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Cross-sectional screening for inflammation in tinnitus with near-normal hearing

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ABSTRACT

Tinnitus is the perception of sound without an external stimulus. Recently, inflammation has been implicated in the pathophysiology of tinnitus. In tinnitus animal models, cytokine levels are increased throughout the whole auditory pathway, and microglia and astrocytes are activated. However, only a few human studies on inflammation in tinnitus were conducted, which generally did not account for confounders such as hearing loss, anxiety and depression. The current study therefore aimed to evaluate the association between inflammation and tinnitus specifically in participants with (near-)normal hearing and without signs of anxiety or depression.

In this cross-sectional study, fifty tinnitus participants and fifty healthy controls completed a tinnitus questionnaire and underwent audiometric testing. Complete blood count measures were determined in blood plasma, as well as cytokine concentrations by using the enzyme-linked immunosorbent assay (ELISA) technique.

Platelet count and cytokine concentrations of IL-10 and IFN-γ were lower in participants with tinnitus compared to controls, and male sex, lower MCV, lower platelet count, and lower IL-10 and IFN-γ concentrations were significant predictors of tinnitus presence.

The current study shows that inflammatory parameters are altered in tinnitus patients after exclusion of important confounders such as hearing loss, anxiety, depression, and inflammatory diseases.

1. Introduction

Subjective tinnitus is the perception of sound without an external stimulus. It is present in four to 37 % of the general population, and it negatively affects quality of life in one to four percent (Eggermont and Roberts, 2004; McCormack et al., 2016; Jarach et al., 2022). Tinnitus patients frequently suffer from depression, anxiety, insomnia, concentration difficulties, and greater mean days of work missed (Savage and Waddell, 2014; Bhatt et al., 2017). Currently, treatment is focused on reducing tinnitus perception and developing new coping strategies, but a curative treatment does not yet exist (Piccirillo et al., 2020).

The pathophysiology of tinnitus is not fully understood. It is thought that aberrant neural activity underlies the phantom perception, which presumably is generated in the brain due to sensory deprivation (Berliner et al., 1992; Baguley et al., 2013). Accumulating evidence suggests that inflammation may play a role in the pathophysiology of tinnitus (Mennink et al., 2022). Inflammation is the body's response to harmful stimuli, which is mediated by numerous cell types, such as microglia, astrocytes, and cytokines. Whereas inflammation is initially protective, a sustained inflammatory response can be detrimental and

Abbreviations: AUC, area under the curve; CBC, complete blood count; ELISA, enzyme-linked immunosorbent assay; HADS, hospital anxiety and depression score; Hb, hemoglobin; Ht, hematocrit; IFN, interferon; IL, interleukine; IQR, interquartile range; MCV, mean corpuscular volume; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PTA, pure tone average; ROC, receiver operating curve; TFI, tinnitus functional index; TNF, tumor necrosis factor.

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lead to neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease (Kwon and Koh, 2020). This may also be the case in tinnitus. Inflammatory agents can affect neurotransmission and consequently cause an imbalance in excitatory and inhibitory neurotransmission, which is implicated in tinnitus (Mennink et al., 2022). Animal models of tinnitus showed that the expression of cytokines, specifically Tumor Necrosis Factor (TNF)-α and Interleukin (IL)−1β, is increased throughout the whole auditory pathway (Mennink et al., 2022). Moreover, infusion of TNF-α in the auditory cortex of healthy mice resulted in tinnitus (Wang et al., 2019). Additionally, microglia and astrocytes were activated, and pharmacological depletion of microglia prevented TNF-α increase and the occurrence of noise-induced tinnitus (Wang et al., 2019). Thus, there are clear indications that inflammation is implicated in animal models of tinnitus.

Human data on inflammation in tinnitus is scarce and inconsistent. An increase of IL-6 and a decrease of IL-10 concentrations were found, as well as a correlation between serum TNF-α levels and tinnitus loudness (C. Weber et al., 2002; Szczepek et al., 2014; H.F. Haider et al., 2020). However, the mayor pitfall of these studies is that important confounders are not (enough) accounted for. Neither study included tinnitus patients based on the degree of hearing loss, or accounted for hearing loss in the analysis, even though inflammation has also been implicated in hearing loss (Fuentes-Santamaría et al., 2017; Frye et al., 2019). Moreover, tinnitus patients are more prone to developing anxiety and depression, and those are known to modulate tinnitus (severity) and are on itself related to inflammation as well (C. Weber et al., 2002; Ahmed et al., 2022; Meijers et al., 2022; Turner, 2024). This was also not accounted for in previous studies. It therefor remains unclear whether previous results on inflammatory markers are specific to tinnitus, and whether potential effects remained unsignificant because of the presence of confounders. Therefore, the aim of this study is to evaluate inflammatory markers in blood samples of tinnitus patients with (near-) normal hearing and without signs of anxiety or depression, and to relate inflammatory markers to tinnitus severity.

2. Methods

2.1. Participants

Fifty tinnitus patients and fifty healthy volunteers were included. Participants from both groups were recruited via locally distributed flyers. Additionally, tinnitus patients were recruited from the tinnitus outpatient clinic at the University Medical Center Groningen. The inclusion criteria were i) adult; ii) pure tone average threshold (PTA) of $\leq$25 dB across the frequencies 1, 2 and 4 kHz for each ear; iii) Hospital Anxiety and Depression Score (HADS) of $\leq$7 for both anxiety and depression individually; and iv) sufficient mastery of the Dutch language to fill in the questionnaires.

Exclusion criteria were history of a) objective tinnitus; b) neurological disease; c) inflammatory disease; d) malignancy; e) chronic ear disease (such as Ménière's disease, otosclerosis, chronic otitis media); f) coagulation disorder; g) psychiatric disorder; h) other systemic diseases in which inflammation is implicated (for instance Diabetes Mellitus); and i) current pregnancy;

2.2. Clinical evaluation

Data collected from participants included detailed clinical history. Anxiety and depression scores, ranging from 0 to 21, were assessed using the 14-item HADS questionnaire (Zigmond and Snaith, 1983). Hearing thresholds were determined for all participants using pure tone audiometry in a soundproof booth using an Interacoustic Clinical Audiometer (model AC40) at octave frequencies ranging from 0.125 to 8 kHz.

2.3. Tinnitus assessment

After audiometric testing, tinnitus pitch and loudness were determined with a matching procedure for each tinnitus participant. It was first determined whether the tinnitus percept was more similar to a tone or a noise. The evaluation of the tinnitus frequency was performed by offering frequencies from 0.125 to 8 kHz (two stimuli at a time) and asking the participant what frequency was closest to their tinnitus. Frequencies above 8 kHz were not possible to present. Tinnitus loudness was then determined in a similar manner.

Tinnitus participants were also asked to fill in the Tinnitus Functional Index (TFI), which is a questionnaire to assess tinnitus severity based on its impact on the quality of life. It consists of 25 items, and the total score ranges from 0 (low severity) to 100 (high severity) (Santacruz et al., 2021).

2.4. Inflammatory markers

Complete blood count (CBC) measurements were performed using an automatic blood counter immediately after collection. Platelet, neutrophil, lymphocyte and monocyte count, as well as hemoglobin (Hb), hematocrit (Ht) platelet distribution width (PDW), mean platelet volume (MPV) and mean corpuscular volume (MCV) were measured. Platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were calculated.

For cytokine measurements, venous blood samples were collected in EDTA tubes and stored on ice during transport. The blood was centrifuged in the collection tubes (1800 g, 10 min, 4 °C), after which plasma was divided between two cups. These were stored at −80 °C until further processing. The enzyme-linked immunosorbent assay (ELISA) technique was employed for the quantification of cytokines, using the human Quantikine kits specific for IFN-γ (Quantikine® HS Human IFN-γ Immunoassay), IL-1β (Quantikine™ HS Human IL-1β/IL-1F2 Immunoassay), IL-6 (Quantikine® HS Human IL-6 Immunoassay), IL-10 (Quantikine™ HS Human IL-10 Immunoassay) and TNF-α (Quantikine™ HS Human TNF-α Immunoassay), purchased from R and D Systems GmbH (Wiesbaden, Germany). ELISA was performed according to the standard protocol provided by the company.

2.5. Statistical analysis

All statistical analyses were performed in R (v4.3.3) software. Extreme outliers of outcome variables (values more than three times the interquartile range (IQR) above or below the IQR boundaries) were removed. Statistical significance was established at $p < .05$, and Bonferroni-corrections were applied to correct for multiple comparisons when necessary. Differences between the tinnitus and non-tinnitus groups were assessed with T-tests or Mann Whitney U tests, depending on normality as tested by the Shapiro-Wilk test.

2.5.1. Regression analyses

The relation between inflammatory markers and the presence and severity of tinnitus were evaluated by employing multiple logistic and linear regression, respectively. Missing data of predictive variables was handled by multiple imputation using the R-package {mice} before running the regression analyses, as complete case analysis used by regression would lead to loss of statistical power and might introduce bias (van der Heijden et al., 2006). Following the method of Zhang et al. (2016) , a multiple logistic regression was employed with the presence of tinnitus as outcome variable. First, multiple univariate logistic regression analyses were performed, and variables with a p-value smaller than 0.25 were included in the further multivariate analysis. These variables were sex, age, HADS anxiety score, MCV, platelet count, monocytes, IL-1β, IL-10 and IFN-γ. The final multivariate model was constructed through backwards selection, eliminating the variable with the highest p-value at each step, confirmed by a partial likelihood ratio

test, until all remaining variables were statistically significant. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test, and the model's predictive performance was evaluated by calculating the area under the curve (AUC) of the receiver operating curve (ROC).

A multiple linear regression was utilized to investigate the relationship between tinnitus severity and inflammatory markers, using a similar approach. The variables identified through univariate analyses for inclusion in the multivariate analysis were age, mean PTA, HADS anxiety score, HADS depression score, leukocytes, lymphocytes, and monocytes. The model was constructed by backwards selection. Model accuracy was determined by calculating the adjusted R^2.

2.5.2. Sensitivity analyses

Both regressions are based on the results of the imputed dataset. However, understanding the extent to which these results are influenced by the imputation process is crucial. To assess the impact of imputation on the results and thereby evaluate the robustness of the derived conclusions, a sensitivity analysis was conducted. In the sensitivity analyses, the same procedure for model building was employed for both the multiple logistic and linear regression but on the original dataset instead of the imputed dataset

3. Results

3.1. Participant characteristics

Participants with tinnitus were of similar age as participants without tinnitus, but they were more often male (X^2 (1, $N = 100$) = 9.004, $p = .003$) (Table 1). The mean PTA of each ear was similar in both groups. The audiogram per group and ear is shown in Fig. 1. The hearing thresholds of all frequencies for each ear were similar in tinnitus participants compared to the control group after correcting for multiple comparisons (significance threshold of p=.05/12=0.0042) (Left ear: (0.25kHz: $W = 1123$, $p = .368$; 0.5kHz: $W = 1207$, $p = .755$; 1kHz: $W = 956$, $p = .035$; 2kHz: $W = 1014$, $p = .095$; 4kHz: $W = 1065$, $p = .200$; 8kHz: $W = 943$, $p = .033$); Right ear: (0.25kHz: $W = 1053$, $p = .162$; 0.5kHz: $W = 1117$, $p = .345$; 1kHz: $W = 1195$, $p = .695$; 2kHz: $W = 1122$, $p = .366$; 4kHz: $W = 1097$, $p = .287$; 8kHz: $W = 910$, $p = .019$)). No difference was present in HADS anxiety and HADS depression scores between participants with and without tinnitus. Mean PTA was correlated with age [rs(98) = 0.635, $p < .001$].

Table 1
Participant characteristics.

Characteristics	Participants			Test	p – value
	All (n = 100)	*Tinnitus (n = 50)*	*No tinnitus (n = 50)*		
Age	51 (23–76)	53 (23–75)	46 (23–76)	Mann-Whitney U	0.187
Sex (male)	49 (49 %)	32 (64 %)	17 (34 %)	Chi-squared	0.003
PTA (1, 2 & 4 kHz)					
Left (kHz)	6 (−7–25)	7 (−7–22)	3 (−7–25)	Mann-Whitney U	0.084
Right (kHz)	7 (−7–25)	7 (−7–20)	4 (−7–25)	Mann-Whitney U	0.326
HADS score					
Anxiety	2 (0–7)	3 (0–7)	2 (0–7)	Mann-Whitney U	0.244
Depression	1 (0–6)	1 (0–6)	0 (0–6)	Mann-Whitney U	0.358
Tinnitus					
TFI score		21 (1–61)			
Pitch (kHz)		6 (0.25–8)			
Loudness (dB)		27 (14–61)			
Laterality					
Central/ bilateral		24 (48 %)			
Left		16 (32 %)			
Right		10 (20 %)			
Duration		6y (2m-60y)			

Data is presented as median (range) or n (%).

Participants with tinnitus had a median TFI-score of 21. Their tinnitus was most often central/bilateral, and median loudness and pitch were 27 dB and 6 kHz, respectively. All participants except for two experienced tinnitus for at least two years. TFI-score was correlated with both HADS anxiety [rs(98) = 0.369, $p = .008$] and HADS depression [rs (98) = 0.502, $p < .001$], but not with age or mean PTA.

3.2. Inflammatory markers

3.2.1. Complete blood count

Platelet count was lower in tinnitus participants than in non-tinnitus participants (Table 2). No differences were present in the other CBC measures.

3.2.2. Cytokines

Both IL-10 and IFN-γ levels were lower in tinnitus participants than in non-tinnitus participants (Table 3). No differences were present in TNF-α, IL-1β and IL-6 concentrations.

3.3. The relation between tinnitus presence and inflammatory markers

Before running a multiple logistic regression, univariate analyses were performed with the presence of tinnitus as outcome variable (Table 4). The variables sex, age, HADS anxiety score, MCV, platelet count, monocytes, PLR, IL-1β, IL-10 and IFN-γ all had a p-value below 0.25 and were thus included in the multiple logistic regression. After backwards selection, the final model consisted of sex, MCV, platelet count, IL-10 and IFN-γ. Male sex was associated with the presence of tinnitus, and all numeric variables were negatively associated with the presence of tinnitus. The model showed good discrimination (area under the ROC curve: 0.856, 95 % confidence interval: 0.756–0.920) and good calibration (Hosmer-Lemeshow p-value = 0.690) (Nahm, 2022).

3.3.1. Sensitivity analysis

The multiple logistic regression based on the original, non-imputed data is presented in Table 5. The variables MCV, platelet count, IL10 and IFN-γ were included and thus related to the presence of tinnitus, consistent with the model based on the imputed dataset. However, unlike the model derived from the imputed data, sex was not incorporated into this analysis. Additionally, this model includes monocytes, a variable absent from the imputed dataset model.

3.4. The relation between tinnitus severity and inflammatory markers

The univariate analyses revealed the variables age, mean PTA, HADS anxiety score, HADS depression score, leukocytes, lymphocytes and monocytes as potential predictors of the TFI-score and were thus included in the multivariate analysis (Table 6). After backwards selection, the final multiple linear regression model existed only of HADS depression score and monocytes. The adjusted R^2 was merely 0.339 (95 % CI: 0.131 - 0.538), which means that the predictive power of the model is very limited.

3.4.1. Sensitivity analysis

The multiple linear model constructed from the original data was identical to the model derived from the imputed data (Table 7).

4. Discussion

Neuroinflammation is a complex and dynamic process that involves

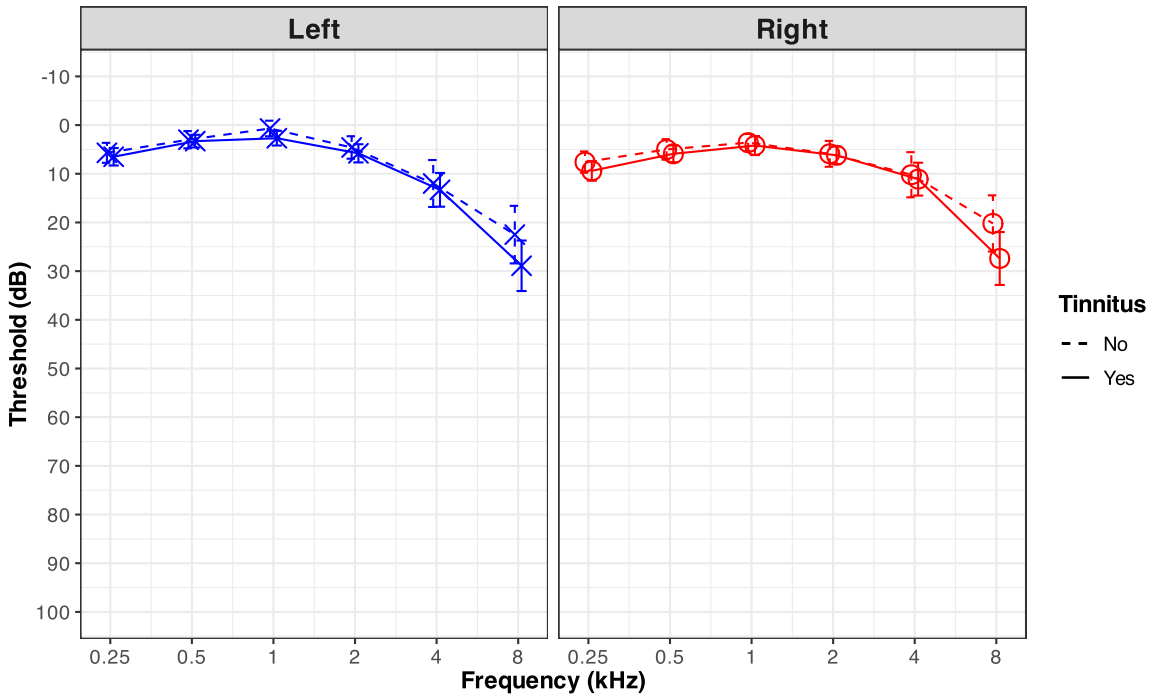

Fig. 1. Mean audiogram of the left and right ear from participants with and without tinnitus. The error bars represent the 95 %-confidence interval.

Table 2
Differences in complete blood count measures between participants with and without tinnitus.

Characteristics	Participants			Test	*p* – value
	All (n = 100)	*Tinnitus (n = 50)*	*No tinnitus (n = 50)*		
Hb (mmol/L)	8.9 (0.8)	9.0 (0.9)	8.8 (0.7)	T-test	.118
Ht (L/L)	.42 (0.03)	.43 (0.04)	.42 (0.03)	T-test	.293
MCV (fL)	91 (3.6)	90 (3.8)	91 (3.4)	T-test	.101
Platelet count (10^9/L)	274 (63)	257 (53)	292 (68)	T-test	.004*
Neutrophils (10^9/L)	3.3 (1.7–6.7)	3.3 (1.7–6.7)	3.3 (2.0–6.0)	Mann-Whitney U	.715
Lymphocytes (10^9/L)	1.9 (0.8–4.4)	1.9 (1.0–4.1)	1.9 (0.8–3.6)	Mann-Whitney U	.685
Monocytes (10^9/L)	.51 (0.14)	.52 (0.15)	.48 (0.13)	T-test	.163
PDW (fL)	12 (9–18)	12 (9–17)	11 (9–18)	Mann-Whitney U	.276
MPV (fL)	10 (9–12)	10 (9–12)	10 (9–12)	Mann-Whitney U	.329
NLR	1.7 (0.8–4.5)	1.8 (0.8–4.5)	1.6 (0.8–4.0)	Mann-Whitney U	.380
PLR	143 (63–269)	142 (63–258)	144 (91–269)	Mann-Whitney U	.178

Data is presented as mean (sd) or median (range). * = significant.

reciprocal interaction of a wide range of cellular and molecular components, including microglia, astrocytes, and various types of immune cells, for instance cytokines. It can have both protective and detrimental effects, depending on the context and duration of the inflammatory stimulus. The current study aimed to evaluate the association between inflammation and tinnitus, excluding the important confounders hearing loss, anxiety and depression. We found that a few inflammatory markers were altered in tinnitus patients: platelet count, IL-10, and IFN-γ were significantly lower and male sex, lower MCV, lower platelet count, and lower IL-10 and IFN-γ concentrations were found to be significant predictors of tinnitus presence. These results support that inflammation may play a role in tinnitus.

4.1. Complete blood count levels and tinnitus

Alterations in blood parameters, including neutrophil count, lymphocyte count, and platelet count, as well as the derived ratios NLR and PLR, are commonly observed during systemic inflammatory responses. A meta-analysis demonstrated that only elevated MPV and PDW differed between tinnitus and non-tinnitus subjects (Mennink et al., 2022). Both elevated MPV and PDW are indicative of activated platelets, and they are potent inducers of the acute inflammatory response, as they release several cytokines and chemokines after activation (Chen et al., 2020; Chrbolka et al., 2020). Contradictory to earlier findings, the current study did not find any difference in MPV or PDW between both groups, but it did reveal a lower platelet count in participants with tinnitus which was never shown before. Previous studies

Table 3
Differences in cytokine concentrations between participants with and without tinnitus.

Characteristics	Participants			Test	*p* – value
	All (n = 100)	*Tinnitus (n = 50)*	*No tinnitus (n = 50)*		
TNF-α (pg/mL)	.55 (0.21–1.50)	.51 (0.21–1.00)	.56 (0.25–1.47)	Mann-Whitney U	.521
IL-1β (pg/mL)	.41 (0.02–1.62)	.37 (0.02–1.19)	.52 (0.03–1.62)	Mann-Whitney U	.091
IL-6 (pg/mL)	.86 (0.10–3.52)	.81 (0.25–3.52)	.86 (0.10 – 3.04)	Mann-Whitney U	.457
IL-10 (pg/mL)	.53 (0.14–0.92)	.51 (0.14–0.73)	.54 (0.42–0.92)	Mann-Whitney U	.017*
IFN-γ (pg/mL)	.49 (0.25–1.02)	.45 (0.25–1.02)	.54 (0.34–0.98)	Mann-Whitney U	< 0.001*

Data is presented as median (range). * = significant.

Table 4
Univariate logistic regression analyses and final model (multivariate analysis) on the imputed dataset with tinnitus presence as outcome variable.

	Univariate analyses			Multivariate analysis		
Variable	Coefficient	Standard error	*p*-value	Coefficient	Standard error	*p*-value
Sex✣	−1.239	.420	.003^	−1.438	.550	.011
Age	.020	.014	.139^			
Mean PTA	.022	.029	.455			
Smoking status	−0.427	.936	.650			
HADS anxiety score	.115	.097	.238^			
HADS depression score	.162	.141	.254			
MCV	−0.094	.058	.107^	−0.167	.080	.041
Platelet count	−0.010	.004	.008^	−0.010	.005	.025
MPV	.152	.236	.521			
PDW	.048	.111	.667			
Leukocytes	.028	.134	.836			
Neutrophils	.048	.190	.801			
Lymphocytes	−0.161	.348	.644			
Monocytes	2.010	1.443	.167^			
NLR	.186	.263	.482			
PLR	−0.008	.005	.127^			
TNF- α	−0.537	.988	.588			
IL-1β	−1.262	.684	.068^			
IL-6	.205	.288	.477			
IL-10	−6.083	1.856	.001^	−6.806	2.600	.011
IFN-γ	−5.265	1.826	.006^	−5.197	2.347	.034

After univariate analysis, variables with a *p*-value below 0.25 were included in the multivariate analysis (^). Next, variables were removed using backwards selection with $p < 0.05$ as threshold. ✣Sex was coded as follows: Female = 1, male = 2.

Table 5
Final multiple logistic regression model based on the original dataset.

Variable	Coefficient	Standard error	*p*-value
MCV	−0.174	.085	.040
Platelet count	−0.014	.005	.003
Monocytes	5.722	2.107	.007
IL-10	−5.324	2.365	.024
IFN-γ	−7.032	2.354	.003

show extensive heterogeneity, as noted in the meta-analysis, which could potentially be explained by differences in inclusion criteria (e.g. in hearing loss, medical history, duration of tinnitus or stress levels). These may also explain the discrepancies between previous literature and the current study. First, the duration of tinnitus at the time of the study may be of importance. In our cohort, all participants except for two experienced tinnitus for at least two years. Although many studies did not report tinnitus duration, some studies may have included more participants who more recently developed tinnitus. Since platelets are involved in acute inflammation, any platelet activation related to acute tinnitus could have been subsided by the time it became chronic, as in our subjects. Second, the lower platelet count observed could be related to

Table 7
Final multiple linear regression model based on the original dataset.

Variable	Coefficient	Standard error	*p*-value
HADS depression score	5.086	1.196	<0.001
Monocytes	26.718	12.344	.036

Table 6
Univariate regression analyses and final model (multivariate analysis) on the imputed dataset with TFI score as outcome variable.

	Univariate analyses			Multivariate analysis		
Variable	Coefficient	Standard error	*p*-value	Coefficient	Standard error	*p*-value
Sex✣	−3.91	4.740	.414			
Age	.276	.171	.112^			
Mean PTA	.527	.398	.192^			
Smoking status	10.164	11.599	.385			
HADS anxiety score	2.914	.981	.005^			
HADS depression score	5.562	1.220	<0.001^	5.086	1.196	<0.001
MCV	−0.426	.604	.484			
Platelet count	.029	.043	.499			
MPV	3.015	2.950	.312			
PDW	.688	1.517	.652			
Leukocytes	2.179	1.387	.123^			
Neutrophils	2.070	2.067	.322			
Lymphocytes	5.811	3.765	.130^			
Monocytes	36.373	14.130	.013^	26.718	12.344	.036
NLR	−2.018	2.738	.465			
PLR	−0.037	.058	.532			
TNF- α	−9.289	11.504	.424			
IL-1β	−4.413	8.728	.616			
IL-6	1.962	3.170	.539			
IL-10	−3.194	14.571	.828			
IFN-γ	−17.264	17.262	.323			

After univariate analysis, variables with a *p*-value below 0.25 were included in the multivariate analysis (^). Next, variables were removed using backwards selection with $p < 0.05$ as threshold. ✣Sex was coded as follows: female = 1, male = 2.

the demographic characteristics of the study participants. Both male gender and advanced age are associated with reduced platelet count (Balduini and Noris, 2014; De Gaetano et al., 2023). In our cohort, the tinnitus group had a higher proportion of males and was slightly older, although the latter was not significant. Nonetheless, together these demographic differences might contribute to the observed lower platelet count in our tinnitus group.

Additionally, lower MCV emerged as a significant predictor of tinnitus presence. This finding contradicts the only prior study on MCV, that demonstrated higher MCV levels in tinnitus patients compared to controls (Çeçen et al., 2021). Monocyte count appeared to be a predictor of the TFI-score, but the clinical significance is unsure since the model only exhibited weak predictive power. Monocyte count had not previously been identified as a predictor of tinnitus severity; however, one study did report elevated monocyte counts in subjects with tinnitus (Koçak et al., 2017). Under pathological conditions, monocytes are believed to fail in removing neurotoxins from the central nervous system, interact with astrocytes to alter synapse development and plasticity, and infiltrate the central nervous system, exacerbating neuroinflammation (Garré and Yang, 2018). Given that both MCV and monocytes have not been extensively studied in the context of tinnitus, it remains necessary to elucidate whether they play a role in the pathophysiology of tinnitus and, if so, to determine their precise roles.

4.2. Cytokine levels and tinnitus

Cytokines are classically classified according to their immune response as pro-inflammatory (e.g. IL-1α, IL-1β, IL-6, TNF-α, and IFN-γ) or anti-inflammatory (e.g. IL-12 and IL-10). In the current tinnitus patients, concentrations of the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine IFN-γ were decreased, and they were significant predictors of tinnitus presence in the regression model. The pro-inflammatory cytokines TNF-α, IL-1β and IL-6 showed no difference.

Drawing conclusions about the inflammatory process in tinnitus based on a few cytokines is challenging due to the complex nature of neuroinflammation. The effect of cytokines is highly context-dependent, as they can have different effects on various cell types and under different conditions. Moreover, multiple cytokines can perform similar roles, making it difficult to isolate the impact of one. Changes in cytokine concentrations can even be compensatory. It is therefore impossible to draw a straightforward conclusion.

Nevertheless, the current results show altered inflammatory markers in tinnitus. Few other human studies have investigated cytokine concentrations in subjects with tinnitus. With regard to IL-10, H.F. also reported decreased levels of IL-10 in tinnitus patients. On the other hand C. found no difference in IL-10 concentration, but they did not account for hearing loss in their study. IL-10 is known to strongly inhibit activation of several immunologic cells, including monocytes, dendritic cells, macrophages, and granulocytes, and is therefore seen as an 'dampener' of inflammation (Beebe et al., 2018). Increased levels of IL-10 have even been shown to suppress neurodegenerative diseases such as multiple sclerosis and ALS (Gravel et al., 2016; Beebe et al., 2018). Lower IL-10 levels could thus lead to decreased inhibition of pro-inflammatory cells and therefore to persistence of neuroinflammation, which could also apply for tinnitus.

Similar to the current study, the concentration of IFN-γ was reduced in afternoon samples of tinnitus subjects in the study of H.F. . The sole animal study examining IFN-γ concentrations also demonstrated a decrease in the auditory cortex of animals with tinnitus (Chen and Zheng, 2017). Since the latter indicated this effect during the acute phase of tinnitus, and human studies typically focus on chronic tinnitus, IFN-γ might be decreased in tinnitus subjects during both the acute and the chronic phase. IFN-γ is traditionally considered a pro-inflammatory cytokine with potential damaging effects on the central nervous system, but it also entails a protective and regulatory role in neuroinflammation (Ottum et al., 2015). For example, IFN-γ is able to regulate the dual activity of microglia. At low concentrations, IFN-γ enables microglia to perform neuroprotective functions, but high concentrations of IFN-γ caused microglia to be cytotoxic and impaired their neuroprotective activities. The contradictory roles of IFN-γ can even be implicated within a single disease, depending on disease stage (Ottum et al., 2015). Given that few studies have investigated the role of IFN-γ in tinnitus and considering its dual roles (i.e., pro-inflammatory and anti-inflammatory), a definitive conclusion regarding its role in tinnitus cannot yet be made.

Interestingly, TNF-α and IL-1β were similar in individuals with and without tinnitus. This observation aligns with previous human studies, despite elevated levels of TNF-α and IL-1β in tinnitus across five animal studies (Hwang et al., 2013; Hu et al., 2014; Chen and Zheng, 2017; Wang et al., 2019; Xia et al., 2020). This may be due to that fact that these cytokines might be mainly associated with the acute inflammatory response at the onset of tinnitus, which may normalize as the condition becomes chronic. In animal studies, cytokine levels were measured shortly after the induction of tinnitus, whereas human studies are usually focused on chronic tinnitus. Another explanation could be that in human studies cytokine concentrations are determined in blood samples, whereas in animal studies cytokine concentrations are determined in brain tissue. Therefore, the comparison between both animal and human studies, and acute and chronic tinnitus, can be made with more certainty when potential confounders, such as duration of tinnitus and tissue of interest, are more comparable between human and animal studies. Thus, a next step in tinnitus research could be to study cytokine levels in human cerebrospinal fluid or even brain tissue obtained during surgery.

4.3. Strengths and limitations

The current study provides new insights into the pathophysiology of tinnitus. Due to excluding individuals with hearing loss or signs of anxiety or depression, it is less likely that the observed results are attributable to these confounding factors rather than to tinnitus itself. To our knowledge, this is the first inflammation study in tinnitus that excluded participants with hearing loss or signs of anxiety or depression. However, the study does have a limitation. Despite the strict inclusion criteria for hearing thresholds, the hearing threshold at 8 kHz was frequently above 20 dB. Nonetheless, since there were no significant differences in hearing thresholds between both groups, and we accounted for hearing threshold in the analyses, the impact of this limitation is considered minimal.

5. Conclusion

The current study provides novel insights into the association between inflammation and tinnitus, revealing alterations in inflammatory markers in tinnitus patients, such as lower platelet count, IL-10, and IFN-γ. These findings suggest a potential role of inflammation in tinnitus, independent of confounding factors like hearing loss, anxiety, and depression. This underscores the need for further research to elucidate the underlying mechanisms and explore targeted therapeutic interventions for tinnitus management.

6. Ethical considerations

This study was conducted in accordance with the declaration of Helsinki, and was approved by the Ethics Committee of the University Medical Center Groningen (METc 2022/143). Written informed consent was obtained from all participants.

CRediT authorship contribution statement

Lilian M. Mennink: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration,

Visualization, Writing – original draft. **Lina B.M. Albakri:** Investigation, Project administration, Writing – original draft. **Marlien W. Aalbers:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Pim van Dijk:** Conceptualization, Methodology, Supervision, Writing – review & editing. **J.Marc C. van Dijk:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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