NLRP3 Activation With Bisphosphonate Use and the Risk of Incident Age-Related Macular Degeneration

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Purpose. To determine whether bisphosphonate use increases the risk of age-related macular degeneration (AMD), thereby providing evidence for the involvement of the NLRP3 inflammasome in AMD pathogenesis.

Methods. Retrospective cohort study among US veterans who had undergone dual-energy x-ray absorptiometry (DEXA) scans. Time-dependent Cox models were used to assess the association between cumulative bisphosphonate exposure and AMD incidence. Propensity score matching was applied to balance characteristics between bisphosphonate users and nonusers. A secondary analysis examined the impact of NLRP3 inhibitors (fluoxetine and fluvoxamine) on AMD risk among bisphosphonate users.

Results. After propensity score matching, each additional year of bisphosphonate use was associated with a 4.7% increased hazard of AMD (hazard ratio [HR], 1.047; 95% confidence interval [CI], 1.020–1.074). In the secondary analysis, fluoxetine or fluvoxamine use among bisphosphonate users was linked to a reduced hazard of incident AMD (HR, 0.814; 95% CI, 0.676–0.98) in the matched sample.

Conclusions. Bisphosphonate use increases AMD risk, while NLRP3 inhibitors mitigate this effect. These findings support the hypothesis that the NLRP3 inflammasome is involved in AMD pathogenesis and represents a potential therapeutic target.

Keywords: NLRP3, bisphosphonates, age-related macular degeneration, fluoxetine, inflammation

Age-related macular degeneration (AMD) is a leading cause of blindness worldwide, affecting the macula and leading to the loss of central vision. AMD pharmacotherapy involves anti–vascular growth factor (VEGF) injections that target neo-angiogenesis in advanced disease. Although VEGF injections are effective at slowing disease progression, they require frequent administration and can be burdensome for patients and the health care system because of the high cost and need for regular follow-up. Recently, the NLRP3 inflammasome (nucleotide oligomerization domain [NOD]-, leucine-rich repeat [LRR]-, and pyrin domain-containing protein 3) has been implicated in the pathogenesis of AMD through its release of proinflammatory cytokines, including IL-1B.[1–4] Mechanistically, chronic activation of the NLRP3 inflammasome in retinal pigment epithelial (RPE) cells leads to inflammation, cell death, and impaired ability to support photoreceptors, driving AMD progression.[1] Interestingly, there is a presumed mechanistic convergence among lipid dysmetabolism, autophagy, and NLRP3 biology in the pathogenesis of AMD.[5,6] Dry AMD is noted by an abundance of noncoding, cytotoxic *Alu* RNAs, which activate the NLRP3 inflammasome in both RPE cells and macrophages.[7,8] Excessive inflammasome activation contributes to the progressive loss of RPE and photoreceptors, leading to geographic atrophy (GA), the advanced form of dry AMD. In neovascular AMD, inflammasome activation within macrophages and microglia promotes angiogenesis via inflammatory mediators like IL-1β and growth factors like VEGF.[9] Fluoxetine and nucleoside reverse transcriptase inhibitors have been demonstrated to inhibit NLRP3 and serve as a possible treatment for AMD.[10,11] However, there are medications that can activate NLRP3; therefore, can NLRP3 activation increase the risk of AMD?

Bisphosphonates are a class of pyrophosphate analogs used for the treatment of skeletal disorders, most commonly, osteoporosis. Preclinical evidence suggests that bispho-

sphonates can activate NLRP3, increasing proinflammatory cytokines.[12–17] Zoledronic acid was found to increase IL-1B inflammatory cytokines via the NLRP3 signaling pathway in THP-1 cells[12] and is consistently associated with proinflammatory cytokine release via the NLRP3 pathway.[13–15] Alendronate, another bisphosphonate, has also been found to increase proinflammatory cytokines via NLRP3 signaling,[16,17] suggesting a class-wide mechanism. Bisphosphonates have been associated with rare side effects, such as osteonecrosis of the jaw, now believed to be induced via proinflammatory NLRP3 signaling,[13] including toll-like receptors upstream of NLRP3.[18] While bisphosphonates have the potential to increase NLRP3 expression and NLRP3 is considered a pharmacologic target for AMD, there are currently no data demonstrating a link between medication-related NLRP3 expression and an increased risk of AMD. Based on preclinical research suggesting bisphosphonates exacerbate NLRP3 expression in immune cells, we hypothesize that patients treated with these medications have an increased risk of developing AMD. We test this hypothesis using a national cohort of US veterans initiating bisphosphonate therapy.

Prior research also identified commonly used medications such as fluoxetine (FLX) and fluvoxamine (FLV) as direct NLRP3 inhibitors.[10,11] Therefore, if bisphosphonates are observed to be positively associated with AMD incidence, could the use of FLX or FLV modify the risk of AMD among patients taking bisphosphonates? To test this hypothesis, we conducted a subanalysis among the study participants exposed to a bisphosphonate.

Materials and Methods

Data Source

This retrospective cohort study investigated the relationship between AMD development and bisphosphonate use among patients within the US Department of Veterans Affairs (VA). Demographic information, medical history, hospitalization records, and outpatient medication data were sourced from the Veterans Affairs Informatics and Computing Infrastructure (VINCI).[19,20] VINCI was used to access structured clinical data from outpatient and inpatient encounters documented from all VA health care facilities. The research adhered to VA protocols and received approval from the VA Dorn Research Institute Institutional Review Board (IRB No. 1139248) and Research and Development. The study followed Strengthening the Reporting of Observational Studies in Epidemiology guidelines for reporting observational studies.

Cohort Creation and Outcome

The study cohort was created using patients with a dual-energy x-ray absorptiometry (DEXA) scan, extracted from the outpatient data using Current Procedural Terminology (CPT) code 77080. The first scan for each patient was used as the index date for the study. The study period spans the years 2005 through 2024. Patients were followed from study index, the initial DEXA scan to the first incidence of AMD diagnosis, or the last date of an ophthalmology clinic visit or eye examination. AMD was extracted from outpatient or inpatient claims data with International Classification of Diseases, Ninth/Tenth Revision, Clinical Modification (ICD-9/10-CM) codes of 362.51x, 362.52x, H35.31x, and H35.32x. The first instance of any of the above codes was set as the AMD diagnosis date. Ophthalmology clinic visits were extracted from VA stop (clinic) codes indicating ophthalmology or optometry. Eye examinations were extracted using CPT codes (S0620, S0621, 92004, 92014, 92002, and 92012). Patients with a diagnosis of AMD prior to the index were excluded. Patients were required to have an ophthalmology visit, eye exam, or AMD after the index.

Exposure Data

Study bisphosphonates include zoledronic acid, alendronate, risedronate, and ibandronate. Study medications were extracted from the outpatient pharmacy data. Pamidronate was not included as a study drug because of its indication in cancer therapy. Patients with use of any study medication prior to the index were excluded. Bisphosphonate exposure was coded time-dependently, and the cumulative number of exposure days, based on the days' supply dispensed, was calculated and annualized. Cohort data were organized longitudinally, with each row corresponding to a 6-month period, starting at the index, and followed to study end. Cumulative bisphosphonate exposure was recorded at the start of every 6-month period.

Statistical Analysis

Patients are categorized if they were ever exposed to a study bisphosphonate, and baseline summaries compare treated to untreated patients. Key demographic covariates such as age and year at initial DEXA scan (herein referred to as index year), race, sex are included. Clinical characteristics are captured using the Charlson comorbidity index, body mass index (BMI), and smoking status. The Charlson index was calculated using all inpatient and outpatient claims in the 1 year prior to the DEXA scan. Height and weight data are used to calculate BMI. Smoking status was flagged if patients had a health factor screening that indicates tobacco usage during the study follow-up. Health factor screening results were obtained via keyword search. ICD-9/10-CM diagnosis codes are used to identify other comorbid diagnoses such as hypertriglyceridemia, hypertension, and pure hypercholesterolemia within one year prior to the index date. Baseline characteristics were compared between the two groups using the Wilcoxon rank-sum test for continuous variables (age, index year, and Charlson comorbidity index) and the χ^2 test for categorical variables. In addition to conducting statistical tests, we use the standardized difference (std.diff) to quantify the similarity between cohorts for each variable. Smaller std.diff values indicate greater similarity, with values below 0.2 suggesting acceptable balance and values below 0.1 indicating minimal differences between cohorts, which is the ideal threshold for comparability. For categorical variables with more than two levels, we use the maximum standardized difference (std.diff) value across all levels to assess imbalance.

Time-dependent Cox models are fit to examine the association between cumulative bisphosphonate exposure and AMD incidence accounting for baseline covariates. Propensity score matching is used because bisphosphonate treatment was nonrandomized. A 1:1 ratio matching was used with a greedy nearest-neighbor algorithm. Matched patient characteristics are then presented alongside standardized differences to assess balance between groups.

Time-dependent Cox models are subsequently fit to the matched sample. Hazard ratios (HRs) and associated 95% confidence intervals (CIs) estimated from Cox models are presented. All data management and analysis are performed using SAS 9.4 (SAS Institute, Cary, NC, USA) and R (R Project for Statistical Computing, Vienna, Austria).

Secondary Analysis

Among bisphosphonate users, we extracted all FLX and FLV prescriptions from the VA outpatient pharmacy data. FLX and FLV exposure was treated time-dependently, with patients coded as unexposed until their first exposure, after which they were coded as exposed through the end of the study. Patients with FLX and FLV prior to the study were coded as exposed through the study. Propensity score matching was used to balance the FLX and FLV exposed and unexposed cohorts based on their baseline characteristics. Time-dependent Cox models were fit to the original and propensity score matched data.

Results

Primary Analysis

A total of 291,921 patients were included in the study, with 265,137 unexposed and 26,784 treated with a study bisphosphonate. Baseline characteristics are presented in Table 1. The average age of patients treated with bisphosphonate was 69.15 compared to 65.3 years in the nonexposed ($P < 0.001$, std.diff = 0.33). Patients in the bisphosphonate-treated group were 80.966% White, and patients without bisphosphonate exposure were 71.715% White. Among the bisphosphonate-treated patients, 11.492% were Black, and among the untreated, 21.182% were Black. Both cohort groups were predominantly male, 84.0% in the treated and 78.613% among those without treatment. On average, Charlson comorbidity index scores were 1.98 in the treated and 2.04 in the untreated ($P < 0.001$, std.diff = 0.02). The treated group had 34.0% of patients in the 18.5 to 24.9 BMI category and 26.785% in the 30+ BMI category. For the nonexposed group, these percentages were 19% and 45.1%, respectively. Smokers comprised 26.9% of the treated group and 20.6% of the nonexposed group ($P < 0.001$, std.diff = 0.14). The proportions of hypertriglyceridemia, hypertension, and hypercholesterolemia were similar in both groups, with standardized differences of less than 0.1.

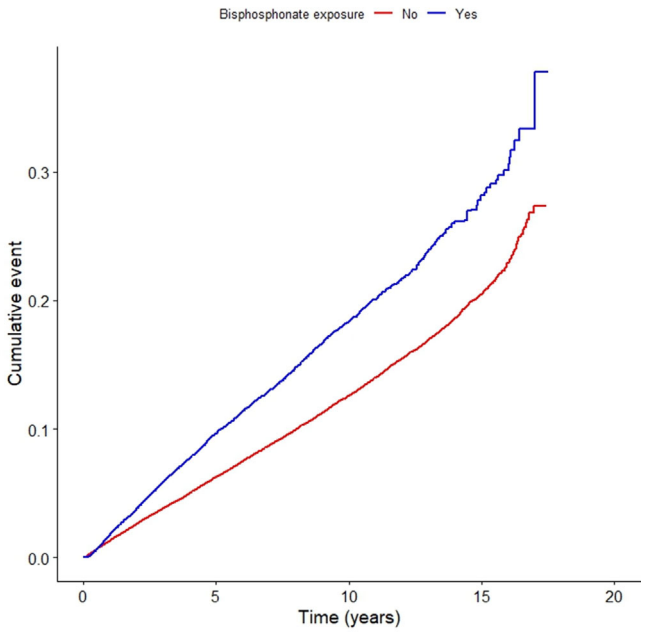

Figure 1. Extended Kaplan–Meier.

Figure 1 displays the extended Kaplan–Meier survival cumulative incidence curves. Across the study follow-up period, patients treated with bisphosphonates had a higher cumulative incidence of AMD. Table 2 presents HRs and

Table 1. Baseline Characteristics

Variable	No Bisphosphonate (n = 265,137)	Bisphosphonate Exposed (n = 26,784)	*P* Value	Std.diff
Age*	65.3 (11.911)	69.15 (11.305)	<0.001	0.33180268
Race				
Black	56,162 (21.182%)	3078 (11.492%)	<0.001	0.26457394
Other/unknown	18,831 (7.102%)	2020 (7.542%)	<0.001	0.26457394
White	190,144 (71.715%)	21,686 (80.966%)	<0.001	0.26457394
Sex				
Female	56,706 (21.387%)	4285 (15.998%)	<0.001	0.1388262
Male	208,431 (78.613%)	22,499 (84.002%)	<0.001	0.1388262
Charlson*	2.04 (2.337)	1.98 (2.138)	<0.001	0.02287483
BMI				
<18.5	3003 (1.133%)	1049 (3.917%)	<0.001	0.38849432
18.5–24.9	50,455 (19.03%)	9108 (34.005%)	<0.001	0.38849432
25–29.9	91,476 (34.501%)	9383 (35.032%)	<0.001	0.38849432
30+	119,615 (45.114%)	7174 (26.785%)	<0.001	0.38849432
Missing	588 (0.222%)	70 (0.261%)	<0.001	0.38849432
Smoker	54,630 (20.604%)	7207 (26.908%)	<0.001	0.14845058
Hypertriglyceridemia	30,811 (11.621%)	2566 (9.58%)	<0.001	0.06500362
Hypertension	174,499 (65.815%)	17,268 (64.471%)	<0.001	0.02728508
Pure hypercholesterolemia	14,920 (5.627%)	1760 (6.571%)	<0.001	0.04179238
Index year*	2015.86 (4.831)	2014.29 (4.84)	<0.001	0.32417537
AMD diagnosis	17,317 (6.56%)	2307 (8.649%)	<0.001	0.075526

* Indicates variables analyzed with Wilcoxon-rank sum test; all other variables were analyzed as categorical variables with the χ^2 test.

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Table 2. Cox Model: Hazard of AMD

Variable	HR (95% CI)
Bisphosphonate use per year	1.053 (1.029–1.078)
Age	1.061 (1.059–1.062)
Race other/unknown vs. Black	2.867 (2.651–3.102)
White vs. Black	3.43 (3.235–3.636)
Male	1.062 (1.014–1.113)
Charlson comorbidity index	1.024 (1.017–1.031)
BMI 18.5–24.9 vs. <18.5	0.95 (0.827–1.092)
25–29.9 vs. <18.5	0.832 (0.725–0.955)
30+ vs. <18.5	0.764 (0.665–0.877)
Missing vs. <18.5	1.12 (0.786–1.596)
Hypertension	1.126 (1.089–1.163)
Hyperlipidemia	0.948 (0.921–0.977)
Pure hypercholesterolemia	0.953 (0.902–1.006)
Smoker	0.818 (0.787–0.849)
Index year	1.039 (1.035–1.043)

associated CIs estimated from the time-dependent Cox model. Each additional year of bisphosphonate treatment was associated with a 5.3% increased hazard of AMD (HR, 1.053; 95% CI, 1.029–1.078). Other factors such as older age, male sex, White race, and hypertension were also associated with a higher hazard of AMD.

Propensity score (PS)–matched sample characteristics are presented in Table 3. Overall good balance was achieved between the groups, as reflected in the low standardized differences. However, BMI was still relatively unbalanced, with the maximum standardized difference being 0.25. Figure 2 displays the Kaplan–Meier cumulative incidence curves. After matching, the curves are noticeably closer, but cumulative incidences remain higher in the treated group. The PS-matched Cox model results are presented in Table 4. The results are consistent with the unmatched analysis, with bisphosphonate exposure associated with an increased hazard of AMD (HR, 1.047; 95% CI, 1.02–1.074). Older age, White race, and hypertension are all positively associated with AMD.

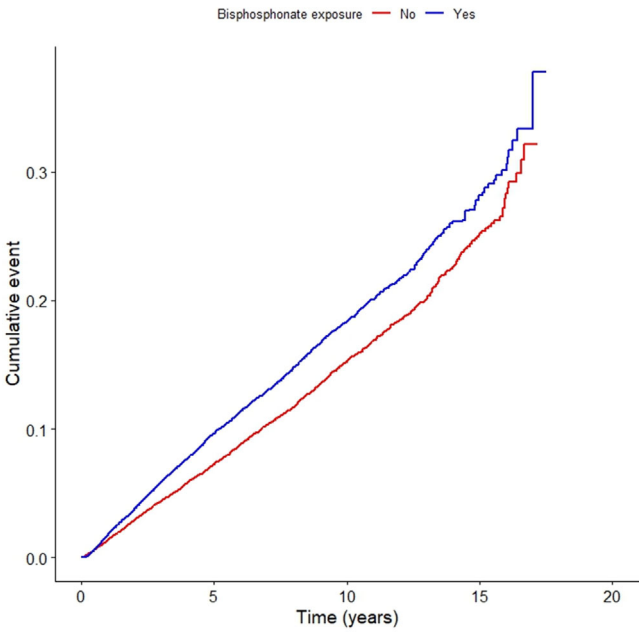

Figure 2. PS-matched extended Kaplan–Meier.

Secondary Analysis

Supplementary Table S1 presents baseline characteristics for the bisphosphonate-exposed patients categorized by their exposure to FLX or FLV. Patients have similar baseline characteristics, with FLX- and FLV-exposed patients being slightly younger (mean 65 vs. 69.9 years in the unexposed; std.diff = 0.44). After propensity score matching, standardized differences for all characteristics, including age, are less than 0.1, indicating excellent balance between the groups (Supplementary Table S2). Time-dependent Cox models are fit to both the original and matched samples, and hazard ratios with corresponding 95% confidence intervals are presented in Table 5. FLX or FLV exposure among bispho-

Table 3. PS-Matched Characteristics

Variable	No Bisphosphonate (n = 26,784)	Bisphosphonate Exposed (n = 26,784)	P Value	Std.diff
Age	68.98 (11.519)	69.15 (11.305)	0.117	0.015062
Race				
Black	2911 (10.868%)	3078 (11.492%)	<0.001	0.030973
Other/unknown	1857 (6.933%)	2020 (7.542%)	<0.001	0.030973
White	22,016 (82.198%)	21,686 (80.966%)	<0.001	0.030973
Sex				
Female	4335 (16.185%)	4285 (15.998%)	0.565	0.005442
Male	22,449 (83.815%)	22,499 (84.002%)	0.565	0.005442
Charlson	1.9 (2.202)	1.98 (2.138)	<0.001	0.039891
BMI				
<18.5	386 (1.441%)	1049 (3.917%)	<0.001	0.255631
18.5–24.9	6071 (22.667%)	9108 (34.005%)	<0.001	0.255631
25–29.9	9897 (36.951%)	9383 (35.032%)	<0.001	0.255631
30+	10,373 (38.728%)	7174 (26.785%)	<0.001	0.255631
Missing	57 (0.213%)	70 (0.261%)	<0.001	0.255631
Smoker	7128 (26.613%)	7207 (26.908%)	0.447	0.006777
Hypertriglyceridemia	2337 (8.725%)	2566 (9.58%)	<0.001	0.031219
Hypertension	17,239 (64.363%)	17,268 (64.471%)	0.801	0.002089
Pure hypercholesterolemia	1666 (6.22%)	1760 (6.571%)	0.101	0.016344
Index year	2014.27 (4.781)	2014.29 (4.84)	0.807	0.004532

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Table 4. PS-Matched Cox Model: Hazard of AMD

Variable	HR (95% CI)
Bisphosphonate use per year	1.047 (1.02–1.074)
Age	1.059 (1.056–1.062)
Race other/unknown vs. Black	3.266 (2.691–3.965)
White vs. Black	3.973 (3.377–4.674)
Male	1.07 (0.968–1.183)
Charlson comorbidity index	1.021 (1.006–1.037)
BMI 18.5–24.9 vs. <18.5	0.871 (0.709–1.071)
25–29.9 vs. <18.5	0.769 (0.626–0.944)
30+ vs. <18.5	0.679 (0.552–0.836)
Missing vs. <18.5	1.205 (0.675–2.151)
Hypertension	1.123 (1.052–1.198)
Hyperlipidemia	0.966 (0.911–1.025)
Pure hypercholesterolemia	0.918 (0.824–1.022)
Smoker	0.802 (0.746–0.862)
Index year	1.023 (1.016–1.031)

sphonate users is associated with a lower hazard of incident AMD in the original sample (HR, 0.81; 95% CI, 0.701–0.937) and in the PS-matched sample (HR, 0.814; 95% CI, 0.676–0.98).

Discussion

The NLRP3 inflammasome may play a key role in AMD pathogenesis, making it important to understand how commonly prescribed medications that influence NLRP3 activation could affect AMD risk. NLRP3 inhibitors have been suggested as possible treatments for AMD.[7,8] Conversely, we sought to evaluate if chronic administration of medications that stimulate NLRP3 activation could accelerate the pathogenesis of AMD.

NLRP3 inflammasome activation in RPE cells and macrophages drives caspase-1–mediated processing and secretion of proinflammatory cytokines, particularly IL-1β. In dry AMD, this inflammatory cascade is triggered by cytotoxic *Alu* RNAs,[7,8] leading to RPE cell death and impaired photoreceptor support. Sustained NLRP3 activation results in chronic inflammation within the retinal microenvironment, accelerating photoreceptor degeneration and the development of geographic atrophy. In neovascular AMD, NLRP3-mediated inflammation in macrophages and microglia promotes pathological angiogenesis through the release of IL-1β and VEGF.[9] Therefore, chronic use of medications that exacerbate NLRP3 activity could theoretically accelerate these pathological processes in both forms of AMD, potentially increasing disease risk and progression.

Bisphosphonates, widely used to treat osteoporosis, have been shown to increase NLRP3 expression and promote the release of proinflammatory cytokines. Preclinical studies have consistently demonstrated that bisphosphonates enhance NLRP3 activity in immune cells.[9–15] However, the clinical impact of this potential NLRP3 activation on AMD risk remains unknown. To address this gap, we investigated the potential association between bisphosphonate use and the risk of developing AMD.

Using a large national cohort of US veterans with a DEXA scan, we observed a significant increase in the hazard for incident AMD among patients treated with bisphosphonates. Results were consistent before and after propensity score matching, with hazard ratios suggesting an approximate 5% increase in the hazard for each additional year of bisphosphonate treatment. Moreover, our secondary analysis results suggests that the use of direct NLRP3 inhibitors such as fluoxetine and fluvoxamine can reduce the risk of AMD among patients taking bisphosphonates. Collectively, the results of this study provide more data supporting the notion that the NLRP3 inflammasome is an important therapeutic target for AMD.

The present study has important limitations that warrant careful consideration when interpreting the results. The use of a nonactive comparator design, due to smaller sample sizes of potential comparators (e.g., denosumab), represents a significant methodological constraint, as it hampers our ability to fully account for confounding by indication. The observed differences in AMD risk between treatment groups may partially reflect underlying variations in patient characteristics and disease severity rather than true treatment effects. Additionally, the inherent relationship between bisphosphonate therapy and more advanced-stage osteoporosis—itself independently associated with AMD—introduces potential residual confounding that could amplify the observed association between bisphosphonate exposure and AMD risk. Importantly, however, our secondary analysis only looks at patients with bisphosphonate expo-

Table 5. FLX/FLV Use Among Bisphosphonate Users: Hazard of AMD

Variable	Original, HR (95% CI)	PS-Matched, HR (95% CI)
Fluoxetine/fluvoxamine use	0.81 (0.701–0.937)	0.814 (0.676–0.98)
Age	1.061 (1.057–1.066)	1.063 (1.054–1.072)
Race other/unknown vs. Black	3.362 (2.568–4.401)	2.819 (1.554–5.114)
White vs. Black	3.812 (3.038–4.784)	2.88 (1.792–4.63)
Male	1.054 (0.913–1.218)	1.12 (0.848–1.48)
Charlson comorbidity index	1.011 (0.989–1.033)	1.033 (0.986–1.082)
BMI 18.5–24.9 vs. <18.5	0.831 (0.652–1.058)	0.915 (0.529–1.584)
25–29.9 vs. <18.5	0.715 (0.56–0.912)	0.685 (0.393–1.192)
30+ vs. <18.5	0.687 (0.535–0.881)	0.67 (0.382–1.177)
Missing vs. <18.5	0.968 (0.42–2.229)	0.972 (0.127–7.423)
Hypertension	1.173 (1.067–1.288)	1.226 (0.99–1.518)
Hyperlipidemia	0.96 (0.882–1.045)	1.022 (0.844–1.238)
Pure hypercholesterolemia	0.868 (0.741–1.017)	0.744 (0.5–1.105)
Smoker	0.865 (0.778–0.961)	0.851 (0.692–1.047)
Index year	1.024 (1.013–1.035)	1.025 (1–1.05)

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sure comparing the use of known NLRP3 inhibitors, fluoxetine and fluvoxamine. The results of the secondary analysis are consistent with prior research showing these medications are associated with lower AMD risks.[6] Moreover, despite our attempts to control for known confounders, the observational nature of our study design limits our ability to establish causal relationships definitively. To address these limitations, future research should utilize active comparator designs and adopt more advanced methods to control for disease severity, leveraging the availability of DEXA scan results to better account for potential confounding. Furthermore, future studies should investigate the association between bisphosphonate use and the progression to advanced disease in patients with early-stage AMD.

Long-term use of bisphosphonates may be associated with elevated risk of AMD. Subsequent research would need to confirm the results of this study. Our sample comprised US veterans and may not be generalized to other patient groups. Moreover, the results of this study should not be used clinically in the decision to use or continue to use bisphosphonates. Rather, this study was undertaken to evaluate NLRP3 as a possible target for AMD in the context of drug development.

Conclusions

The NLRP3 inflammasome inhibitors have been proposed as a pharmacologic therapy for AMD. The goal of this article was to evaluate the NLRP3 inflammasome as a therapeutic target for AMD by examining the effect of drugs that increase NLRP3 and inflammatory cytokine expression. Bisphosphonates, commonly used for osteoporosis, also increase NLRP3 expression. Our study results found that increased exposure to these medications results in a higher hazard of AMD. The results of this study provided evidence supporting the importance of the NLRP3 inflammasome in the pathogenesis of AMD.

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