

BONE RESORPTION MARKER RANKL IN THE DIAGNOSIS AND TREATMENT OF PATIENTS WITH MAXILLARY SINUS CYSTS

Shkorbotun V.O., Nachesa Y.S.

Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine

<https://doi.org/10.35339/ic.11.3.shn>

ABSTRACT

Background. RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) is a protein belonging to the tumor necrosis factor superfamily serving as a specific marker for osteoclast activation and osteolysis.

Aim. To determine RANKL levels in the homogenates of Maxillary Sinus Cysts (MSC) at the time of their removal, investigate the correlation of these indicators with the presence of pathology in the upper row of projection teeth in the respective patients, and refine the timing for potential dental implantation or sinus lifting after sinusotomy based on these findings.

Materials & Methods. The study included 25 patients aged 20–65 years who underwent surgical treatment for MSCs located on the inferior wall of the sinus. The research methods comprised general clinical, otorhinolaryngological, radiological, and pathohistological techniques and Enzyme-Linked ImmunoSorbent Assay (ELISA) for RANKL in the tissues of the removed cysts. Pathohistological examination revealed retention cysts in 11 patients and pseudocysts in 14 patients. The presence of dental pathology and active odontogenic processes at the time of cyst removal was established based on prospective clinical and radiological evaluations. The dynamics of mucoperiosteal defect regeneration in the MSC, considering RANKL levels, were assessed 3–6 months post-surgery.

Results. In the postoperative period, 8 (32%) out of 25 patients exhibited odontogenic alteration processes in the tissues of the maxilla adjacent to the cysts. The RANKL concentration in these patients was $[80.65 \pm 34.22]$ pg/ μ g of protein, while in the other 17 patients without signs of bone damage, it was $[33.10 \pm 4.35]$ pg/ μ g of protein ($p < 0.05$). The threshold RANKL concentration distinguishing these groups in this study was 51.50 pg/ μ g of protein.

Conclusions. In patients with peri-apical bone changes in the maxilla in the projection area of the cysts (alveolar recess), the RANKL level in their homogenates is higher compared to patients without such pathology. Measuring RANKL levels in the homogenates of cysts removed during endonasal maxillary sinusotomy can serve as an additional indicator for planning postoperative dental treatment in these patients.

Keywords: ENT-pathology, endoscopic rhinosurgery, histology, postoperative period, paranasal sinus x-ray, treatment.

Introduction

Recent advancements in the management of Maxillary Sinus Cysts (MSC) include the introduction of highly informative imaging modalities and endoscopic surgical methods, leading to significant improvements in treatment outcomes [1].

However, a debate has emerged in recent years regarding the necessity of removing asymptomatic cysts, especially those smaller than 20 mm or 15 mm in diameter, in patients undergoing sinus lift procedures [2; 3]. This issue is particularly relevant for patients scheduled for dental implantation involving subantral bone augmentation. In these cases, the decision to remove a cyst is influenced not only by its size but also by its location. Research has shown that cysts directly above the planned augmentation site increase the risk of complications, such as Schneiderian membrane rupture during sinus lifting [4].

Corresponding Author:

Yaroslav Nachesa – MD, PhD student of the Department of Otorhinolaryngology, Shupyk National Healthcare University of Ukraine.

✉ Ukraine, 04112, Kyiv, Dorohozhytska str., 9.
E-mail: doctor.yaroslavnachesa@gmail.com

The cyst size remains a critical factor due to the potential for ostium blockage caused by cyst elevation during the sinus lift and the risk of reactive rhinosinusitis [4]. Nonetheless, the key determinant of successful surgical management of MSCs is the risk of recurrence [5].

Currently, endoscopic endonasal microdebrider-assisted maxillary sinusotomy, adhering to periosteum-preserving techniques, represents the gold standard for sinus surgery [6]. This minimally invasive approach allows for effective sanitation of the maxillary sinus while preserving the integrity of the periosteal layer of the mucoperiosteum.

A stable outcome in sinusotomy largely depends on the surgeon's ability to ensure complete sanitation of the tissues surrounding the cyst attachment site. This is particularly important for odontogenic cysts, as the absence of visible dental pathology does not always rule out inflammation in adjacent tissues of the maxilla. The completeness of bone tissue sanitation near the cyst is evaluated through imaging studies, primarily Computed Tomography (CT), and monitoring its post-operative dynamics [7]. However, awaiting positive CT changes often requires prolonged observation and follow-up scans, which may not be practical in clinical settings.

This highlights the need to identify inflammation markers in bone tissue at different stages of MSC management to detect localized inflammatory processes. One promising marker is the Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), which indicates bone destruction and could serve as a valuable diagnostic tool in these cases.

Aim

To enhance the effectiveness of treatment for patients with maxillary sinus cysts (MSCs) by developing a differentiated approach to determining the necessity for further sanitation of maxillary teeth adjacent to the cysts, based on the application of the RANKL immunoassay.

Materials and Methods

The study included 25 patients aged 20–65 who underwent surgical treatment for MSCs. The diagnosis was established through rhinological examinations, including endorhinoscopy and CT of the paranasal sinuses. General clinical investigations were also performed for all patients.

The cohort consisted of 11 women and 14 men, with an average age of $[31 \pm 12.2]$ years. The examinations were conducted at the Department of Otorhinolaryngology of Shupyk National Health-

care University of Ukraine, the clinical facilities of Kyiv City Clinical Hospital No.9, and the Department of Fundamental and Applied Problems of Endocrinology at the "V.P. Komissarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine", where immunoassay analyses were performed.

This prospective study evaluated the dynamics of CT data obtained 3–6 months after endoscopic sinus surgery, correlating the findings with the levels of RANKL, a bone destruction marker, measured in tissue samples from cyst walls located on the floor of the maxillary sinus.

Inclusion criteria were the presence of cystic lesions of the maxillary sinuses in patients who required surgical treatment.

Exclusion criteria were radiological signs of facial bone resorption unrelated to sinus pathology, the presence of carious lesions of the mandibular teeth, skull fractures within the past 6 months, and a confirmed oncological diagnosis.

All patients underwent endoscopic endonasal maxillary sinusotomy with cyst removal. Radiological studies were performed using the NewTom GO CT scanner (Cefla Group, Italy), equipped with a "CANON/TOSHIBA D-067SB" tube (serial number 0D14854-J4241A3) (Canon/Toshiba, Japan). Measurement ranges were $[155–172]$ mGy/cm $\pm 10.00\%$, with an error margin of $\pm 4.24\%$. The analysis focused on the condition of the mucoperiosteum of the maxillary sinus and the maxillary teeth in the region of the cyst (presence and extent of caries, apical changes, and periapical alterations in the bone adjacent to the cyst).

Histological examination of the removed cysts revealed that 11 cases (44.0%) were retention cysts, while 14 cases (56.0%) were lymphangiectatic cysts (pseudocysts).

RANKL levels in the fragments of cyst walls removed from the maxillary sinus were determined using Enzyme-Linked Immunosorbent Assay (ELISA). After weighing the biopsy material and adding phosphate buffer, homogenization was performed, followed by centrifugation of the homogenate to isolate the supernatant for analysis. The human TNFSF11 (RANKL) ELISA kit ("Abcam", UK) was used, employing a colorimetric method with sensitivity < 10 pg/mL and a detection range of $[78–5000]$ pg/mL [8].

All patients were informed about the study objectives, provided written informed consent for participation, and were allowed to withdraw at any stage. The study adhered to the ethical principles

of the Declaration of Helsinki, the Constitution of Ukraine, and national healthcare regulations.

Statistical analysis was performed using Statistica v.12.6 software (StatSoft, USA), employing non-parametric methods such as the Mann-Whitney U-test and Fisher's exact test. Qualitative variables were described using absolute and percentage values with 95% confidence intervals (CI). A p-value of <0.05 was considered statistically significant.

Results

The average RANKL level in the cyst walls of the studied patients was $[56.07 \pm 29.72]$ pg/ μ g of protein. Among patients with true cysts, the level was $[32.2 \pm 5.1]$ pg/ μ g of protein, while in individuals with pseudocysts, it was significantly higher, at $[71.7 \pm 31.1]$ pg/ μ g of protein ($p < 0.05$, Mann-Whitney U-test).

CT findings, considering the dental status of patients prior to endorhinosurgical intervention and cyst type, are presented in *Table 1*.

Analysis of *Table 1* demonstrates that periapical bone changes in areas adjacent to the walls of removed cysts were significantly more frequent in patients with pseudocysts. Moreover, the proportion of patients with healthy teeth or superficial caries (not affecting deeper structures) was notably lower in this group. Additionally, endodontic treatment with root canal filling was more com-

mon among patients with pseudocysts (43%) than with retention cysts (18%), though this difference did not reach statistical significance.

Our prospective study focused on detecting odontogenic inflammation and analyzing CT dynamics in patients who underwent maxillary sinusotomy. The evaluation considered RANKL levels as markers of inflammation in bone tissue and residual inflammatory processes in maxillary structures. CT findings 3–6 months post-surgery were used to assess the mucoperiosteum regeneration and the state of the maxillary sinus in the areas of former cyst localization (*Table 2*).

Table 2 data indicate that no cyst recurrence was observed in patients, regardless of cyst type, suggesting the effectiveness of endoscopic surgical intervention. However, mucoperiosteal thickening at the cyst removal site was observed in 6 patients (42.8%) with pseudocysts and in 1 patient (9.1%) with a true cyst. In lymphangiectatic cyst cases, these changes were localized near the apices of the teeth, in the patient with a retention cyst, they were found in the lateral sections of the maxillary sinus, excluding odontogenic origin.

Signs of negative dynamics in bone tissue in the periapical areas of teeth adjacent to removed MSCs were identified in one patient, alongside mucoperiosteal thickening. In contrast, two patients after endodontic treatment before cyst removal

Table 1. Results of the study of maxillary structures in patients with maxillary sinus cysts based on computed tomography data

Radiological feature	Share of individuals with identified radiological signs						p*
	Patients with retention cysts (n=11)		Patients with lymphangiectatic cysts (n=14)		Total (n=25)		
	abs., n	% [95% CI]	abs., n	% [95% CI]	abs., n	% [95% CI]	
Absence of caries signs	5	45.5 [21.3–72.0]	1	7.1 [1.3–31.5]	6	24.0 [11.5–43.4]	≥0.05
Caries without pulp involvement	4	36.4 [15.2–64.6]	0	0.0 [0.0–13.0]	4	16.0 [6.4–34.7]	<0.05
Caries extending to the pulp	2	18.1 [5.1–47.7]	1	7.1 [1.3–31.5]	3	12.0 [4.2–30]	≥0.05
Periapical bone changes near maxillary teeth	0	0.0 [0.0–16.0]	8	57.2 [32.6–78.6]	8	32.0 [17.2–51.6]	<0.05
Edentulism in the area adjacent to the cyst	0	0.0 [0.0–16.0]	4	28.6 [11.7–54.6]	4	16.0 [6.4–34.7]	≥0.05
Endodontically treated teeth adjacent to the cyst	2	18.2 [5.1–47.7]	6	42.9 [21.4–67.4]	8	32.0 [17.2–51.6]	≥0.05

Note: for determine p* (1–2) the Fisher's exact test used.

Table 2. CT data of patients who underwent surgery for maxillary sinus cysts

Clinical signs considered	Share of individuals with identified clinical signs n (% [95% CI])		p ^{***}
	Patients with retention cysts (n=11)	Patients with lymphangiectatic cysts (n=14)	
Recurrence of the cyst	0 (0.0 [0.0–16.0])	0 (0.0 [0.0–13.0])	0.688
Thickening of the mucous membrane at the site of the intervention	1 (9.1 [1.6–37.7])	6 (42.8 [21.4–67.4])	0.090
Presence of exudate in the sinus cavity	0 (0.0 [0.0–16.0])	1 (7.1 [1.3–31.5])	0.366
Periapical decrease in bone density of the upper jaw	0 (0% [0.0–16.0])	8 (57.1% [32.6–78.6]) ^{*,**}	0.003

Notes:

* – feature observed alongside thickened sinus mucosa;

** – in two patients, a reduction in periapical changes was noted, while in one case, the area of changes increased;

*** – to determine p the Fisher's exact test used.

demonstrated a reduction of periapical bone density loss. These two patients, exhibiting advanced caries with pulp involvement and periapical bone density reduction near the cyst-associated teeth, had elevated RANKL levels of 80.85 pg/μg and 73.88 pg/μg of protein, respectively. Subsequent dental evaluations confirmed the presence of inflammation in the roots of these teeth.

A total of 8 patients (32.0%) were identified with dental pathology associated with bone destruction in areas adjacent to the cysts. Analysis of RANKL levels in these patients revealed levels of 51.50 pg/μg or higher. *Figure 1* illustrates the relationship between dental pathology and RANKL levels in the homogenates of removed cyst walls.

Statistical analysis of *Figure 1* data revealed that the mean RANKL level in cyst walls of patients with confirmed advanced dental pathology near the cyst site was [80.65±34.22] pg/μg of protein, significantly higher than the level in patients without such pathology, as determined by clinical and radiological assessments ([33.10±4.35] pg/μg of protein, p<0.05).

Discussion

The study data regarding CT characteristics in patients with MSCs can be explained by the peculiarities of their pathogenesis. It is well known that lymphangiectatic cysts are more often of odontogenic origin, which is supported by the higher frequency of dental pathology detected in this group.

However, it is not always possible to identify pathology based on CT findings during intervention. In 6 (42.8%) cases, inflammatory processes were detected only through mucosal thickening in the cyst removal area during follow-up. In all these cases, RANKL levels were elevated, and inflammation in the bone tissue was confirmed by dental evaluation and subsequent endodontic treatment. These findings corroborate the results of experimental studies by Daisuke Nishida et al. (2021), which indicated the significance of RANKL as a marker in osteolysis processes [9]. Furthermore, Natalia Asquino et al. (2021) emphasized the importance of investigating the RANKL system in patients with dental pathologies, particularly periodontitis, in their review [10].

A critical aspect of this study is determining the threshold RANKL level in MSC walls that indicates bone remodeling processes in adjacent maxillary areas. In our study, this threshold was established at 51.50 pg/μg of protein, which has significant practical implications.

Conclusions

1. Mean RANKL level in the cyst walls of patients with dental pathology was significantly higher than in those without deep tooth structure involvement, amounting to [80.65±34.22] pg/μg of protein and [33.10±4.35] pg/μg of protein, respectively.

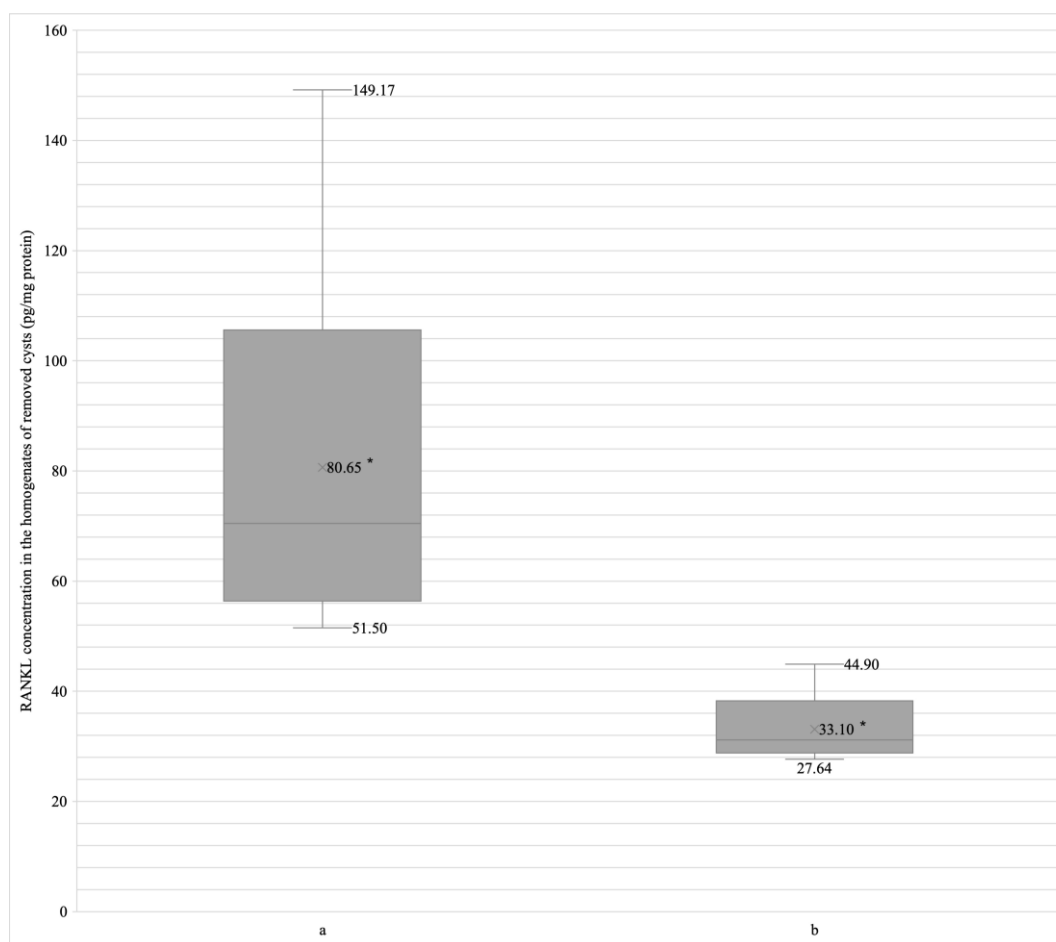


Fig. RANKL levels in cyst homogenates were relative to signs of active inflammation in the adjacent maxillary bone:

*a – signs of a verified inflammation in the adjacent maxillary bone (n=8);
b – no signs of inflammation of the adjacent maxillary bone (n=17).*

Notes: * – $p < 0.05$; MB – maxillary bone.

2. The threshold RANKL level in the MSC wall indicative of bone destruction and remodeling processes in adjacent maxillary areas is 51.50 pg/ μ g of protein.

3. Measuring RANKL levels in cyst walls during their removal can be recommended as a laboratory method for identifying patients requiring sanitation of adjacent teeth.

Prospects for further research

Determining the significance of RANKL levels in the soft tissues adjacent to teeth requires further investigation in larger cohorts. This could potentially lead to the development of an algorithm for detecting hidden dental pathologies with osteolysis in patients with inflammatory MSC conditions.

Study limitations

This study is based on a limited sample size and a single pathological condition (MSC). Fur-

ther research is needed to refine our understanding of the RANKL regulatory system in other nosological entities.

DECLARATIONS:

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

Data Transparency

The data can be requested from the authors.

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Funding Sources

There are no external sources of funding.

Consent for publication

All authors give their consent to publication.

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Received: 26 Jul 2024

Accepted: 25 Sep 2024

Published: 30 Sep 2024

Cite in Vancouver style as: Shkorbotun VO, Nachesa YS. Bone resorption marker RANKL in the diagnosis and treatment of patients with maxillary sinus cysts. *Inter Collegas*. 2024;11(3):29-34. <https://doi.org/10.35339/ic.11.3.shn>

Archived: <https://doi.org/10.5281/zenodo.14294192>

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