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Biomarkers of Systemic Inflammation and Risk of Incident Hearing Loss

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Abstract

Background: Chronic inflammation may lead to cochlear damage, and the only longitudinal study that examined biomarkers of systemic inflammation and risk of hearing loss found an association with a single biomarker in individuals <60 years of age. The purpose of our study was to determine whether plasma inflammatory markers are associated with incident hearing loss in two large prospective cohorts, Nurses' Health Studies (NHS) I and II.

Methods: We examined the independent associations between plasma levels of markers of systemic inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor receptor 2 (TNFR-2)) and self-reported hearing loss. The participants in NHS I (n=6,194 women) were 42 to 69 years of age at the start of the analysis in 1990, while the participants in NHS II (n=2,885 women) were 32 to 53 years in 1995. After excluding women with self-reported hearing loss prior to the time of blood-draw, incident cases of hearing loss were defined as those women who reported hearing loss on questionnaires administered in 2012 in NHS I and 2009 or 2013 in NHS II. The primary outcome was hearing loss that was reported as moderate or worse in severity, pooled across the NHS I and NHS II cohorts. We also examined the pooled multivariable (MV)-adjusted hazard ratios for mild or worse hearing loss. Cox proportional hazards regression was used to adjust for potential confounders.

Results: At baseline, women ranged from age 42 to 69 years in NHS I and 32 to 53 years in NHS II. Among the NHS I and II women with measured plasma CRP, there were 628 incident cases of moderate or worse hearing loss during 100,277 person-years of follow-up. There was no significant association between the plasma levels of any of the three inflammatory markers and

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Conflict of Interest:

Shruti Gupta has no conflicts of interest to report

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incident moderate or worse hearing loss (MV-adjusted pooled p-trend for CRP=0.33; p-trend IL-6=0.54; p-trend TNFR-2=0.70). There was also no significant relation between inflammatory marker levels and mild or worse hearing loss. While there was no significant effect modification by age for CRP or IL-6 in NHS I, there was a statistically significant higher risk of moderate or worse hearing loss (p-interaction=0.02) as well as mild or worse hearing loss (p-interaction=0.004) in women aged ≥60 who had higher plasma TNFR-2 levels.

Conclusion: Overall, there was no significant association between plasma markers of inflammation and risk of hearing loss.

INTRODUCTION

The inner ear was once thought to be immune-privileged due to the presence of tight junctions in the stria vascularis(McCabe 1989); however, inflammatory cells have been shown to infiltrate the cochlea in animal studies (Hirose 2008; Okano et al. 2008; Fujioka et al. 2014). Furthermore, experimental studies of inner ear inflammation have demonstrated the *in vivo* production of tumor necrosis factor-alpha (TNF- α), interleukin-1β and interleukin-6 (IL-6) after ototoxic insults (Satoh et al. 2003; Fujioka et al. 2006). These inflammatory markers have the ability to elicit potent secondary inflammatory responses in the ear, inducing local leukocyte infiltration and scarring.

On a systemic level, higher plasma levels of inflammatory markers have been consistently associated with age-related chronic disease and disability, such as cardiovascular disease (Ridker et al. 2000) and dementia (Mancinella et al. 2009). C-reactive protein (CRP), IL-6, and TNF- α are thought to reflect the systemic burden of inflammation, and have therefore become recognized as potential therapeutic targets (Satoh et al. 2003; Karadag et al. 2008; Rincon et al. 2012). A number of mechanisms of chronic inflammation leading to sensorineural hearing loss have been posited, including inflammation-related microvascular disease (Ohlemiller 2009). These proposed mechanisms provide a compelling case for further investigating the association between CRP, IL-6, and soluble tumor necrosis factor receptor 2 (TNFR-2), and hearing loss.

While cross-sectional studies have observed an association between plasma inflammatory markers and hearing loss (Bainbridge et al. 2010; Verschuur et al. 2012), there is a dearth of longitudinal data. In the only previous longitudinal study of plasma inflammatory markers, individuals aged <60 years with higher CRP had a 96% higher 10-year incidence of hearing loss, but no significant associations were observed among older individuals and no associations were observed for IL-6 and TNFR-2 (Nash et al. 2014). The aim of our study was to examine whether levels of CRP, IL-6, and TNFR-2 were associated with incident hearing loss in two large cohorts, Nurses' Health Studies (NHS) I and II (n=9,079).

MATERIALS AND METHODS

Study Participants

The NHS I is a prospective cohort study of 121,700 married, female registered nurses aged 30–55 at enrollment in 1976. NHS II is a similar cohort of 116,430 female participants aged

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25–42 at enrollment in 1989. Participants in both cohorts complete questionnaires every 2 years, with a follow-up rate of more than 90% of the eligible person-time.

Those women who had levels of CRP, IL-6, and/or TNFR-2 already measured as part of other projects were selected for inclusion in this study. We also limited the study to women in NHS I who provided information on their hearing in 2012, and NHS II participants who answered questions about their hearing on the 2009 or 2013 questionnaires. There were 6,194 NHS I women and 2,885 NHS II women included.

The baseline of our NHS I analysis was 1990, as the first blood collection occurred between 1990 and 1992. The baseline of the NHS II analysis was 1995, the year closest to the first blood collection (1996–1999), when covariate data were available.

Ascertainment of Inflammatory Marker Levels

Plasma CRP, IL-6, and TNFR-2 concentrations were measured in previously performed nested case–control and validation studies in the NHS I and II cohorts using stored blood samples (Pai et al. 2002). The years of the blood draws for NHS I were 1990–1992 and 2000–2002. The years of the blood draws for the NHS II cohort were 1996–1999 and 2010–2011. Not all participants had values for each biomarker. Of the 6,194 NHS I women included, 5,216 had CRP levels measured, 4,178 had IL-6 levels, and 4,000 had TNFR-2 levels. Of the 2,885 NHS II women included, 2,675 had CRP levels, 2,339 had IL-6 levels, and 499 had TNFR-2 levels.

All assays were conducted at laboratories that satisfied rigorous blinded quality control procedures. Coefficients of variation were <3.0% for CRP, <6.1% for IL-6, and <11.6% for TNFR-2. CRP (mg/L) was measured using a high-sensitivity latex-enhanced immunonephelometric assay on a BNII analyzer. IL-6 (pg/mL) was measured via a quantitative sandwich enzyme immunoassay technique using a Quantikine HS kit (R&D Systems, Minneapolis, Minnesota), and soluble TNFR-2 (pg/mL) was assessed via an enzyme-linked immunosorbent assay kit utilizing immobilized monoclonal antibody to human TNFR-2 (Genzyme, Cambridge, Massachusetts). Stability of CRP, IL-6, and TNFR-2 was assessed in 17 fresh blood samples from the NHS I at receipt and after a delay in processing of 24 and 36 hours. The intraclass correlation coefficients (ICC) between the results of the two collections were >0.75 for all three inflammatory markers (Pai et al. 2002). Blood samples were taken in a subsample of study participants 4 years apart, and the ICCs for the inflammatory markers were estimated (0.68 for CRP; 0.47 for IL-6; 0.78 for TNFR-2) (Pischon et al. 2003), thus each single measure reasonably represents longer-term exposure.

Ascertainment of Hearing Loss

The primary outcome was self-reported hearing loss that was moderate or worse in severity. For the NHS I cohort, information was obtained from the 2012 long-questionnaire on which participants were asked, “Do you have a hearing problem?” (response options: none, mild, moderate, severe). If they reported hearing loss, they were then asked at what age they first noticed a change in their hearing. Similarly in NHS II, the primary outcome was determined based on responses to the 2009 and 2013 questionnaires. On the 2009 main questionnaire,

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participants were asked, “Do you have a hearing problem?” (no, mild, moderate, severe), and “At what age did you first notice a change in your hearing?” On the 2013 main questionnaire, participants were asked, “Which best describes your hearing?” (excellent, good, a little hearing trouble, moderate hearing trouble, deaf), and “Have you noticed a change in your hearing?” and, if the response was “Yes,” “At what age did you first notice a change in your hearing?”

Incident cases of hearing loss were defined as those women in NHS I who reported a hearing problem starting after the first blood draw. We excluded women who noticed a change in their hearing prior to the years of the first blood draws and those with a history of cancer other than non-melanoma skin cancer due to the possible exposure to ototoxic chemotherapeutic drugs.

For this study, we chose *a priori* to examine moderate or worse hearing loss as the primary outcome to focus on hearing loss that is likely to be the most clinically meaningful and to minimize misclassification. The use of questionnaires to assess hearing loss has been found to be reliable (Schow et al. 1990; Ferrite et al. 2011), and has been effective in detecting significant associations in this and similar cohorts (Schow et al. 1990; Schow & Gatehouse 1990; Coren & Hakstian 1992; Nondahl et al., 1998; Gomez et al. 2001). In a validation study of self-reported hearing loss as compared with audiometrically measured hearing loss in Australia, the sensitivity of a single question to assess hearing loss among women <70 years of age was 95% for detecting moderate hearing loss (better ear pure tone average [PTA] 0.5,1,2,4 kilohertz [kHz] >40 dB HL) and 100% for detecting marked hearing loss (better ear $PTA_{0.5,1,2,4\ kHz} > 60$ dB HL), and the specificity was 65% and 64%, respectively (Sindhusake et al. 2001).

As a secondary outcome, we also assessed the incidence of mild or worse hearing loss. Mild hearing loss was defined as self-reported “mild” hearing loss based on the 2009 and 2012 questionnaires. On the NHS II questionnaire from 2013, women with “mild” hearing loss were those who described having “a little hearing trouble.”

Ascertainment of Covariates

We considered the following covariates based on risk factors for hearing loss that have been reported in previous studies. These factors include age (Agrawal et al. 2008); race (Agrawal et al. 2008); menopausal status (Hederstierna et al. 2007; Curhan et al., 2017); tinnitus (Nondahl et al. 2002; Shargorodsky et al. 2010); body mass index (BMI) (Curhan et al. 2013); waist circumference (Curhan et al. 2013); alcohol consumption (Curhan et al. 2015); intake of folate (Durga et al. 2007; Curhan et al. 2015), β-cryptoxanthin (Curhan et al. 2015), β-carotene (Choi et al. 2014; Curhan et al. 2015), long chain omega-3 fatty acids (Curhan et al. 2014), vitamin B12 (Houston et al. 1999; Durga et al. 2007), vitamin C (Curhan et al. 2015), potassium (Wangemann 2006), and magnesium (Haupt et al. 2003); physical activity level (Li et al. 2006; Curhan et al. 2013); smoking (Itoh et al. 2001); diabetes (Bainbridge et al. 2010); hypertension (Lin et al. 2016); and regular use of non-steroidal anti-inflammatory agents (NSAIDs), acetaminophen, and aspirin (Curhan et al. 2012; Lin et al. 2017).

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Updated covariate data were obtained from biennial questionnaires. Dietary intake data were derived from semi-quantitative food frequency questionnaires, which are administered every 4 years. Covariate information obtained from these questionnaires has been reported to be valid and reproducible (Willett et al.1985; Rimm et al. 1992).

Statistical Analysis

Analyses were performed prospectively using exposure information collected before the reported onset of hearing loss. Levels of CRP were categorized based on clinically-relevant cut-points established by the American Heart Association/Centers for Disease Control (<1 mg/L low risk group, 1–3 mg/L average risk group, and >3 mg/L high risk group) (Pearson et al. 2003). We further categorized CRP into 3 to < 6 and ≥6 according to the distribution in our cohorts to see if more extreme values were associated with a different magnitude of risk. Thus, for CRP, the categories were <1 mg/L, 1 to <3 mg/L, 3 to <6 mg/L, and ≥ 6 mg/L. In the literature, clinically relevant cut-points for IL-6 and TNFR-2 vary, though some studies suggest that >3 pg/mL constitutes high levels of IL-6 (Lai et al., 2002). IL-6 was categorized as <1 pg/mL, 1 to <2 pg/mL, 2 to <3 pg/mL, and ≥ 3 pg/mL. For TNFR-2, the categories were <2000 pg/mL, 2000 to <3000 pg/mL, and ≥ 3000 pg/mL. Categories of IL-6 and TNFR-2 were created based on the distribution of the data in our cohorts in order to ensure adequate discrimination between different cut-points. Exposure for each participant was assigned based on the baseline and updated inflammatory marker category, as some participants had the same biomarker measured at both time periods.

We examined the risk of hearing loss that was moderate or worse in severity in each cohort. In NHS I, person-time was calculated from 1990 until the date of self-reported hearing loss or end of follow up in 2012. In NHS II, person-time of follow-up was calculated from 1995 until the date of self-reported hearing loss or end of follow-up in 2013. Women who reported mild hearing loss were skipped starting from the date of onset for that time period and reentered the analysis as a case if they subsequently reported moderate or worse hearing loss.

In the primary analysis, we pooled the multivariable-adjusted hazard ratios from NHS I and NHS II for the risk of moderate or worse hearing loss. The pooled multivariable-adjusted hazard ratios of moderate or worse hearing loss were calculated based on inflammatory categories. Because of the different timings of blood collection, we ran separate models for each cohort. We then used a meta-analysis macro to obtain the pooled parameter estimates and hazard ratios after controlling for the same covariates in both cohorts. Participants were censored at the reported onset of hearing loss or new cancer diagnosis, other than non-melanoma skin cancer. The outcome definition was changed to mild or worse hearing loss and the analysis was repeated.

Multivariable-adjusted hazard ratios of hearing loss by inflammatory marker category were calculated using Cox proportional hazards regression models. We tested for the proportional hazards assumption by evaluating the interaction of each biomarker with time-varying age. None of the interaction terms were significant; thus the constant proportional hazards assumption was not violated. Covariate data that had been collected biennially was included in the Cox regression model as time-varying. We considered covariates that might be

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potential confounders and removed those not found to be statistically significant using backward selection, setting a p-value threshold of < 0.10. For consistency, we retained the same covariates in the NHS I and NHS II models (BMI, age, hypertension, and acetaminophen use). We also tested for effect modification by age in the NHS I cohort (categorized as <60 and ≥ 60 years). These analyses were not performed in NHS II due to the younger ages of the participants. We calculated 95% confidence intervals for all hazard ratios. Tests for linear trend for CRP, IL-6, and TNFR-2 were performed by assigning the median value for each category to all participants in that group. *P* values are all two-sided. SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina) was used for all statistical analyses.

This study was approved by the Partners Healthcare Institutional Review Board.

RESULTS

Baseline Characteristics

The characteristics of participants at baseline according to categories of CRP in NHS I and II are shown in Tables 1 and 2. Most participants were white. Those with the highest levels of CRP also tended to have a higher BMI, larger waist circumference, lower physical activity, more regular analgesic use (>3 days/week of acetaminophen, aspirin, and NSAID use), and more hypertension. In NHS I, participants with higher levels of CRP were more likely to have diabetes. Other health, dietary, and lifestyle characteristics did not vary substantially according to category of CRP.

Baseline characteristics for participants according to IL-6 and TNFR-2 categories for each cohort are shown in Supplementary Tables 1-4. Similar relations were seen with regards to age, hypertension, BMI, and analgesic use. In NHS I, participants with higher levels of IL-6 were more likely to have diabetes and lower mean intake of folate, β-carotene, magnesium, and potassium. With the exception of β-carotene, these relations were not seen in the NHS II cohort.

The median time to age-adjusted moderate or worse hearing loss in both cohorts was 12 years.

CRP

Among the 7,891 participants with measured plasma CRP, 628 incident cases of hearing loss that were moderate or worse in severity were reported to have occurred during 100,277 person-years of follow-up (Table 3). There was no significant association between plasma levels of CRP and incident hearing loss after multivariable adjustment (p-trend=0.33) (Table 3). The cohort-specific results for moderate or worse hearing loss by plasma CRP level are shown in Supplementary Table 5.

Among participants with measured plasma CRP, there were 1,849 incident cases of mild or worse hearing loss during 101,447 person-years of follow up (Table 4). There was no significant association between CRP level and mild or worse hearing loss (MV-adjusted p-

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trend=0.23). Cohort-specific results for mild or worse hearing loss by CRP level are shown in Supplementary Table 6.

IL-6

Among the 6,517 women in NHS I and NHS II with measured plasma IL-6 levels, 458 incident cases of hearing loss that were moderate or worse in severity were reported to have occurred during 79,307 person-years of follow-up (Table 3). There was no independent association between IL-6 and hearing loss that was moderate or worse in severity when the results were pooled for NHS I and NHS II (p-trend=0.54).

There were 1,306 incident cases of mild or worse hearing loss during 79,267 person-years of follow up (Table 4). There was no significant association between IL-6 level and mild or worse hearing loss (p-trend=0.45). Cohort specific results for moderate or worse and mild or worse hearing loss by IL-6 level are presented in Supplementary Tables 7 and 8.

TNFR-2

Among the 4,000 NHS I women with plasma TNFR-2 levels , there were 452 incident cases of moderate or worse hearing loss reported during 52,809 person-years of follow-up (Table 3). There was no independent association between TNFR-2 levels and moderate or worse hearing loss in NHS I (p-trend=0.70). We could not examine the multivariable-adjusted hazard ratio of moderate or worse hearing loss in NHS II, because there were only 9 incident cases of moderate or worse hearing loss.

When evaluating the risk of mild or worse hearing loss according to TNFR-2 levels in NHS I and NHS II, there were 981 cases of hearing loss during 55,582 person-years of follow up (Table 4). There was no significant association between TNFR-2 category and mild, moderate, or worse hearing loss (p-trend=0.61). Cohort specific results for mild or worse hearing loss by TNFR-2 level are depicted in Supplementary Table 9.

Stratification by Age

After stratifying by age ≥60 and <60 years in NHS I, no significant associations were observed between levels of CRP and IL-6 and the risk of moderate or worse hearing loss in younger or older women (p-interaction for CRP =0.54; p-interaction for IL-6 = 0.21). In participants ≥60 years, the risk of moderate or worse hearing loss was highest in those with plasma TNFR-2 levels ≥3000 pg/mL (HR 1.52, 95% CI: 1.02–2.25) compared with <2000 pg/mL (p-interaction=0.02) (Table 5). There was no significant association between plasma TNFR-2 levels of 2000 to <3000 pg/mL and moderate or worse hearing loss compared with levels <2000 pg/mL (HR 1.20, 95% CI: 0.83–1.74).

When we examined the outcome defined as mild or worse hearing loss in NHS I (Table 6), the risk of hearing loss was similarly highest among those in the highest category of TNFR-2 levels compared with those who were in the lowest category (HR 1.39, 95% CI: 1.07–1.82) For participants <60, there was a significant inverse association between TNFR-2 level and the risk of mild or worse hearing loss among those women with TNFR-2 levels ≥3000 compared with those with TNFR-2 levels <2000 (HR 0.70, 95 % CI: 0.50–0.97).

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Sensitivity Analysis

We examined the change in CRP, IL-6, and TNFR-2 levels among participants who had biomarkers measured in both periods by subtracting levels at the second time period from the first time period. The median change in levels was <15% for all 3 biomarkers.

When data were missing, we created “missing” indicator categories for the inflammatory markers and covariates, and we ran models with and without the missing categories. Because the results did not change, we left out the missing categories for the inflammatory markers in the final models.

DISCUSSION

In this longitudinal study of 9,079 women, we found no overall association between plasma levels of inflammatory markers and the risk of self-reported hearing loss.

Local inflammation in the cochlea has been demonstrated to lead to cell injury and hearing loss in animal studies. Within the lateral wall of the cochlea, bone marrow-derived resident macrophages are activated in response to noise exposure, ischemia, and surgical stress (Fujioka et al. 2014). In vivo production of TNF-α and IL-6 occurs after directly challenging the ear with a specific antigen presenting cell, resulting in an extensive immune response in the cochlea (Satoh et al. 2003). TNF-α has also been shown to be activated in noise induced hearing loss, as it controls cochlear microcirculation through its effects on spiral modiolar arterial vasoconstriction (Arpornchayanon et al. 2013).

While inflammation has been shown to occur locally at the level of the inner ear, animal and in vitro studies have also demonstrated that circulating inflammatory molecules can cause hearing loss by disrupting the endothelial cell integrity of the stria vascularis, leading to loss of the blood-labyrinth barrier (Trune & Nguyen-Huynh 2012). Circulating IL-6 and TNF-α have been found to strip off the protective glycocalyx, thereby exposing the cell surface to circulating immune factors (Iversen et al. 1999; Henry & Duling, 2000; Trune & Nguyen-Huynh, 2012). The loss of tight junctions in the ear exposes the blood-labyrinth barrier, leading to decreased endolymph production and endolymphatic potential. Compromise of the barrier with vascular leakage can therefore lead to hearing loss.

Neuro-inflammation and neurodegeneration may also be associated with sensorineural hearing loss. A number of neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s disease, and multiple sclerosis have been associated with inflammation (Glass et al. 2010; Smith et al. 2012). In these disorders, microglia secrete inflammatory mediators like TNF-α that then trigger astrocytes to induce secondary inflammatory responses, leading to oxidative stress and accumulation of reactive oxygen species (Glass et al. 2010). In Alzheimer’s disease, TNF-α and IL-6 are found in excess in the serum; they cross the blood-brain barrier and activate the central immune response (Walters et al. 2016). Age-related hearing loss, which is characterized by loss of hair cells and atrophy of the stria vascularis, is also thought to occur secondary to oxidative stress and an overactive neuro-inflammatory response (Riva et al. 2007; Fujimoto & Yamasoba 2014). Mice models of age-related hearing loss suggest that microglia assume a primed state in the presence of age-related

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hearing loss and release pro-inflammatory cytokines (Bowl & Dawson, 2015; Norden & Godbout, 2013). The elderly may be less able to down-regulate cytokine production and neuro-inflammation (El Assar et al. 2013).

Elevated plasma levels of inflammatory markers have also been identified in a variety of forms of hearing loss (Trune & Nguyen-Huynh, 2012). A small cross-sectional study (n=611) reported that higher levels of plasma CRP and IL-6 were associated with poorer hearing sensitivities in older adults (Verschuur et al. 2012). Cross-sectional results from NHANES showed that higher CRP levels were associated with a higher odds of hearing loss as defined by pure-tone audiometry, with a HR 1.98, 95% CI: 1.26–3.10 at mid/low frequencies (0.5, 1, and 2 kHz) and HR 1.50, 95% CI: 1.01–2.23 at high frequencies (3, 4, 6, and 8 kHz) (Bainbridge et al. 2010).

Prospective data on the relation between inflammation and hearing decline are limited. To date, there is only one other study that has longitudinally assessed the association between CRP, IL-6, and TNFR-2 levels and hearing loss (Nash et al., 2014). Nash et al. used the Epidemiology of Hearing Loss Study (EHLS) to examine whether levels of inflammatory markers are related to measured hearing loss over 10-year follow-up (n=1073). They found no significant association between higher levels of baseline CRP, IL-6, or TNFR-α and hearing loss, defined as $PTA_{(0.5,1,2,4)}$ >25 dB HL. In our study, there was also no statistically significant association between higher CRP, IL-6, and TNFR-2 categories and hearing loss. Our finding that the median changes in biomarker levels were low when we subtracted each level in the second time period from the first time period, suggest that even if individuals changed categories between the two periods, this is unlikely to explain the null findings.

Nash et al. did find a statistically significant association between CRP and the incidence of hearing impairment in participants aged <60 years at baseline (HR 1.98, 95% CI: 1.22–3.20) but not in those ≥60 years and older (p-interaction = 0.03). These findings were independent of sex, obesity, smoking, and alcohol use. In our NHS I cohort, there was no interaction with the same age categories for CRP but there was a significant interaction for TNFR-2. The risk of moderate or worse hearing loss or mild or worse hearing loss was higher with higher plasma levels of TNFR-2, but only in those women who were aged 60 or older. The reasons why our findings differ from the Nash study are unclear. The EHLS results were unchanged after stratifying by sex and included many of the same potential confounders in their models. We evaluated hearing based on self-report, whereas Nash et al. used hearing thresholds to gauge change in hearing. Our sample size was also much larger.

Our finding of a higher risk of hearing loss in higher categories of TNFR-2 among those ≥60 could mean that older subsets of the population with higher degrees of inflammation are more susceptible to hearing loss than their similarly aged counterparts; however, it could also have been a chance finding. TNF-α is more closely involved in neural signaling pathways, and is specifically produced by spiral ligament fibrocytes following noise exposure (Fujioka et al. 2006). Furthermore, infusion of TNF-α is into guinea pig cochlea leads to infiltration of leukocytes in the setting of acoustic trauma (Hirose et al. 2005). These findings that are specific to TNF-α may explain why the same associations were not seen

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with CRP and IL-6. Our findings of a lower risk of hearing loss in higher categories of TNFR-2 among those women <60 was unexpected and may have been a chance finding.

Efforts are underway to target both local and systemic inflammatory pathways in order to prevent or reverse hearing loss. Locally, treatment with TNF-α blockers has led to inconsistent results in the treatment of hearing loss (Matteson et al. 2005; Van Wijk et al. 2006). On a systemic level, the ASPREE-HEARING trial is now recruiting patients to determine whether daily aspirin slows the progression of age-related hearing loss in healthy older adults (Lowthian et al. 2016). In this three-year double-blind, randomized controlled trial, investigators are enrolling 1262 Australians who are aged 70 to assess whether 100 mg of aspirin results in a difference in $PTA_{(0.5,1,2,4)}$ over a 3-year period. However, in the male Health Professionals Follow-up Study, there was a higher risk of self-reported hearing impairment among males who were regular aspirin users (n=26,917) (Curhan et al. 2010). Furthermore, the results of our study suggest that systemic inflammation, as assessed by CRP, IL-6, and TNFR-2 levels, may not modify the risk of hearing impairment, though local inflammation could still play a role.

While our study found no significant association between inflammatory markers and incident hearing loss, it is possible that CRP, IL-6, and TNFR-2 are not the optimal markers, and serum levels may not be representative of inflammation occurring at the level of the cochlea. This raises the question of whether addressing systemic inflammation would influence hearing loss risk. Furthermore, there may be other as yet unidentified mediators of the relation between inflammation and hearing loss; for instance, one Japanese study implicated certain polymorphisms of genes encoding inflammatory mediators as risk factors for presbycusis (Uchida et al. 2014).

This is the largest prospective study to date that has examined the risk of incident hearing loss in relation to levels of inflammatory markers. Furthermore, the information obtained from the NHS I and II cohorts is highly reliable, and follow-up rates are high. However, this study has limitations. Hearing loss assessment was based on self-report. Nonetheless, while pure-tone audiometry is considered the gold standard for evaluating hearing loss, studies have shown that self-reported hearing loss is a reliable indicator of hearing loss (Gomez et al. 2001; Ferrite et al. 2011; Kamil et al. 2015). Several prior studies have been conducted in NHS I and NHS II that have identified important risk factors for self-reported hearing loss (Curhan et al. 2011; Curhan et al. 2012; Curhan et al. 2015; Lin et al. 2016; Lin et al. 2017; Curhan et al. 2017). We chose to examine moderate or worse hearing loss as the primary outcome to minimize misclassification. The sensitivity of a single question to detect moderate or worse hearing loss has been shown to be high among women (Sindhusake et al. 2001). The study consisted of mostly non-Hispanic white women; therefore, the findings may not be generalizable to men or other racial groups.

Based on the findings from this large longitudinal study, plasma markers of inflammation are not associated with risk of hearing loss. Additional studies are needed to elucidate other modifiable risk factors, given the public health burden of hearing loss.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Baseline Characteristics of NHS I Participants in 1990 According to CRP Categories

	1 0<CRP<1 (n= 2321)	2 1≤CRP<3 (n=1454)	3 3≤CRP<6 (n= 714)	4 CRP≥6 (n= 727)
Age, yrs	54.6 (6.7)	56.4 (6.5)	56.4 (6.1)	55.7 (6.4)
BMI	24.6 (4.5)	25.4 (3.9)	27.1 (5.0)	28.3 (5.9)
Waist Circumference, ≥80 cm	478 (20.6)	445 (30.6)	269 (37.7)	278 (38.2)
White Race	2196 (94.6)	1400 (96.3)	689 (96.5)	682 (93.8)
History of HTN	515 (22.2)	393 (27.0)	233 (32.6)	274 (37.7)
History of DM	55 (2.4)	81 (5.6)	40 (5.6)	76 (10.5)
Post-Menopausal	1703 (73.4)	1216 (83.6)	612 (85.7)	622 (85.6)
History of Tinnitus	442 (19.0)	279 (19.2)	128 (17.9)	149 (20.5)
Smoking				
Never	1112 (47.9)	656 (45.1)	312 (43.7)	360 (49.5)
Past	975 (42.0)	636 (43.7)	311 (43.6)	285 (39.2)
Current	230 (9.9)	160 (11.0)	89 (12.5)	80 (11.0)
Alcohol, g/d	5.3 (8.9)	5.5 (9.5)	5.1 (9.4)	4.3 (8.7)
Physical Activity, total METs/week	17.3 (26.9)	16.4 (24.2)	15.6 (18.0)	13.8 (16.9)
Folate, mcg/d	427 (215)	434 (218)	434 (233)	424 (223)
Vitamin C, mg/d	289 (320)	304 (340)	318 (351)	275 (305)
β-cryptoxanthin, mcg/d	171 (104)	177 (108)	167 (97.9)	171 (105)
β-carotene, mcg/d	4306 (2529)	4437 (2615)	4376 (2556)	4350 (2374)
B12, mcg/d	9.2 (10.5)	9.5 (13.1)	9.7 (13.6)	9.6 (9.7)
Omega-3 fatty acids, g/d	0.3 (0.3)	0.3 (0.3)	0.3 (0.2)	0.3 (0.2)
Potassium, mg/d	2883 (504)	2896 (488)	2904 (594)	2858 (514)
Magnesium, mg/d	309 (80.3)	310 (73.4)	310 (100)	305 (78.3)
Aspirin, >3 d/week	283 (12.2)	239 (16.4)	105 (14.7)	131 (18.0)
Acetaminophen, >3 d/week	83 (3.6)	68 (4.7)	44 (6.2)	55 (7.6)
NSAIDs, >3 d/week	197 (8.5)	165 (11.4)	104 (14.6)	92 (12.7)

Note: Values represent baseline characteristics in first time period. Values are mean (SD) for continuous variables or n (%) for categorical variables.

Abbreviations: NHS= Nurses' Health Study; CRP= C-reactive protein; yrs= years; BMI= body mass index; HTN= hypertension; DM= diabetes mellitus; g= grams; mg=milligrams; d= day; METs= metabolic equivalents; mcg= micrograms; NSAIDs= non-steroidal anti-inflammatory drugs

Units for CRP = mg/L

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Table 2:

Baseline Characteristics of NHS II Participants in 1995 According to CRP Categories

	1 0<CRP<1 (n= 1376)	2 1≤CRP<3 (n=725)	3 3≤CRP<6 (n= 325)	4 CRP ≥6 (n= 249)
Age, yrs	42.4 (4.4)	43.5 (4.5)	45.0 (4.6)	44.7 (4.4)
BMI	23.0 (3.1)	26.4 (4.8)	28.8 (5.7)	32.4 (8.1)
Waist Circumference, ≥80 cm	186 (13.5)	204 (28.1)	140 (43.1)	107 (43.0)
White Race	1342 (97.5)	716 (98.8)	317 (97.5)	244 (98.0)
History of HTN	89 (6.5)	90 (12.4)	63 (19.4)	64 (25.7)
History of DM	6 (0.4)	6 (0.8)	2 (0.6)	10 (4.0)
Post-Menopausal	141 (10.3)	131 (18.1)	89 (27.4)	67 (26.9)
History of Tinnitus	145 (10.5)	725 (11.0)	47 (14.5)	26 (10.4)
Smoking				
Never	967 (70.3)	509 (70.2)	227 (70.0)	157 (63.1)
Past	330 (24.0)	158 (21.8)	79 (24.3)	70 (28.1)
Current	77 (5.6)	58 (8.0)	19 (5.9)	22 (8.8)
Alcohol, g/d	4.9 (8.1)	4.0 (6.8)	4.4 (9.8)	4.0 (9.5)
Physical Activity, total METs/week	20.6 (22.0)	16.8 (19.3)	16.4 (21.3)	13.0 (13.8)
Folate, mcg/d	551 (274)	566 (272)	579 (271)	595 (276)
Vitamin C, mg/d	372 (453)	357 (407)	376 (404)	323 (332)
β-cryptoxanthin, mcg/d	147 (88.3)	145 (93.1)	159 (122)	155 (100)
β-carotene, mcg/d	4899 (3960)	4692 (3557)	4398 (3728)	4668 (3126)
B12, mcg/d	8.9 (11.4)	8.8 (9.9)	7.7 (4.9)	8.3 (5.7)
Omega-3 fatty acids, g/d	0.2 (0.1)	0.2 (0.1)	0.2 (0.2)	0.2 (0.1)
Potassium, mg/d	3296 (596)	3298 (594)	3256 (590)	3310(590)
Magnesium, mg/d	333 (78.6)	328 (69.1)	318 (71.1)	334 (70.9)
Aspirin, >3 d/week	75 (5.5)	54 (7.5)	28 (8.6)	31 (12.5)
Acetaminophen, >3 d/week	27 (2.0)	12 (1.7)	12 (3.7)	13 (5.2)
NSAIDs, >3 d/week	103 (7.5)	56 (7.7)	40 (12.3)	42 (16.9)

Note: Values represent baseline characteristics in first time period. Values are mean (SD) for continuous variables or n (%) for categorical variables.

Abbreviations: NHS= Nurses' Health Study; CRP= C-reactive protein; yrs= years; BMI= body mass index; HTN= hypertension; DM= diabetes mellitus; g= grams; mg=milligrams; d= day; METs= metabolic equivalents; mcg= micrograms; NSAIDs= non-steroidal anti-inflammatory drugs

Units for CRP = mg/L

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Table 3:

Pooled Results for Age-Adjusted and Multivariable-Adjusted Hazard Ratios for Moderate or Worse Hearing Loss, by Categories of Inflammatory Markers, Among Women in the Nurses' Health Studies

	1 0<CRP<1	**2 1≤CRP<3**	**3 3≤CRP<6**	**4 CRP≥6**	**P for trend**
Cases (n)	229	177	110	112	
Person-years	44940	28404	14175	12758	
Age-adjusted HR, 95% CI	1.00	0.84 [0.63–1.12]	1.03 [0.84–1.27]	1.17 [0.95–1.43]	0.08
Multivariable-adjusted HR, 95% CI	1.00	0.87 [0.70–1.07]	1.01 [0.82–1.24]	1.13 [0.92–1.39]	0.33
	1 0<IL-6<1	**2 1≤IL-6<2**	**3 2≤IL-6<3**	**4 IL-6 ≥3**	**P for trend**
Cases (n)	181	174	46	57	
Person-years	38105	26747	7189	7266	
Age-adjusted HR, 95% CI	1.00	1.10 [0.89–1.36]	1.01 [0.73–1.41]	1.21 [0.90–1.64]	0.25
Multivariable-adjusted HR, 95% CI	1.00	1.05 [0.85–1.30]	0.94 [0.67–1.31]	1.13 [0.83–1.54]	0.54
	1 0<TNFR-2<2000	**2 2000≤TNFR-2<3000**	**3 TNFR-2≥3000**		**P for trend**
Cases (n)**	73	245	134		
Person-years	11829	30026	10954		
Age-adjusted HR, 95% CI	1.00	0.98 [0.75–1.28]	1.11 [0.82–1.48]		0.40
NHS I Multivariable-HR, 95% CI	1.00	0.95 [0.73–1.25]	1.03 [0.77–1.39]		0.70

Outcome: moderate or worse hearing loss

Abbreviations: NHS=Nurses' Health Study; CI=confidence intervals; HR=hazard ratio

Units for CRP = mg/L; Units for IL-6 = pg/mL; Units for TNFR-2= pg/mL

Covariates: Adjusted for age, BMI, history of hypertension, and acetaminophen use (removed missing values for acetaminophen due to non-convergence of model)

** Note: results for TNFR-2 only shown for NHS I, as in NHS II, as there were only 9 cases with moderate or worse hearing loss

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Table 4:

Pooled Results for Age-Adjusted and Multivariable-Adjusted Hazard Ratios for Mild or Worse Hearing Loss, by Categories of Inflammatory Markers, Among Women in the Nurses' Health Studies

	1 0<CRP<1	**2 1≤CRP<3**	**3 3≤CRP<6**	**4 CRP≥6**	**P for trend**
Cases (n)	674	552	331	292	
Person-years	44700	28711	14801	13235	
Age-adjusted HR, 95% CI	1.00	1.00 [0.89–1.12]	1.09 [0.91–1.30]	1.15 [0.97–1.38]	0.14
Multivariable-adjusted HR, 95% CI	1.00	0.98 [0.87–1.10]	1.02 [0.89–1.17]	1.06 [0.91–1.23]	0.23
	1 0<IL-6<1	**2 1≤IL-6<2**	**3 2≤IL-6<3**	**4 IL-6 ≥3**	**P for trend**
Cases (n)	604	444	127	131	
Person-years	37637	26962	7333	7335	
Age-adjusted HR, 95% CI	1.00	1.00 [0.88–1.13]	1.07 [0.88–1.29]	1.12 [0.93–1.36]	0.19
Multivariable-adjusted HR, 95% CI	1.00	0.97 [0.85–1.10]	1.02 [0.83–1.25]	1.09 [0.89–1.33]	0.45
	1 0<TNFR-2<2000	**2 2000≤TNFR-2<3000**	**3 TNFR-2≥3000**		**P for trend**
Cases (n)	183	508	290		
Person-years	12280	31286	12016		
Age-adjusted HR, 95% CI	1.00	0.95 [0.74–1.24]	1.04 [0.86–1.26]		0.39
Multivariable-adjusted HR, 95% CI	1.00	0.93 [0.75–1.15]	1.01 [0.83–1.23]		0.61

Outcome: mild or worse hearing loss (mild, moderate, or severe)

Abbreviations: NHS=Nurses' Health Study; CI=confidence intervals; HR=hazard ratio

Units for CRP = mg/L; Units for IL-6 = pg/mL; Units for TNFR-2= pg/mL

Covariates: Adjusted for age, BMI, history of hypertension, and acetaminophen use (removed missing values for acetaminophen due to non-convergence of model)

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Table 5:

Multivariable -Adjusted Hazard Ratios for Moderate or Worse Hearing Loss by Age <60 versus ≥60, Among Women in NHS I

	Age<60		Age≥60		P for interaction
	No. of Cases	HR with 95% CI	No. of Cases	HR with 95% CI	
CRP Category					
1 0<CRP<1	91	1.00	106	1.0-	P = 0.54
2 1≤CRP<3	68	0.83 [0.60–1.14]	96	0.93 [0.70–1.22]	
3 3≤CRP<6	46	0.86 [0.59–1.26]	53	1.07 [0.76–1.51]	
4 CRP≥6	49	0.99 [0.68–1.44]	55	1.20 [0.85–1.70]	
IL-6 Category					
1 0<IL-6<1	82	1.00	67	1.00	P = 0.21
2 1≤IL-6<2	62	0.91 [0.64–1.27]	90	1.10 [0.79–1.53]	
3 2≤IL-6<3	17	0.83 [0.48–1.42]	26	1.07 [0.67–1.72]	
4 IL-6 ≥3	23	1.18 [0.73–1.91]	31	1.16 [0.75–1.80]	
TNFR-2 Category					
1 0<TNFR-2<2000	35	1.00	35	1.00	P = 0.02
2 2000≤TNFR-2<3000	78	0.74 [0.49–1.12]	161	1.20 [0.83–1.74]	
3 TNFR-2≥3000	35	0.64 [0.38–1.06]	99	1.52 [1.02–2.25]	

Outcome: moderate or severe hearing loss

Abbreviations: NHS=Nurses' Health Study; CI=confidence intervals; HR=hazard ratio

Covariates: adjusted for age, BMI, HTN, acetaminophen use (removed missing values for acetaminophen due to non-convergence of model)

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Table 6:

Multivariable-Adjusted Hazard Ratios for Mild or Worse Hearing Loss by Age <60 versus ≥60, Among Women in NHS I

	Age<60		Age≥60		P for interaction
	No. of Cases	HR with 95% CI	No. of Cases	HR with 95% CI	
CRP Category					
1 0<CRP<1	202	1.00	170	1.00	P = 0.27
2 1≤CRP<3	208	0.99 [0.81–1.21]	173	0.97 [0.78–1.20]	
3 3≤CRP<6	152	1.00 [0.80–1.25]	89	0.95 [0.72–1.24]	
4 CRP≥6	123	0.95 [0.74–1.21]	97	1.15 [0.88–1.50]	
IL-6 Category					
1 0<IL-6<1	198	1.00	120	1.00	P = 0.14
2 1≤IL-6<2	139	0.89 [0.71–1.12]	148	1.00 [0.78–1.28]	
3 2≤IL-6<3	47	0.98 [0.71–1.37]	43	0.97 [0.68–1.39]	
4 IL-6 ≥3	42	0.99 [0.70–1.39]	50	1.17 [0.83–1.65]	
TNFR-2 Category					
1 0<TNFR-2<2000	74	1.00	82	1.00	P = 0.004
2 2000≤TNFR-2<3000	151	0.65 [0.49–0.87]	302	1.12 [0.88–1.44]	
3 TNFR-2≥3000	99	0.70 [0.50–0.97]	181	1.39 [1.07–1.82]	

Outcome: mild, moderate, or severe hearing loss

Abbreviations: NHS=Nurses' Health Study; CI=confidence intervals; HR=hazard ratio

Covariates: adjusted for age, BMI, HTN, acetaminophen use (removed missing values for acetaminophen due to non-convergence of model)