from follow-up consultations.

DISCUSSION

Femoral heads are a valuable source of bone tissue for tissue banks. According to guidelines of the Belgian Health Council (*2,3,4*), tissue intended for transplantation should bear no microorganisms making it harmful to the recipient. With this concern in mind it is mandatory to perform a microbiological screening to check the sterility of the bone. In the literature, the rate of contamination of retrieved femoral heads (living donors) ranges from 1% to 22% (*1,5,9*). This large range may possibly be explained by the various number of samples taken and diverse culture methods used in different institutions.

In our study, the rate of contamination was 0% both with the conventional swab culture and with the bone tissue fragment in rich nutrient broth. This high sterility level points at the sterile conditions

while working in a laminar air flow theatre. Surgeons and scrub nurses are equipped with a special helmet covering their entire head and which is connected to the outside of the chamber with a tube through which exhaled air is removed by negative pressure. In a recent more modern version, microair filters are being used instead of the former exhaust system. Staff also wear a gown covering the entire body surface. Previous authors have already shown that ultra-clean air, occlusive clothing, strict theatre discipline and careful surgical techniques can reduce the rate of deep infections of prostheses (*6*).

The contamination rate of a single femoral head surface swab (conventional method) used at our University Hospital between 1994 through 2003, was 1.4% (9/661 donors – data not shown). This is in agreement with the data from Sommerville *et al* (*8*) reporting the results of a femoral head surface swab culture only (1.3%). The same authors also found that, when additionally an acetabular swab was taken, the contamination rate raised to 14.2% and when collecting a culture of capsular tissue as well, the rate increased to 22% (*8*). They stated that taking various specimens from different locations in the hip joint more accurately assessed the contaminating bioburden, while reducing the number of false negatives (*8*). Our study, however, showed that increasing the number of samples also increases the number of false positive samples. As such, when taking into account the multiple swab cultures performed in our study, there were 9 (23%) donors with a positive swab on agar plate and/or in broth. These very likely represent contamination from manipulation since none of the donors showed any signs of an infectious complication after the femoral head was retrieved and a prosthesis was implanted.

Our study failed to determine which of the two culture techniques – swab or bone tissue fragment- is most sensitive in screening femoral heads, because not one of the methods yielded positive results. This study was limited in that the low number of study cases did not allow statistical analysis to have a sufficient power for discrimination between both test methods. We suggest though that inoculating a tissue fragment in Wilkins Chalgren broth is more accurate in avoiding false positive and/or false negative results than simply taking a surface swab. One of the reasons is that swabs have to be transported to the laboratory, and in the mean time, microorganisms could starve. Moreover, microorganisms may reside on the swab without being transferred to the agar plate at streaking. This may lead to false negative results. Conversely extra manipulations by streaking the swab onto an agar plate and inoculating it in thioglycolate broth enhances the risk for false positive results. With the multiple cultures performed from the two swabs, we demonstrated that the risk of contamination is substantial. A tissue fragment put into a nutrient broth immediately during the operating has two advantages : It reduces the risk of false positive results by avoiding additional manipulations required to inoculate swabs onto and into additional culture media. Furthermore potential starvation of microorganisms linked to the delay in transport of the specimen and inoculation in the lab is avoided, thus reducing the risk of false negative results.

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