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Surveillance of Oral Cultures for *Enterobacteriaceae* During Bone Marrow Transplantation

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Bone marrow-transplanted patients can suffer from severe life-threatening infections. Oral bacterial cultures were collected from a group of 40 patients prior to and following bone marrow transplantation every 3 days, following initial preparation and eradication of oral infections. The samples were grown on the Titertek-Enterobac kit specific for *Enterobacteriaceae*. In 426 oral cultures 30.5% grew gram-negative bacteria, 76.6% of them were *Enterobacteriaceae*. Young male patients had 8.3% positive cultures at the study start, a percentage which constantly increased during later periods. Older patients did not follow the same pattern. Also, the allogeneic transplantation group had a higher percentage of *Enterobacteriaceae* than the autologous group (49.0 versus 19.5%). In blood cultures 18 out of the 94 positive ones showed the presence of *Enterobacteriaceae*. The most commonly found microorganisms in oral cultures were *Klebsiella oxytoca* (23%), *Enterobacter cloacae* (18%) and *Klebsiella pneumoniae* (15%). The decrease in the positive oral cultures from 35.0% during the pretransplantation period to 5.4% close to the transplantation, demonstrates that the preparatory protocol used for the prevention of oral infections was highly effective.

Keywords: bone marrow transplantation, chemotherapy, *Enterobacteriaceae*, immunocompromised patients, oral lesions, oral flora, oral health

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INTRODUCTION

MOST ORAL infections in hospitalised patients receiving chemotherapy are either opportunistic or nosocomial, they might be life threatening while the host's defences are at their lowest.

A study performed on a group of non-lymphocytic leukaemia patients revealed that all developed infections with hospital pathogens, especially with *Pseudomonas aeruginosa*. Some of the patients died as a result of infection and bacteraemia, and 14 out of a total of 111 infection episodes were demonstrated in the pharynx [1]. An increased prevalence of gram-negative bacilli in the oral–pharyngeal flora was found in such patients, apparently due to increased adherence of these bacteria to epithelial cells [2]. In another study conducted on 1000 hospitalised patients with leukaemia, the oral infection rate was 32.9%, and 25% of these infections were produced by gram-negative bacilli [3].

Infection in bone marrow-transplanted patients is a multifactorial condition. Prior to the reconstitution of the immune system following transplantation, both neutropenia, lymphocytopenia and the destruction of the oral mucosa barriers are primary risk factors for bacterial infections [4]. The period of severe granulocytopenia is approximately 18 days [5] but is longer in patients receiving T-cell-depleted or autologous bone marrow transplant [6, 7].

Enterobacteriaceae were found in 20% of blood cultures taken from patients with bacteraemia, either before or immediately after engraftment [4]. Herpetic infections of the oral cavity occurring during the period of nadir may lead to severe secondary bacterial infection [8]. Furthermore, bacterial infections during the period which follows the bone marrow transplantation are reported at a high rate, 64% with gram-positive species and 20% with gram-negative species [4]. Solberg *et al.* [9] in 1971 suggested that gram-negative bacilli are the most common causative organisms in systemic infections. In their report 9 out of 11 patients had gram-negative infections and 6 died. Similar findings were described by Greenberg *et al.* in 1982 [10]. Oral infections were also studied by Heimdahl *et al.* [11] who found episodes of septicemia with *Klebsiella* spp. and *Pseudomonas* spp, the latter resulting in death. Almost all the patients developed fever secondary to severe granulocytopenia prior to the transplantation or immediately after it. A shift in the oral flora from the saprophytic gram-positive aerobes and anaerobes to the gram-

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negative aerobic bacilli was demonstrated in the immunocompromised patients. In these cases, subclinical infections might exacerbate [12, 13].

The objective of the present study was to conduct surveillance cultures for *Enterobacteriaceae* at different periods of time during the clinical course of bone marrow transplantation. The need for this evaluation arose from the systemic implications of oral infection and their nosocomial nature, in patients at high risk. Members of this family of microorganisms frequently colonise the nasopharynx and oropharynx of the seriously ill debilitated patients [14].

PATIENTS AND METHODS

A group of 40 patients was examined, each a mean of 10.5 times during the study period.

The patients were divided into two main groups, according to their diagnosis and the preparatory therapy. A first group of 29 leukaemia patients included 17 patients with acute myelocytic leukaemia, 5 patients with acute lymphocytic leukaemia and 7 patients with chronic myelocytic leukaemia. In this group, the conditioning included total body irradiation (TBI) with 1200 rad or total lymph node irradiation (TLI) with 600 rad. The chemotherapy consisted of cyclophosphamide 1500 mg/m^2, melphalan 60 mg/m^2, etoposide (VP-16) 1500 mg/m^2. Adjacent to the transplantation, cytosine arabinoside was given intrathecally. A second group of 9 patients consisted of 6 patients with Hodgkin's disease and 3 patients with non-Hodgkin's lymphoma. To the preparatory protocol of these patients, carmustine (BCNU) 300 mg/m^2 was added, without any irradiation. 2 more examined patients suffered from severe aplastic anaemia. They received TLI 1800 rad and chemotherapy with cyclophosphamide 200 mg/kg and busulfan 4 mg/kg. As a rule, the leukaemia patients received T-cell-depleted (CAMPATH-treated) allogeneic bone marrow, whereas the lymphoma patients were transplanted with autologous bone marrow [15].

A group of 10 patients hospitalised for at least 1 week in the Medicine Department served as an indicator for the presence of *Enterobacteriaceae* in the oral flora of immune-competent hospitalised patients. Their diagnoses were ischaemic heart disease or obstructive lung disease with no systemic infections or antibiotic therapy.

In order to minimise the chances of oral infections, all of the patients received an oral examination and basic dental treatment aimed at eradication of oral infections, which included scaling, curettage, oral hygiene instructions, treatment of carious lesions, root canal treatment and extractions when needed. Oral rinses with chlorhexidine 0.2% 5 ml twice daily for 1 min, and administration of nystatin or amphotericin B were instituted on a routine basis. The patients rinsed with a normal saline solution three to four times a day. Patients' compliance with this protocol was closely watched.

Patient examination

The patients were examined every 3 days by the same examiner. Special attention was paid for the presence of mucositis and clinical signs of oral bacterial, fungal or viral infections. The patients' general status was recorded for fever, blood cultures results and peripheral blood counts, including differential counts.

Oral bacteriological cultures

Oral bacteriological samples were taken every 3 days by a routine standardised method of passing a sterile swab through the oral tissues, including the existing ulcerated areas. The cultures were taken at least 5 h following the last chlorhexidine rinse.

The swabs were immediately transferred to the Microbiology Laboratory and cultured on MacConkey medium (specific for gram-negative bacteria), incubated at 37°C for 18–24 h and examined for bacterial growth. Bacterial identification of *Enterobacteriaceae* was performed with the Titertek-Enterobac kit (Flow Lab, Ltd. Irvine, Ayrshire, U.K.), which is mainly aimed at identifying *Enterobacteriaceae*.

In order to facilitate the statistical analysis, the data were grouped according to five time periods: days -11 to -8, the baseline period, when 32 examinations were performed; days -7 to -3, the pretransplantation period, 56 examinations; days -2 to 2, peritransplantation period, 56 examinations; days 3 to 8, immediate post-transplantation period, 75 examinations; days 9 to maximum (day 46), post-transplantation period, 207 examinations, which was the longest and obviously involved the largest population of patients.

Statistical analysis of results

The results were analysed by two statistical tests. For a parametric sample with a normal distribution a Student's *t*-test was used. When the distribution of the sample was not known (aparametric), the Wilcoxon matched pairs signs rank test was employed. For the analysis of the correlation between groups of data, the Pearson's product-moment correlation coefficient and the Spearman's rank correlation coefficient tests were used.

Comparisons between subgroups of the study population with the background parameters, e.g. age, sex, primary disease, type of transplantation and survival were conducted. The correlation between the background and the patients' laboratory results and clinical signs was related to the oral bacteriological and blood cultures.

RESULTS

The patients distribution by sex was 19 females and 21 males. The age range of the examined group was 5–42 years old with a mean age of 24.5 years (S.D. $\pm$9.87).

Two types of transplantation were performed: autologous (14 patients) and allogeneic (26 patients). Out of the patients included in the study, 11 expired while still being hospitalised.

Oral cultures

A total of 426 oral microbiological cultures were performed. In 128 (30.5%) samples, gram-negative bacteria were identified. In 98 (76.6% of these cultures) samples *Enterobacteriaceae* could be detected (Table 1).

In 11 cases, two different microorganisms were isolated in the same culture. Thus 22 isolates out of the 98 showed a combination of two strains whereas the majority of 76 showed single *Enterobacteriaceae* growth (Table 2). In a relatively large number of cultures with the combination of bacteria, *Enterobacter agglomerans* was present (five cultures out of 11) however, this microorganism was found in only seven cultures out of the total 98. In a number of patients, the same strain was repeatedly found. *Klebsiella* spp. were found four times in 9 patients, whereas *Enterobacter* spp. were isolated between two and four repeated times in 10 patients.

Table 1. Gram-negative bacteria isolated from oral cultures, identified with the Titertek-Enterobac kit

Bacteria	No. of cultures
Klebsiella oxytoca	23
Klebsiella pneumoniae	15
Klebsiella ozaenae	2
Total *Klebsiella* spp.	40
Enterobacter cloacae	18
Enterobacter aerogenes	7
Enterobacter agglomerans	7
Enterobacter sakazakii	6
Enterobacter vulneris	2
Total *Enterobacter* spp.	40
Citrobacter freundii	3
Citrobacter diversus	2
Total *Citrobacter* spp.	5
Escherichia coli	7
Acinetobacter anitratus	3
Yersinia enterocolitica	2
Serratia liquefaciens	1
Subtotal	98
Unidentified	30
Total	128

Table 2. Combinations of two Enterobacteriaceae *in oral cultures*

	Frequency of combination
Enterobacter agglomerans + *Klebsiella oxytoca*	3
Enterobacter sakazakii + *Enterobacter cloacae*	2
Acinetobacter anitratus + *Escherichia coli*	2
Enterobacter agglomerans + *Citrobacter freundii*	2
Enterobacter sakazakii + *Klebsiella pneumoniae*	1
Serratia liquefaciens + *Enterobacter aerogenes*	1

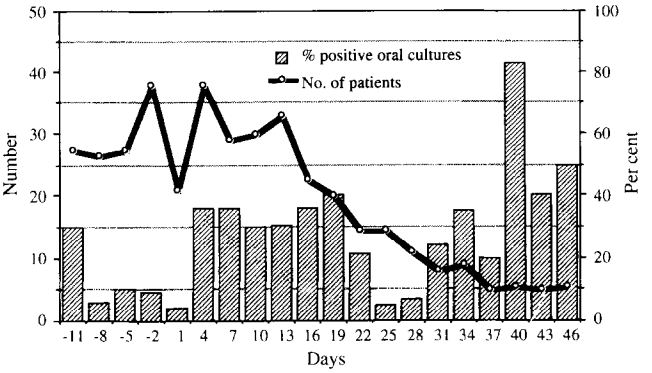

Fig. 1. Number of patients and percentages of positive oral cultures by time.

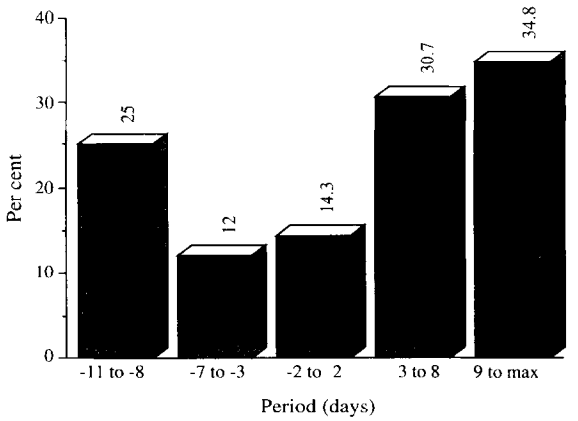

Fig. 2. Percent of patients with positive oral cultures according to five time periods.

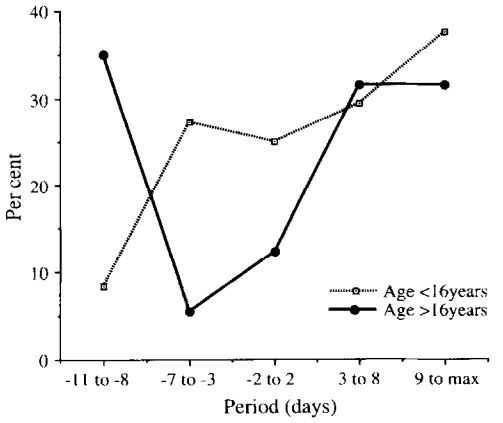

Fig. 3. Positive oral cultures by age.

While during the baseline period 25.0% of the oral cultures were positive, a marked decrease to 12.0 and 14.3% was found during the pretransplantation and peritransplantation periods, respectively, followed by a 30.7 and 34.8% increase during the immediate post-transplantation and post-transplantation periods, respectively (Figs 1, 2).

A positive correlation was found between the percentage of positive oral cultures and the patients' age, younger than 16 years and older than 16 years, according to the five described periods: only 8.3% of the younger group showed positive cultures compared to 35.0% in the older group at the baseline period. A constant rise in that percentage was found in the younger group to 27.3%, 25.0%, 29.4% and 37.5% during the consecutive periods. The older group showed a marked decline in positive cultures at the pretransplantation period (5.4%) followed by a constant increase to up to 31.5% during the last period of examination. These differences were not statistically significant (Fig. 3).

When the patients were divided according to the two types of transplantation, virtually identical percentages of positive oral cultures were obtained. However, during the post-transplantation period a higher percentage was observed in the allogeneic group (43.0%) as compared with a low one in the autologous group (19.5%). These differences were not statistically significant. Male patients showed a remarkably higher percentage of positive cultures at the baseline than females, 36.8 versus 7.7%, respectively, however this was not statistically significant. No differences between those two groups were found during the further study period.

The follow-up of the oral cultures in the patients who survived was not different from the patients who expired during the study period.

A significant difference was found between the positive oral cultures in patients with leukaemia when compared to the lymphoma patients, throughout the period of study ($P=0.06$, t-test). During the post-transplantation period, the leukaemia group showed 37.6% positive oral cultures, while only 15.4% of the lymphoma group had similar findings. It should be taken into consideration that the leukaemia patients received either TLI or TBI whereas the lymphoma patients were not at all irradiated according to the protocol. Only one of the oral bacterial cultures taken from the control group of 10 cultures grew gram-negative bacteria.

Blood cultures

Ninety-four positive bacterial blood cultures were obtained from the 40 patients, during the study period. Eighteen of these cultures were positive for *Enterobacteriaceae* (Table 3).

Positive blood cultures showed an almost constant percentage in the patients examined, relative to time since transplantation (Fig. 4). There was no change in the time distribution when the *Enterobacteriaceae* were analysed. Patients younger than 16 showed a significantly higher percentage of positive blood cultures than the older ones ($P=0.007$), starting at the peritransplantation period, 16.7 versus 14.6% and increasing to 23.5 versus 17.3% during the immediate post-transplantation period. During the post-transplantation period a decrease in positive blood cultures was detected but still with a higher percentage of 19.4% in the younger group versus 13.3% in the older group.

The patients who survived at the end of the study had fewer positive blood cultures than the ones who expired, best seen during the last two periods, 25.0 and 21.0% compared with 17.0 and 11.9% ($P=0.025$).

*Table 3. Positive isolates from blood cultures**

Bacteria	No. of cases
Staphylococcus coagulase negative	49
Staphylococcus coagulase positive	7
Total *Staphylococcus* spp.	56
Streptococcus viridans	8
Streptococcus faecalis	3
Total *Streptococcus* spp.	11
Klebsiella oxytoca	1
Klebsiella pneumoniae	3
Enterobacter cloacae	5
Escherichia coli	4
Citrobacter freundii	3
Acinetobacter anitratus	1
Proteus mirabilis	1
Total *Enterobacteriaceae*	18
Pseudomonas aeruginosa	7
Diphteroides	2
Total	94

*Four blood cultures were positive foryeasts.

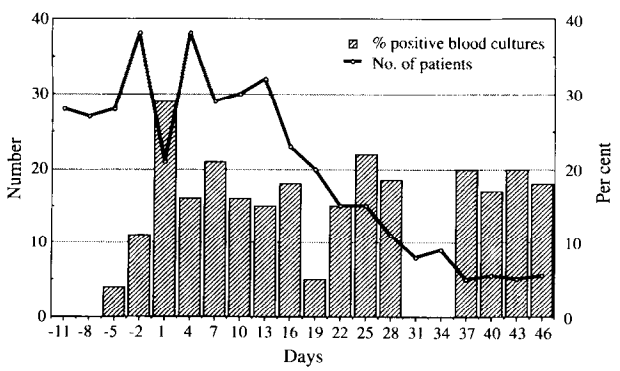

Fig. 4. Number of patients and percentages of positive blood cultures by time.

DISCUSSION

According to the data reported in the literature, gram-negative bacteria are detected in about half of the oral cultures taken from patients undergoing chemotherapy [3, 16–18], but data relating to bone marrow-transplanted patients is sparse. In our study, a lower prevalence of only 30.5% was detected, and approximately 80% of these were identifiable.

The possible explanation for this difference is the fact that all of our patients were following a meticulous oral hygiene protocol which included local mechanical eradication of possible sources of infection accompanied by 0.2% chlorhexidine mouth rinses. The three most common microorganisms detected were *Klebsiella oxytoca* (23%), *Enterobacter cloacae* (18%) and *Klebsiella pneumoniae* (15%) (Table 1). (The latter is recognised as one of the most common causes of gram-negative bacteraemia in granulocytopaenic patients with acute leukaemia.) *Pseudomonas aeruginosa*, which was also suggested to cause bacteraemia in these patients [19], was found by us only in 7 cases (of 94 positive cultures). The 30% unidentified positive oral cultures in our study most probably contained *Pseudomonas*, however, the identifying kit used was not aimed at this agent.

Enterobacter agglomerans seems to be an example of oral commensalism with other *Enterobacteriaceae*, being found in five of the 11 polyinfection cases (Table 2). Repeated colonisation, mainly with *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *E. coli*, suggests that these microorganisms were harder to eradicate from the oral microflora than the other *Enterobacteriaceae*.

The three peaks of positive oral cultures, seen in Fig. 1, are related to: (1) the pretransplantation period, i.e. previous to the treatment of oral infection; (2) the immediate post-transplantation period; and (3) at a much later stage, the post-transplantation period, when the patients could develop graft-versus-host disease. Analysis of the first peak disclosed that the majority of the patients were males older than 16 years, probably as a result of the fact that their oral health status prior to hospitalisation was poor. Another possible explanation for the relatively high incidence of gram-negative bacteria in these patients was mentioned by Johanson *et al.* [23] who showed that both the illness and the antimicrobial agents used for treatment create a shift towards such infections; a fact suggested in our study by the lack of similar gram-negative infections in the immunocompetent hospitalised patients.

The decrease in the positive oral cultures from 35% during the pretransplantation period to 5.4% adjacent to the transplantation itself demonstrates that the preparatory protocol used for prevention of oral infections was highly effective. However, the steady increase noticed during the following periods points to the fact that the worsening of the oral health status, including mucositis together with possible nosocomial infections, may have certain correlative significance. Indeed, the post-transplantation period (days 9–50) showed the return to the baseline period incidence of oral cultures positive for

gram-negative bacteria. The second peak may be explained by the nadir in granulocyte counts and the development of mucositis. This period is known to be most fulminant, due to extreme immunological deficiency [21]. Oral and systemic conditions lead to dysphagia, oral hygiene is uncontrollable, salivary secretion is diminished and the incidence of oral infections increases significantly. The third peak was detected in the group of patients hospitalised for a longer period of time (up to 46 days) due to medical complications. Of these patients, 39.5% expired and all of them had oral cultures positive for gram-negative microorganisms. The observed differences in oral cultures between the allogeneic transplantation group and the autologous one (such as in leukaemias versus lymphomas) could be explained by the more severe immunosuppression of the first group following the more aggressive chemotherapy protocol and the added irradiations. As such, these patients should be more prone to the development of infections with gram-negative bacteria.

The daily blood cultures taken from all the patients who participated in the study showed positive results for a variety of microorganisms, concomitant with the decrease in the number of peripheral granulocytes. Throughout the study an almost constant percentage of patients showed positive blood cultures but only 26.6% grew gram-negative bacteria. In this same group, 18 out of the 94 (19.1%) showed *Enterobacteriaceae*. Of those, only 2 cases were positive oral isolates with similar microorganisms detected previous to the bacteremia. It is possible that the additional 16 cases originated from sources other than the oral cavity, such as the urinary tract or respiratory system. The discrepancy between the oral infections with *Enterobacteriaceae* and bacteremia could be explained by the prevention of the spread of infection due to the careful eradication of oral infections and the strict oral hygiene protocol followed by our hospitalised patients. In 9 leukaemia patients who did not receive dental treatment prior to chemotherapy, Greenberg *et al.* [10] reported that 77% developed septicemia and 4 of the septicemia cases were related to oral bacterial infection. This study strongly suggests that this risk was significantly reduced in bone marrow-transplanted patients who followed our protocol.

In conclusion, members of *Enterobacteriaceae* are a clinically significant component of the oral flora in patients undergoing bone marrow transplantation. Male patients and/or patients younger than 16 years showed a higher percentage of oral positive isolates prior to treatment. Peaks in positive oral cultures were found during the pretransplantation period, the immediate post-transplantation and during the late post-transplantation periods. In only a few incidents septicemia could be directly related to oral infections with *Enterobacteriaceae*. It seems that eradication of oral infection contributes to the prevention of systemic infections with microorganisms originating from the oral cavity in bone marrow recipient patients.

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