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ORIGINAL RESEARCH

Expression and Clinical Significance of IL-33 and IL-25 in Post-Irradiation Otitis Media with Effusion

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Purpose: This study investigates IL-33 and IL-25 expression in post-radiation otitis media with effusion (POME), along with classic oxidative stress markers (MDA and SOD), to investigate radiation-induced oxidative stress and its association with ILC2-mediated chronic inflammation, providing basis for targeted therapies.

Methods: Middle ear effusions (MEE) were collected from 35 irradiated nasopharyngeal carcinoma patients with otitis media with effusion (POME, 43 ears) and 20 non-irradiated conventional chronic otitis media with effusion (CCOME, 20 ears) patients. IL-33, IL-25, IL-6, SOD, and MDA levels were measured by ELISA. Eustachian tube function was evaluated using the Endoscopic Evaluation of the Eustachian Tube (3ET) scoring system.

Results: Comparative analysis revealed distinct molecular profiles between POME and CCOME, with POME showing significantly reduced IL-33 levels ($p=0.046$) but elevated SOD activity ($p=0.015$), along with a non-significant trend toward higher MDA ($p=0.083$). Temporal analysis demonstrated peak expression of both IL-25 and IL-33 at 6 months post-radiation. Correlation studies identified significant associations between IL-33 ($r=0.391$) and IL-6, as well as between IL-25 ($r=0.483$) and IL-6 (both $p<0.01$). Clinically, IL-25 levels showed positive correlation with 3ET endoscopic scores ($r=0.407$, $p=0.021$) and were significantly reduced following tympanostomy tube placement ($p=0.024$). Notably, no direct correlation was observed between IL-33 and IL-25 ($p>0.05$), nor were any significant associations found with allergic comorbidities (all $p>0.05$).

Conclusion: IL-33 and IL-25 synergistically drive ILC2-mediated chronic inflammation in radiation-induced OME, with IL-25 emerging as a biomarker for radiotherapy-associated Eustachian tube dysfunction. The 6-month cytokine surge post-radiation highlights a therapeutic window for targeted interventions to mitigate long-term complications.

Keywords: post-irradiation, otitis media with effusion, nasopharyngeal carcinoma, interleukin-33, interleukin-25

Introduction

Post-irradiation otitis media with effusion (POME) is one of the most common ear complications after radiotherapy for nasopharyngeal carcinoma (NPC), with an incidence of 29.4% to 52.9%.[1–3] Patients typically manifest recurrent symptoms such as tinnitus, stuffiness, deafness, and middle ear effusion (MEE) for a considerable period after radiotherapy, significantly compromises long-term quality of life and imposes substantial socioeconomic burdens. Currently, there are no precise and effective treatment protocols or clinical guidelines to guide the management of patients with POME. The treatment for these patients is typically similar to that of conventional chronic otitis media with effusion (CCOME) patients, including repeated puncture and suctioning of the tympanic membrane, tympanotomy and tube placement, tympanostomy, and balloon dilatation of the eustachian tube (ET). However, these approaches yield suboptimal outcomes in POME, offering only transient

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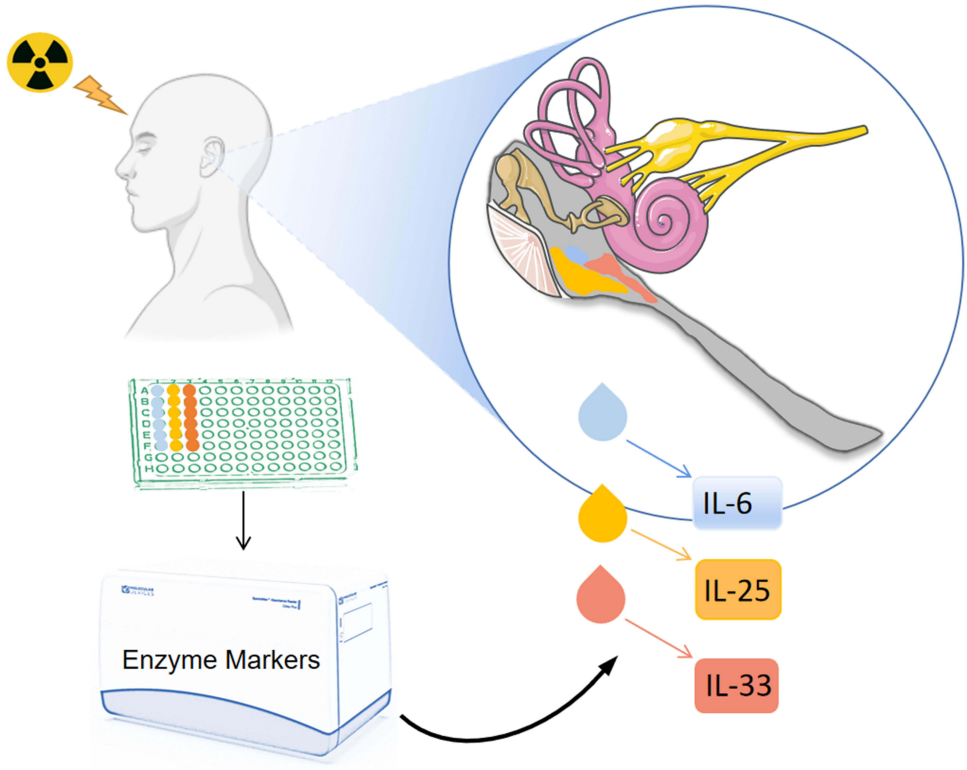
Graphical Abstract



symptom relief and carrying high risks of complications (eg, tympanic membrane perforation, otorrhea; incidence: 15.6–42.2%).[4] Despite its clinical urgency, the pathophysiology of POME remains poorly elucidated, with research predominantly limited to incremental refinements of existing therapies rather than mechanism-driven interventions.

Currently understanding, local inflammation of the middle ear, which may be caused by infectious inflammation or allergic reactions, is recognized as a key contributor to be one of the main factors in otitis media with effusion (OME).[5] Of particular interest are cytokine dysregulation and oxidative stress mechanisms, which have garnered significant attention in OME research. Emerging evidence highlights group 2 innate lymphoid cells (ILC2) emerged as critical regulators in the type 2 inflammatory response.[6] Mechanistically, ILC2s are primarily activated by interleukin-25 (IL-25) and interleukin-33 (IL-33), and then releases large quantities of type 2 cytokines, resulting in an imbalance between type 1 and type 2 cytokines immune responses, thereby amplifying disease progression.[7]

To date, the functional roles of IL-25, IL-33, and ILC2s in POME remain unexplored. IL-25 (interleukin-17E), a pleiotropic member of the IL-17 cytokine family, exerts potent pro-inflammatory properties across mucosal barriers. In the airway epithelium, IL-25 triggers three key pathological processes: Induction of pro-allergic chemokines (eg, CCL17, CCL22); Expansion of goblet cell populations and mucus hypersecretion (paralleling its effects in gastrointestinal epithelia); Epithelial remodeling through hyperplasia and airway hyperreactivity.[8]

Mechanistically, IL-25 serves as a master coordinator of mucosal immunity, bridging innate and adaptive responses via dual activation of ILC2s and Th2 cells. This synergistic axis perpetuates a Th2-polarized mucosal milieu characterized by sustained type 2 inflammation and disrupted Th1/Th2 homeostasis.[9] IL-33, belonging to the IL-1 family, is a multifunctional cytokine that plays an important role in varieties of biological processes, including the generation and regulation of immune responses, maintenance of tissue homeostasis, growth, and repairment.[10]

Accumulating evidence underscores oxidative stress as a pivotal driver in OME pathogenesis. The presence of oxidative stress biomarkers such as reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide, and catalase have been consistently detected in MEE.[11,12] Radiotherapy exacerbates this imbalance by generating excessive ROS, thereby amplifying oxidative stress damage.[13] Among these markers, SOD serves as the primary defense against ROS and their reactive products induced by abiotic stress.[14] Conversely, MDA, as the end product of membrane lipid peroxidation, reflects the severity of radiation damage.[15] Clinically, SOD and MDA are commonly established gold-standard biomarkers for monitoring oxidative stress levels following radiotherapy due to their diagnostic reproducibility and temporal stability.[16,17] Notably, intensity-modulated radiotherapy induces persistent immunomodulatory effects in nasopharyngeal carcinoma patients, reprogramming cytokine/chemokine networks and sustaining inflammatory cascades.[18] This suggests that cytokine levels and oxidative stress levels in patients with POME after radiotherapy for NPC may be different from those in patients with CCOME.

Guided by the temporal dynamics of post-radiotherapy cytokine reprogramming and the well-characterized pathological axis of oxidative stress in OME, this study aims to unravel the potential involvement of IL-25 and IL-33 in the development of POME in nasopharyngeal carcinoma patients. By decoding the crosstalk between these cytokines and oxidative stress biomarkers, we systematically evaluate their clinical significance as inflammatory mediators and predictive indicators for disease chronicity.

Materials and Methods

Study Group

This cross-sectional study investigated MEE collected over a seven-month period (November 2022-May 2023) from patients with POME and CCOME controls. For POME patients, disease duration was calculated from radiotherapy completion rather than symptom onset due to the characteristic delayed presentation of otologic symptoms in this population, where tumor surveillance typically takes precedence over ear complaints, and because radiation-induced mucosal damage represents the definitive initiating event. Concurrently, age- and sex-matched CCOME patients were enrolled as controls, with their disease duration measured from initial symptom onset, thereby enabling comparative analysis of inflammatory mediator profiles between radiation-induced and conventional chronic middle ear inflammation.

The patients included in this study must meet the following conditions: (1) adult patients diagnosed with OME in the Fifth Affiliated Hospital of Sun Yat-sen University; (2) the course of the disease was at least 3 months at the time of MEE collection; (3) MEE was found endoscopically; (4) there were no signs or symptoms of acute otitis media; and (5) no antibiotics or hormone therapy was received within 1 month. Additionally, relevant information about all patients was recorded, such as gender, age, side of MEE, history of tympanotomy tube placement, and history of allergic rhinitis.

Patients with POME were excluded if they had MEE before radiotherapy, severe systemic disease, or tumor recurrence. Patients with POME were divided into two groups based on the presence or absence of endoscopic manifestations of allergic rhinitis and previous diagnostic and clinical manifestations. In order to minimize the damage and medical expenditure on patients after radiotherapy, we did not test each patient for allergens. Patients with POME were divided into two groups based on the history of tympanic tube placement. Patients with CCOME were excluded if they had nasopharyngeal tumors. Patients with OME were also excluded if they had too little MEE for testing or their MEE was mixed with blood.

Collection of Samples

For POME patients, MEE collection was initiated ≥3 months post-radiotherapy (range: 3–125 months; 11 ears at ≤6 months, 32 ears at >6 months). For CCOME patients, MEE specimens were collected only after ≥3 months of effusion duration. All samples were obtained during clinical visits when patients presented with persistent symptomatic effusion. After disinfecting the external auditory canal with 70% alcohol, the tympanic membrane was anesthetized with a 2% tetracaine cotton pad for 20±1 minutes (timer-monitored). Under the guidance of a 0-degree endoscope (3000 lux fixed light source), the anterior-inferior quadrant of the tympanic membrane was punctured, and MEE was collected into pre-chilled EP tubes using a standard suction device, with the side and volume of effusion recorded.

MEE samples were collected aseptically, immediately placed on ice, and processed within 30 min. After centrifugation (2,000 g, 10 min, 4°C), supernatants were aliquoted: 100 μL for SOD activity, 200 μL with butylated hydroxytoluene for MDA analysis (light-protected), and the remainder with protease inhibitors for cytokine (IL-25/IL-33/IL-6) measurements. All aliquots were stored at −80°C and analyzed within 3 months, with ≤1 freeze-thaw cycle for SOD/MDA samples and ≤2 for cytokines.

Enzyme-Linked Immunosorbent Assay

The levels of IL-33 in the MEE were measured by enzyme-linked immunosorbent assay (ELISA) kits (435907, Biolegend, San Diego, USA). The levels of IL-25, and IL-6 were analyzed by standard quantitative ELISA kits (E-EL-H1648c, E-EL-H6156, ElabScience, Wuhan, China). The levels of SOD and MDA were measured by standard quantitative ELISA kits (A001-3-2, A003-1-2, Jiancheng, Nanjing, China). The lowest detection limit for each cytokine and chemokine were set as follows: Biolegend: IL-33, 4.14 pg/mL. ElabScience: IL-25, 31.25 pg/mL; IL-6, 1.56 pg/mL. Jiancheng: SOD, 0.5 U/mL; MDA, 0.5 nmol/mL. The optical density value was read and analyzed on a spectrophotometer (Synergy LX, Agilent, USA). All assays were conducted according to the manufacturer's protocols. All ELISA assays were validated using standard curves.

The Endoscopic Evaluation of the Eustachian Tube Score

The Endoscopic Evaluation of the Eustachian Tube (3ET) score, a standardized assessment of nasopharyngeal inflammation observed by nasal endoscopy, was highly specific and correlated linearly with the 7-item Eustachian Tube Dysfunction Questionnaire (ETDQ-7) score of pharyngeal tube dysfunction, as well as with Eustachian tube dysfunction (ETD) symptoms.[19] The 3ET scoring system has been proposed to assess the severity of inflammation at the ET orifice and has demonstrated good interrater and intrarater reliability.[20]

Eustachian tube function in POME patients was assessed using the 3ET scoring system by three independent otolaryngologists: one senior specialist with extensive experience in otology and two attending physicians with several years of ENT practice. Prior to evaluation, all raters completed standardized training involving review of representative endoscopic cases and calibration exercises, achieving excellent inter-rater agreement (Kappa >0.8) in reliability testing.

The investigators reviewing the endoscopic images were blinded from details of the participant, including the time since radiotherapy and the levels of the test indicators. Patients were excluded from analysis in the event of compromised image quality or inadequate visualization of the ET orifice. The 3ET score consists of evaluation of 4 components of the nasopharyngeal ET orifice: edema of the ET torus, erythema of the ET torus, exudate at the ET orifice, and presence of tubal tonsil.[19] Each component is scored from 0 to 2 and left and right sides are scored separately. A score of 0 represents a normal exam for each of the categories, a score of 1 indicates an intermediate amount of edema, erythema, exudate, or the presence of adenoid tissue on the torus tubarius that does not involve the lumen of the ET, and a score of 2 represents severe edema, erythema, exudate, or the presence of adenoid tissue that enters the lumen of the ET.

Statistical Analysis

The P value of less than 0.05 was considered statistically significant. The Kolmogorov–Smirnov test was used to assess the normality of the data distribution. Statistical differences in numerical data were analysed using the Mann–Whitney *U*-test. The chi-square test was used to analyze categorical data and Linear regression was applied to adjust for covariate. Spearman correlation was performed to determine correlations. All statistical analyses were performed with Prism 9.0 software (GraphPad Software Inc, La Jolla, California, USA).

Results

Clinical Characteristics

A total of 43 ears with POME and 20 ears with CCOME were included in this study. The participants' clinical characteristics were presented in Table 1.

Table 1 Clinical Characteristics of Enrolled Patients

	CCOME Group (n=20)	POME Group (n=43)	P-value
Female, n(%)	5 (25%)	10 (23%)	0.880
Age (years), (range)	49 (28–79)	51 (29–73)	0.323
Previous ventilation tube insertions, n (%)	6 (30%)	7 (16%)	0.210
Allergic rhinitis, n (%)	5 (25%)	8 (19%)	0.559

Notes: Categorical variables (sex, ventilation tube history, allergic rhinitis) were compared using χ^2-tests, while continuous variables (age) were analyzed with Mann–Whitney *U*-tests for non-normally distributed data.
Abbreviations: CCOME, conventional chronic otitis media with effusion; POME, post-irradiation otitis media with effusion.

Indicator Levels in MEE in POME and CCOME Patients

Comparative analysis of inflammatory/oxidative markers in MEE (n=43 POME vs n=20 CCOME) using nonparametric Mann–Whitney *U*-tests (all data non-normally distributed) revealed distinct profiles: IL-33 levels were significantly lower in POME (p=0.046), while SOD activity was elevated (p=0.015) (Figure 1A). No intergroup differences were detected for IL-25 (p=0.843), IL-6 (p=0.241), or MDA (p=0.130) (Figure 1B–E). These results suggest distinct inflammatory (IL-33) and antioxidant (SOD) responses in POME pathogenesis.

Indicator Levels in POME Patients with or Without a Past History of Allergic Rhinitis

Further stratification of POME patients by allergic rhinitis (AR) comorbidity (AR+: n=8 vs AR−: n=35) using nonparametric Mann–Whitney *U*-tests revealed no significant differences in MEE biomarker levels: IL-33 (p=0.771), IL-25

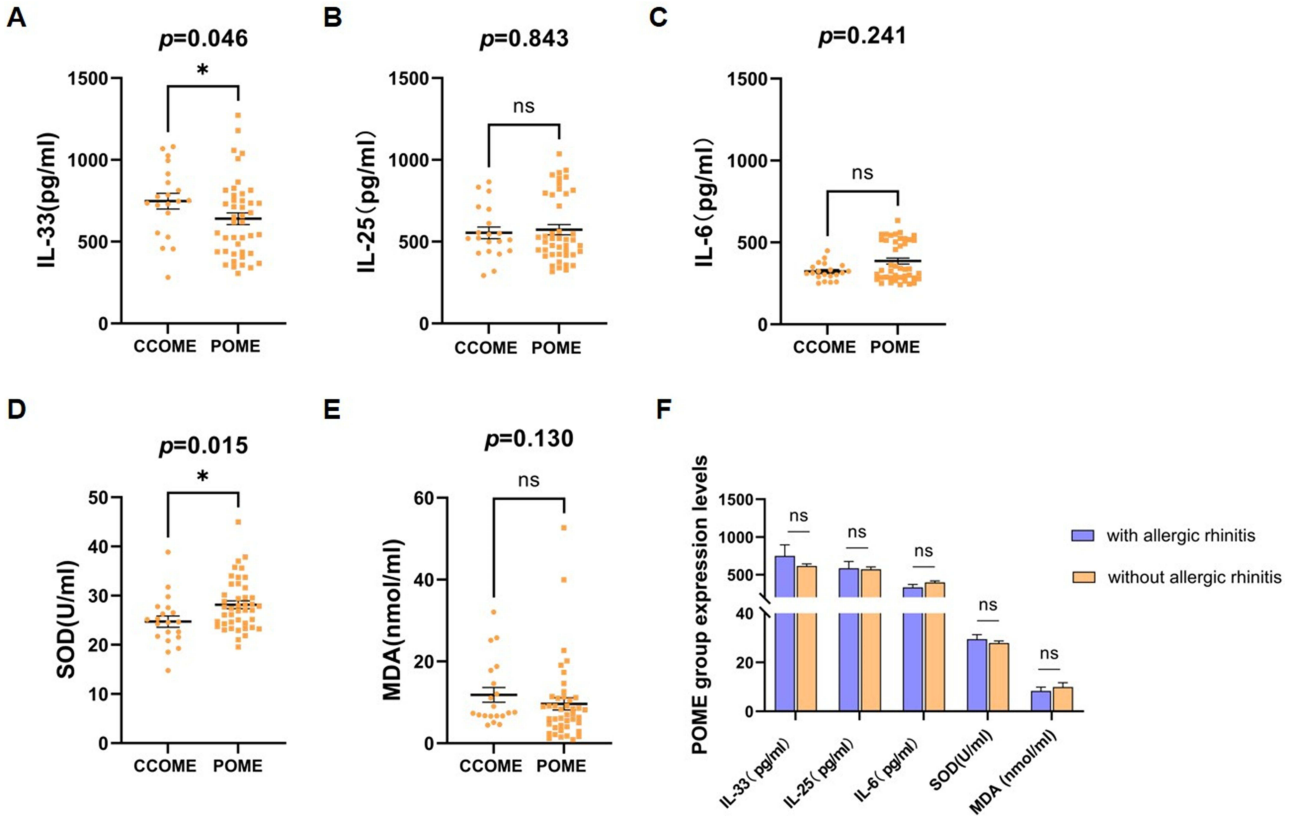


Figure 1 Biomarker profiles in MEE of POME (n=43) versus CCOME (n=20) patients. (**A**) IL-33, (**B**) IL-25, (**C**) IL-6, (**D**) SOD, and (**E**) MDA levels in POME and CCOME groups; (**F**) Subgroup analysis of POME patients with (n=8) or without (n=35) allergic rhinitis history. Mann–Whitney *U*-tests; *p<0.05, **p<0.01.
Abbreviations: ns, not significant; MEE, middle ear effusion; POME, post-irradiation otitis media with effusion; CCOME, conventional chronic otitis media with effusion.

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(p>0.999), IL-6 (p=0.095), SOD (p=0.385), or MDA (p=0.795) (Figure 1F). These findings suggest that AR comorbidity does not substantially modulate these inflammatory/oxidative mediators in radiation-induced otitis media.

Relationship Between Indicator Levels and Disease Duration in MEE of Patients with POME

Longitudinal analysis using Spearman's rank correlation (nonparametric data distribution) revealed time-dependent cytokine dynamics in POME patients. Both IL-33 and IL-25 levels exhibited biphasic peaks at 6 months and 21–24 months post-radiotherapy (Figure 2A and B). Significant negative correlations with disease duration were observed for inflammatory markers: IL-33 (r=−0.350, p=0.022), IL-25 (r=−0.511, p<0.001), and IL-6 (r=−0.457, p=0.002) (Figure 2C–E). In contrast, oxidative stress markers showed no temporal associations (SOD: r=0.031, p=0.845; MDA: r=0.167, p=0.285), suggesting potential differences in kinetic patterns between inflammatory and oxidative pathways.

Correlation Analysis of Indicator Expression in MEE in POME Patients

Pairwise Spearman correlation analysis of MEE biomarkers in POME patients (n=43) demonstrated significant positive associations between IL-33 and IL-6 (r=0.391, p=0.010) (Figure 3B) and between IL-25 and IL-6 (r=0.483, p=0.001) (Figure 3A), while remaining marker combinations showed no significant correlations (all p>0.05) (Figure 3C–J). These findings suggest potential coregulation of IL-33/IL-25 with IL-6, which appears distinct from oxidative stress responses (SOD/MDA).

Association Between Inflammatory Markers and Eustachian Tube Function in POME Patients

Following exclusion of 11 patients with suboptimal imaging quality, Spearman correlation analysis revealed a significant positive association between IL-25 levels and 3ET scores (r=0.407, p=0.021) (Figure 4A), suggesting IL-25's potential role as a biomarker for radiation-induced Eustachian tube dysfunction in POME. No significant correlations were found for other biomarkers: IL-33 (r=0.325, p=0.069), IL-6 (r=0.186, p=0.309), SOD (r=0.309, p=0.086), or MDA (r=−0.330, p=0.065) (Figure 4B–E). All analyses were performed using nonparametric methods appropriate for the non-normally distributed data.

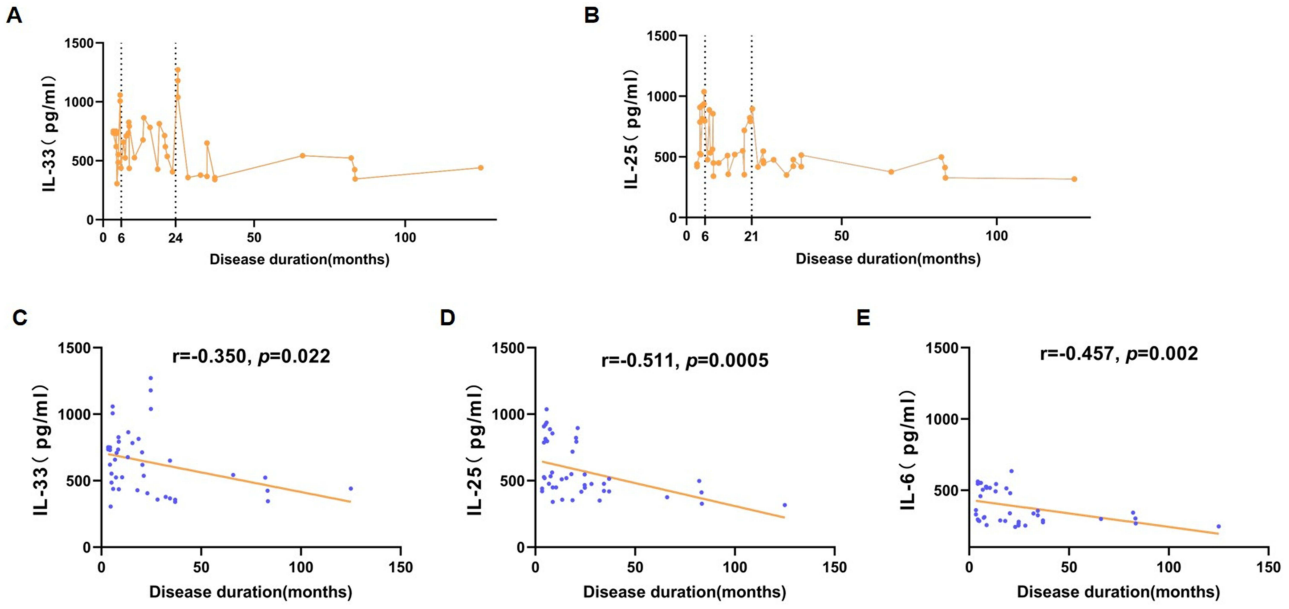

Figure 2 Temporal dynamics of biomarkers in MEE from POME patients. (**A** and **B**) IL-33 and IL-25 levels across disease duration phases. (**C**–**E**) Spearman correlation analysis between (**C**) IL-33, (**D**) IL-25, (**E**) IL-6 and post-radiotherapy disease duration. Disease duration calculated from radiotherapy completion date.

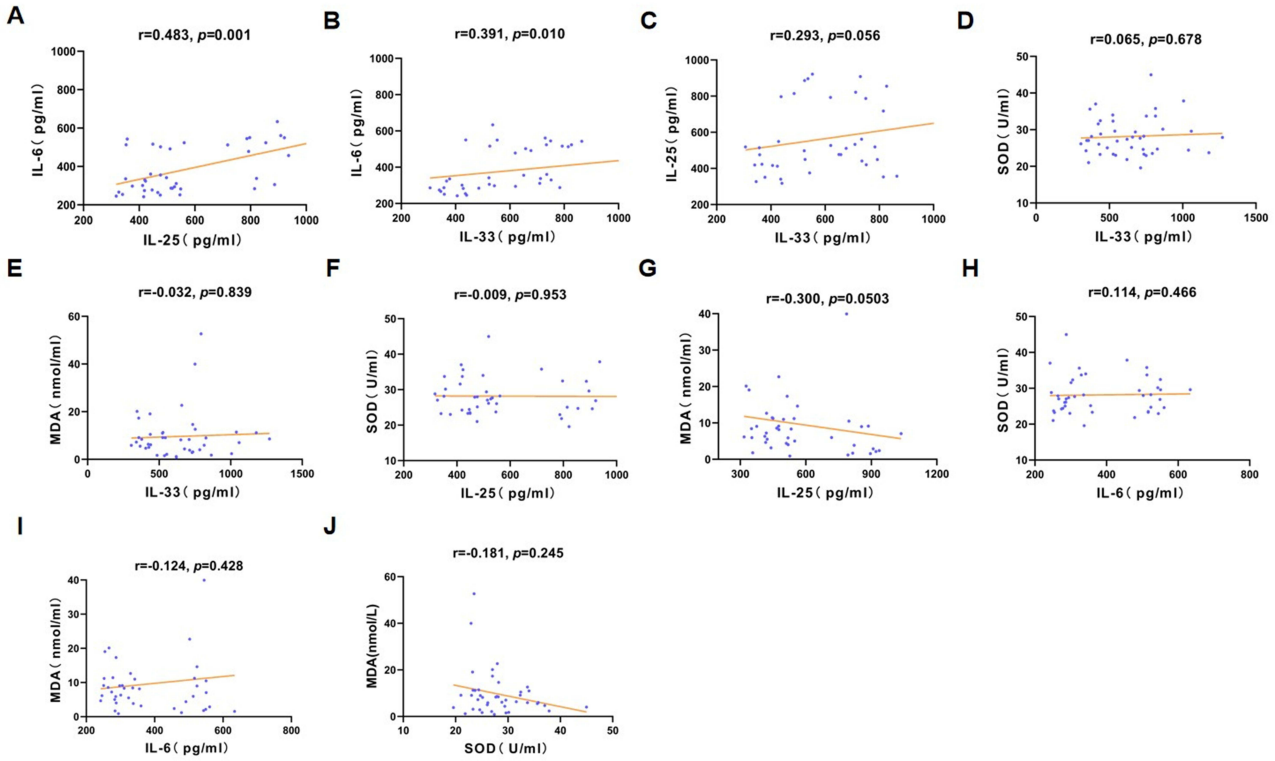

Figure 3 Pairwise biomarker correlations in MEE from POME patients (n=43). Subfigures (**A**–**J**) represent Spearman correlation analyses between inflammatory and oxidative stress markers, with significant positive correlations observed for IL-25 vs IL-6 ((**A**) r=0.483, p=0.001) and IL-33 vs IL-6 ((**B**) r=0.391, p=0.010), while non-significant associations ((**C**–**J**) IL-33/IL-25, IL-6/SOD, IL-6/MDA, IL-25/SOD, IL-25/MDA, IL-33/SOD, IL-33/MDA, SOD/MDA; all p ≥0.05) are indicated.

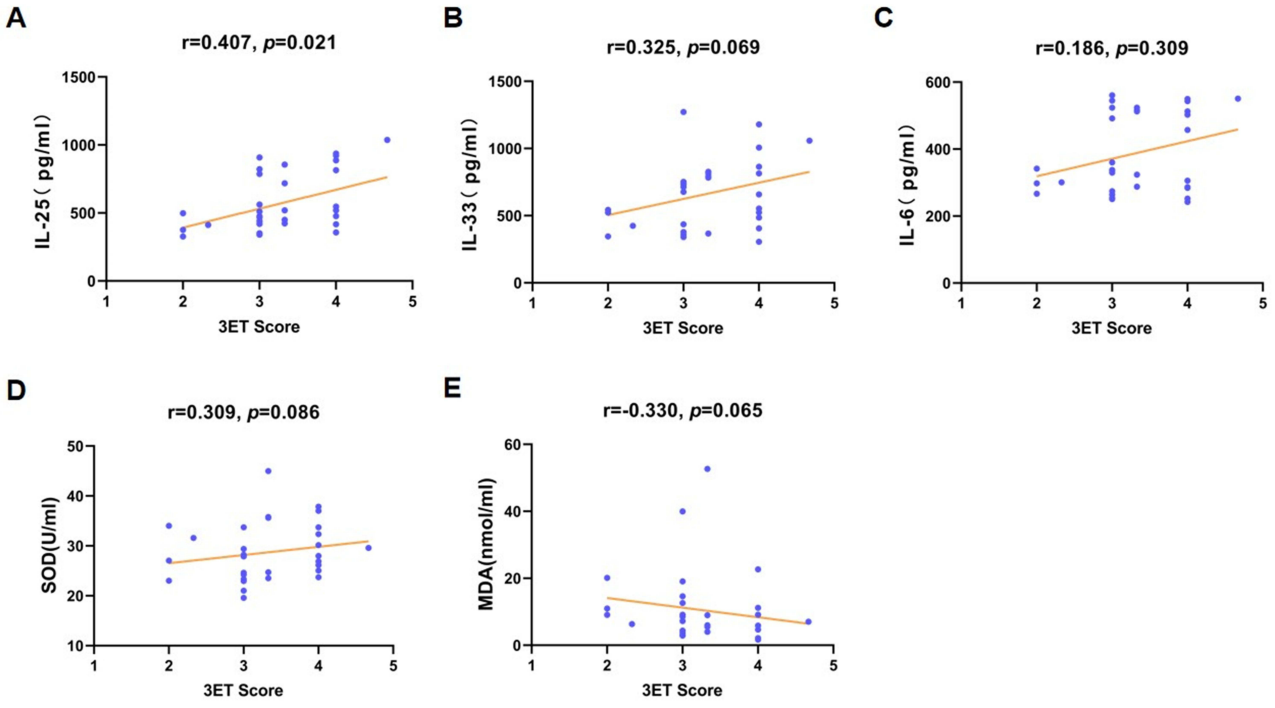

Figure 4 Spearman correlation analysis between biomarker levels in MEE and 3ET scores in POME patients (n=43). (**A**) IL-25, (**B**) IL-33, (**C**) IL-6, (**D**) SOD, and (**E**) MDA levels are shown with their respective correlation coefficients (r) and p-values. See Methods for 3ET scoring criteria.
Abbreviation: 3ET, Endoscopic Evaluation of the Eustachian Tube.

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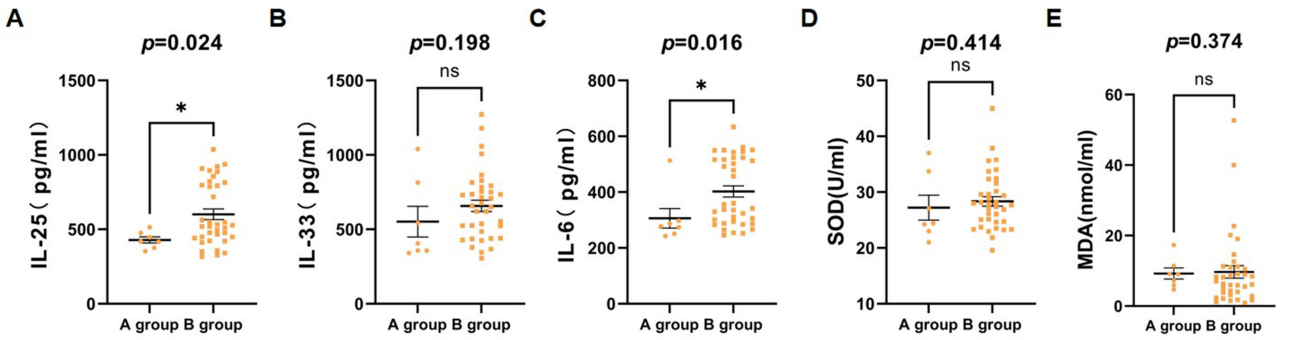

Figure 5 Comparison of biomarker levels in MEE between POME patients with (Group A, n=7) and without (Group B, n=36) ventilation tube insertion history. (**A**) IL-33, (**B**) IL-25, (**C**) IL-6, (**D**) SOD, (**E**) MDA. Mann–Whitney *U*-tests; *p<0.05.

Impact of Ventilation Tube History on Middle Ear Inflammation

Comparative analysis using Mann–Whitney *U*-tests (for non-normally distributed data) showed significantly elevated MEE levels of IL-25 (p=0.024) (Figure 5A) and IL-6 (p=0.016) (Figure 5C) in POME patients without prior ventilation tube placement (n=7) compared to those with tube history (n=36). No significant intergroup differences were observed for IL-33 (p=0.198) (Figure 5B), SOD (p=0.414) (Figure 5D), or MDA (p=0.374) (Figure 5E), suggesting that tube placement may attenuate specific inflammatory responses in radiation-induced otitis media.

Discussion

This study reveals the unique pathogenic mechanisms of POME mediated through IL-25/IL-33 signaling and oxidative stress dynamics. As an extension of the unified airway, the middle ear mucosa exhibits cytokine expression patterns similar to those of the upper respiratory mucosa but with distinct radiation-specific modifications. Compared to CCOME, POME patients demonstrated significantly reduced IL-33 levels ($p = 0.046$). Based on reported characteristics of radiation-induced mucosal damage—including congestion, edema, ciliary loss, epithelial atrophy, and fibrosis[21] —we propose that radiation disrupts middle ear microenvironmental homeostasis, thereby impairing IL-33 expression, a hypothesis requiring further functional validation. Notably, despite decreased IL-33 expression, post-radiotherapy IL-25 expression levels remains stable, likely originating from radiation-resistant mast cells or myeloid cell populations,[22,23] independent of epithelial integrity. This finding suggests that POME exhibits inflammatory regulatory mechanisms fundamentally distinct from conventional otitis media, potentially driven by radiation-induced selective damage and activation of specific cell subsets. Supporting this concept, Hamour et al demonstrated that renal mast cells retain radiation resistance and persistently secrete IL-17 under irradiation.[22]

When analyzing the pathological specificity of POME, it is crucial to note that while allergic factors contribute to Eustachian tube dysfunction-related otitis media,[24] their role in radiation-induced otitis media is distinctly different. Our findings demonstrated no significant differences in IL-25 and IL-33 expression levels in MEE between POME patients with and without allergic rhinitis (all $P > 0.05$), indicating that radiation injury drives inflammation through mechanisms independent of allergic responses. Further analysis revealed a strong positive correlation between IL-25 expression and 3ET endoscopic scores ($r = 0.407$, $p = 0.021$), absent in IL-33 or IL-6 (all $p > 0.05$), establishing IL-25 as a key mediator of post-radiotherapy ETD. Concurrently, IL-6, a downstream factor ILC2 effector[8] —showed strong correlations with both IL-25 ($r = 0.483$, $p < 0.01$) and IL-33 ($r = 0.391$, $p < 0.01$), indicating its role in amplifying inflammation via ILC2-dependent pathways. However, the temporal dissociation between IL-6 and IL-25/IL-33 peaks suggests it primarily contributes to synergistic inflammatory regulation rather than primary pathogenesis.

Oxidative stress profiling revealed another critical feature: significantly increased SOD activity ($p = 0.015$) contrasted with stable MDA levels ($p > 0.05$), indicating preferential activation of superoxide anion (O_2^-) scavenging over lipid peroxidation post-radiotherapy. Notably, IL-25 and IL-33 expression showed no correlation with SOD or MDA (all $p > 0.05$), suggesting independent regulatory pathways for inflammation and oxidative stress. In contrast, TGF-β, a pivotal inflammatory mediator in post-radiotherapy fibrosis, drives ROS generation through distinct mechanisms. The elevated

ROS stimulates collagen synthesis and further upregulates TGF-β, driving fibroblast-to-myofibroblast differentiation and creating a vicious cycle that exacerbates both inflammation and fibrosis.[25–27] Moreover, TGF-β enhances the expression of major mucins in MEE, thereby prolonging inflammatory responses.[28] Integrating these findings with previous studies,[8,29] we propose that IL-25/IL-33 may participate in the inflammatory and fibrotic processes occurring in the Eustachian tube and surrounding tissues following radiotherapy. However, persistent oxidative stress coupled with chronic inflammation[30,31] appears to exacerbate tissue damage through parallel mechanisms.

Temporal analysis provides critical validation: IL-33/IL-25 levels peaked at 6 months post-radiotherapy, coinciding with both the functional nadir of Eustachian tube in nasopharyngeal carcinoma patients and the optimal therapeutic window for balloon dilation interventions.[32,33] These observations strongly implicate IL-33/IL-25 in the POME pathogenesis, although their exact mechanisms remain to be further investigation. In striking contrast, VEGF exhibits phase-specific activity, showing exclusive association with MEE formation during acute-phase OME, with no significant involvement in chronic or recurrent stages.[34] This acute-phase effect operates through HIF-1α-mediated vascular permeability enhancement, directly driving early effusion development.[35]

Notably, tympanostomy significantly reduced IL-25 levels ($p = 0.024$), suggesting that mechanical interventions modulate inflammatory factors by improving microenvironmental conditions. Secondary IL-33/IL-25 peaks at 21–24 months may reflect delayed immune activation, while persistently stable SOD/MDA levels (all $p > 0.05$) underscore the persistence of oxidative damage and its relative independence from inflammatory pathways.

Based on these findings, we propose a dual-modality therapeutic strategy: targeting the IL-25 pathway to suppress inflammation and fibrosis while restoring Eustachian tube biomechanics through interventions such as balloon dilation.[33] This integrated strategy combines biological therapy (targeting inflammatory pathways) with physical rehabilitation (restoring luminal function), inspired by successful precedents in chronic airway diseases—such as the synergistic use of biologics and bronchial thermoplasty to improve lung function in asthma.[36] Sun et al validated that balloon dilation at 6 months post-radiotherapy intervention window significantly improves ETDQ-7 scores and air-bone gaps,[33] providing direct evidence for mechanical intervention window. However, the translational potential of combined IL-25 inhibition and mechanical interventions in radiation-induced otitis media necessitates rigorous validation through prospective clinical trials.

This study establishes the involvement of IL-33 and IL-25 in POME pathogenesis, but the key limitations require acknowledgment: The relatively small sample size limits statistical robustness; the cross-sectional design with heterogeneous sampling intervals restricts temporal dynamic analysis; and the absent cellular localization data hinders mechanistic interpretation. Future investigations must prioritize larger cohorts, longitudinal designs with standardized radiotherapy protocols, multi-omics analytical technologies, and controlled animal models validation. Of particular importance are large-scale investigations combining IL-25/IL-33 pathway modulation with mechanical interventions are needed to explore potential therapeutic synergies.

Conclusion

This study delineates distinct roles for IL-33 and IL-25 in POME pathogenesis. The reduced IL-33 levels ($p=0.046$) may reflect radiation-induced mucosal damage and epithelial radiosensitivity. In contrast, IL-25 demonstrates sustained overexpression post-radiotherapy and shows significant correlation with Eustachian tube dysfunction severity ($r=0.407$), supporting its potential as a predictive biomarker for ETD. These findings provide a rationale for IL-25-targeted therapeutic strategies, with consideration for combined mechanical interventions during the 6-month post-radiation inflammatory window to control both inflammatory responses and fibrotic progression.

Abbreviations

POME, post-irradiation otitis media with effusion; NPC, nasopharyngeal carcinoma; MEE, middle ear effusion; CCOME, conventional chronic otitis media with effusion; OME, otitis media with effusion; ILC2, group 2 innate lymphocytes; IL-25, interleukin-25; IL-33, interleukin-33; ELISA, enzyme-linked immunosorbent assay; SOD, superoxide dismutase; MDA, malondialdehyde; ET, Eustachian tube; ETD, Eustachian tube dysfunction; 3ET, The Endoscopic Evaluation of the Eustachian Tube; AR, allergic rhinitis.

Data Sharing Statement

Related data and materials are available upon request to Zhihe Lin and Shaoyan Feng.

Ethical Considerations

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University, China (NO: K217-1). Written informed consent was obtained from all subjects prior to their study enrollment and collection of MEE.

Informed Consent

Informed consents were obtained from all individual participants included in the study.

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Mr. Zhihe Lin's current affiliation is the Department of Otolaryngology, Head and Neck Surgery, Longhua District People's Hospital of Shenzhen, Shenzhen, Guangdong, China.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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