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Investigation of bacteremia induced by removal of orthodontic mini-implants

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SUMMARY The aim of this study was to investigate potential occurrence of bacteremia in orthodontic patients after removal of miniscrews. The study group comprised 30 healthy subjects (17 males, 13 females) with a mean age of 24.1 years treated with self-ligating fixed appliances and mini-implant anchorage. Two 20 ml venous blood samples were obtained prior to and 30–60 seconds after miniscrew explantation following an aseptic technique. Blood culturing in aerobic and anaerobic conditions was carried out by means of the BACTEC blood culture analyzer. Microbiological analysis showed that none of the pre- and post-operative samples exhibited detectable bacteremia. Future research should be focused on determining the collective bacteremic effect of a sequence of orthodontic procedures including miniscrew placement or removal, typically performed during a single treatment session.

Introduction

Transient bacteremia commonly results from dental operative procedures and routine daily activities like toothbrushing, flossing and food chewing (Wilson *et al.*, 2007). Subsequent dissemination of microorganisms in various target organs may provoke focal infections, including infective endocarditis (IE) (Tomás *et al.*, 2012).

The considerable morbidity–mortality attributed to IE urged domestic and international expert committees to periodically analyze the available evidence and publish preventive guidelines such as antimicrobial prophylaxis. According to the newly revised statement of American Heart Association (AHA) on IE-related dental procedures, antibiotic administration should be reserved for those involving management of the gingival or periapical region of teeth or perforation of the oral mucosa. Such a prophylactic regimen is strictly recommended to patients with underlying cardiac conditions associated with the highest risk of reverse outcome from IE, i.e. patients with prosthetic cardiac valve, history of IE, congenital heart disease or cardiac transplantation recipients that develop cardiac valvulopathy (Wilson *et al.*, 2007). Likewise, the Working Party of the British Society for Antimicrobial Chemotherapy (Gould *et al.*, 2006) and the European Society of Cardiology (Habib *et al.*, 2009) advise prophylaxis for patients susceptible to IE undergoing dental treatment that implies dentogingival manipulation, and endodontics.

Reports of bacterial endocarditis in orthodontic patients have been so far sparse in the literature (Biancaniello and Romero, 1991; Dajani, 1991; Hobson and Clark, 1993; Ziolkowska *et al.*, 2010). Despite a direct relationship has not been proven, fixed appliance adjustment, likely implicated with mucosal injury that forewent the onset of symptoms, might have contributed from a theoretic perspective in the appearance of IE. Since Degling first evidenced absence of bacteremia in full-banded orthodontic patients (Degling, 1972), a number of researchers attempted to elucidate the link between several orthodontic procedures and bacteremia (Table 1). Apparently, the only orthodontic procedure established to be significantly associated with bacteremia is the placement of elastic separators (Lucas *et al.*, 2002).

The introduction of mini-implants in orthodontics (Kanomi, 1997, Costa *et al.*, 1998) simplified maximum anchorage achievement, and enabled, due to the advantageous technical characteristics, clinical application on a routine basis (Livas *et al.*, 2006). Nowadays miniscrews are being widespread used worldwide with the US numbers estimated to approximate 83% of residency programs and 69% of private practices (Shirck *et al.*, 2011). It is also acknowledged that oral bacteria may inhabit the peri-implant sulcus causing infection of surrounding soft and hard tissues, especially in case of poor oral hygiene after implantation (Apel *et al.*, 2009). In particular, bacterial colonization of the implant surface within a 3 week post-placement period was confirmed in miniscrews retrieved from orthodontic patients (Apel *et al.*, 2009; Tortamano *et al.*, 2012). Furthermore, vascular injuries adjacent to plaque biofilm triggered by periodontal probing, scaling,

Table 1 Studies investigating prevalence of bacteremia (in percentages) in orthodontic patients before and after therapeutic and preventive procedures ([-]: no preoperative blood sample withdrawn).

Procedures	Studies												
	Burden *et al.*, 2004	Chung *et al.*, 1986	Degling 1972	Erverdi *et al.*, 1999	Erverdi *et al.*, 2000	Erverdi *et al.*, 2001	Gürel *et al.*, 2009	Lucas *et al.* 2002	Lucas *et al.*, 2007	McLaughlin *et al.*, 1996	Rosa *et al.*, 2005	Schlein *et al.*, 1991	Uysal *et al.*, 2010
Archwire adjustment								33–19.4					
Banding			[-]–0	0–7.5				36–44		3.3–10			
Debanding			[-]–0		6.6–6.6				19–26				
Banding/Chlorhexidine rinsing						0–2.5							
Debanding/Chlorhexidine rinsing						2.5–2.5							
Debonding/Debanding	3–13												
Bonded RME appliance removal							0–32						
Haas palatal expander removal											0–50		
Gold chain adjustment									57–57				
Mini-implant insertion													0–2.5
Separator placement								27–36					
Toothbrushing		66.7–20										0–25	
Upper alginate impression								23–31					

root planning, or tooth extractions may lead to microbial seeding into the bloodstream (Forner *et al.*, 2006). Hence, given that that mini-implant anchorage may be maintained for several months, it is conceivable to presume a comparable effect might take place after miniscrew explantation.

Therefore, the purpose of this study was to examine the prevalence of bacteremia in a sample of orthodontic patients following mini-implant removal.

Materials and methods

Thirty subjects (17 males, 13 females) with an average age of 24.1 years (standard deviation: 10.7) treated between January and July 2012 at the orthodontic clinic of 251 Hellenic Air Force VA General Hospital utilizing skeletal anchorage were enrolled in this study. Full fixed orthodontic appliances treatment and implantation procedures have been performed out by one experienced specialist. All patients were bearing in interradicular sites for various anchorage requirements at least one self-drilling mini-implant of 1.4 mm diameter and 10 mm length (Dual-Top® Anchor System, Jeil Medical Corporation, Seoul, South Korea) for an average period of 0.8 years (standard deviation: 0.7) (Table 2). The exclusion criteria applied for sample selection are displayed in Table 3. From the literature and after piloting none of the patients had bacteremia before implant insertion. It was assumed that an increase in the prevalence of bacteremia to 35% from an initial prevalence of 10% or less before implant insertion would be of clinical importance. The assumption of 10% bacteremia before the placement of the implant was taken by averaging findings from other studies cited in the manuscript. At an alpha level of 5% and power of 80% it was calculated that 24 patients would be enough to allow us to detect a difference in prevalence of at least 25% in bacteremia before and after implant removal if such difference exists. To allow for potential losses to follow-up, it was decided to recruit 30 patients.

Ethical approval was granted by the Institutional Scientific Committee of 251 Hellenic Air Force VA General Hospital (# 035519122011). The participants and their guardians, in case of minors, were informed verbally and in writing, and a written informed consent was obtained. Food consumption and toothbrushing was instructed to be avoided 2 hours before the scheduled sampling session.

Blood collection and implant management preceded fixed appliances adjustment. In case of subjects with two mini-implants, blood sampling procedures were carried out for the first miniscrew determined to be removed. The right antecubital fossa of each patient was prepared with 1% povidone iodine solution and a 20G sterile plastic cannula (Bio-Med Healthcare Products Pvt. Ltd, Haryana, India) was inserted into the antecubital vein. The cannula was fitted with a sterile three-way valve device (B. Braun Melsungen AG, Melsungen, Germany) to facilitate intended blood sampling. Immediately before mini-implant removal, a preoperative blood sample of 20 ml was obtained through the cannula and the three-way valve device adjusting a 21G sterile needleless syringe (Shandong Qiaopai Group Co., Ltd, Shandong, China) following a strict aseptic technique. When sufficient blood volume had been withdrawn, the syringe was removed, and the intact sterile 21G needle was fixed to allow blood inoculation into culture bottles. Prior inoculation, the rubber seals of the bottles were swabbed with alcohol to ensure the asepsis of the technique. In each blood sampling two bottles were used, one for aerobic (BD BACTEC Plus Aerobic Culture Vial, Becton, Dickinson and Company, Shannon, County Clare, Ireland) and one for anaerobic culture (BD BACTEC Plus

Table 2 Patient gender, age, and mini-implant placement details.

Subjects	Gender	Age (yrs)	Implant maintenance (yrs)	Number of implants	Insertion site	Implant-supported movement
No. 1	F	33.9	1.1	2	15–16, 42–43	mesialization/distalization
No. 2	F	16.0	0.5	1	43–44	mesialization
No. 3	F	15.4	1.1	1	13–14	distalization
No. 4	F	34.6	1.3	2	33–34, 43–44	mesialization
No 5	F	17.9	1.2	1	13–14	distalization
No. 6	F	12.0	0.4	1	12–13	mesialization
No. 7	F	35.5	0.5	2	33–34, 43–44	mesialization
No. 8	F	15.1	0.3	2	33–34, 43–44	intrusion
No. 9	F	17.0	0.2	1	42–43	mesialization
No. 10	F	23.0	0.5	1	26–27	distalization
No. 11	F	17.4	1.1	1	36–37	distalization
No. 12	F	15.8	0.2	2	15–16, 16–17	buccal movement
No. 13	F	16.6	0.6	1	33–34	mesialization
No. 14	M	14.9	0.9	1	44–45	intrusion/distalization
No. 15	M	17.9	1.4	1	16–17	distalization
No. 16	M	51.7	1.3	2	33–34, 43–44	mesialization
No. 17	M	28.8	0.7	1	43–44	mesialization
No. 18	M	32.6	0.7	1	22–23	mesialization
No. 19	M	21.7	0.5	1	26–27	mesialization
No. 20	M	42.8	2.7	2	33–34, 43–44	mesialization
No. 21	M	18.7	1.1	2	26–27, 36–37	distalization
No 22	M	40.9	0.3	2	11–13, 21–23	mesialization
No. 23	M	19.3	0.5	1	25–27	mesialization
No. 24	M	15.9	0.3	1	32–33	mesialization
No. 25	M	16.1	0.2	2	32–33, 42–43	mesialization
No. 26	M	45.8	0.2	1	43–44	mesialization
No. 27	M	17.2	3.3	1	16–17	distalization
No. 28	M	14.9	0.2	2	32–33, 42–43	mesialization
No. 29	M	17.9	0.3	2	35–36, 45–46	distalization
No. 30	M	51.7	0.2	1	13–14	mesialization

Table 3 Exclusion criteria applied for sample selection.

Exclusion criteria
Congenital heart disease
History of rheumatic fever
Aortic stenosis, mitral stenosis, or both
Prosthetic heart valves
History of subacute bacterial endocarditis
Hypertrophic cardiomyopathy
Surgically constructed systemic-pulmonary shunts
Vascular and joint prostheses
Immunosuppression
Diabetes mellitus
Bleeding disorder
Pregnancy
Antibiotic usage within the past 3 months
Regular use of antiseptic mouthwash

Anaerobic Culture Vial, Becton, Dickinson and Company, Shannon, County Clare, Ireland). Each culture bottle was inoculated with 10 ml of blood. After mini-implant was unscrewed with the corresponding manufacturer's screwdriver, 20 ml of blood was collected by the abovementioned protocol, and finally inoculated into aerobic and anaerobic culture bottles. Post-operative sample was taken between 30 and 60 seconds after miniscrew manipulation. Blood culturing was achieved using the BACTEC blood culture analyzer (Becton–Dickinson Diagnostic Instrument Systems, MD, USA), a device that produces a qualitative aerobic or anaerobic culture calculating a colormetric detection algorithm. The incubation of the blood samples took place at 37°C for seven days. When the BACTEC blood culture analyzer had provided a growth alert, the positive bottle culture was subcultured onto blood agar (Bio-Rad, Marnes-la-Coquette, France), blood agar with hemin and menadione (Sigma Chemical Co., St. Louis, United States), chocolate agar (Bio-Rad, Marnes-la-Coquette, France), and MacConkey agar (Bio-Rad, Marnes-la-Coquette, France) plates. The incubation of all agar plates was executed aerobically (blood agar and MacConkey agar), anaerobically (blood agar with hemin and menadione), and in a microaerophilic environment (chocolate agar) containing 5–10% carbon dioxide. Additionally, at the end of every 7 day incubation period, samples of all negative blood cultures were obtained from the respective bottles, inoculated onto the above agar plates and incubated at 37°C for another 2 days as a cross-check with the BACTEC blood culture analyzer. Colonies arised from any culture were undergone a Gram staining procedure as a first step of morphological identification. Species classification was designed to be accomplished by

contemporary standard methods such as semi-automatic identification system (Microscan, Siemens Healthcare Diagnostics, Deerfield, IL, USA) (Jin *et al.*, 2011) and manual biochemical testing techniques (API System, BioMérieux SA, Lyon, France) (http://www.biomerieux.com/servlet/srt/bio/portail/home). Susceptibility profile to a wide variety of antimicrobial agents was intended to be determined by the disk diffusion method in accordance with the current Clinical Laboratory Standards Institute guidelines and the Minimal Inhibitory Concentration definition (Etest, BioMérieux, Marcy l'Etoile, France).

Results

The microbiological cultures of the preoperative specimen, produced by the technique of choice, were free of aerobes and anaerobes. Negative results were also acquired from aerobic and anaerobic culturing of the blood sample received after mini-implant removal. The absence of bacteria was cross-checked by the supplementary 2 day cultivation of the negative cultures.

Discussion

Albeit scientific consensus has not been met, the frequency and density of bacteremias are considered to be influenced by the degree of inflammation or infection at the site of the trauma (Wilson *et al.*, 2007). Moreover, a significant association between the increase of plaque accumulation and gingival inflammation parameters and the development of bacteremia following toothbrushing has been recently substantiated (Tomás *et al.*, 2012). Given that the implant surface roughness contributes in bacterial adhesion (Chin *et al.*, 2007), and prolonged plaque retention in the peri-implant gingival tissue and mucosa triggers the development of localized cell inflammation (Sebbar *et al.*, 2012), we conducted this study to investigate whether bacteremia occurs after miniscrew explantation. Interestingly, our culture-based microbiological methods confirmed absolute lack of bacteremia in all sixty blood samples. Negative results have been also obtained from the baseline samples before miniscrew installation in 40 orthodontic patients (Uysal *et al.*, 2010). Nevertheless, in the latter investigation bacteremia was not developed in all but one individual after mini-implant insertion.

Orthodontic studies dedicated to bacteremic occurrence after miscellaneous interventions have yielded rates reaching up to 57% in the instance of gold chain adjustment (Lucas *et al.*, 2007). However, a closer examination of the results reveals that in specific studies bacteremia percentages did not increase in post-operative samples, and either maintained (Erverdi *et al.*, 2000, 2001; Lucas *et al.*, 2007) or even declined (Chung *et al.*, 1986; Lucas *et al.*, 2002). These findings, not discussed by the authors in the respective papers, pose questions about the study design and methodology. Reviewers of microbiological studies, published between the years 1950–77 and 2006–10, discerned greater precision in detection and identification of bacterial isolates in the later papers (Tomás *et al.*, 2012). The timing of the second blood sampling is of crucial importance for accurate determination of bacteremia. Based on robust data that the peak value of bacteremia is attained between 30 and 60 seconds after dental extraction (Roberts *et al.*, 1992), the aforementioned period was selected as method of choice. Afterwards, the number of positive blood cultures drops rapidly, whereas small prevalence percentages may be observed in the second half-hour post-procedure (Wilson *et al.*, 2007). Of note, times ranging from 30 seconds (Burden *et al.*, 2004; Lucas *et al.*, 2002, 2007) to 15 minutes (Chung *et al.*, 1986) have been cited in similar articles and this may stand for the different outcomes. Queries may be also raised for studies that did not provide time details (Degling, 1972; Erverdi *et al.*, 2001), or whether the procedure of interest can be consistently completed within limited time (Burden *et al.*, 2004). Standard treatment performed with bonded brackets on incisors, canines, premolars and bands placed with glass-ionomer cement on first molars instigates dental plaque accumulation and gingival inflammation as well as growth of pathogenic bacteria and anaerobes (Ristic *et al.*, 2007; Liu *et al.*, 2011). It is generally recognized that inflammatory reaction of gingival tissue and calculus apposition in fixed orthodontics are related to retentive surfaces around bonded attachments (Alexander, 1991). Oral hygiene status was set as inclusion criterion elsewhere (McLaughlin *et al.*, 1996; Erverdi *et al.*, 1999, 2000, 2001), probably with the intention to identify bacteremia directly derived from the intervention under investigation. Intuitively, such a decision might have led to erroneous research design with recruitment of not typical orthodontic patients.

Besides the evidence-based timing in blood collection and carefully elected sample, this study offers further methodological advantages such as utilization of a single miniscrew type and one orthodontist involvement in treatment procedures. In contrast, the age of participants and mini-implant location may imply confounding. Though optimal in terms of study design, stratification of a larger study group with solid characteristics would have been impractical and ethically sensitive. Nonetheless, the number of patients coincides or is even greater than in the majority of previous bacteremic-orthodontic investigations (Table 1). As regards the microbiological technique of the study, the advocates of molecular sequence-based methods may argue for the higher sensitivity relative to culture-based methods (Parahitiyawa *et al.*, 2009). Still, the lack of validation and use of molecular methods in prospective clinical trials of oral bacteremia needs to be addressed beforehand (Tomás *et al.*, 2012).

The prognostic role of bleeding for bacteremia is ambiguous. Substantial bacteremia may occur regardless of presence of clinically discernible bleeding (Roberts, 1999). In this context, the AHA writing group (Wilson *et al.*, 2007)

revisited antecedent recommendations that warranted antibiotics for oral interventions for which bleeding was anticipated (Dajani *et al.*, 1997). On the other hand, generalized bleeding after toothbrushing was correlated with an almost eightfold increase in risk of developing bacteremia (Lockhart *et al.*, 2009). In the current study, notwithstanding bleeding of some extent was evident in all subjects after mini-implant had been removed, it did not affect bacteremia acquisition.

In the last years, the concept of cumulative exposure over time has been introduced to assess the risk of bacteremias arising from various activities. It has been calculated that the collective exposure to random bacteremias caused by mastication and everyday oral hygiene measures largely exceeds the duration of bacteremia related to tooth extraction (Guntheroth, 1984; Roberts, 1999). With reference to orthodontics, though, the nature and frequency of visits call for attention when organizing research on the potential bacteremic impact of treatment procedures. In effect, a regular orthodontic treatment session does not necessarily mean a single-appointment procedure. On annual basis, the number of fixed appliances controls (Fleming *et al.*, 2010; DiBiase *et al.*, 2011; Johansson and Lundström, 2012) may be 7 to 10 times greater than the average attendance of dental offices (Wall and Brown, 2003).

In an era where the orthodontic armamentarium is increasingly upgraded with novel therapeutic systems, it is the task of the professionals to illuminate all relevant aspects encountered in the clinical practice. While antimicrobial prophylactic therapy is endorsed merely for patients with predisposing to IE cardiac conditions planned to receive bands (Wilson *et al.*, 2007), our results do not rationalize preoperative administration of antibiotics for miniscrew removal. Future research design based upon the aggregated investigation of mini-implants in conjunction with other orthodontic procedures customarily performed during a single visit may advance our comprehension over orthodontics-related bacteremia.

Conclusions

Our study clearly demonstrates that none of the 30 patients presented bacteremia following removal of orthodontic miniscrews. Prospective clinical studies should aim to inquire the cumulative bacteremic capacity of mini-implants combined with additional orthodontic treatment techniques.

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