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Inflammatory stimulation of osteoblasts and keratinocytes from a SAPHO patient for implant risk evaluation

KEYWORDS

SAPHO
Lipopolysaccharides
Cell viability
Pro-inflammatory cytokines

SUMMARY

The present report exemplifies a translational method that could assist the clinical pre-evaluation of patients at risk before surgical interventions. In this study, a presurgical implant decision in a case of SAPHO (synovitis, acne, palmoplantar pustulosis, hyperostosis, osteitis) is described. Since the etiology of this syndrome is likely to involve genetic, infectious and immunological components, lipopolysaccharides (LPS) may conceptually trigger cytokine production leading to a specific chronic inflammation and immunological host response. This may hamper proper healing or accentuate the destruction of periodontal host tissues. In our approach, we examined the ex vivo cell viability and immune responses of primary osteoblasts and keratinocytes under sterile inflammation induced by *P. gingivalis* LPS. Keratinocytes and osteoblasts were obtained from biopsies of the keratinized gingiva and alveolar

bone tissues of a SAPHO human subject. Enzymatically dissociated cells were thus cultured and incubated to LPS at different concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml and 1 µg/ml) for 24 h in order to test inflammatory cytokine response (quantitative real-time PCR) and toxicity (cell viability). Healthy primary keratinocytes and osteoblasts were used as control cells. The highest concentration of LPS (1 µg/ml) significantly reduced cell viability ($p < 0.05$). Meanwhile, all tested LPS concentrations similarly enhanced the mRNA expressions of selected inflammatory cytokines (*TNF α* , *IL-6*, *IL-8*, *IL-1 β* and *IL-1 α*) up to ≈ 3.5 -fold, when compared to the healthy cell controls ($p < 0.05$). This study demonstrated a valuable inflammatory risk evaluation before implant placement, which was successfully performed based on the presented laboratory diagnostic/prognostic approach.

Introduction

Placement of dental implants has become a routine therapeutic option to replace one or more missing teeth. However, there are still a plethora of critical systemic and/or environmental factors that may leave the short- or long-term success of implant therapy at considerable risk (CLEMENTINI ET AL. 2014). Especially medical and environmental conditions that negatively affect the local tissue blood circulation, defense mechanisms and tissue homeostasis should be critically assessed since they can indirectly or directly influence the overall wound healing and potential of the bone regeneration (INSUA ET AL. 2017). The list of well-documented influencing parameters such as periodontitis, diabetes and smoking should be mentioned as quintessential examples in this context. They are not only related to early failures but also increase the risk of late biological complications, i.e. peri-implantitis. These core elements are already known from the model of periodontal disease pathogenesis (PAGE & KORNMAN 1997) and should always be thoughtfully taken into consideration when planning implants. The combination of biofilm composition, host inflammatory immune response and disease tolerance also need to be taken into account. The quantity and quality of healthy bone reflects the basis for a successful and predictable implantation and functional osseointegration, and any potentially impairing metabolic or tissue-based conditions might jeopardize the load-bearing bone substrate for the implants (MEYLE & CHAPPLE 2015, STAVROPOULOS ET AL. 2018).

In recent years, a primary chronic although rare osteomyelitis has become a threatening pathologic condition of the jaws. In most cases, it is known to be a non-suppurative, chronic inflammatory disease whose etiology is not always identifiable. Based on the University of Zurich data set and classification, primary chronic osteomyelitis can be a subset to two main groups of the onset of symptoms, i.e. an early onset (adolescence) and a second group with a late onset (peaking after the fifth decade of age with a more active clinical character in the younger patients when considering clinical presentation and course of the disease, radiological patterns and histologic findings) (BALTENSBERGER ET AL. 2004). This classification thus mainly recommends a subclassification into early- and adult-onset primary chronic osteomyelitis but advocates in cases with an isolated mandibular involvement a differentiation from cases with further dermatoskeletal manifestations (SAPHO syndrome) or chronic recurrent multifocal osteomyelitis. The SAPHO syndrome represents a heterogeneous, benign inflammatory acquired complex of different symptoms that can be manifested as subacute, recurrent or process-like chronic disease process occurring in all age groups with a common skin-bone association. The acronym SAPHO refers to pathological changes in the bone-joint system under the technical terms osteitis (bone inflammation) and the associated hyperostosis (increase in bone substance) and synovitis (joint inflammation). Symptoms of the skin may include pustulosis (purulent vesicles), psoriasis (mostly on the palms of the hands and soles of the feet) and acne (*acne conglobata* or *fulminans*). SAPHO disease cases heal with a relatively favorable prognosis, only rarely at risk for spinal or visceral complications but not infrequently with functional disability or defect healing. The diagnosis is mainly confirmed by magnetic resonance imaging and/or histology. The therapy includes non-steroidal anti-inflammatory drugs and analgesics as first-line agents. Systemic corticosteroids, disease-modifying anti-rheumatic drugs, biologicals targeting tumor necrosis factor- α and interleukin-1, and bis-

phosphonates have all been beneficial in some patients but ineffective in others (NGUYEN ET AL. 2012).

By mapping the pathogenesis of peri-implantitis, the oral pathogenic bacteria that accumulate on the implant surface as plaque/biofilm have been identified as one of the primary causes of the tissue inflammation. In addition, there is a multitude of other causative factors such as the environment and host immune response that together are responsible for the destruction of the supporting tissues through unresolved inflammation. Moreover, oral infection can expand beyond the oral cavity and reach distant organs, which will implicate systemic health conditions such as cardiovascular disease, diabetes, etc. In vitro laboratorial tests for the long-term safety evaluation of implants may offer certain advantages and could be helpful specially for patients with chronic inflammatory bone disorders such as SAPHO syndrome. Specific oral tissue reactions can be identified including inflammatory bacterial reactions. The mechanisms leading to cell toxicity can be assessed and different tissue types can be examined.

The aim of the present study was to assess the suitability of placement of a dental implant in a SAPHO patient. To mimic the natural host inflammatory and immune response, we utilized induced sterile inflammation by lipopolysaccharides (LPS) from pathogenic bacteria in an ex vivo model to stimulate cytokine production, which is known to induce chronic inflammation. We challenged isolated osteoblasts and keratinocytes from the SAPHO patient with different LPS concentrations (RAMENZONI ET AL. 2020, RAMENZONI ET AL. 2022A) in order to evaluate their viability and inflammatory response. With this report, we can observe whether cells obtained from the patient react normally to LPS stimulation, and we can assess whether the ex vivo observations are supported by the clinical outcome. Additionally, with this exemplary ex vivo model we can in fact change many variables and stimulate the environment by changing cell types, medium conditions, bioactive growth factors, etc. (Fig.1). We hypothesized that – provided with comparable results to healthy control samples of respective tissues – the cells obtained from the patient should correspondingly react to bacterial endotoxin LPS, which still remains the greatest risk for inflammation around teeth and implants (SCHMID ET AL. 2021, RAMENZONI ET AL. 2019).

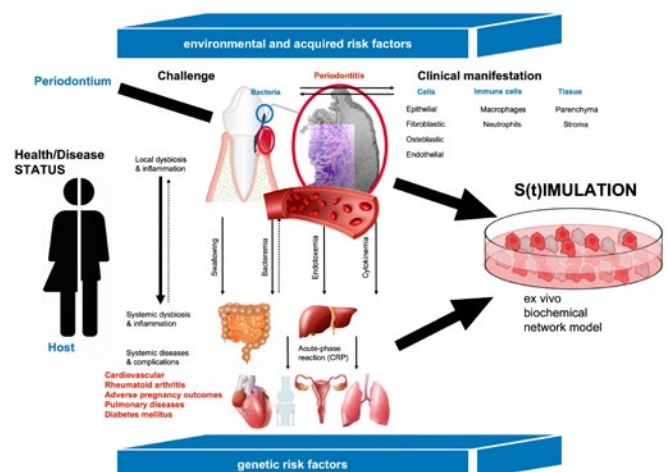


Fig. 1 Ex vivo simulation/stimulation model. Illustration of the immune response with the use of LPS pathogenic bacteria to stimulate cytokine production.

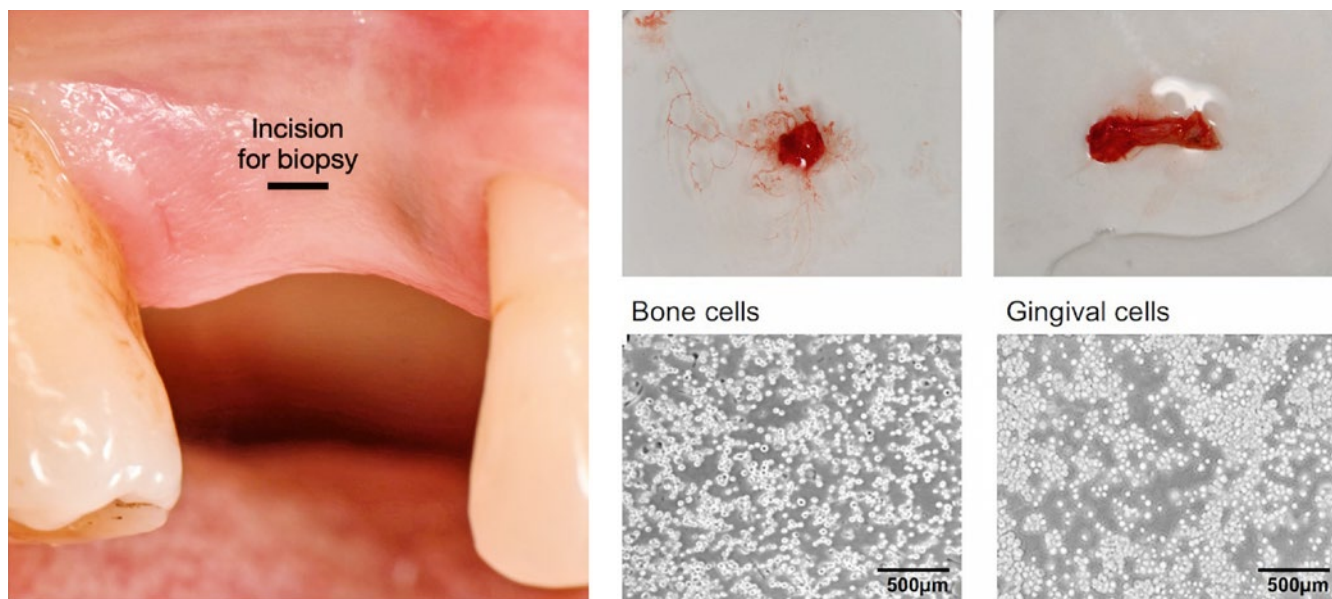


Fig. 2 Tissue isolation and cultivation of primary epithelial and bone cells. (A) This panel shows representative images of gingival and bone tissues after collection in the clinic. The scale bars = 500 µm.

Material and method

Tissue isolation and cultivation

The patients in this study provided informed oral and written consent for inclusion in accordance with the Declaration of Helsinki. The primary gingival keratinocytes and alveolar osteoblasts were obtained from minimally invasive biopsy of gingiva and bone under local anesthesia from the SAPHO patient and healthy volunteers as previously described (RAMENZONI ET AL. 2019, KEDJARUDE ET AL 2001). Cells were cultured with Dulbecco's minimal essential medium (DMEM, Invitrogen, Karlsruhe, California, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Karlsruhe, California, USA), 100 units penicillin and 100 µg/ml Streptomycin (Biochrom, Berlin, Germany) at 37°C, 95% air and 5% CO₂. The cells were then seeded onto T75 culture dishes (Falcon BD, Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at 37°C in 5% CO₂. After confluence was achieved, the cells were washed with phosphate-buffered saline (PBS 1X, Seromond Biochrom, Berlin, Germany) and resuspended with 0.25% trypsin to enable further passages for experiments. Cells at passage P2-P3 were used in the experiments (Fig. 2).

Cell viability

The influence of LPS on osteoblasts and keratinocytes viability was determined by non-radioactive, colorimetric MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide colorimetric, Sigma-Aldrich, Steinheim, Germany). The cells were seeded on 24-well plates and incubated for 24 h at 37°C. Then, the cells were separately treated for 24 h with different concentrations of LPS (50 ng/ml, 200 ng/ml, 500 ng/ml and 1 µg/ml). Next, MTT (0.45 mg/ml) was added and the cells were incubated for further 4 h at 37°C. Finally, after removal of MTT, dimethyl sulfoxide as a solubilization reagent was added and the cells were additionally incubated for 2 h. The test absorbance at 570 nm and reference absorbance at 630 nm were measured using a spectrophotometer reader (Tecan, Austria and USA). In total, three independent experiments were replicated at least three times.

Gene expression analysis

Gene expression analysis at the mRNA level was performed for interleukin-1α (*IL-1α*), interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNFα*), interleukin-6 (*IL-6*) and interleukin-8 (*IL-8*) by using quantitative real-time PCR (RT-PCR). Osteoblasts and keratinocytes were first seeded on titanium discs (1 × 10⁵ cells/well, 24-well plates) for 1 week to enable adherence and confluence. Then, the medium was replaced with medium containing LPS (1 µg/ml) and cultured for a further 24 h. Total mRNA (2 µg) was isolated with trizol (Invitrogen, Grand Island, New York, USA) and converted into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland). The RT-PCR reactions were performed using the 7500 real-time PCR System (Applied Biosystems, Grand Island, New York, USA), the Power SYBR Green PCR Master Mix and cDNA equivalent to 30 ng total mRNA. Three independent experiments were performed for genes with the following specific primers: *GAPDH* (forward primer: 5'-AAT CCC ATC ACC ATC TTC CA-3', reverse primer: 5'-TGG ACT CCA CGA CGT ACT CA-3'), *TNFα* (forward primer: 5'-AGG CGC TCC CCA AGA CA-3', reverse primer: 5'-TCC TTG GCA AAA CTG CAC CT-3'), *IL-1β* (forward primer: 5'-ACA GAT GAA GTG CTC CTT CCA-3', reverse primer: 5'-GTC GGA GAT TCG TAG CTG GAT -3'), *IL-6* (forward primer: 5'-GGT ACA TCC TCG ACG GCA TCT-3', reverse primer: 5'-GTG CCT CTT TGC TGC TTT CAC-3'), *IL-8* (forward primer: 5'-AAG AGA GCT CTG TCT GGA CC, reverse primer: 5'-GAT ATT CTC TTG GCC CTT GG) and *IL-1α* (forward primer: 5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA-3', reverse primer: 5'-ATT GAA AGG AGG GGA GGA TGA CAG AAA TGT-3'). The relative mRNA expression of genes was normalized to the housekeeping gene *GAPDH* and was analyzed using the comparative Ct method (2^{-ΔΔCt} formula). The results were presented in means ± standard deviations.

Statistical methods

The mean values and standard deviations were computed for the MTT test, and a multiple comparison analysis of variance (ANOVA) with Bonferroni adjustment with a global significance

level of 5% was conducted to assess the statistical significance of the differences between the experimental groups using IBM SPSS software (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, New York, USA). Differences were considered significant at $p < 0.05$ and all experiments were performed in triplicate and repeated at least three times at the same conditions.

SAPHO patient case description

The female patient, who was born in 1963 and a non-smoker, was reported with no other medical conditions besides the diagnosis of SAPHO syndrome. The patient was regularly treated with intravenously-applied bisphosphonates from 2001 to 2006 (Pamidronic acid, Aredia, Novartis Pharma Schweiz AG, Rotkreuz, Switzerland, every three to six months). At first visit at our clinic in 2021, the patient was regularly taking Naproxen p.o. as a non-steroidal anti-inflammatory drug. The medical history revealed no other diseases but an antibiotic allergy to sulfamethoxazole and trimethoprim (Bactrim). Intraorally, the patient had two pronounced mandibular tori bilaterally, and the orthopantomogram revealed a stable round bone-dense radiographic change in mandibular premolar/molar (region 35/36) compared to images taken in previous years. In 2019, she experienced acute pain in a conservatively heavily pretreated 26, which had to be extracted. After a traumatic tooth extraction, the patient had to be hospitalized. Understandably, she was afraid of being treated with a dental implant due to her underlying disease. After uneventful extraction of tooth 14 in 2021 (under this time atraumatic extraction and strict antibiotic prophylaxis regimen), the patient desired a fixed prosthetic solution in this esthetically critical area since a gap was not tol-

erable in the long term. Finally, she was referred to us for risk assessment. In this context, we report – according to our best knowledge – for the first time on a distinct laboratory diagnostic/prognostic approach, which could provide useful information before implant decision. After presentation and discussion of the ex vivo results of the experiments, the patient and referring dentists agreed on a minimally invasive treatment with a diameter-reduced implant in order to avoid any complications in combination with augmentation procedures during healing in our clinic (PRS, Fig. 3). In short: A diameter-reduced tissue level implant (Straumann AG, Basel, Switzerland) was placed under systemic prophylaxis (penicillin, 500 mg, 7 days) in a standard procedure approach and open healing. Two months after uneventful healing and successful clinical and radiographic osseointegration, a long-term screw-retained crown was fabricated chairside (Cerec, Dentsply Sirona, Bensheim, Germany) using a hybrid ceramics material (Cerasmart, GC Europe, Lucerne, Switzerland).

Results

For the keratinocytes, the MTT assay indicated significant decrease in cell viability of control and SAPHO cells when exposed to the highest LPS concentration (1 µg/ml) as compared to the untreated respective cells ($p = 0.0025$). Overall, the keratinocytes treated with lower LPS concentrations at 50, 200 and 500 ng/ml showed similar degrees of viability and no significant change compared to control keratinocytes ($p > 0.05$, Fig. 4). In addition, osteoblasts demonstrated significant decrease in viability on control and SAPHO-derived cells when exposed to 1 µg/ml LPS compared to the untreated control ($p = 0.0016$), while no significant change was observed at LPS concentrations of 50, 200 and 500 ng/ml ($p < 0.05$, Fig. 4), respectively.

Analyses on the expression levels of the *TNFα*, *IL-1β*, *IL1α*, *IL-6* and *IL-8* genes in keratinocytes osteoblasts cultured with LPS at all concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml and 1 µg/ml) showed similar upregulation of expression levels of transcripts up to 3.5-fold as compared to the untreated control ($p = 0.0015$). Comparably, the osteoblasts presented the upregulation of same genes to be up to 3.4-fold higher when cells were exposed to any of the tested LPS concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml and 1 µg/ml) compared to untreated control ($p = 0.0035$, Fig. 5).

Discussion

In this study, we aimed to evaluate risk of biological complications before placement of a dental implant in a SAPHO patient. Considering the key role of LPS as bacterial endotoxins in peri-implantitis disease, the purpose of this study was to ascertain the effect of different concentrations of *P. gingivalis* LPS on cell viability and inflammatory response of primary keratinocytes and osteoblasts isolated from a SAPHO syndrome patient following previously established methodology (RAMENZONI ET AL. 2019, KEDJARUNE ET AL. 2001, RAMENZONI ET AL. 2022B). The results showed that for the SAPHO patient-derived cells, LPS at 1 µg/ml concentration decreased cell viability up to 45% compared to untreated control cells. The similar result was shown by the control cells treated with LPS at 1 µg/ml concentration. The inflammatory response of SAPHO-derived cells and control cells was also similarly increased with all LPS concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml and 1 µg/ml) compared to untreated controls. The findings showed that these changes in cell response were most likely dependent on LPS concentration and

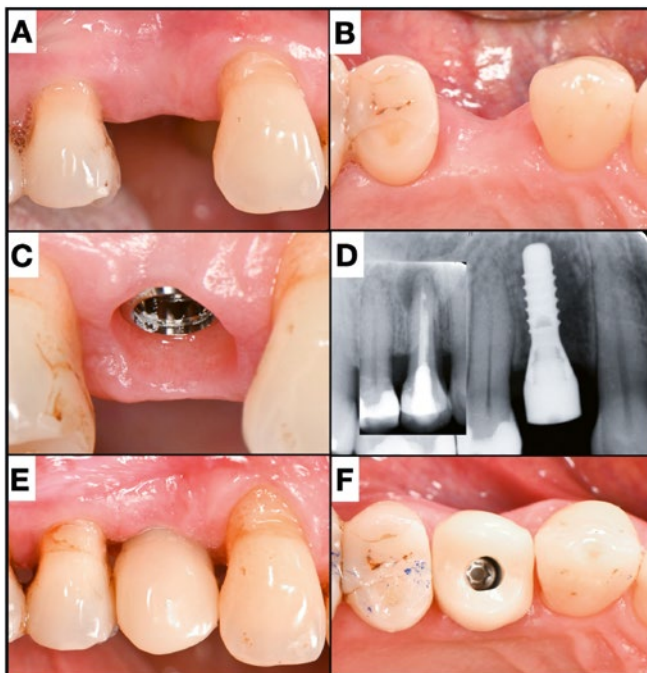


Fig. 3 Clinical documentation of the implant treatment. A and B) Situation after tooth extraction and healing before implant surgery (note the loss of buccal bone width). C) Situation after two months healing and removal of the healing abutment showing healthy tissues. D) Radiographic situation and X-Ray of the site before extraction (note that a diameter-reduced implant was placed in order to avoid additional augmentation). E and F) Prosthetic long term provisional solution with a chairside fabricated screw-retained CEREC restoration.

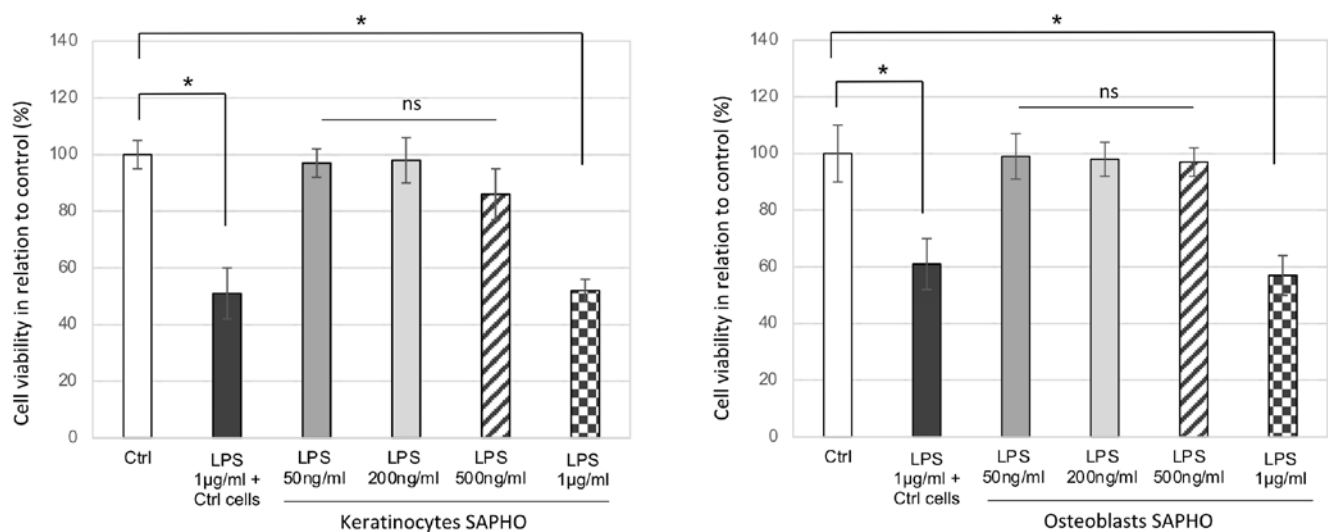


Fig. 4 Evaluation of cellular viability using MTT assay of SAPHO-derived primary osteoblasts and keratinocytes cells over 24 h for the control and four LPS concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml, 1 µg/ml). Control cells: HEGK16 (human epithelial gingival keratinocytes) and HOAS (human alveolar osteoblasts), ns: not significant, * $p < 0.05$. Y-axis = OD (optical density).

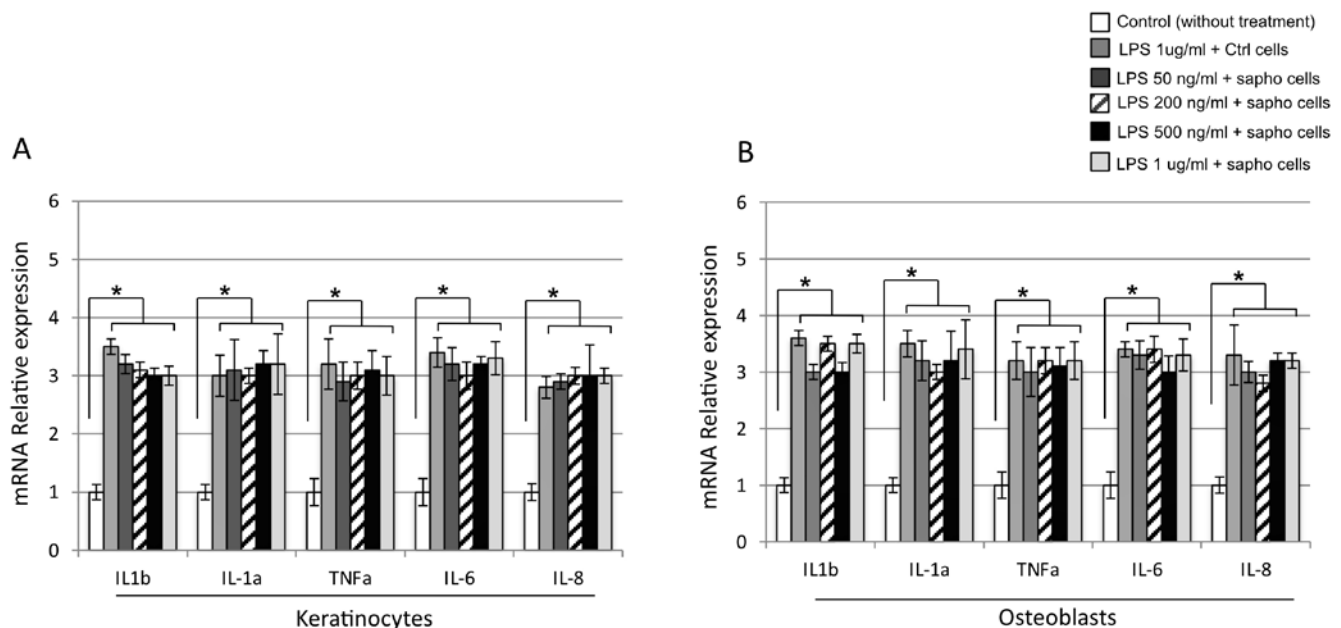


Fig. 5 Inflammatory cytokine gene expression by SAPHO human alveolar osteoblasts and keratinocytes cultured with four LPS concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml, 1 µg/ml). A) Keratinocytes, B) Osteoblasts. Data were obtained from real-time polymerase chain-reaction analysis and are shown as means \pm SD expressed relative to GAPDH. Control cells: HEGK16 (human epithelial gingival keratinocytes) and HOAS (human alveolar osteoblasts), ns: not significant, * $p < 0.05$.

LPS-stimulated cytokine expression in SAPHO cells and control cells to the same extent. Based on these results, it appears that the benefit of placing an implant in this SAPHO patient outweighs its risk.

The pathogenesis of the SAPHO syndrome is probably multifactorial and involves a combination of genetic, infectious and immunological components (KHANNA & EL-KHOURY 2012). In theory, infectious reaction to a virulent pathogen such as *P. gingivalis* could act as a trigger and then it could maintain the progression of the oral disease through subsequent autoimmune response (ASSMANN & SIMON 2011). Periodontal bacteria were shown to induce *IL-1*, *IL-8* and *TNF- α* (MCPHILLIPS ET AL. 2010). Genetic background may also influence the progression of the autoimmune response symptoms (HURTADO-NEDELEC ET AL. 2008). The most likely explanation for the origin of the SAPHO

syndrome is that it is brought on by autoimmune reactions in people with a genetic predisposition who have been exposed to an infectious pathogen (ASSMANN & SIMON 2011). Other authors have proposed that the periosteum is the primary location of osteomyelitis in the SAPHO condition (SUEI ET AL. 2005). The osteoblastic and osteoclastic cells are provoked by some substances (likely cytokines like interleukin and tumor necrosis factor) released in the inflammatory periosteum, which promotes periosteal bone growth in addition to cortical bone resorption (SUEI ET AL. 2003). An earlier study found that dental operations may increase the probability that the mandibular region may be affected by SAPHO syndrome, indicating that transitory bacteremia and tissue injury may play a significant role (WANG ET AL. 2020). A case of a patient who acquired SAPHO syndrome and had mandibular involvement in the region corresponding to a

tooth with recurrent caries was described in a prior study (WANG ET AL. 2020). *Prevotella ssp.*, a mucous gram-negative bacterium genus associated with chronic inflammatory disease such as periodontitis and rheumatoid arthritis (COTTI ET AL. 2015), was discovered to control the inflammatory response in a mouse model of chronic multifocal osteomyelitis (LARSEN 2017, LUKENS ET AL. 2014).

This study has taken a first step in the direction of defining the relationship between SAPHO syndrome features and periodontal disease. Although SAPHO is a rare disease, more cases are being documented as public awareness grows. When considering dental implant therapies for patients with lytic, sclerotic or hyperostotic bone lesions and pain, SAPHO should be suspected. This paper is an attempt to increase awareness about SAPHO syndrome among dental clinicians and prompt recognition. In addition, it could help to avoid unnecessary dental surgical treatments, antibiotic therapy or possible physical and psychological impairments associated with the disease. Laboratory findings as presented here may be of great value, as it could show specific elevation of different inflammatory indices with special exacerbations due to LPS inflammatory oral stimulation. However, in vitro testing may be individually indicated when SAPHO-induced changes may adversely affect bone mechanics on an implant. It is a reasonable consideration when bone destruction increases the risk of pathologic fractures in the SAPHO patient. However, it is important to emphasize that methodological problems in the research may limit interpretations. Thus, the methodological approach outlined in this study should be replicated in the future and deserves further empirical study. The final results of this study indicate a possible useful laboratory method for implant placement risk assessment. However, the cost/benefit ratio of the ex vivo assessment should be considered since many of the ex vivo approaches still require specialized testing capabilities and are mainly used in research. Additionally, the cost of measuring these specific methods is yet high, which may further limit their application in routine clinical practice. The further development of implant risk assessment tests may in the future enable dental practitioners to measure inflammatory load using a rapid or non-invasive chairside approach, as opposed to relying on clinical parameters alone.

In conclusion, we encountered a case of previous osteomyelitis in a patient with diagnosed SAPHO syndrome that could possibly develop further future complications after implant surgery. In order to conveniently evaluate the patient's inflammatory response, we successfully used laboratorial methodology to confirm the positive cytokine and toxicity increase upon LPS treatment. As implant treatment in SAPHO patients are still not recommended, the methods used in this study could facilitate future patient evaluation for further implant placement.

Acknowledgements

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Zusammenfassung

Einleitung

Der vorliegende Fallbericht beschreibt eine neue translationale Methode, um die klinische Vorbewertung von Risikopatienten

vor chirurgischen Eingriffen mit Ex-vivo-Daten zu unterstützen. In diesem Fall wurde damit die präoperative Entscheidungsfindung in einem Fall von SAPHO (Synovitis, Akne, palmo-plantare Pustulose, Hyperostose, Osteitis) ergänzt. Da die Ätiologie dieses Syndroms wahrscheinlich genetische, infektiöse und immunologische Komponenten umfasst, wurde ein Simulations-/Stimulations-Set-up mit Lipopolysacchariden (LPS) angewendet, um die individuelle Zytokinproduktion der Patientin zu untersuchen und mit gesunden Proben zu vergleichen, da dies klinisch zu einer spezifischen chronischen Entzündung und einer immunologischen Reaktion der Wirtsperson führen kann; dies wiederum kann dann eine ordnungsgemäße Heilung behindern oder die Zerstörung des Wirtsgewebes verstärken.

Material und Methoden

Konkret untersuchten wir in unserem Ansatz die Ex-vivo-Zelllebensfähigkeit und die Immunreaktionen von primären Osteoblasten und Keratinozyten unter quasi steriler Entzündung, die durch LPS von *P. gingivalis* ausgelöst wurde. Die Zellen wurden minimal-invasiv aus Biopsien keratinisiert-er Gingiva und des Alveolarknochens der Patientin gewonnen. Enzymatisch dissoziierte Zellen wurden kultiviert und 24 Stunden lang mit LPS in verschiedenen Konzentrationen (50 ng/ml, 200 ng/ml, 500 ng/ml und 1 µg/ml) inkubiert, um die Reaktion auf die Bildung von proinflammatorischen Zytokinen mit quantitativer Echtzeit-PCR und die Toxizität (Zellviabilität) zu testen. Gesunde primäre Keratinozyten und Osteoblasten wurden zum Vergleich als Kontrollzellen gleichermaßen untersucht.

Resultate

Die höchste LPS-Konzentration (1 µg/ml) verringerte die Zelllebensfähigkeit signifikant ($p < 0,05$). Gleichzeitig erhöhten alle getesteten LPS-Konzentrationen in ähnlicher Weise die mRNA-Expressionen ausgewählter entzündlicher Zytokine (TNF α , IL-6, IL-8, IL-1 β und IL-1 α) um das bis zu $\approx 3,5$ -Fache im Vergleich zu den gesunden Zellkontrollen ($p < 0,05$).

Diskussion

Diese Studie konnte in der Tat ein potenziell erhöhtes entzündliches Risikoprofil der Patientin aufzeigen, das in der Folge mit der Patientin besprochen wurde. Das Modell kann für die individuelle Prognose bei Implantaten berücksichtigt werden. Weitere Untersuchungen sind im Gange, und methodologische Anpassungen und Verbesserungen werden angestrebt.

Résumé

Introduction

Le présent rapport de cas décrit une nouvelle méthode de recherche translationnelle visant à étayer, sur la base de données ex vivo, l'évaluation clinique préliminaire de patients à risque avant une intervention chirurgicale. Dans le cas qui nous intéresse, cela a permis de compléter les éléments décisionnels préopératoires chez une patiente présentant un syndrome SAPHO (synovite, acné, pustulose palmo-plantaire, hyperostose, ostéite). Étant donné que l'étiologie de ce syndrome comprend vraisemblablement des composantes génétiques, infectieuses et immunologiques, une configuration de simulation/stimulation avec des lipopolysaccharides (LPS) a été appliquée, afin d'investiguer la production individuelle de cytokines par la patiente et de la comparer à des échantillons sains, car cela peut

conduire cliniquement à une inflammation chronique spécifique et à une réponse immunologique de l'hôte ; cela peut alors empêcher une guérison normale ou augmenter les destructions tissulaires de l'hôte.

Matériel et méthodes

Concrètement, notre approche a consisté à étudier la viabilité cellulaire *ex vivo* et les réponses immunitaires d'ostéoblastes et de kératinocytes primaires dans le cadre d'une inflammation quasi stérile induite par des LPS de *P. gingivalis*. Les cellules ont été prélevées de manière minimalement invasive à partir de biopsies de la gencive kératinisée et de l'os alvéolaire de la patiente. Des cellules dissociées par voie enzymatique ont été cultivées et incubées pendant 24 heures avec des LPS à différentes concentrations (50 ng/ml, 200 ng/ml, 500 ng/ml et 1 µg/ml) afin de tester la réponse à la formation de cytokines pro-inflammatoires par PCR quantitative en temps réel, ainsi que la toxicité (viabilité cellulaire). Des kératinocytes primaires et des os-

téoblastes sains ont été examinés de la même manière en tant que cellules témoins à des fins de comparaison.

Résultats

La concentration la plus élevée de LPS (1 µg/ml) a réduit significativement la viabilité des cellules ($p < 0,05$). Parallèlement, toutes les concentrations de LPS testées ont augmenté de manière similaire les expressions d'ARNm des cytokines inflammatoires sélectionnées (TNF α , IL-6, IL-8, IL-1 β et IL-1 α) jusqu'à $\approx 3,5$ fois par rapport aux témoins cellulaires sains ($p < 0,05$).

Discussion

Cette étude a permis de mettre en évidence chez cette patiente un profil de risque inflammatoire potentiellement augmenté, qui a été discuté avec elle par la suite. Le modèle utilisé peut être pris en compte pour le pronostic individuel en cas d'implants. D'autres études sont en cours et des ajustements et améliorations méthodologiques sont recherchés.

References

- ASSMANN G, SIMON P: The SAPHO syndrome—are microbes involved? *Best Pract Res Clin Rheumatol* 25: 423–434 (2011)
- BALTENSBERGER M, GRATZ K, BRUDER E, LEBEDA R, MAKEK M, EYRICH G: Is primary chronic osteomyelitis a uniform disease? Proposal of a classification based on a retrospective analysis of patients treated in the past 30 years. *J Craniomaxillofac Surg* 32: 43–50 (2004)
- CLEMENTINI M, ROSSETTI P H, PENARROCHA D, MICARELLI C, BONACHELA W C, CANULLO L: Systemic risk factors for peri-implant bone loss: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 43: 323–334 (2014)
- COTTI E, CAREDDU R, SCHIRRU E, MARONGIU S, BARCA M P, MANCONI P E, MERCURIO G: A case of SAPHO syndrome with endodontic implications and treatment with biologic drugs. *J Endod* 41: 1565–1570 (2015)
- HURTADO-NEDELEC M, CHOLLET-MARTIN S, NICAISE-ROLAND P, GROOTENBOER-MIGNOT S, RUIMY R, MEYER O, HAYEM G: Characterization of the immune response in the synovitis, acne, pustulosis, hyperostosis, osteitis (SAPHO) syndrome. *Rheumatology* 7: 1160–1167 (2008)
- INSUA A, MONJE A, WANG H L, MIRON R J: Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. *J Biomed Mater Res A* 105: 2075–2089 (2017)
- KEDJARUNE U, PONGPRERACHOK S, ARPORNMARK-LONG P, UNGKUSONMONGKHON K: Culturing primary human gingival epithelial cells: comparison of two isolation techniques. *J Craniomaxillofac Surg*. 2001 Aug;29(4):224–31.
- KHANNA L, EL-KHOURY G Y: SAPHO syndrome—a pictorial assay. *Iowa Orthop J* 32: 189–195 (2012)
- LARSEN J M: The immune response to *Prevotella* bacteria in chronic inflammatory disease. *Immunology* 151: 363–374 (2017)
- LUKENS J R, GURUNG P, VOGEL P, JOHNSON G R, CARTER R A, MCGOLDRICK D J, BANDI S R, CALABRESE C R, WALLE L V, LAMKANFI M, KANNEGANTI T D: Dietary modulation of the microbiome affects autoimmune inflammatory disease. *Nature* 516: 246–249 (2014)
- MACPHILLIPS A, WOLFORD L M, RODRIGUES D B: SAPHO syndrome with TMJ involvement: review of the literature and case presentation. *Int J Oral Maxillofac Surg* 39: 1160–1167 (2010)
- MEYLE J, CHAPPLE I: Molecular aspects of the pathogenesis of periodontitis. *Periodontol* 2000 69: 7–17 (2015)
- NYGUYEN M T, BORCHERS A, SELMI C, NAGUWA S M, CHEEMA G, GERSHWIN M E: The SAPHO syndrome. *Semin Arthritis Rheum* 42: 254–265 (2012)
- PAGE R C, KORNMANN K S: The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 14: 9–11 (1997)
- RAMENZONI L L, ANNASOHN L, MIRON R J, ATTIN T, SCHMIDLIN P R: Combination of enamel matrix derivative and hyaluronic acid inhibits lipopolysaccharide-induced inflammatory response on human epithelial and bone cells. *Clin Oral Investig* 26: 1773–1783 (2022a)
- RAMENZONI L L, BOSCH A, PROKSCH S, ATTIN T, SCHMIDLIN P R: Effect of high glucose levels and lipopolysaccharides-induced inflammation on osteoblast mineralization over sandblasted/acid-etched titanium surface. *Clin Implant Dent Relat Res* 22: 213–219 (2020)
- RAMENZONI L L, SCHNEIDER A, FOX S C, MEYER M, MEBOLDT M, ATTIN T, SCHMIDLIN P R: Cytotoxic and Inflammatory Effects of Electronic and Traditional Cigarettes on Oral Gingival Cells Using a Novel Automated Smoking Instrument: An In Vitro Study. *Toxics* 10: 179 (2022b)
- RAMENZONI L L, ZUELLIG R A, HUSSAIN A, LEHMANN R, HEUMANN C, ATTIN T, SCHMIDLIN P R: Bacterial supernatants elevate glucose-dependent insulin secretion in rat pancreatic INS-1 line and islet β -cells via PI3K/AKT signaling. *Mol Cell Biochem* 452: 17–27 (2019)
- RUKAVINA I: SAPHO syndrome: a review. *J Child Orthop* 9: 19–27 (2015)
- SCHMID E, EICK S, SCULEAN A, SALVI G E: Peri-Implant Diseases: Characteristics of the Microbiota and of the Host Response in Humans – A Narrative Review. *Monogr Oral Sci* 29: 98–104 (2021)
- STAVROPOULOS A, BERTL K, PIESCHMANN P, PANDIS N, SCHIÖDT M, KLINGE B: The effect of antiresorptive drugs on implant therapy: Systematic review and meta-analysis. *Clin Oral Implants Res* 18: 54–92 (2018)
- SUEI Y, TAGUCHI A, TANIMOTO K: Diagnosis and classification of mandibular osteomyelitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology* 100: 207–214 (2005)
- SUEI Y, TAGUCHI A, TANIMOTO K: Diagnostic points and possible origin of osteomyelitis in synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome: a radiographic study of 77 mandibular osteomyelitis cases. *Rheumatology* 42: 1398–1403 (2003)
- WANG M, LI Y, CAO Y, LU X, LIU Y, ZHAO J, ZHANG W, LI C: Mandibular involvement in SAPHO syndrome: a retrospective study. *Orphanet J Rare Dis* 15: 312 (2020)

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¹⁾Wasser G., Joao-Souza S., Lussi A., Carvalho TS., 2018, Erosion-protecting effect of oral-care products available on the Swiss market. Swiss Dental J. SSO, 2018, 128, 290-296.