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Association between Tonsillar Core Microflora and Post-tonsillectomy Bacteremia

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The aim of the study was to determine whether the population levels of aerobic and anaerobic bacteria in the core tonsils of adults with recurrent acute tonsillitis influence the development of post-tonsillectomy bacteremia. For that purpose we performed qualitative and quantitative bacteriology of 24 excised tonsillar core specimens, together with aerobic and anaerobic perioperative blood cultures. The blood cultures from ten non-surgical patients with recurrent acute tonsillitis served as controls to determine the occurrence of baseline bacteremia. Post-tonsillectomy bacteremia was found in nine (38%) patients. Isolated bacteria were α-hemolytic streptococci ($n = 2$), group C β-hemolytic streptococci ($n = 2$), group A β-hemolytic streptococcus (GAS) ($n = 1$), *Peptostreptococcus* sp. ($n = 1$), *Bacteroides* sp. ($n = 1$) and *Prevotella* sp. ($n = 2$). None of the blood cultures from the controls were positive. Mixed aerobic and anaerobic bacterial flora was found in the tonsil cores from both the patients with blood culture positive or negative results. All blood culture isolates were also recovered in the corresponding tonsillar core, except for the GAS. However, its presence was subsequently demonstrated by PCR. In further analysis, we found no correlation between the bacterial population levels and the development of post-tonsillectomy bacteremia. The absence of GAS growth from the core tonsil of a patient with GAS bacteremia also suggests that translocation of certain bacteria may occur in spite of low counts. In conclusion, the occurrence of post-tonsillectomy bacteremia is quite frequent in adults and the spectrum of invading agents is variable. *Key words*: post-tonsillectomy bacteremia, tonsil core cultures.

INTRODUCTION

The passage of viable indigenous bacteria across the mucosal barrier to local mesenteric lymph nodes and to other normally sterile body sites has been defined as bacterial translocation (1). Several factors, such as disruption of the mucosal barrier, a compromised defence system of the host and alteration of the indigenous microflora promote bacterial translocation both in animals and humans (1–3). In animal models, bacterial overgrowth in the indigenous microflora promotes translocation of aerobic and facultative bacteria across the intact mucosal barrier, whereas the translocation of obligate anaerobes has been reported to stay low (1, 2, 4, 5). On the other hand, in the situation of altered mucosal barrier increased incidence of bacterial translocation has been found, and strict anaerobes appear to translocate along with aerobic and facultative species (1, 2). However, these conclusions have not been consistently supported by clinical observations (6, 7).

In experimental and clinical studies, most interest has been focused on the gastrointestinal tract as potentially the most important source of translocating bacteria (1–3); however, bacterial translocation can take place through several mucosal membranes. Dissemination of bacteria via the bloodstream during a number of surgical and diagnostic procedures is called transitory bacteremia, which is particularly common during dental extractions in patients with periodontal disease, ranging from 10 to 100% in different studies (4, 8). The positive blood culture following tonsillectomy is also a well-recognised phenomenon, with reported incidences of 20–40% in children (9–12). In adults, the incidence of post-tonsillectomy bacteremia has not been assessed by advanced aerobic and anaerobic blood culture bottle techniques (13). The relation between the abundant mixed aerobic and anaerobic bacterial flora, which has been shown in the core tonsils of children and adults with recurrent acute tonsillitis (14, 15) and the development of post-tonsillectomy bacteremia is not yet clear. In children, no relation has been found between the aforementioned tonsillar flora and bacteremia, while unfortunately only assessment of aerobic species in blood cultures was performed (10). Therefore, this issue deserves a more sophisticated approach by also paying attention to anaerobic species.

The present study was undertaken to determine whether the population levels of aerobic and anaerobic bacteria in the core tonsils of adults with recurrent acute tonsillitis influence the development of post-tonsillectomy bacteremia. For that purpose we performed qualitative and

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quantitative bacteriology of the excised tonsillar core specimens, together with aerobic and anaerobic perioperative blood cultures. For detection of group A β-hemolytic streptococcus (GAS) in tonsillar core specimens, the PCR method was simultaneously applied.

MATERIALS AND METHODS

Patients

The study involved 34 adult patients (21 females and 13 males ranging in age from 15 to 42, mean 24 years) who underwent elective tonsillectomy for recurrent acute tonsillitis at the Department of Otorhinolaryngology. The adult patient was defined as 15-years-old and over (9). The decision for tonsillectomy had been made previously by a member of the department and was defined as five or more attacks of tonsillitis within the previous year (9). The recruitment was made at random from the respective population of patients of adult age on certain days of week. Exclusion criteria included those who had received antibiotics in the 5 weeks prior to hospitalisation and the medical conditions that would require antibiotic treatment in the perioperative period. The study was approved by the Local Research Ethics Committee and in each case written informed consent was obtained from the patient. In 24 patients the qualitative and quantitative bacteriology of the excised tonsillar core specimens were performed, together with aerobic and anaerobic perioperative blood cultures (study group). These patients were followed up for postoperative infectious complications for 1 week. Another ten patients without surgery served as a control group to determine the occurrence of baseline bacteremia. All patients served as a part of a larger ongoing study to determine the clinical preoperative risk factors for the development of post-tonsillectomy bacteremia.

Procedures

All operations were carried out under general anaesthesia with the use of orotracheal intubation by a standard dissection technique. During removal of the second tonsil (approximately 5 min after removal of the first), the peripheral blood cultures were drawn aseptically into a BACTEC Plus Aerobic/F and a BACTEC Plus Anaerobic/F blood culture bottles (Becton Dickinson, USA). The blood cultures of the control group were drawn a day before the operation, before any oro-pharyngeal manipulations, administration of oral and parenteral drugs or having a meal. All blood culture bottles were promptly taken to the laboratory and incubated at 36°C in a fully automated blood culture instrument (Bactec 9050™, Becton Dickinson, USA). The blood culture bottles were cultured totally in 7 days. When evidence of growth was noted, Gram staining and subculture on relevant plates were performed. All strains were identified according to standard laboratory methods (17). Among gram-negative anaerobic isolates yielding black colonies, the indole positive, colistine sensitive isolate was presumptively identified as *Prevotella intermedia*, and the indole negative, colistine resistant isolate as *P. melaninogenica*.

Bacteriological analyses

Sample preparation. Immediately after excision, one of the tonsils was placed in a sterile Petri dish and taken on ice to the laboratory. One side of the tonsil was cauterised with a heated scalpel, and an incision was made through that area cutting the tonsil in half. For a tonsillar core culture (representing the microbial flora of the tonsillar crypts) approximately 0.2 g of tissue was aseptically excised and homogenised in a sterile mortar with a known amount of pre-reduced phosphate-buffered saline (PBS; pH 7.2) in the anaerobic glove box (Sheldon Manufacturing Inc., USA, with a gas mixture: 5% CO_2, 5% H_2, 90% N_2) and was further serially diluted (10^{-2}–10^{-7}).

Isolation of tonsillar microbes. Serial dilutions of tonsillar tissue were seeded on eight freshly prepared media. Blood agar was employed for total aerobes count, Columbia agar with streptococcus selective supplement for hemolytic streptococci, chocolate agar with Vitox supplement for fastidious bacteria, Endo and McConkey agar for enterobacteria and non-fermenting microorganisms, de Man–Rogosa–Sharpe agar for lactobacilli, Wilkins–Chalgren agar with vancomycin and nalidixic acid supplement for gram-negative anaerobes and Wilkins–Chalgren agar with colistin sulphate and nalidixic acid for gram-positive anaerobes. All these media and supplements were purchased from Oxoid Ldt., UK. The anaerobic plates were incubated for 5–6 days at 36°C in an anaerobic glove box; blood, chocolate, Columbia and MRS agar plates were incubated for 48 h at 36°C in an atmosphere enriched with 10% CO_2 in Jouan IG150 incubator (Jouan, France), and McConkey and Endo agar plates were incubated for 48 h at 36°C in an ambient atmosphere. Colonies with different morphology growing on the plates with the highest dilutions of bacteria were Gram stained and subjected to microscopy. The microorganisms were identified mostly on genus or species level with the use of conventional methods (17).

PCR amplification

Total genomic DNA was extracted from the tonsillar tissue sample by the method of Louie et al. (18). For the amplification of the specific GAS mitogenic factor gene (19), the following primers were used: forward, 5′-CTA CTT GGA TCA AGA CGG-3′; and reverse, 5′-TTA GGG TTT CCA GTC CAT CC-3′. The PCR was performed in a 25 μl volume with $\sim$10 ng DNA sample, in an automated thermal cycler (Biometra, Eppendorf) by using a Ready-To-Go PCR Bead (Amersham Pharmacia Biotech Inc., USA). Extracted DNA of *Streptococcus pyogenes* ATCC 19615 was served as a positive control.

Statistical methods

The total count ($\log_{10}$ CFUs/g—colony forming units per gram of core tonsil) of microorganisms and the counts of various genera and species were calculated for each patient by using growth results from serial dilutions. The detection level of the various microorganisms was 3 $\log_{10}$ CFUs/g. In addition, the relative amounts of the particular microbes were expressed as a proportion of the total count (%). The microorganisms were considered potentially predominant if they made up more than 10% of the total population (20).

Statistical analyses were performed by using 'Statgraphics' (Statistical Graphics Corp.) and 'EXCEL' (Microsoft Corp.) software programs. In comparison of bacteriological analyses the following tests were employed: χ^2 test (prevalence of colonisation), Mann–Whitney rank sum test for unpaired data and Student's t-test for paired data (counts). The significance level was $p < 0.05$.

RESULTS

Prevalence of bacteremia

Nine of the 24 (38%) patients in the study group demonstrated post-tonsillectomy bacteremia. None of the blood cultures from the controls were positive. Different aerobic and anaerobic bacteria were recovered from positive blood culture bottles (Table I), one isolate from each. Patients in the study group were further subdivided into blood culture positive ($n = 9$) and negative ($n = 15$) groups, indicating the presence or absence, respectively, of post-tonsillectomy bacteremia. None of the patients in both subgroups had major infectious complications during the follow up period.

Tonsillar core microflora

Mixed aerobic and anaerobic bacterial flora was present in all core tonsils of the study group patients. Details of the tonsillar core bacteriology are given in Table II. The most frequently isolated aerobic microorganisms were α- and β-hemolytic streptococci, *Staphylococcus aureus*, coagulase negative staphylococci and *Corynebacterium* spp. The most prevalent anaerobic bacteria were *Peptostreptococcus*, *Propionibacterium*, *Actinomyces*, *Prevotella*, *Bacteroides* and *Fusobacterium* species. We found no differences in the total number of isolates (mean 14.6 ± 2.1 vs 14.5 ± 4.3, $p = 0.732$) per tonsil, and the number of aerobic (mean 7.4 ± 1.9 vs 7.5 ± 3.9, $p = 0.980$) or anaerobic bacteria (mean 7.2 ± 2.4 vs 7.0 ± 4.8, $p = 0.581$) between the blood culture positive and negative groups. All bacteria recovered from the positive blood cultures were also present in the corresponding tonsillar cores, except for the patient in whom GAS was isolated from the blood. Therefore, the PCR method was simultaneously applied for more objective assessment of GAS in the tonsillar core specimens. Although none of the core tonsils showed GAS growth by culture method, PCR revealed the presence of GAS DNA in seven of 24 (29%) tonsillar tissue specimens, including the patient growing GAS from the blood.

Table I

Microorganisms isolated from positive blood cultures of 24 patients during tonsillectomy

Aerobes	
α-Hemolytic streptococci	2
Group A β-hemolytic streptococci	1
Group C β-hemolytic streptococci	2
Anaerobes	
Peptostreptococcus sp.	1
Bacteroides non-fragilis group	1
P. intermedia	1
P. melaninogenica	1
Total	9

Tissue concentration of bacteria in relation to bacteremia

In the quantitative analysis of the tonsillar core microflora there were no significant differences in the mean count of isolated bacterial species/groups between two subgroups. However, the total count of anaerobes was higher in the blood culture positive than in the blood culture negative group ($\log_{10}$ 8.2 ± 1.3 CFU/g vs 7.5 ± 0.8 CFU/g, $p = 0.036$), whilst the mean count of aerobes was equal in both subgroups ($\log_{10}$ 7.1 ± 0.8 CFU/g vs 7.0 ± 0.9 CFU/g, $p = 0.669$). In further analysis, the proportions (%) of bacteria from total population of microorganisms in the core tonsils were calculated. We found that *Peptostreptococcus* and *Fusobacterium* species had the highest proportions in the core tonsils of the study group as a whole (Table II). Fig. 1 demonstrates the proportions of the blood culture isolates in the core tonsils of the blood culture positive and negative groups. In both subgroups, the predominating bacteria were *Peptostreptococcus* and *Prevotella* species, while *Bacteroides* species predominated also in the blood culture negative group. Only in cases of *Peptostreptococcus* sp. and *Bacteroides* sp. bacteremia were their proportion in the corresponding core tonsil predominating (26.3 vs 62.5%), while isolated α- and β-hemolytic streptococci and *Prevotella* species were proportionally less numerous ($\leq 3\%$).

DISCUSSION

In the present study the occurrence of post-tonsillectomy bacteremia in adult patients (38%) was comparable with that of children described in previous studies (9–12). We chose to investigate adults because of the lack of recent data (13), although tonsillectomies are also commonly performed at the age of 15–35 years. It has been reported that pre-tonsillectomy blood cultures were always sterile and the duration of procedure-related bacteremias is short

Table II

Microorganisms recovered in tonsillar cores from 24 patients

Microorganisms	Number of isolates	Mean counts of organisms/g ($\log_{10}$ CFU $\pm$ SD)	Mean proportion of total count (%)
Aerobic and facultative bacteria			
α-Hemolytic streptococci	24	6.3 ± 0.9	1.8
Group C β-hemolytic streptococci	11	6.4 ± 1.0	2.2
Group F β-hemolytic streptococci	12	6.0 ± 1.1	0.8
Group G β-hemolytic streptococci	2	5.2 ± 1.3	0.1
S. aureus	15	5.9 ± 0.9	0.7
Coagulasnegative staphylococci	13	6.4 ± 1.6	2.2
Stomatococcus sp.	10	4.2 ± 0.6	0.01
Enterococcus sp.	2	4.5	0.02
Moraxella sp.	8	5.2 ± 1.1	0.1
M. catarrhalis	11	5.3 ± 1.1	0.2
Neisseria sp.	10	5.4 ± 1.0	0.2
Corynebacterium sp.	16	5.7 ± 1.1	0.5
Lactobacillus sp.	2	6.0 ± 0.9	0.8
H. influenzae	8	6.3 ± 0.9	1.8
H. parainfluenzae	11	5.0 ± 0.6	0.1
Eikenella corrodens	9	5.6 ± 1.0	0.4
Other non-fermentative	4	4.7 ± 0.5	0.1
Escherichia coli	1	5.2	0.1
Capnocytophaga sp.	6	4.9 ± 0.8	0.1
Anaerobic bacteria			
Peptostreptococcus sp.	22	7.4 ± 1.1	22.4
Veillonella sp.	1	3.6	0.004
Propionibacterium sp.	21	6.8 ± 1.1	5.6
Bifidobacterium sp.	2	6.7 ± 2.1	4.5
Eubacterium sp.	6	6.6 ± 1.0	3.6
Actinomyces sp.	16	6.9 ± 1.0	7.1
Prevotella sp.	22	7.0 ± 1.2	8.9
Porphyromonas sp.	3	6.6 ± 1.0	3.6
Bacteroides sp.	21	7.0 ± 1.2	8.9
Fusobacterium sp.	22	7.4 ± 1.6	22.4
Leptotrichia sp.	6	5.9 ± 1.1	0.7
Total aerobic isolates	175	7.2 ± 0.9	12.3
Total anaerobic isolates	142	8.0 ± 0.9	87.7
Total isolates	317	8.1 ± 0.9	100.0
Aerobes:anaerobes			1:7.1

lived (8, 9, 11). Therefore, the blood cultures directly before tonsillectomy (after intubation) and also during the follow up period were not performed. Every microorganism recovered from the positive blood culture in this study was normally found in the core tonsils of patients with recurrent acute tonsillitis. However, many of these bacteria also belong to the normal microflora of several mucosal membranes, and therefore, it is difficult to consider if a microorganism causing a given case of transient bacteremia originated from the tonsils. On the other hand, the recovery of positive blood cultures only in the study group patients strongly supports the dissemination of bacteria from the tonsils in relation to surgical procedure. Seemingly, fingerprinting of collected strains could provide further help, unfortunately not done in the present study.

In children, the most common blood culture isolate after tonsillectomy has been *Haemophilus influenzae* (9, 10). In adults, we found that α- and β-hemolytic streptococci and *Prevotella* species were the most common blood culture isolates. An explanation why *H. influenzae* was not found in the present material is that its prevalence is higher in children and consequently lower in adults (16, 21). Of note was the frequent recovery of anaerobes (four of the nine) from the blood cultures in our series. Such high incidence of anaerobes has not been reported in previous studies in children (9–12). A possible explanation for this finding is that the ability of bacteria to survive in the bloodstream has perhaps greater importance in determining differences between the isolation rates of aerobes and anaerobes in the blood cultures than their ability to pass through mucosal barriers. It has been shown that aerobes survive better in the bloodstream than anaerobes (7). Therefore, the high incidence of anaerobic post-tonsillectomy bacteremia in the present study supports the effec-

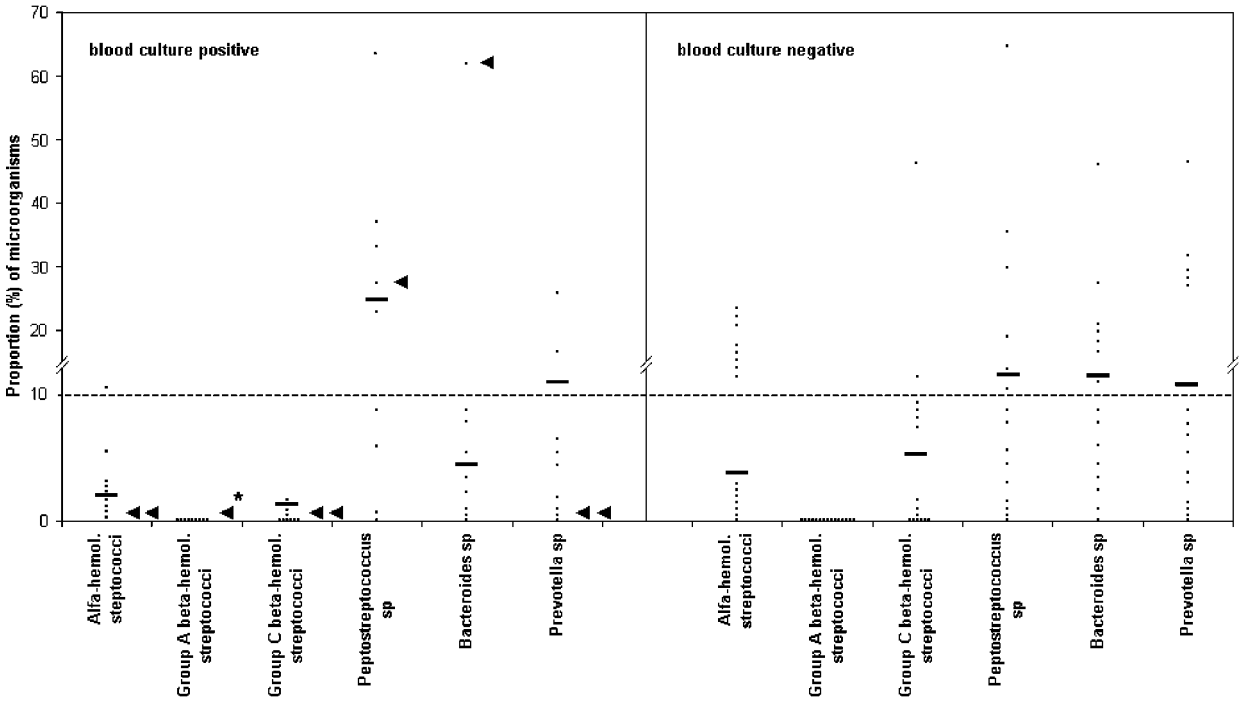

Fig. 1. Predominance of blood culture isolates in the core tonsils of patients in blood culture positive ($n = 9$) and negative ($n = 15$) groups. Each dot represents the proportion (%) of the bacterium of the total bacterial count in a single core tonsil. The short lines represent the mean of the proportions of the bacteria. The dotted line represents the 10% cut-off value. Arrowheads indicate the proportion of the blood culture isolates in the corresponding core tonsil (* GAS was detected by PCR).

tiveness of the blood sampling and culture technique used in this study.

We chose to culture the tonsillar core rather than the surface because of higher recovery of anaerobes from the core and the findings that the surface tonsillar flora may not always reflect the content of the deep tonsillar flora (15, 16). Similar to previous studies (14–16), we found abundant mixed aerobic and anaerobic bacterial flora in the core tonsils of the study group patients. One of the most considerable findings in the present study was the total absence of GAS growth from the tonsillar cores by conventional culture method, while revealing the presence of GAS DNA in 29% of the tonsillar tissue specimens by PCR method. In the tonsillar core culture the incidence of GAS among the >15-year-old group has only been 5%, which is significantly lower than 15% among the 2–14-year-old group (15). An explanation for this decreasing incidence of GAS in different age groups is not clear. The growth inhibition by oral α-hemolytic streptococci and some anaerobic bacteria (22) or intracellular penetration of GAS, thus being non-cultureable (23), has been speculated. Therefore, DNA-based identification of some non-cultureable pathogens may favour the assessment of etiology of recurrent acute tonsillitis, including the virtual role of GAS, as our present study indicated.

In the present study, the proportions (%) of bacteria from the total population of microorganisms in the core tonsils were calculated. We suggest that the total count of bacteria may vary between individuals and is therefore, an unreliable characteristic (20). Thus, to establish the association between individually different counts of tonsillar bacteria and their translocation, we considered proportions to be more appropriate. We found that all α- and β-hemolytic streptococci and *Prevotella* species recovered from blood cultures were present at subordinate proportions in the corresponding core tonsil, while only in cases of *Peptostreptococcus* sp. and *Bacteroides* sp. bacteremia were their proportions predominating. This is consistent with the study of children (10) where no association was found between mean tonsillar colony counts of blood culture isolates and the occurrence of post-tonsillectomy bacteremia. The occasional relation between the bacterial proportions and their translocation, as well as the lack of baseline bacteremia in controls, reflects that the physical disruption of the mucous barrier during surgery has perhaps greater importance in the development of post-tonsillectomy bacteremia than the contents of the tonsillar core microflora. The absence of GAS growth from the core tonsil of one patient growing GAS from the blood support the suggestion that translocation of certain bacteria during surgery occurs in spite of low counts of the bacterium in the tonsillar tissue.

We conclude that the post-tonsillectomy bacteremia in adults with recurrent acute tonsillitis occurs in a significant

percentage by both aerobic and anaerobic bacteria. We found that the bacterial population levels have little or no influence on the genesis of post-tonsillectomy bacteremia, and that it may occur in spite of very low counts of bacterium in the core tonsils. The sensitive DNA-based identification methods help to reveal some hidden, difficult-to-culture microorganisms in the core tonsillar tissue, as with GAS in our present study.

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