Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

Breast Cancer: Basic and Clinical Research

Hyperactivated Signaling Pathways of Chemokine RANTES/CCL5 in Osteopathies of Jawbone in Breast Cancer Patients—Case Report and Research

Johann Lechner[1] and Volker von Baehr[2]

[1]Head of Clinic for Integrative Dentistry, Munich, Germany. [2]Medical Head of Compartment of Immunology and Allergology, Institute for Medical Diagnostics in MVZ GbR, Berlin, Germany.

ABSTRACT

BACKGROUND: Hollow spaces in the jawbone have been defined as fatty degenerative osteonecrosis of jawbone (FDOJ) and have been linked with a dysregulated immune system. Little is known about the underlying relationship.

OBJECTIVES: Samples of FDOJ were analyzed to assess expression of cytokines which can play a role in the pathogenesis of breast cancer (MaCa).

MATERIAL AND METHODS: Samples of FDOJ extracted from 23 patients with MaCa and 19 healthy control jawbone samples were analyzed for 7 immune messengers.

RESULTS: RANTES was found to be highly overexpressed in disease samples. No change was observed in expression levels of the other immune mediators.

DISCUSSION: This data provides a compelling confirmation that FDOJ produces high levels of RANTES, a cytokine implicated in MaCa and metastasis. Levels detected in FDOJ are five-fold higher than that previously reported for MaCa tissue suggesting its role as a cytokine source in MaCa.

CONCLUSION: We thus hypothesize that FDOJ may serve as an expeditor of MaCa progression, through RANTES production.

KEYWORDS: RANTES/CCL5, breast cancer, jawbone, osteonecrosis, metastasis, signaling pathways

CITATION: Lechner and von Baehr. Hyperactivated Signaling Pathways of Chemokine RANTES/CCL5 in Osteopathies of Jawbone in Breast Cancer Patients—Case Report and Research. *Breast Cancer: Basic and Clinical Research* 2014:8 89–96 doi:10.4137/BCBCR.S15119.

RECEIVED: March 2, 2014. **RESUBMITTED:** April 27, 2014. **ACCEPTED FOR PUBLICATION:** April 28, 2014.

ACADEMIC EDITOR: Goberdhan P. Dimri, Editor in Chief

TYPE: Original Research

FUNDING: Authors disclose no funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: drlechner@aol.com

This paper was subject to independent, expert peer review by a minimum of two blind peer reviewers. All editorial decisions were made by the independent academic editor. All authors have provided signed confirmation of their compliance with ethical and legal obligations including (but not limited to) use of any copyrighted material, compliance with ICMJE authorship and competing interests disclosure guidelines and, where applicable, compliance with legal and ethical guidelines on human and animal research participants.

Background of the Study

Osteopathies of the bone and, in particular, of the jawbone, are subject to different definitions and classifications.[1] It has become increasingly obvious that intravascular coagulation plays a significant role in the pathogenesis of osteonecrosis; however, the precise pathophysiology and underlying cellular biology remain difficult to define. The term "osteonecrosis" is used to describe a condition that is influenced by a wide range of factors, and further research is required to fully elucidate the causal factors.[2] Jawbone cavitations are hollow dead spaces in the jawbone (JB) where the bone marrow is dying or dead. These areas of fatty degenerative osteonecrosis of the jaw (FDOJ), or "cavitations", can be silent and they can remain for years as an asymptomatic process. There is a clear difference between FDOJ and typical acute or chronic osteomyelitis. FDOJ is similar to "silent or subclinical inflammation" that is often painless. The histopathology and neurological effects of FDOJ were defined by Bouquot et al[3] and Bouquot and Christian.[4] Areas of FDOJ have long been believed to interfere with the immune system, and they have been suspected to be a factor in many chronic illnesses, including breast cancer (MaCa).[11] However, the possible systemic effects and the

status of cytokine-triggered immune activation in samples of FDOJ have not been widely assessed.[5]

Diagnostic problems of FDOJ lesions in jawbone. The existence of FDOJ is largely neglected today in mainstream dentistry; the reason is that conventional X-ray techniques have limited ability to diagnose the location and extent of FDOJ. To aid the practitioner in diagnosing the debilitating effects of bone marrow softening inside the FDOJ lesions, a computer-assisted through-transmission alveolar ultrasound (TAU) device was developed.[6] TAU precisely images and identifies cavitational porosity in the JB. Studies have shown that in 84% of cases, FDOJ lesions in TAU images were more obvious and more readily identified than in radiographs of the same site.[7] TAU imaging proved significantly superior to radiology in the detection of microscopically confirmed FDOJ.[7] The efficiency and reliability of TAU in the diagnosis and imaging of FDOJ have been presented in numerous publications.[4] Due to these diagnostic difficulties when using conventional methods, JB disease is often underdiagnosed by dentists.

Morphological definition of fatty degenerative osteonecrosis of jawbone/FDOJ. The macroscopic features of the FDOJ bone samples obtained from 23 MaCa patients are consistent throughout the cohort; due to the softening of the medullary bone, the marrow space can be curetted easily. Degeneration of the cancellous bone extends into the mandibular areas, as far as the canal of the inferior alveolar nerve. We documented the extent of these lesions in an earlier publication.[8] Figure 1 shows a typical FDOJ specimen with a yellowish and predominantly fatty transformation of the bone marrow (black arrow).

Pathohistological definition of fatty degenerative osteonecrosis of the jawbone/FDOJ. Each of the FDOJ samples were examined histopathologically (Dr Zwicknagel and Dr Assmus; Institute for Pathology and Cytology, Freising, Germany). Based on over 1,000 histological observations of these types of osteolytic osteopathies, a characteristic definition of FDOJ could be derived; the trabeculae are thin, and they are characterized by a loss of their bony interconnections. The fatty marrow shows mucoid degeneration with interstitial edema. These chronic degenerative changes are intermingled with foci of recent areactive adipocyte necrosis with granular dissolution of the cytoplasm. The amount of fat cells is consistently and strikingly increased. The typical signs of inflammation, especially with respect to inflammatory cell responses, are missing. The fatty degenerative and osteolytic process might be due to insufficient metabolic supply. Widened intertrabecular spaces often contain small necrotic bone fragments. Fatty microvesicles and pools of liquefied fat that are similar to oil cysts, with an almost complete loss of adipocyte nuclei and residual fatty degenerated marrow, are present. Small nerve fibers are a striking feature in most biopsies of FDOJ, as they are situated in close contact to degenerated and necrotic fatty tissue.[9] Figure 2 shows the typical pathohistological structure in the FDOJ samples with inconspicuous fatty tissue (green arrow), which lie directly beside the fatty degenerative changed bone marrow (red arrows).

Hypothesis and objectives. As FDOJ exhibits many structural characteristics of a pathoimmunological process, we hypothesized that hidden immune messengers in FDOJ could support a mediating link between FDOJ lesions and MaCa, and this could be of importance in MaCa. Therefore, we examined different immunological signaling pathways. Among them were RANTES (regulated on activation, normal T-cell expressed and secreted), fibroblast growth factor (FGF)-2, and other cytokines, which we analyzed both in FDOJ and in the serum of MaCa patients. The study is patient centered, with samples and data obtained directly from patients from the corresponding author's dental clinic.

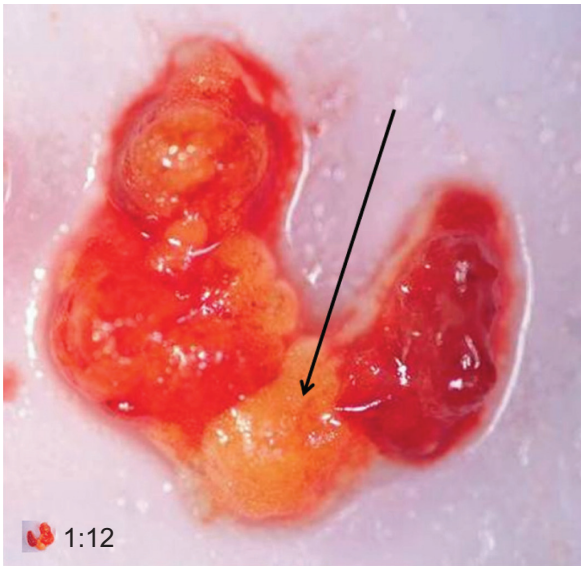

Figure 1. FDOJ sample completely converted the marrow of the JB to fatty tissue.

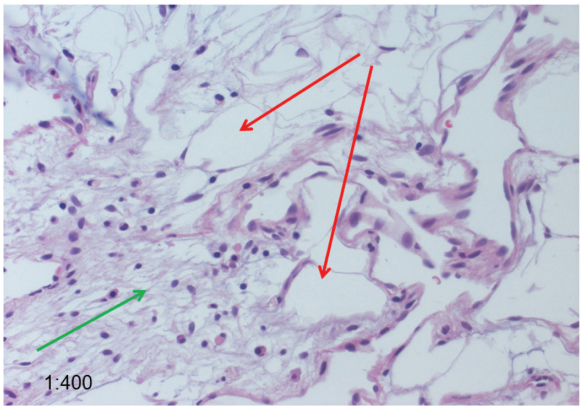

Figure 2. Pathohistological structure of typical FDOJ—inconspicuous tissue in direct comparison with degenerative changed fatty tissue (Hematoxylin eosin stain HE 400-fold).

The Case Report: Metastasis of Adenocarcinoma in Jawbone/FDOJ

A 47-year-old female patient presented with a request for the diagnosis and sanitation of possible areas of FDOJ because she was diagnosed with an adenocarcinoma of her right breast. Conventional X-ray technologies did not show any FDOJ. We have previously highlighted the difficulties of diagnosing FDOJ using conventional methodologies in an earlier publication.[10] Inconspicuous findings in the two-dimensional orthopantomogram (2D-OPG) and three-dimensional cone beam (3D-digital volume tomogram/DVT) can be confirmed using a TAU measurement. The development of a TAU imaging system, which allows for the measurement of bone density in the area of an assumed FDOJ, is a substantial step forward.[6,7]

After the administration of local anesthesia, and following the folding away of a mucoperiostal flap in the edentulous area of the lower right wisdom tooth area, a cortical lid was removed. Below, fatty degenerative bone marrow was found instead of a normally structured medullary bone. The pathological examination of this FDOJ sample found hallmarks of osteonecrotic metastases of an adenocarcinoma of the breast. Figure 3 demonstrates the remarkably close accumulation and direct proximity of the tumor cells (red arrows) and necrotic adipocytes (green arrows), which we consider as a source of immunological networking by the FDOJ.

In parallel to histopathology, we performed a Luminex®-based cytokine analysis (Luminex Corporation, Austin, TX, USA) on the FDOJ of the lower right retromolar area 48/49 (in US terminology area 32), which is structurally similar to what was shown in Figure 1. Results of the cytokine assay are presented in Figure 4.

The most striking result of this analysis is the high concentration of RANTES. No other proinflammatory messenger, such as interferon-gamma, interleukin (IL)-6, IL-8, or tumor necrosis factor (TNF)-alpha, was detected at such elevated levels. Contrary to the inconspicuous radiographs, the pathohistological examination showed fatty degeneration, while the Luminex® analysis showed substantial overexpression of RANTES in FDOJ of the retromolar area. Following a pilot study conducted with only six patients and 27 cytokines,[5] the compelling data generated with this specific method brought us to select and analyze seven FDOJ cytokines in a further 23 MaCa patients. In a previous publication, we have discussed the role of RANTES in immunological diseases.[11]

Immunhistochemical marking of Ma carcinoma cells (Antibodies against Panzytokeratin, magnification 400 fold)

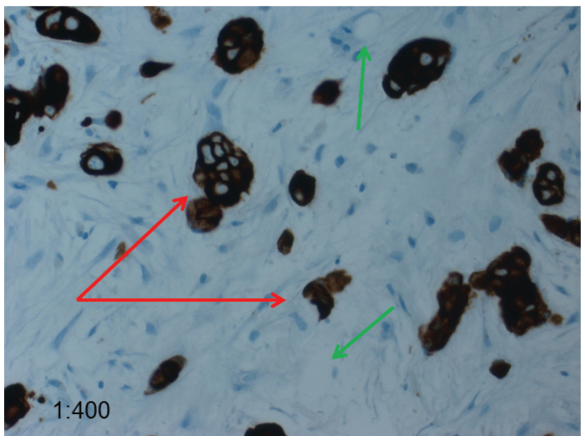

Figure 3. Tumor cells in the retromolar area of FDOJ tissue (HE 400-fold).

7 cytokines in retromolar area 48/49 (US 32) with metastasis of MaCa compared to normal jawbone (n = 19)

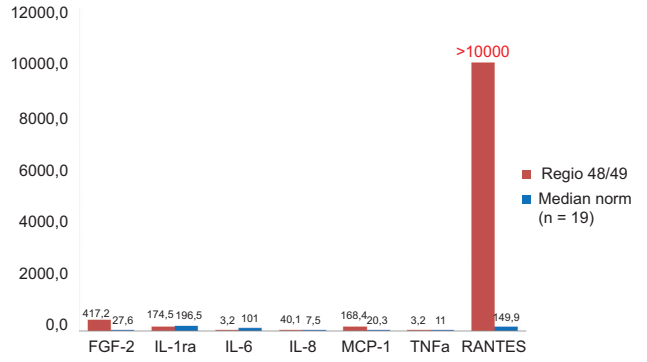

Figure 4. Analysis of seven cytokines in excised FDOJ tissue (retromolar area of the right mandible) from a MaCa patient in comparison to a normal JB.

Materials and Methods

Study structure. From 23 MaCa patients, we retrospectively analyzed FDOJ tissue samples collected at the time of routine surgery. From 13 out of the 23 MaCa patients, we had also collected serum samples. A further 19 patients volunteered to provide samples of healthy JB, after giving their written, informed consent. The local ethics committee waived the ethical approval process for this retrospective study.

Cohort of patients. The study included samples from 23 MaCa patients, as outlined above. The medication status of patients, as based on the presence of a systemic disease, was not used as an exclusion criterion. The inclusion criterion was the local diagnosis of FDOJ in the JB; the medical indication for FDOJ surgery in these patients was based on orthopantomograms (2D-OPG) and an additional cone beam X-ray (3D-DVT). Multiple and irregular remnants of the lamina dura were still present against a subtle radiolucent background, which led to the suspicion of the presence of FDOJ. The definitive indication for FDOJ surgery was derived from the additional measurement of bone density via the TAU technique. Peer reviewed studies that highlight the efficiency and reliability of TAU in comparison to conventional radiographs in the diagnosis of FDOJ are available.[6,7] The demographic data of 23 cases in the FDOJ cohort were as follows: average age, 60.5 years; range, 46–77 years; sex (female/male), 23/0.

Moreover, a control group of 19 patients volunteered to provide samples of healthy JB, which were taken via drill cores performed during dental implantation surgery. The inclusion criteria for this group were: no radiologically distinctive features in 2D-OPG and 3D-DVT; inconspicuous TAU measurements of bone density in the implantation range; and the absence of MaCa. The use of biphosphonate medication was the central exclusion criterion for both groups. The demographic data of the 19 cases in the FDOJ control group were: average age, 51.4 years; range, 33–72 years; sex (female/male): 10/9.

Sampling of FDOJ tissue. In the corresponding author's dental clinic, 23 patients diagnosed with FDOJ had surgery in the affected part of the jaw following the administration of local anesthesia and after the mucoperiostal flap was folded. The cortical layer was removed. All 23 patients showed osteolytic spongial areas and degenerative fatty tissue, as described in the first section. In all cases, surgery was performed in edentulous jaw areas, primarily in the areas where there were former wisdom teeth and in the adjacent retromolar areas (nine cases in the upper and 14 in the lower FDOJ areas).

Processing of necrotic tissue samples and measurement of RANTES in FDOJ samples and serum. The necrotic tissue samples (number [n] = 23) (see Figs. 1 and 2) with a volume up to 0.5 cm^3 were stored in a dry, sterile, 2 mL air-tight collecting vial (Sarstedt AG & Co, Nümbrecht, Germany) and frozen at –20°C. In 13 of these patients, their serum was taken intravenously before or concomitantly after FDOJ surgery with a heparinized 7.5 mL collection tube (Sarstedt AG & Co), and the samples were immediately frozen.

In the laboratory (Institute for Medical Diagnostics, Berlin, Germany; certified by DAKKS/Deutsche Akkreditierungsstelle GmbH, Berlin, Germany; accreditation following DIN EN ISO/IEC 17025:2005 and DIN EN ISO 15189:2007; www.IMD-Berlin.de), tissue samples were homogenized by mechanical force in 200 µL of cold protease inhibitor buffer (cOmplete, Mini Protease Inhibitor Cocktail; Roche Diagnostics, Basel, Switzerland). The homogenate was centrifuged for 15 minutes at 13,400 × g. Afterwards, the supernatant was collected and centrifuged for another 25 minutes at 13,400 × g.

In 42 samples of JB tissue homogenate (23 MaCa cases of FDOJ samples plus 19 healthy JB samples), we measured RANTES/chemokine ligand (CCL)5, FGF-2, IL-1 receptor antagonist (IL-1ra), IL-6, IL-8, monocyte chemotactic protein-1, and TNF-alpha. In 13 patients of the FDOJ cohort, RANTES/CCL5 was additionally analyzed in serum. For the measurement of RANTES in serum, samples were prediluted to 1:100 in a sample buffer according to the manufacturer's instructions. Measurements were performed using the Human Cytokine/Chemokine Panel 1 (MPXHCYTO-60K; EMD Millipore, Billerica, MA, USA) according to the manufacturer's instructions and analyzed using the Luminex® 200™ with xPonent® software (Luminex Corporation) (Fig. 5).

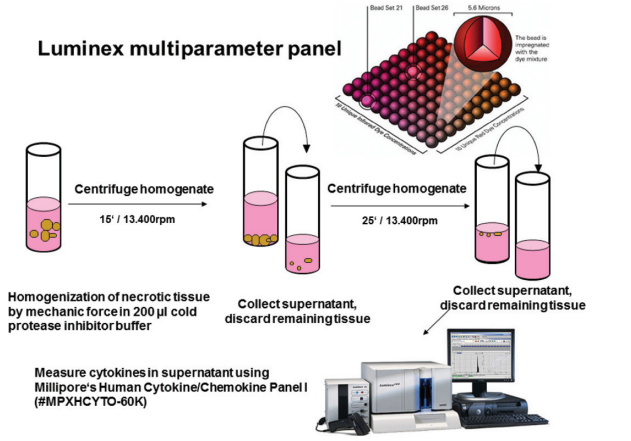

Figure 5. Bead-based processing of FDOJ samples.

Results of Seven Cytokine Panel Evaluations in Osteonecrotic and Healthy Jawbone

The median and standard deviation (SD) results of the seven cytokine panel evaluations in FDOJ among the MaCa cohort (n = 23) are shown in Figure 6. Elevated levels of IL-1ra, RANTES/CCL5, and FGF-2 (pg/mL) were observed; IL-1ra showed a median level of 589 pg/mL (SD ± 502), RANTES showed a median level of 5,123 pg/mL (SD ± 2,860), and FGF-2 showed a median level of 741 pg/mL (SD ± 604). There was a correlation between FGF-2 and RANTES in the FDOJ tissue ($P < 0.01$; correlation coefficient, 0.607). No correlation was observed between the other mediators. The activity of the proinflammatory cytokine, RANTES, is counterbalanced by high levels of IL-1ra.

The results of the 19 samples of normal JB were as follows: IL-1ra, 195 pg/mL (SD ± 0 pg/mL); RANTES, 149 pg/mL (SD ± 127 pg/mL); and FGF-2, 27 pg/mL (SD ± 59 pg/mL). The values for healthy patients and normal JB were not available for comparison from the literature. Figure 6 shows the mean healthy values in comparison with

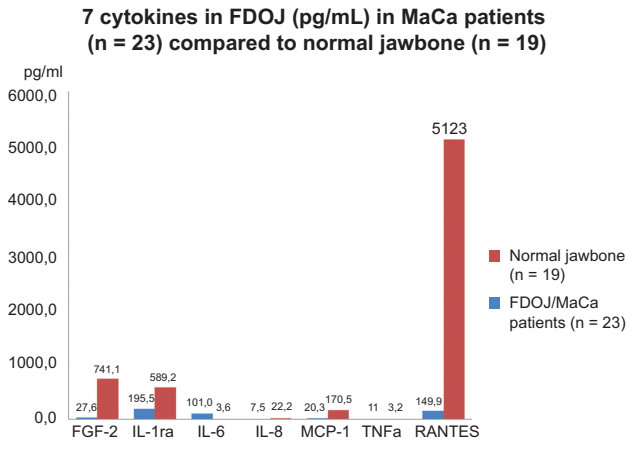

Figure 6. Distribution of seven cytokines in FDOJ in MaCa cases and in a normal JB (n = 23 and n = 19, respectively) (pg/mL).

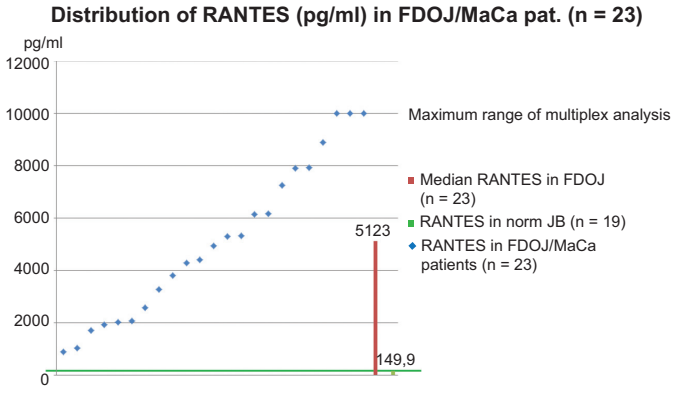

Figure 7. Distribution of individual RANTES values in FDOJ MaCa cases compared to the normal JB (in green; baseline) (n = 23 and n = 19, respectively) (pg/mL).

seven cytokines in FDOJ and the striking difference in the RANTES values. Figure 7 shows that each individual RANTES value in FDOJ is higher than the RANTES range in the normal JB cohort.

The mean value of RANTES in the serum from 13 MaCa patients was 56.4 (ng/mL) with a SD of ± 36.1 ng/mL.

Discussion

It is generally claimed that an imbalance between cytokines and their respective inhibitors is characteristic of chronic inflammatory conditions.[12] The aim of this study was to obtain initial insights into whether the cytokine levels in FDOJ are conspicuous. FDOJ is a chronic insidious and subtle process. This is supported by the fact that typical acute proinflammatory cytokines, such as TNF-alpha and IL-6, are not increased in these processes. Missing acute cytokines—such as IL-6 and TNF-alpha—in the FDOJ samples, we hypothesized that RANTES signaling is a chronic disturbance that might contribute to the RANTES-propelled development of MaCa and metastasis. The absence of acute inflammation denotes the subliminal and hidden proliferation of chronic immunological processes under the guidance of RANTES.

The role of RANTES in diseases. RANTES belongs to the family of chemotactic cytokines known as chemokines. RANTES is produced by circulating T-cells and it plays an active role in recruiting leukocytes to inflammatory sites. Studies have demonstrated that RANTES is actually implicated in many serious illnesses: the chemotactic activities of RANTES route T-cells, dendritic cells, eosinophils, natural killer cells, mast cells, and basophils to sites of inflammation and infection.[13] However, RANTES can have detrimental effects via the recruitment of immune cells that enhance inflammatory processes such as arthritis, atopic dermatitis, nephritis, colitis, and other disorders.[14] RANTES targets the central nervous system and is able to promote multiple sclerosis and Parkinson's disease. In targeting the mast cells, RANTES is involved in allergies, alopecia, and thyroid disorders.[15] RANTES is also excreted by human melanoma cells and has been shown to accelerate tumor growth in a murine disease model.[16] In Hodgkin lymphoma malignant Reed–Sternberg cells (HRS) produce RANTES, which provokes chemotactic migration of mast cells into the tumor tissues. Hodgkin lymphoma cell lines mainly express RANTES in vivo.[17]

In contrast to RANTES, IL-1ra acts as a strong antiinflammatory mediator by blocking signal transduction at the IL-1 receptor level. Due to the antiinflammatory effects of IL-1ra, we never found common hallmarks of inflammation in the histopathology of FDOJ samples (see the section titled "Pathohistological definition of fatty degenerative osteonecrosis of jawbone/FDOJ"). The striking discovery from the data presented is that RANTES is found at high levels in all 23 FDOJ tissue samples investigated (see Fig. 6). The high levels of RANTES indicate that FDOJ can be specified by a derailed metabolic pattern, causing similar and mutually reinforcing pathogenic signaling pathways towards other organs. The immune system seems to be activated in response to "danger signals," which evoke various innate molecular pathways that culminate in inflammatory cytokine production and in the possible activation of the adaptive immune system. This integrative aspect requires us to have a closer look at RANTES and its role in MaCa.

Role of RANTES in breast cancer. The initially outlined question on the role of RANTES in FDOJ can be elucidated when evaluated in the context of patients with MaCa. RANTES has been associated with both the induction or promotion of cancer.[18] The development of MaCa may, in part, be due to the ability of RANTES to act directly on the tumor cells and to promote tumor progression.[19] The high incidence and intensity of RANTES expression has been noted in advanced breast carcinoma; RANTES expression was analyzed in parallel to disease progression, which was positive upon diagnosis and was predictive of clinical course. These results suggest that the assessment of RANTES expression may be a useful prognostic indicator for the identification of patients with an apparently poor prognosis.[20]

Origin of RANTES in FDOJ: fatty tissue and adipocytes. Fatty tissue in general is seen today as being endocrine active and as part of the immune system. Our findings regarding RANTES secretion by FDOJ fatty tissue deserve further discussion; reduced blood flow and capillary density followed by ischemia may lead to hypoxia.[21] The diameter of adipocytes in obesity exceeds the range of the diffusion of oxygen inside the tissues (~120 nm), and this alone can trigger local hypoxia. Adipocytes are triggers of "silent inflammation;" it is well known that fat cells release inflammatory cytokines.[22] In our own histological examination of FDOJ tissue, "necrobiotic modified adipocytes" are regularly found (see the section titled "Pathohistological definition of fatty degenerative osteonecrosis of jawbone/FDOJ"). Interestingly, a remarkable difference in the cytokine patterns of body fat and FDOJ exists; in

obesity, increased levels of TNF-alpha and IL-6 play an important role in systemic effects, while these mediators do not show up in FDOJ samples (see Fig. 6). The existence of high levels of RANTES in FDOJ is confirmed by a recent study conducted on macrophages and RANTES in fatty tissue; it was found that macrophages are not the only inflammatory cells that are found in adipose tissue in obesity.[23] Recently T-lymphocytes were also detected in adipose tissue in obese patients and in mouse models of obesity.[23] T-lymphocytes are also attracted by chemokines.[24] Huber et al[25] found an increased expression of RANTES in fatty tissue in obese patients. The transference of these immune effects on the understanding of FDOJ adipose tissue (see Figs. 1 and 2) is a clear issue that should be further illuminated in the discussion.

Comparison of RANTES expression in FDOJ to RANTES expression in MaCa tissues. RANTES is considered a key mediator in MaCa, but what can we learn from published research about the levels of RANTES in MaCa tissue? Niwa et al[19] measured RANTES content in primary malignancies. Increased RANTES levels were found in all of the examined breast and cervical tumors. In twelve cases of breast cancer tissue, 1,032 ± 120 pg/mg, and in cervical collar tumors 984 ± 115 pg/mg of RANTES were reported.[19] Comparably high levels of RANTES have been found in breast cancer cells at 797.6 ± 3.96 pg/mL.[26] Figure 8 compares the means of the RANTES FDOJ levels (n = 23) with the 5,123 pg/mL RANTES levels of patients with breast cancer. It becomes obvious that areas of FDOJ contain three-fold higher levels of destructive RANTES signaling, as are found in MaCa tissue samples in corresponding papers.[19,26] If RANTES plays an important role in MaCa, then RANTES levels in FDOJ could also be included in pathogenic considerations and therapeutic objectives.

RANTES and metastasis of MaCa. The simultaneous occurrence of high RANTES levels and MaCa metastases inside the FDOJ sample of the MaCa case described in the case report section gives rise to the consideration of the possible role of RANTES in metastasizing MaCa. The nature of the cells producing chemokines or harboring chemokine receptors appears to be crucial for the infiltration of the primary tumor by leukocytes and via angiogenesis. In addition, chemokines and their receptors are key players in the homing of cancer cells to distant metastasis sites. Evidence suggests that the mechanism of action of chemokines in cancer development involves the modulation of proliferation, apoptosis, invasion, leukocyte recruitment, or angiogenesis.[26] Thus, there is significant interest in developing strategies to antagonize chemokine function, and an opportunity to interfere with metastasis, which is the leading cause of death in most MaCa patients.[27] RANTES levels were markedly elevated in the primary tumor and metastatic lesions from all patients with MaCa who were examined. This study suggests that there is an as-yet-undefined, but important, role played by RANTES in carcinogenesis.[28] RANTES was increased in MaCa cases without metastasis, while levels were significantly increased in metastatic patients when compared to controls.[29] RANTES may also be involved in the formation of MaCa metastasis; stem cells transform individual tumor cells into metastasizing cells using transmitters like chemokine RANTES/CCL5.[30] This increased propensity towards metastasis is reversible and dependent on RANTES signaling.[31] Karnoub et al[32] assume that those stem cells transmute tumor cells into metastasizing cells with the aid of messengers like CCL5–RANTES stimulates metastasis. MaCa cells stimulate the de novo secretion of RANTES from mesenchymal stem cells, which then act in a paracrine fashion on cancer cells to enhance their motility, invasion, and metastasis.[32]

RANTES/CCL5 in serum and their correlation to MaCa. In Figure 9, we compare the RANTES values in the serum of 13 MaCa patients to those of normal/healthy controls, as obtained from two papers. In a study conducted on type 2 diabetes, the RANTES levels at baseline in noncases were 23,807 pg/mL = 24,6 ng/mL (n = 1,872) for comparison.[33] Others found that RANTES levels fell in a normal range of 34.98 ± 7.43 ng/mL (n = 19).[34] Taken together, we refer to a normal RANTES serum value of 29 ng/mL or 29,000 pg/mL.

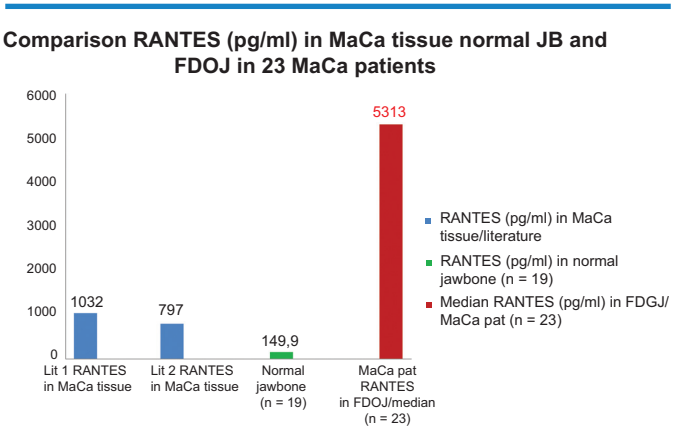

Figure 8. Comparison of RANTES expression in MaCa tissues taken from the literature and in FDOJ among 23 MaCa patients.

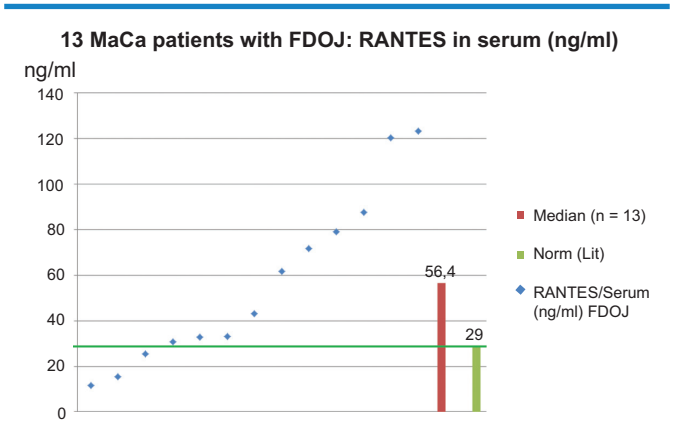

Figure 9. RANTES in the serum of 13 MaCa patients compared to normal values.

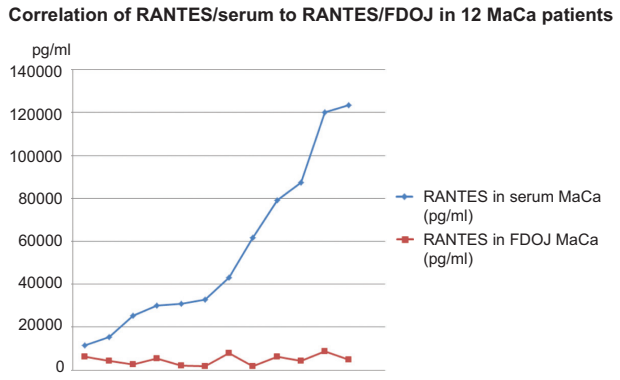

Figure 10. Matched correlations of RANTES in FDOJ and in serum.

To correlate the levels of RANTES in the serum of breast cancer patients with bone or other organ metastases, as compared to breast cancer patients without metastasis and healthy controls, Eissa et al[29] estimated the role of RANTES as a prognostic marker for MaCa. The study was conducted on 25 MaCa cases with no metastasis, 35 cases with metastasis, and 30 apparently healthy controls. There was no significant increase in the level of RANTES in metastatic patients when compared to nonmetastatic patients. RANTES levels were higher in cancer patients, as compared to controls.[35] High RANTES serum levels on their own may not be predictive of metastasis, but they are highly indicative in MaCa. This statement also addresses the distribution noted in our cohort of 13 serum samples; the corresponding RANTES values in FDOJ are shown in Figure 10. A comparison of matched serum and FDOJ samples indicates a nonsignificant trend towards higher levels of RANTES in serum. The correlation coefficient between RANTES tissue levels and serum levels was not significant (p = -,130). Nevertheless, Figure 10 shows that RANTES serum levels were excessively high in six cases (50%).

FDOJ and RANTES—partners of inflammatory systemic networking? The data presented in this study support the hypothesis that FDOJ sites in JBs promote the pathogenesis of MaCa by immune mediator expression.

In chronic inflammation, the production of cytokines by infiltrating and resident tissue cells escapes regulatory mechanisms, while indirectly leading to tissue destruction via the activation of immune and inflammatory cells. Cytokines are involved in the triggering of the immune response, the induction of acute inflammatory events, and the transition to or persistence of chronic inflammation. Thus, the findings presented in FDOJ-derived RANTES signaling might play a part as a possible building block in MaCa. This body of data poses the question of whether the dentist/jaw surgeon could be responsible for causing such alarming reactions, according to Selye.[36]

Synopsis. FDOJ areas tend to dispel, and not heal, without surgical curettage. The misjudged and actual existence of FDOJ seems to be proven by the following aspects: 1) FDOJ is characterized morphologically and macroscopically by fatty degenerative softening and osteonecrosis of the JB (see the section titled "Morphological definition of fatty degenerative osteonecrosis of jawbone/FDOJ"); 2) FDOJ is characterized in histopathological and microscopic examinations by the resolution and propagation of adipocytes, jelly-like substances inside the jawbone, metabolic disturbance, and a total lack of typical leukocytic inflammation (see the section titled "Pathohistological definition of fatty degenerative osteonecrosis of jawbone/FDOJ"); 3) FDOJ is biochemically characterized by high levels of proinflammatory chemokine RANTES in 23 MaCa samples and, in comparison to 19 normal JB samples, by their significantly higher values of these mediators (see the Results section); and 4) FDOJ is biochemically characterized by the absence of acute cytokines such as TNF-alpha and IL-6. This explains the painless and cryptic nature of FDOJ, which make its clinical acceptance in daily practice difficult (see the Results section).

Sections 1) to 4) show that FDOJ areas are not regular, and that they are pathological states of the JB. Thus, the presented data and contexts described herein suggest that areas of FDOJ could hold significant pathogenic potential across the overall system. Adipocytes and the necrotic parts of fat cells are considered by many studies to be immunologically effective ingredients (see the section titled "Origin of RANTES in FDOJ: fatty tissue and adipocytes"). The mean RANTES level in 23 FDOJ samples is about five times as high as the RANTES level in MaCa tissue (see the section titled "Comparison of RANTES expression in FDOJ to RANTES expression in MaCa tissues"). Surgical debridement of FDOJ may be a key to reversing MaCa.

Limitations of the study. The significance of the study lies in the relatively high number of 23 FDOJ and 19 normal JB samples, and in the significantly overexpressed RANTES levels in FDOJ. One limitation of the study is the fact that MaCa has multiple causes, which does not allow for the evaluation of the clinical efficacy of FDOJ surgery in this study.

Conclusion

Although the extent to which the increased expression of RANTES in FDOJ contributes to immune-mediated diseases is unknown, the results of this study provide evidence for the possible interaction between RANTES signaling derived from FDOJ with MaCa. The hypothesis presented in this study is based on the pathogenic aspect of FDOJ: the permanently increased expression of RANTES in FDOJ sites could exert inflammatory signaling in breast tissue. A comprehensive understanding of the complex networks outlined in this paper will require further research into the fundamental properties of cytokines and ILs. The results submitted by the authors are, therefore, of a preliminary nature, but they nevertheless provide new insights into deregulated RANTES expression within FDOJ. The challenge posed by these discoveries is the need to raise awareness of FDOJ throughout the medical and

dental community. In order to clarify reliable backgrounds of RANTES signaling pathways in FDOJ, this research can only highlight the need for a prospective multicenter study.

Acknowledgements

We would like to thank the peer reviewers for spending their time and experience.

Author Contributions

Conceived and designed the experiments: JL. Analysed the data: JL, VvB. Wrote the first draft of the manuscript: JL. Contributed to the writing of the manuscript: JL, VvB. Agree with manuscript results and conclusions: JL. Jointly developed the structure and arguments for the paper: JL. Made critical revisions and approved final version: JL. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Glueck CJ, Freiberg RA, Wang P. Role of thrombosis in osteonecrosis. *Curr Hematol Rep*. 2003;2(5):417–422.
2. Wilke I, Becker S. Osteonekrose—pathologische grundlagen. *Journal für Mineralstoffwechsel*. 2007;14(1):3–6. German.
3. Bouquot JE, Roberts AM, Person P, Christian J. Neuralgia-inducing cavitational osteonecrosis (NICO). Osteomyelitis in 224 jawbone samples from patients with facial neuralgia. *Oral Surg Oral Med Oral Pathol*. 1992;73(3):307–319; discussion 319–320.
4. Bouquot JE, Christian J. Long-term effects of jawbone cutterage on the pain of facial neuralgia; treatment results in neuralgia-inducing cavitational osteonecrosis. *Oral Surg Oral Med Oral Pathol*. 1991;72:582.
5. Lechner J, Mayer W. Immune messengers in neuralgia inducing cavitational osteonecrosis (NICO) in jaw bone and systemic interference. *Eur J Integr Med*. 2010;2(2):71–77.
6. Bouquot J, Shankland W, Margolis M. Through-transmission alveolar ultrasonography (TAU) B new technology for evaluation of bone density and desiccation. Comparison with radiology of 170 biopsied alveolar sites of osteoporitic and ischemic damage. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;93:413–414.
7. Bouquot J, Martin W, Wrobleski G. Computer-based thru-transmission sonography (CTS) imaging of ischemic osteonecrosis of the jaws—a preliminary investigation of 6 cadaver jaws and 15 pain patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001;92:550.
8. Lechner J. *Herd, Regulation and Information*. Heidelberg, Germany: Hüthig-Verlag; 1993.
9. Bouquot J, LaMarche M. Subpontic osteonecrosis: imaging and microscopic features in 38 patients with "idiopathic" chronic pain. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;86:209–210.
10. Lechner J. NICO—1st fehlende röntgenologische Evidenz Beweis fehlender klinischer Existenz? *ZWR—Das Deutsche Zahnärzteblatt*. 2010;119(10):578–592.
11. Lechner J, von Baehr V. RANTES and fibroblast growth factor 2 in jawbone cavitations: triggers for systemic disease? *Int J Gen Med*. 2013;6:277–290.
12. Burger D, Dayer JM. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev*. 2002;1(1–2):111–117.
13. Levy JA. The unexpected pleiotropic activities of RANTES. *J Immunol*. 2009;182(7):3945–3946.
14. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol*. 2000;18:217–242.
15. Bischoff SC, Krieger M, Brunner T, et al. RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. *Eur J Immunol*. 1993;23(3):761–767.
16. Mrowietz U, Schwenk U, Maune S, et al. The chemokine RANTES is secreted by human melanoma cells and is associated with enhanced tumour formation in nude mice. *Br J Cancer*. 1999;79(7–8):1025–1031.
17. Fischer M, Juremalm M, Olsson N, et al. Expression of CCL5/RANTES by Hodgkin and Reed-Sternberg cells and its possible role in the recruitment of mast cells into lymphomatous tissue. *Int J Cancer*. 2003;107(2):197–201.
18. Soria G, Ben-Baruch A. The inflammatory chemokines CCL2 and CCL5 in breast cancer. *Cancer Lett*. 2008;267(2):271–285.
19. Niwa Y, Akamatsu H, Niwa H, Sumi H, Ozaki Y, Abe A. Correlation of tissue and plasma RANTES levels with disease course in patients with breast or cervical cancer. *Clin Cancer Res*. 2001;7(2):285–289.
20. Wigler N, Shina S, Kaplan O, et al. Breast carcinoma: a report on the potential usage of the CC chemokine RANTES as a marker for a progressive disease. *Isr Med Assoc J*. 2002;4(11 Suppl):940–943.
21. Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes (Lond)*. 2009;33(1):54–66.
22. Stulnig TM. Adipositas und die entzündung des fettgewebes. *J Clin Endocrinol Metab*. 2009;2(3):17–21.
23. Kintscher U, Hartge M, Hess K, et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol*. 2008;28(7):1304–1310.
24. Wu H, Ghosh S, Perrard XD, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation*. 2007;115(8):1029–1038.
25. Huber J, Kiefer FW, Zeyda M, et al. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. *J Clin Endocrinol Metab*. 2008;93(8):3215–3221.
26. Eichbaum C, Meyer AS, Wang N, et al. Breast cancer cell-derived cytokines, macrophages and cell adhesion: implications for metastasis. *Anticancer Res*. 2011;31(10):3219–3227.
27. Ali S, Lazennec G. Chemokines: novel targets for breast cancer metastasis. *Cancer Metastasis Rev*. 2007;26(3–4):401–420.
28. Azenshtein E, Luboshits G, Shina S, et al. The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. *Cancer Res*. 2002;62(4):1093–1102.
29. Eissa SA, Zaki SA, El-Maghraby SM, Kadry DY. Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. *J Egypt Natl Canc Inst*. 2005;17(1):51–55.
30. Zischek C, Niess H, Ischenko I, et al. Targeted stem cell based RANTES/TK suicide gene-therapy in a murine pancreatic cancer tumour model. *Deutsche Gesellschaft für Chirurgie*. 2009;38:3–5.
31. Tsukishiro S, Suzumori N, Nishikawa H, Arakawa A, Suzumori K. Elevated serum RANTES levels in patients with ovarian cancer correlate with the extent of the disorder. *Gynecol Oncol*. 2006;102(3):542–545.
32. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature*. 2007;449(7162):557–563.
33. Herder C, Illig T, Baumert J, et al. RANTES/CCL5 gene polymorphisms, serum concentrations, and incident type 2 diabetes: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. *Eur J Endocrinol*. 2008;158(5):R1–R5.
34. Noris M, Daina E, Gamba S, Bonazzola S, Remuzzi G. Interleukin-6 and RANTES in Takayasu arteritis: a guide for therapeutic decisions? *Circulation*. 1999;100(1):55–60.
35. Selye H. *Hormones and Resistance*. Berlin, Germany: Springer-Verlag; 1971.