Comparison of Azithromycin and Amoxicillin for Prophylaxis at Dental Implant Placement: A Randomized Pilot Study of Bioavailability and Anti-inflammatory Effects

THESIS

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Abstract

The rate of early implant failures although low, can be increased in high-risk populations and can be very costly. For this reason, data showing that the implant failure rate can be reduced by a single dose of antibiotic prior to implant surgery has been incorporated into a new standard of care. It is still unknown which antibiotic is the most beneficial. The purpose of this study was to evaluate differences in bio-availability and anti-inflammatory effects between azithromycin (AZM) and amoxicillin (AMX). 18 healthy patients, 21 and older, requiring one-stage placement of a dental implant were recruited from the Graduate Periodontology Clinic at the OSU College of Dentistry. A randomized, double-blinded design was used to eliminate bias. Prior to implant placement surgery, patients were randomly divided into control (AMX) and experimental (AZM) groups. Clinical measurements of gingival inflammation and plaque and samples of GCF, were obtained prior to implant placement on the tooth adjacent to the surgical site. Measurements and samples of GCF and PICF were obtained 6, 13 and 20 days after surgery around the adjacent tooth and the newly placed dental implant. In addition a blood sample was taken at 6 days. Evaluation of bio-availability of AZM and AMX was done using an agar diffusion bio-assay. To evaluate changes in pro-inflammatory mediators between the groups, cytokine and growth factor analysis was carried out using a commercially available multiplex bead-based immunoassay. The results showed that at 6 days AZM concentration in serum, GCF and PICF was $0.015 \pm 0.002 \mu g/ml$, $3.39 \pm 0.73$
µg/ml and 2.77±0.90 µg/ml, respectively, whereas in the AMX group antibiotic concentration was below the level of detection. Changes in GI and GCF volume in the AZM group were not statistically significant when compared to baseline values, while significant changes in both inflammation and GCF volume were present in the AMX group. In GCF samples, content of IL-6 was significantly lower in the AZM group at 6 and 13 days and VEGF was significantly lower at 20 days. In PICF samples, G-CSF content was significantly lower on days 13 and 20 in the AZM group as well as for IL-8, MIP-1β and IP-10 on day 20.

Results suggest that the AZM group had a slightly faster resolution of the post-surgical inflammatory process.
Dedication

This document is dedicated to my family.
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Chapter 1: Introduction

Trends in utilization of dental implants: According to Millennium Research Group, the global authority on medical technology market intelligence, the number of dental implants placed in USA increased 10-fold between 1983 and 2002.\(^1\) Recent data from the same group showed that increasing patient awareness and demand and a widening pool of non-specialist dentists qualifying to perform dental implant procedures will enable the North American dental implant market to grow strongly at an average annual rate of 10 percent.\(^2\) New technologies in implant manufacturing have allowed for high implant survival and success in healthy patients. In a recent retrospective analysis of 511 titanium implants, the 10-year implant survival rate was 98.8\% and the success rate was 97.0\%. In addition, the prevalence of peri-implantitis in this large cohort of orally healthy patients was only 1.8\%.\(^3\) The primary predictors of implant failure are poor bone quality, chronic periodontitis, systemic disease, smoking, advanced age, implant location, parafunctional habits, loss of implant integration and an inappropriate prosthesis.\(^4\)

Implant ossteointegration and primary stability: Osseointegration was first described by Brånemark\(^5\) and the term was first defined by Albrektsson et al.\(^6\) as direct contact at the light microscope level between living bone and implant. Osseointegration is also histologically defined as the direct anchorage of an implant by the formation of bony tissue around the implant without the growth of fibrous tissue at the bone–implant
interface. Since the histological definitions have some limitations, mainly due to their limited clinical application, it has been suggested that osseointegration could be better defined as a process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading. 

One of the main prerequisites for osseointegration is implant primary stability. Several authors have suggested that primary stability may be a useful predictor for osseointegration and that a high primary stability makes immediate loading more predictable. In the past, traditional clinical methods for evaluating bone-implant relationship included radiographic evaluation, tapping the implant with a metallic instrument and assessing the emitted sound and reverse torque application. However, these methods are rather subjective and do not give a linear definition of the level of implant stability. More objective measurements of primary stability have been proposed by several methods like the Periotest (Gulden, Bensheim, Germany) or the Dental Fine Tester (Kyocera, Kyoto, Japan). However, their lack of resolution, poor sensitivity and susceptibility to operator variables have been criticized. Recently, resonance-frequency analysis has been introduced to provide an objective measurement of implant primary stability and to monitor implant stability over the healing period and in the longer term. With this method, implant stability is measured either by determining the resonance frequency of the implant–bone complex or by reading an implant stability quotient (ISQ) value given by the Osstell apparatus (Integration Diagnostics AB, Gothenburg, Sweden). The ISQ measurement is proportional to implant stability and typically ranges between
values of 40 to 80. Resonance frequency analysis is capable of detecting changes in implant stability that cannot be detected by other routine clinical methods.9

**Differences in the attachment complexes of implants and natural teeth:** The structural and biological differences between dental implants and natural teeth have been studied in detail. Although there are many similarities between the peri-implant and periodontal tissues, there are obvious anatomical differences, mainly the lack of periodontal ligament and its associated vascular supply.15,16 The presence of a junctional epithelium similar to that associated with natural teeth has been well documented.15, 17-20 The structure of this epithelium is still a matter of debate.16 The connective tissue fiber orientation represents the most important difference between periodontal and peri-implant tissues. While in the teeth the fibers run perpendicular to the long axis of the tooth, in the peri-implant tissue, the fibers from the bone crest run parallel to the implant surface and are not inserted into the implant surface. As with natural teeth, the establishment of an epithelial junction seems constant around implants. The difference is most consistently seen related to a larger epithelial component at implant sites compared to teeth, ranging from 1.7-2.5mm.20

**Early and late implant failures:** When implant failures occur, they are categorized as either early (defined as occurring prior to or at abutment connection) or late (after occlusal loading).21 A recent retrospective study showed that early implant failures were associated with minimal bone loss and occurred more in women (57.7%). On the other hand late failures were more prevalent in males (58.8%) and were associated with
moderate to severe bone loss. In the early failure group, the main reason for failure was lack of osseointegration (73.2%). In the late failure group, the main reasons were peri-implantitis (32%), overloading (46.4%), and implant fracture (6.2%).

**Clinical signs of implant failure:** The periodontal probe is an essential tool for the diagnosis of peri-implant disease. Experimental peri-implantitis studies have shown that an increase in probing depth over time is associated with attachment loss and bone loss. It has been demonstrated that periodontal probing using a probing force of 0.2–0.3 N is a reliable tool for diagnosing peri-implant health and disease. In health, the probe tip ends at the apical extent of the barrier epithelium, in experimental inflammation around implants probe penetration increased as the degree of inflammation amplified, with up to 1.6mm into the connective tissue in peri-implantitis lesions. In the past, probing around implants was suggested to have a negative impact on the mucosal seal and was not routinely advised. However, a study evaluated the healing following standardized peri-implant probing force (0.25N) and observed that, after 5 days, complete reformation of the mucosal seal was evident. Thus, probing with a conventional periodontal probe with a light pressure (0.25N) is not contraindicated. There are no data regarding the influence of probe material (metal versus plastic) or design on peri-implant probing.

The presence of bleeding on gentle probing (BOP) is a useful parameter for diagnosis of mucosal inflammation. An experimental study showed healthy peri-implant sites had absence of BOP while there was increased BOP at mucositis (67%) and peri-implantitis (91%) sites. The value of BOP as a prognostic factor has also been investigated in a
prospective clinical study evaluating progressive attachment loss in patients with peri-implantitis. BOP was characterized as having a high negative predictive value, thus, the absence of BOP was a good indicator of stable peri-implant conditions. This was confirmed in a prospective clinical study that evaluated the prognostic value of BOP for monitoring peri-implant mucosal tissue conditions during supportive periodontal therapy. This study showed that any site bleeding at more than half of the recall visits over a 2-year period had disease progression. Thus, BOP is considered a valuable parameter for diagnosing peri-implant disease.

**Analysis of peri-implant crevicular fluid (PICF) and saliva:** Since there is no clinical method for detecting site-specific breakdown, the study of inflammatory mediators as possible markers of periodontal disease progression has been of great interest in the periodontal and peri-implant literature. Adonogianaki et al. analyzed GCF and PICF to compare the inflammatory and immunological responses around implants and natural teeth and found no significant component differences between them at either healthy or inflamed sites. This suggested that inflammatory and immune events were similar in the peri-implant mucosa and gingiva in humans, and that PICF and GCF production was governed by similar mechanisms. Similar to the relationship between GCF volume and inflammation around natural teeth, PICF volumes are significantly higher at sites with peri-mucositis and peri-implantitis than at clinically healthy implant sites. Analyses have confirmed a trend of increase in PICF volume with the severity of peri-implant inflammation.
Levels of biochemical mediators in PICF have been studied with the aim of identifying these possible diagnostic markers to monitor peri-implant health. Markers in PICF that have been investigated include host derived enzymes like collagenases, inflammatory mediators like cytokines and prostaglandins and tissue breakdown products. Cytokine analysis of PICF from implants with peri-implantitis and peri-mucositis has detected higher amounts of IL-1β at peri-implantitis sites with shallow or deep probing depths than at peri-mucositis sites. Levels of IL-6 and IL-10 were higher at peri-implantitis sites with shallow probing depths than in those with deep probing depths.\textsuperscript{33}

The use of quantitative polymerase chain reaction gene expression analysis of PICF after one-stage implants has shown promise in a recent pilot study. Patients were randomly assigned to a roughened or smooth surface abutment group and evaluated at 2, 14, 28 and 90 days after surgery. High expression of IL-1β, TNF-α and alkaline phosphatase was observed. Their levels of expression were correlated with several clinical parameters, including bleeding on probing, wound healing index, ISQ and incidence of implant complication (mobility or implant loss). In particular, there was a relatively strong correlation between the level of TNF-α expression at 2 and 14 days and the incidence of implant complications at 90 days.\textsuperscript{34}

More recently samples of saliva, which are simpler to collect than PICF, have been evaluated. Fonseca et al. studied the difference in cytokines present in patients with peri-implantitis and peri-mucositis and found significantly higher levels of IL-8 and IL-12 in patients with peri-implantitis compared to patients with peri-mucositis.\textsuperscript{35}
While this body of evidence indicates potential for diagnostic tests for peri-implant disease, prospective longitudinal studies are required to correlate disease progression with biochemical markers. The search for a sensitive diagnostic test to detect reversible changes before the clinical changes of peri-implant disease continues. This is not surprising, considering there are to date no biochemical diagnostic tests available for periodontitis progression.22

Microbial diagnostic testing has also been used as a possible prognostic marker of disease progression. Luterbacher et al. evaluated a microbiological test in conjunction with BOP, in recall patients over a 2 year period. At each recall visit a microbiological sample for different periodontopathic bacteria, BOP and probing depths where taken at an implant and a natural tooth. They concluded that the addition of the microbiological test enhanced the prognostic characteristics of BOP alone for identifying disease progression at implants.29

**Suppuration:** The presence of pus is the direct result of inflammation and infection. In a report of 218 patients who were examined for biological complications at existing implants 9–14 years after implant placement, the presence of pus was usually accompanied by bone loss (peri-implantitis) up to or past the third thread. 36 This finding was later confirmed in a larger group of subjects with at least one implant that had undergone progressive bone loss.37
Implant mobility: The presence of mobility of an implant indicates complete lack of osseointegration and therefore the implant should be removed. Thus, mobility is not useful for early diagnosis of peri-implant diseases but as a sign of implant failure. 22
Infections around biomaterials are among the most difficult to treat. According to the American College of Surgeons, complex oral surgical procedures, including placement of dental implants, may benefit from prophylactic antibiotics. It is feasible that antibiotics play a role in controlling localized infection and allow a better treatment outcome. The routine use of prophylactic antibiotics in conjunction with dental implant surgery has been controversial for many years, but there is now evidence that it is beneficial. In a study of 2973 implants over a 36 months period that encompassed phases of postoperative healing, surgical uncovering, and implant loading and function, there was a significantly higher implant survival rate in patients who had taken preoperative antibiotics. Evidence-based reviews reinforce these findings. A review of four randomized clinical trials concluded there was evidence that 2 g of amoxicillin (AMX) given orally 1 hour prior to implant surgery significantly reduces failures of dental implants placed in ordinary conditions. More recently, a review of eight clinical trials presented evidence that a single preoperative dose of antibiotics has a beneficial effect on lowering implant failure. In light of this evidence, preoperative prophylaxis with AMX is now the standard of care in many clinics that place dental implants. It is uncertain from the most recent reviews whether postoperative antibiotics are beneficial, and it is also unclear which class of antibiotic produces the most favorable protective effects. The most common regimen for implant placement (2 grams of AMX one hour before the
procedure) is also used to protect patients at risk for developing bacterial endocarditis from bacteremias associated with dental treatment.

Systemic use of AMX as an adjunct to non-surgical therapy is accepted in periodontal therapy.\textsuperscript{41} Although there have been only a few clinical studies in which azithromycin (AZM) has been used as an adjunct to periodontal therapy, the available information suggests it is beneficial. AZM is highly effective \textit{in vitro} against the invasive subgingival pathogens \textit{Aggregatibacter actinomycetemcomitans} and \textit{Porphyromonas gingivalis}.\textsuperscript{42, 43} It also exhibits good activity against \textit{Eikenella corrodens}, \textit{Prevotella} species, fusobacteria, spirochetes, and other anaerobic and facultative oral pathogens.\textsuperscript{44, 45} Steady-state AZM concentrations in GCF are typically 40 times higher than in serum, and effective levels can persist in GCF for at least two weeks.\textsuperscript{46} Randomized clinical trials have demonstrated that use of AZM in combination with scaling and root planing enhances pocket reduction and attachment gain in smokers with moderate to advanced attachment loss\textsuperscript{47} and in young patients with aggressive periodontitis.\textsuperscript{48} Moreover, AZM reduces the frequency of \textit{P. gingivalis} detection after scaling and root planing of patients with chronic periodontitis.\textsuperscript{49}

AZM and other macrolides produce several potentially useful immunomodulatory effects. Human neutrophils actively transport clarithromycin, resulting in high intracellular concentrations of this agent. Neutrophils loaded with clarithromycin in this manner exhibit enhanced phagocytic killing of \textit{A. actinomycetemcomitans}.\textsuperscript{50} Macrolides also inhibit pro-inflammatory cytokine production when used to treat chronic inflammatory
The mechanisms by which this occurs are not fully understood, but macrolides inhibit the activation of NF-κB and AP-1, which regulate the expression of IL-8, IL-6, TNF-α and IL-1β and other pro-inflammatory cytokines. Macrolides may also inhibit mitogen-activated protein kinase and extracellular-regulated kinase, resulting in decreased IL-8 production. In periodontology, related anti-inflammatory effects can be observed in patients with minimal bacterial plaque and sub-clinical gingival inflammation. AZM triggers a significant reduction in the amounts of IL-1β, IL-8, TNF-α, and VEGF in GCF. AZM is known to inhibit NF-κB activation, IL-8 mRNA expression, and IL-8 production in cultured oral epithelial cells. Consistent with these effects, wound healing after periodontal surgery is reportedly accelerated in patients taking AZM.

The rate of early implant failures is relatively low, but is increased in high-risk populations, such as patients with diabetes, smokers or those with poor bone conditions. Implant failures can be very costly. For this reason, data showing that the implant failure rate can be reduced by a single dose of antibiotic prior to implant surgery has been incorporated into a new standard of care by many dental clinics.

**Hypothesis and Specific Aims:** Based on previous studies, we hypothesize that a single pre-operative dose of AZM produces relatively high antimicrobial levels in peri-implant crevice fluid (PICF) and gingival crevicular fluid (GCF). During the early phase of post-surgical wound healing, we predict that its concentrations in PICF and GCF will exceed those produced by a single dose of AMX. Moreover, we expect post-surgical
inflammation to be reduced in subjects taking AZM. These hypotheses will be addressed in the following specific aims:

**Aim 1**: Compare the concentrations of AZM and AMX in PICF from a newly-placed single stage implant, GCF from adjacent teeth, and blood serum during the first 20 days after implant placement surgery.

**Aim 2**: Compare postsurgical changes in plaque accumulation, gingival inflammation, implant stability, and the volumes of PICF and GCF in experimental subjects taking AZM and control subjects taking AMX.

**Aim 3**: Compare postsurgical changes in the content of IL-1β, TNF-α, IL-6, G-CSF, GM-CSF, IL-12, IL-17A, IL-8, MIP-1α, MIP-1β, RANTES, eotaxin, IP-10, PDGF-BB, basic FGF, VEGF, IL-10, IL-1 receptor antagonist, IL-4 and IFN-γ in PICF and GCF.
Chapter 3: Materials and Methods

**Study population:** Eighteen healthy subjects were recruited from patients of the College of Dentistry who elected a treatment plan that included one-stage placement of a dental implant in the Graduate Periodontology Clinic between July, 2012 and April, 2013. Only non-smoking volunteers aged 21 or older were enrolled. The subjects had no history of a drug allergy or systemic disease, were not pregnant or nursing, and had not taken any other drugs in the month prior to the study. Subjects had at least one natural tooth adjacent to the site where the implant was placed. Informed consent was obtained from each subject under a protocol approved by the Institutional Review Board.

**Study design:** A randomized, double-blinded design was used to eliminate bias. Prior to implant placement surgery, patients were randomly divided into control (AMX) and experimental (AZM) groups. A research pharmacist compounded 18 packages of similar appearance containing either a single 2 g dose of AMX or a 500 mg dose of AZM. The pharmacist allocated an equal number of subjects to the AZM and AMX groups and the coding of each package remained sealed until completion of clinical data gathering.

One hour prior to surgery, a baseline clinical assessment of Gingival Index (GI),⁵⁹ Plaque Index (PI),⁶⁰ and GCF volume was carried out for the teeth adjacent to the site where the implant was to be placed. Baseline GCF samples were obtained from teeth adjacent to the proposed implant site and stored frozen for later analysis of biological mediators.
The subjects then took their assigned dose of antibiotic. A one-stage implant surgical protocol, which involves placing the implant and the healing abutment in a single procedure, was utilized by the surgeon assigned to the case. The main determinant of whether an implant could be placed in one stage was availability of sufficient alveolar bone to provide implant stability. Implant stability was assessed with an Osstell ISQ implant stability meter (Osstell AB, Goteborg, Sweden). One-stage implant surgery was deemed appropriate if the ISQ measurement of the newly-placed implant was 50 or above. After placement of the implant, the surgical site was closed using expanded polytetrafluoroethylene sutures. To inhibit post-operative accumulation of bacterial plaque, all subjects used an antimicrobial rinse containing 0.12% chlorhexidine twice daily. Patients were provided with acetaminophen tablets (500mg) and instructed to take one tablet every 4 to 6 hours (no more than 6 tablets per day) as needed for pain. They were also issued a prescription for Vicodin 5/500 in the event that acetaminophen alone provided insufficient relief from pain. Patients were instructed to avoid using non-steroidal anti-inflammatory analgesics.

During the first 20 days after implant placement, gingival health and plaque levels were monitored. Subjects returned for follow-up on days 6, 13 and 20. Modified GI and PI (mGI and mPI61) were assessed at each implant site and GI and PI were assessed at adjacent natural tooth sites. Four surfaces of each implant site (distobuccal, distolingual, mesiobuccal, and mesiolingual) and adjacent natural tooth sites were evaluated. GCF or PICF samples were collected from the same four surfaces at the respective sites with filter paper strips (PerioPaper, OraFlow Inc, Smithtown, NY) as previously described.62
Prior to collecting these samples, the implant and tooth sites were isolated with cotton rolls, supragingival plaque (if present) was removed, and the sites were gently dried with air. PICF and GCF were collected for 30 seconds with filter paper strips positioned at the orifice of the crevice. GCF and PICF volumes were measured with a calibrated gingival fluid measurement device (Periotron 6000, IDE Interstate, Amityville, NY, USA). To assure collection of an adequate sample volume, two strips were collected from each surface. There was a delay of at least 10 minutes between consecutive samples to allow time for the crevice to refill with fluid. Thus, a total of 8 strips from the implant and 8 strips from adjacent natural teeth were typically collected at each follow-up visit and pooled separately. In addition, a 3 ml sample of peripheral blood was obtained on day 6 and a second measurement of implant stability was obtained on day 20. Samples of GCF, PICF and serum were stored at -20° C. At the time of analysis, the paper strips containing GCF and PICF were thawed. Samples were eluted from each pool of paper strip samples with 200 µL PBS as previously described 62. For each eluted sample, 25% of the volume was allocated for analysis of cytokine content and 75% was allocated for determination of AZM or AMX content.

**Measurement of biological mediators in PICF and GCF:** Each pooled sample was subjected to analysis by a multiplex bead-based immunoassay for human cytokines (BioPlex catalog #L50-0IF72WN, Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer’s directions. The assay was customized to measure TNF-α, IL-1β, IL-6, G-CSF, GM-CSF, IL-17A, IL-12, IL-8, MIP-1α, MIP-1β, RANTES, Eotaxin, IP-10, IL-10, IL-1 receptor antagonist, IFN-γ, IL-4, PDGF-BB, basic FGF and
VEGF. The content of these mediators was expressed as the total amount (in picograms) recovered from each pool of 30 second GCF or PICF samples. Each sample pool normally consisted of eight filter paper strips.

**Measurement of AMX and AZM content in PICF and GCF:** Approximately 150 µl of the eluted PICF and GCF samples was processed for measurement of AZM or AMX content. In addition, AZM and AMX were assayed in peripheral blood samples obtained on day 6. GCF and PICF eluates and blood serum samples were treated with 40 µl of 0.5 g/ml Na₂CO₃ and extracted three times with 1 ml of diethyl ether. The extracts were dried under streaming nitrogen, reconstituted in acetonitrile, and applied to sterile paper disks (BD Biosciences, Sparks, MD, USA). After evaporation of the acetonitrile, the AZM or AMX content of the disks was determined with an agar diffusion bioassay, using *Kocuria rhizophila* (ATCC 9341, American Type Culture Collection, Manassas, VA, USA) as the indicator organism. The assay was calibrated over the range of 2 to 15 ng with authentic AZM and AMX standards (US Pharmacoepeia, Rockville, MD, USA). Determination of the AZM content of GCF and PICF samples incorporated a correction for the efficiency of its elution from the filter paper collection strips.
Chapter 4: Results

Study population: Eighteen subjects who fulfilled the inclusion criteria were enrolled in the study. Equal numbers of these subjects were randomly allocated to the two treatment groups (Figure 1). In the AMX group, two subjects were eventually lost due to failure to comply with the appointment schedule. In the AZM group, two subjects sustained physical trauma to the surgical site during the first two weeks after implant placement, and one subject was excluded due to lack of primary stability at the time of implant placement. Thus, a total of thirteen subjects completed the study. The characteristics of the subject groups are presented in Table 1. The AMX group had a lower mean age and a higher percentage of female subjects than the AZM group, but the differences were not statistically significant. In both groups, at least 66% of the implants were placed at a mandibular site. The implants originated from several manufacturers, including ASTRA TECH™ (Dentsply implants, York, PA USA), 3i (Palm Beach, FL USA), Straumann (Andover, MA USA) and Zimmer (Warsaw, IN USA).

Antibiotic concentrations in serum, GCF and PICF: The mean AZM concentrations in samples of blood serum, GCF and PICF obtained from the AZM group on day 6 were 0.015±0.002 µg/ml, 3.39±0.73 µg/ml and 2.77±0.90 µg/ml, respectively. AZM concentrations in GCF and PICF were significantly higher than in serum, but not significantly different from each other (P<0.05, Holm-Sidak test). On day 13, the mean
AZM concentrations in GCF and PICF were 2.24±0.70 µg/ml and 2.03±0.38 µg/ml, respectively. The differences between day 6 and day 13 concentrations were not statistically significant. In the AMX group, serum antibiotic concentrations were below the limit of detection on day 6. Since AMX levels are similar in serum and GCF, it was reasonable to presume that antibiotic levels in the much smaller volumes of GCF and PICF were below the limit of detection.

Clinical findings at tooth surfaces adjacent to implant sites: In both groups, Gingival Index (GI) increased on day 6 after implant placement surgery, then decreased on days 13 and 20 (Figure 2). Only the AMX group exhibited statistically significant changes in GI over time. In particular, there was a statistically significant increase relative to baseline (day 0) GI on days 6 and 13. Oddly, the baseline GI for the AZM group was somewhat higher than that of the AMX group. Both groups exhibited an initial decrease in Plaque Index (PI) on day 6 after implant placement, and PI remained below baseline levels on days 13 and 20. Only the AMX group exhibited statistically significant changes in PI over the course of the study.

GCF volume at sites from both groups increased on day 6 and decreased thereafter (Figure 3). In both groups, the changes in volume over time were statistically significant. However, the only statistically significant increase above baseline GCF volume occurred in the AMX group on day 6. Similar to the GI scores, the baseline GCF volume was higher in the AZM group. On days 6, 13 and 20, however, the GCF volumes of the AZM group were consistently lower than those of the AMX group. To examine these
differences more closely and facilitate comparisons, the changes from baseline were analyzed (Figure 3, inset). ANOVA revealed a statistically significant difference in between the two groups.

**Clinical findings at implant sites:** The modified GI (mGI) scores observed in the AZM group were consistently somewhat higher than those of the AMX group (Figure 4, upper panel). In both groups, mGI decreased between days 6 and 20. However, neither group exhibited statistically significant changes in mGI over time. Similarly, neither group exhibited a significant change in their modified PI scores over the course of the study. Both groups started and ended with low plaque levels.

Both groups exhibited a statistically significant decrease in PICF volume after day 6 (Figure 4, lower panel). The PICF sample volumes from the AZM group were somewhat lower than those of the AMX group on days 6 and 13, but their volumes converged on day 20. The differences observed on days 6 and 13 were not statistically significant (P>0.05, t-test).

Implant stability was assessed by Implant Stability Quotient (ISQ) measurements conducted immediately after implant placement on day 0 and on day 20. The baseline ISQ measurements for the AMX and AZM groups were 65.0±1.9 and 57.9±4.8, respectively. On day 20, the respective measurements were 64.6±1.7 and 54.3±5.9. No statistically significant differences were observed between the two groups.
Analysis of pro-inflammatory cytokines in GCF and PICF samples: The TNF-α content in GCF samples from both groups increased from baseline to day 6 (Figure 5, upper panel). GCF TNF-α content in the AMZ group remained relatively constant from 6 to 13 days, then decreased to below baseline levels on day 20. In the AMX group, TNF-α decreased slightly after day 6 but remained above baseline levels. There were no significant differences between groups at any time point. In the PICF, TNF-α content decreased progressively from day 6 to day 20 (Figure 5, lower panel). TNF content in the AZM group was lower than in the AMX group at the 6 and 13 day time points in the AZM group, but converged on day 20. The differences were not statistically significant. On day 6, the amount of TNF-α in PICF was more than twice that found in GCF.

Baseline IL-1β content in GCF was somewhat higher in the AZM group than the AMX group, but was lower than in the AMX group and lower than baseline on day 20 (Figure 6, upper panel). IL-1β content was significantly higher in the AZM group on day 13. In PICF, the IL-1β content of the PICF of the AZM group was somewhat higher on day 6 (Figure 6, lower panel). Both groups exhibited a marked decrease from day 6 to day 13 and reached similar levels on day 20. No significant differences between groups were noted.

Baseline IL-6 content in GCF was similar in both groups (Figure 7, upper panel). IL-6 increased sharply in both groups on day 6, and then decreased to similar levels by day 20. GCF IL-6 content was significantly higher in the AMX group on days 6 and 13. The amount of IL-6 in PICF was somewhat higher in the AMX group on day 6, and decreased
dramatically in both groups from day 6 to day 20 (Figure 7, lower panel). No statistically significant differences between groups were observed. The amount of IL-6 found in PICF was somewhat higher than in GCF on days 6 and 13.

The G-CSF content of GCF samples from the AMX group was essentially unchanged from baseline to day 20 (Figure 8, upper panel). In contrast, the AZM group exhibited somewhat higher levels of GCF G-CSF at baseline and decreased significantly below baseline on days 6, 13 and 20 (P<0.05, Holm-Sidak test). A similar pattern was observed with respect to the G-CSF content in PICF (Figure 8, lower panel). While there was little change in PICF G-CSF content over time in the AMX group, the G-CSF content in the AZM group was somewhat higher on day 6 and significantly lower than in the AMX group on days 13 and 20.

The baseline IL-12 (p70) content of GCF samples from the AMX group was significantly lower than the baseline content of the AZM group (Figure 9, upper panel). IL-12 content of the AMX group exhibited a significant increase above baseline on days 6 and 20, but was very similar to baseline levels on day 13. The IL-12 content of the AZM group increased slightly on day 6, then gradually decreased, ending below baseline levels on day 20. The IL-12 content of PICF samples decreased from day 6 to day 13 in both groups (Figure 9, lower panel). While the IL-12 content of the AMX group increased from day 13 to day 20, there was essentially no change in the AZM group over the same period. Thus, the PICF IL-12 content of the AZM group was higher than that of
the AMX group on day 6 and lower on day 20. There were no statistically significant inter-group differences at any time point.

An attempt was made to analyze GM-CSF and IL-17A in GCF and PICF. Unfortunately, the levels of these pro-inflammatory cytokines were below the assay’s limit of detection in many GCF and PICF samples. For this reason, data on these mediators are not included.

**Analysis of chemokines in GCF and PICF samples:** The baseline IL-8 content of GCF was somewhat higher in the AZM group (Figure 10, upper panel). In both groups, there was an increase in IL-8 content on day 6 that was followed a decrease to similar levels on day 13. On day 20, IL-8 content in the AZM group was below that of the AMX group and below its own baseline levels. However, no statistically significant differences between groups were noted at any time point. In PICF samples, the IL-8 content in both groups was similar on day 6 and decreased dramatically between day 6 and day 13 (Figure 10, lower panel). On day 20, the IL-8 content in PICF from the AZM group was significantly lower than in the AMX group. On day 6, the mean IL-8 content in PICF was at least twice that found in GCF.

The baseline MIP-1α content in GCF was significantly higher in the AZM group than in the AMX group (Figure 11, upper panel). In the AMX group, there was a significant increase from baseline to day 6 (P<0.05, Holm-Sidak test), followed by a decrease towards baseline levels on day 20. In the AZM group, there was a minor increase on day 6, followed by a gradual decrease to slightly below baseline levels. There were no
statistically significant differences between group on days 6, 13 or 20. The PICF MIP-1α content exhibited a steady decrease from day 6 to day 20, and was essentially identical in the two groups at all time points (Figure 11, lower panel).

The MIP-1β content in GCF was similar at baseline in the two groups and exhibited a marked increase to similar levels on day 6 (Figure 12, upper panel). In both groups, the content on day 20 was similar to baseline levels. No statistically significant differences between groups were noted. In both groups, the amount of MIP-1β in PICF samples decreased progressively from day 6 to day 20 (Figure 12, lower panel). The MIP-1β content in PICF samples from the AZM group was consistently lower than that of the AMX group, and this difference was statistically significant on day 20. MIP-1β content was highly variable on day 6, but the mean amounts measured in PICF were roughly twice those found in GCF.

RANTES content in GCF was highly variable, but baseline content was somewhat higher in the AZM group in comparison to the AMX group (Figure 13, upper panel). The AMX group exhibited a marked increase above baseline levels on day 6 that was sustained on days 13 and 20 (P<0.05, Holm-Sidak test). In contrast, the AZM group did not exhibit much of an increase on day 6 and decreased below baseline level thereafter. There were no statistically significant differences in GCF RANTES content between the two groups at any time points. In both groups, PICF RANTES content decreased progressively from day 6 to day 20 (Figure 13, lower panel). Although the amount of RANTES measured in
samples from the AZM group was consistently lower, the differences were not statistically significant.

The eotaxin content in GCF from the AZM group was somewhat higher than in the AMX group at baseline and gradually decreased below baseline levels over time (Figure 14, upper panel). In contrast, GCF exotaxin content in the AMX group remained at or slightly above baseline levels on days 6, 13 and 20. GCF eotaxin content was significantly higher in the AZM group on day 13. PICF exotaxin content decreased slightly from day 6 to day 20 and was similar in both groups at all time points (Figure 14, lower panel).

The IP-10 content of GCF samples from the AZM group was relatively unchanged from baseline to day 20 (Figure 15, upper panel). In contrast, there was a sharp, but highly variable increase in IP-10 on day 6 in the AMX group, followed by a return toward baseline levels. There were no statistically significant differences between groups at any time point. In the PICF, subjects in the AZM group exhibited a gradual decrease in IP-10 content from day 6 to day 20. In the AMX group, IP-10 levels increased from day 6 to day 13 and decreased thereafter. IP-10 content was significantly lower in the AZM group on day 20.

**Analysis of growth factors in GCF and PICF samples:** The PDGF-BB content of GCF samples from the AMX group was significantly lower than those from the AZM group at baseline and remained near baseline levels on days 6, 13 and 20 (Figure 16, upper panel). In the AZM group, PDGF content decreased below baseline levels over
time (P<0.05, repeated measures ANOVA). On days 6, 13 and 20, no significant differences between groups were evident. Similarly, there were no statistically significant differences in the PDGF-BB content of PICF samples between the two groups on days 6, 13 and 20 (Figure 16, lower panel).

The baseline VEGF content of GCF samples from the AZM group was significantly higher than in those from the AMX group (Figure 17, upper panel). In the AZM group, VEGF content was relatively stable between baseline and day 13, but gradually decreased between days 13 and 20. In the AMX group, GCF VEGF content was higher than baseline levels on days 6 and 20 and slightly below baseline on day 13. Although VEGF content was significantly higher in the AZM group at baseline, it ended significantly lower on day 20. With regard to the VEGF content of PICF, similar amounts were detected in the two groups on days 6 and 13 (Figure 17, lower panel). VEGF levels were higher in the AMX group on day 20, but the difference was not statistically significant.

An attempt was made to measure basic FGF in GCF and PICF samples. Unfortunately, the levels of this growth factor were below the assay’s limit of detection in many GCF and PICF samples. For this reason, data on this mediator are not included.

**Analysis of anti-inflammatory cytokines in GCF and PICF samples:** Baseline IL-10 content was significantly higher in GCF samples from the AZM group (Figure 18, upper panel). In the AZM group, there was little change in GCF eotaxin content from baseline to day 20. The AMX group exhibited a significant increase above baseline on day 6 and remained above baseline on days 13 and 20. No significant differences in PICF IL-10
content were observed between groups over the course of the study, although IL-10 content was somewhat higher on day 6 and somewhat lower on day 20 (Figure 18, lower panel).

The IL-1 receptor antagonist content of the GCF and PICF samples was high and exhibited variability. In GCF, IL-1ra content was very similar from baseline to day 13 in both groups (Figure 19, upper panel). After day 13, IL-1ra content increased in the AZM group, but the increase was not statistically significant on day 20. In PICF samples, the IL-1ra content in both groups was very similar on days 6 and 20 (Figure 19, lower panel). IL-1ra was higher in the AZM group on day 13, but the difference between groups was not statistically significant.

**Analysis of T helper cell cytokines:** Low, but relatively consistent levels of the T\(_H2\) cytokine IL-4 were detected in GCF and PICF. In both groups, there was a trend of gradually decreasing IL-4 levels in GCF from baseline to day 20 (Figure 20, upper panel). The IL-4 content of the AZM group was slightly higher at baseline, but essentially identical to the AMX group on day 20. No significant differences between groups were evident at any time point. PICF IL-4 content also decreased slightly from day 6 to day 20, and the differences between the two groups were of small magnitude (Figure 20, lower panel).

An attempt was made to measure the T\(_H1\) cytokine IFN-\(\gamma\) in GCF and PICF. In many samples, it was below the limit of detection. For this reason, data on this mediator are not included.
Chapter 5: Discussion

Comparison of AMX and AZM groups: With respect to mean age, gender distribution, and baseline plaque levels, the two groups were reasonably comparable. Despite the randomization of subjects, the AZM group had a higher Gingival Index (level of gingival inflammation) at natural tooth sites adjacent to the implant site than subjects in the AMX group at baseline.

Bioavailability of AZM and AMX: AMX was not detected in the blood samples from day 6. This finding was not unexpected, since pharmacokinetic studies have shown that oral doses of AMX produce peak blood levels 1 to 2 hours after administration and exhibit a half-life of approximately 1 hour. Approximately 60% of an orally administered dose of AMX is excreted in the urine within 6 to 8 hours. Since AMX yields similar concentrations in blood serum and GCF, it is reasonable to assume that AMX levels in the much smaller volumes of GCF and PICF are well below the limit of detection. This is consistent with the report of Khoury et al., in which GCF AMX levels were below detectable levels several hours after a single 2 g pre-surgical dose of AMX.

In contrast, the serum, GCF and PICF from the AZM group contained measurable amounts of antibiotic on days 6 and 13. The mean concentrations observed on day 6 in serum and GCF (0.015 and 3.39µg/ml, respectively) are slightly lower than those reported in a study in which the subjects received a total dose of 750 mg AZM. The
observed AZM concentrations in PICF were slightly lower than in GCF, but were not significantly different. On day 13, the AZM concentrations in GCF and PICF (2.24 and 2.03 µg/ml, respectively) were nearly identical to each other and slightly lower than the concentrations observed on day 6. Variations in post-surgical healing may have contributed to the relatively high variability of AZM measurements in GCF and PICF. These findings confirm that AZM has a greater potential to favorably influence post-operative healing around a newly-placed implant because it is eliminated less rapidly than AMX.

**Antibiotic effects on plaque and clinical signs of inflammation:** Although the AZM group entered the study with a higher baseline Gingival Index at adjacent natural tooth sites than the AMX group, there was surprisingly little change in Gingival Index in this group over the course of the study. In contrast, the AMX group exhibited significant increases in Gingival Index on days 6 and 13. In parallel with this finding, the changes from baseline GCF volume after implant placement were of significantly lower magnitude in the AZM group. This suggests that the post-surgical inflammatory response was more constrained in the AZM group. Neither group exhibited significant changes in plaque accumulation on natural teeth or implants during the 20 day healing period. This was not surprising, since subjects in both groups were asked to use an antimicrobial mouthrinse to inhibit plaque formation after implant placement.

Similar to the observations at natural tooth sites, the index of clinical gingival inflammation was somewhat higher at peri-implant sites in the AZM group between days
6 and 20, although these differences were not statistically significant. Peri-implant sites, unlike the adjacent periodontal sites, had no pre-existing junctional epithelium to enhance soft tissue adhesion to the implant. However, the AZM group consistently produced lower volumes of PICF that the AMX group on days 6 and 14. Although this difference was not significant, it suggests that post-surgical healing progressed somewhat more rapidly in the AZM group. The observed PICF volumes per 30 second sample are comparable to those previously reported by Khoury et al.\textsuperscript{66} and Günday et al.\textsuperscript{32} Collectively, the data suggest that resolution of post-operative inflammation and healing of the junctional epithelium occurred more rapidly in the AZM group.

**Antibiotic effects on cytokines and growth factors in GCF and PICF:** A previous report has suggested that there is a tendency toward higher cytokine production around dental implants than around natural teeth.\textsuperscript{67} A recent study that compared implants and adjacent teeth with respect to clinical and biological parameters of wound healing also supports this finding. Their results indicate that peri-implant tissue exhibits a more robust response to the surgical trauma than the periodontal tissue surrounding adjacent teeth. The authors observed higher levels of pro-inflammatory cytokines around implants than around periodontal sites.\textsuperscript{68} Results from our study are consistent with these previous reports, specifically for TNF-α, IL-8, MIP-1α and MIP-1β content in PICF on day 6 and IL-6 on day 6 and 13, which were at least 2-fold higher than their respective contents in GCF.
Studies have shown that erythromycin and AZM suppress the activation of nuclear factor- kappa β (NF-κβ) and activating protein- 1 (AP-1).52, 69 NF-κβ is a family of DNA binding proteins that controls the transcription of many pro-inflammatory cytokines, chemokines, and adhesion molecules.69 The effect of macrolide antibiotics on the expression of inflammatory mediators has been characterized in the medical literature, especially in the treatment of respiratory inflammatory diseases. Erythromycin suppresses the activation of IL-6 in human bronchial epithelial cells.52 Several other cytokines, chemokines or growth factors are reportedly inhibited by macrolides, including TNF-α, GM-CSF, macrophage protein 1 (MIP-1), IL-8, RANTES, eotaxin, and vascular endothelial growth factor (VEGF).70, 71 Aside from its effects on NF-κβ, there is also evidence to suggest that down-regulation of cytokine expression by macrolides may be related to inhibition of bacteria as well as alteration of neutrophil activation.72

Nearly all of the cytokines, chemokines, and growth factors examined in GCF and PICF in the current study are target genes for NF-κβ. Results from the previously sited study on early wound healing around teeth and implants, it is known that there is an initial increase, of up to 3 fold, in IL-6, IL-8 and MIP-1β content in GCF of teeth adjacent to the implant site at one week following surgery compared to the GCF of non surgical tooth sites.68 From our results, in GCF it is noteworthy that in the AZM group these exhibited lower levels of VEGF than the AMX group on day 20 and lower levels of IL-6 on days 6 and 13. In PICF from the newly-placed implant, the AZM group exhibited lower levels of IL-8, MIP-1β and IP-10 on day 20 and lower levels of G-CSF on days 13 and 20. Interestingly, the majority of the mediators studied had a lower mean content in
the AZM group on day 20, but didn’t reach statistical significance. In GCF, these include TNF-α, IL-1β, IL-6, G-CSF, IL-12, IL-8, MIP-1α, MIP-1β, RANTES, eotaxin, IP-10 and PDGF-BB. The corresponding mediators in PICF were TNF-α, IL-6, IL-12, RANTES, eotaxin, VEGF and IL-10. It is possible that decreased variability due to formation of a new epithelial attachment and resolution of inflammation made it easier to detect significant differences in biological mediator content on day 20 than at earlier time points.

**Shortcomings of the study:** Although the findings suggest that the design of this study is appropriate for testing the hypotheses, the statistical power of the study was too low to detect significant differences in some comparisons. As an example, approximately 29 subjects per group would be needed to detect a significant difference in PICF volume between the AMX and AZM groups with a statistical power of 0.80. The criteria for subject inclusion were somewhat stringent, which limited the number of subjects who could potentially be recruited. Moreover, not all patients were qualified or able to complete the study, resulting in an uneven distribution of subjects between the two groups. The relatively small number of subjects in both study groups makes it impossible to draw generalized conclusions. Lastly, the apparent difference in baseline inflammation around the natural teeth adjacent to the implant sites complicated comparisons between the AMX and AMX groups.

**Clinical significance:** The primary purpose of prophylactic antibiotics is to produce transient suppression of bacteria in blood and at wound sites in individuals who are
susceptible to infection. Perhaps the most remarkable finding of the current study is that a single prophylactic dose of AZM appeared to alter several potentially important aspects of inflammation and early wound healing after implant surgery. The results indicate that AZM is available at the surgical site for a longer period of time than AMX and appears to exert immunomodulatory effects. The lower levels of pro-inflammatory cytokines and VEGF observed in the AZM group suggest that this group exhibited a more advanced stage of healing with a more regulated angiogenic response. A decrease in infiltrating inflammatory cells and the levels of inflammatory cytokines could contribute to faster healing and lower incidence of scar formation.

To the best of our knowledge, no previous study has evaluated the presence of AZM using an early wound healing protocol around one-stage dental implants. A pilot study has been published using a similar protocol to evaluate the effectiveness of AMX as a prophylactic antibiotic and its role in early wound healing around one-stage dental implants. Unlike the present study, they only reported 1 week findings and compared GCF of adjacent teeth to PICF of the newly placed implants. They observed no significant differences between the GCF volume at baseline and the PICF volume at 1 week in subjects taking AMX or the control group, which was not given a prophylactic antibiotic. They did report a statistically significant difference in GI between the control and experimental groups at one week, in which the GI was lower in the AMX group. Similarly, no previous study has evaluated the effect of AZM on clinical and biochemical parameters of healing around newly placed dental implants. Our results suggest that
AZM produces a favorable influence on healing, but the extent in which AZM alters the complex processes associated with the inflammatory response requires further study.
REFERENCES


58. Alsaadi G, Quirynen M, Michiles K, Teughels W, Komarek A, van Steenberghe D. Impact of local and systemic factors on the incidence of failures up to


APPENDIX A: TABLE
Table 1: Description of Subject Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AMX Group</th>
<th>AZM Group</th>
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<tr>
<td>Number of Subjects</td>
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<tr>
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<td>62.0 ± 4.1</td>
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<td>4 mandibular/2 maxillary</td>
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<td>Straumann (2)</td>
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<td>Zimmer (2)</td>
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<td>3i (1)</td>
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</tbody>
</table>

\(^1\) Difference not statistically significant (P>0.05, t-test)

\(^2\) Proportions not significantly different from that expected from random occurrence (P>0.05, Fisher Exact test)
APPENDIX B: FIGURES AND LEGENDS
**Figure 1**: Flowchart of subject enrollment and allocation.
Figure 2: Changes in median values for Gingival Index (GI) and Plaque Index (PI) at tooth surfaces adjacent to implant placement sites before and after implant placement surgery in subjects administered a single pre-operative dose of either AZM or AMX. Only the AMX group exhibited significant changes in GI and PI over time (P>0.05, Friedman repeated measures ANOVA on ranks). Asterisks indicate comparisons that are significantly different from baseline values (P<0.05, Dunn’s method).
Figure 3: Changes in mean (- SEM) gingival crevicular fluid volume at tooth surfaces adjacent to implant placement sites in subjects administered a single pre-operative dose of either AZM or AMX. Both groups exhibited significant changes in volume over time (P<0.05, repeated measures ANOVA). An asterisk indicates a significant change relative to baseline (P<0.05, Hold-Sidak). Inset: To adjust for differences in baseline values, data were analyzed as change from baseline. A significant group effect was observed (P=0.03, ANOVA).
Figure 4: Changes in clinical findings at implant placement sites in subjects administered a pre-operative dose of either AZM or AMX. In the upper panel, modified Gingival Index (mGI) and Plaque Index (mPI) are presented as median values. Neither group exhibited significant changes in mGI or mPI over time (P>0.05, Friedman repeated measures ANOVA on ranks). In the lower panel, peri-implant crevice fluid (PICF) volumes are presented as mean - SEM. Both groups exhibited significant changes in volume over time (P<0.05, repeated measures ANOVA). The decreases observed after day 6 were statistically significant (P<0.05, Holm-Sidak).
Figure 5: Changes in the TNF-α content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. No statistically significant differences was observed between groups (P>0.05, t-test).
Figure 6: Changes in the IL-1β content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a pre-operative dose of either AZM or AMX. Data are presented as mean ± SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 7: Changes in the IL-6 content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a pre-operative dose of either AZM or AMX. Data are presented as mean ± SEM. Statistically significant differences between groups are denoted by an asterisks (P<0.05, t-test or Mann-Whitney rank sum test).
Figure 8: Changes in the G-CSF content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. Statistically significant differences between groups are denoted by an asterisks (P<0.05, t-test or Mann-Whitney rank sum test).
Figure 9: Changes in the IL-12 (p70) content of pooled GCF (upper panel) and PICF (lower panel) obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
**Figure 10:** Changes in the IL-8 content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 11: Changes in the MIP-1α content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 12: Changes in the MIP-1β content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 13: Changes in the RANTES content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a pre-operative dose of either AZM or AMX. Data are presented as mean -SEM. No statistically significant differences was observed between groups (P>0.05, t-test).
Figure 14: Changes in the eotaxin content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, Mann-Whitney rank sum test).
Figure 15: Changes in the IP-10 content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, Mann-Whitney rank sum test).
Figure 16: Changes in the PDGF-BB content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 17: Changes in the VEGF content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean ± SEM. Statistically significant differences between groups is denoted by an asterisk (P<0.05, t-test).
**Figure 18**: Changes in the IL-10 content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. A statistically significant difference between groups is denoted by an asterisk (P<0.05, t-test).
Figure 19: Changes in the IL-1 receptor antagonist content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. No statistically significant differences between groups were detected (P>0.05, t-test or Mann Whitney rank sum test).
**Figure 20**: Changes in the IL-4 content of pooled GCF (upper panel) and PICF (lower panel) samples obtained before and after one-stage implant surgery in subjects administered a single pre-operative dose of either AZM or AMX. Data are presented as mean - SEM. No statistically significant differences between groups were detected (P>0.05, t-test).