Nitrogen-Based Bisphosphonate Use and Ovarian Cancer Risk in Women Aged 50 Years and Older

Karen M. Tuesley, MEpi1,2, Penelope M. Webb, DPhil1,2, Melinda M. Protani, PhD1, Katrina Spilsbury, PhD3, Sallie-Anne Pearson, PhD4, Michael D. Coory, PhD5, Peter Donovan, MBBS6,7, Christopher Steer, MBBS8,9 Louise M. Stewart, PhD10, Nirmala Pandeya, PhD1,2, Susan J. Jordan, PhD1,2

1School of Public Health, Faculty of Medicine, University of Queensland, Brisbane, Australia
2Population Health Department, QIMR Berghofer Medical Research Institute, Brisbane, Australia
3Institute for Health Research, The University of Notre Dame Australia, Fremantle, Australia
4Centre for Big Data Research in Health, UNSW, Sydney, Australia
5Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, Australia
6Clinical Pharmacology Department, Royal Brisbane and Women’s Hospital, Brisbane, Australia
7Faculty of Medicine, University of Queensland, Brisbane, Australia
8Border Medical Oncology, Albury-Wodonga Regional Cancer Centre, Albury, Australia
9University of NSW Rural Clinical School, Albury Campus, Albury, New South Wales, Australia
10School of Population and Global Health, The University of Western Australia, Perth, Australia

Corresponding Author:
Karen M. Tuesley
School of Public Health
266 Herston Road
The University of Queensland
Herston QLD 4006, Australia
K.Tuesley@uq.edu.au
+61 7 3365 5393

© The Author(s) 2022. Published by Oxford University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Abstract

Background: There are few readily modifiable risk factors for epithelial ovarian cancer; pre-clinical studies suggest bisphosphonates could have chemo-preventive actions. Our study aimed to assess the association between use of nitrogen-based bisphosphonate medicine and risk of epithelial ovarian cancer, overall and by histotype.

Methods: We conducted a case-control study nested within a large linked administrative dataset including all Australian women enrolled for Medicare, Australia’s universal health insurance scheme, between July 2002 and December 2013. We included all women with epithelial ovarian cancer diagnosed at age 50 years and older between 1st July 2004 and 31st December 2013 (n=9,367) and randomly selected up to five controls per case, individually matched to cases by age, state of residence, area-level socioeconomic status, and remoteness of residence category (n=46,830). We used prescription records to ascertain use of nitrogen-based bisphosphonates (ever use and duration of use), raloxifene and other osteoporosis medicines (non-nitrogen-based bisphosphonates, strontium and denosumab). We calculated adjusted odds ratios (OR) and 95% confidence intervals (CI) using conditional logistic regression.

Results: Ever use of nitrogen-based bisphosphonates was associated with a reduced risk of epithelial ovarian cancer compared to non-use (OR=0.81, 95%CI:0.75-0.88). There was a reduced risk of both endometrioid (OR=0.51, 95%CI:0.33-0.79) and serous histotypes (OR=0.84, 95%CI:0.75-0.93), but no association with the mucinous or clear cell histotypes.

Conclusion: Use of nitrogen-based bisphosphonates was associated with a reduced risk of endometrioid and serous ovarian cancer. This suggests the potential for use for prevention, although validation of our findings is required.
Epithelial ovarian cancer (EOC) is the eighth most commonly diagnosed cancer in women,¹ but, in contrast to other common women’s cancers, the five-year survival rate has not improved substantially over time, remaining below 50%.¹ Better treatments and prevention are therefore required to reduce the burden from this disease. Unfortunately, most established risk factors for EOC are not readily modifiable² and thus new avenues for prevention need to be explored.

One potential approach that has received attention is the repurposing of existing chronic diseases medicines for cancer prevention.³,⁴ In EOC, evidence suggests chemopreventive potential for medicines including statins⁵ and aspirin.⁶ Nitrogen containing bisphosphonates constitute another medicine class with anticancer potential,⁷ but these have been infrequently investigated in EOC. Like statins, these osteoporosis medicines act on enzymes in the mevalonate pathway, potentially inhibiting cancer cell proliferation.⁷ Some preclinical work suggests they inhibit growth in EOC cell lines,⁸,⁹ and in murine models, nitrogen-based bisphosphonates have been shown to suppress EOC.⁹ Some epidemiological studies also suggest bisphosphonate use might be inversely associated with EOC incidence;¹⁰ however, most did not separate nitrogen-based and non-nitrogen-based bisphosphonates, or EOC histotypes, which are known to have differing etiology.¹¹

To investigate the potential chemopreventive effects of nitrogen-based bisphosphonates for EOC we aimed to comprehensively assess the association between nitrogen-based bisphosphonate use and risk of EOC overall and by histotype using a large national linked health dataset with accurate medicine dispensing information, comparing and contrasting the associations observed for other osteoporosis medicines.

**Methods**

**Data source and study population**

We conducted a nested case-control study using linked administrative data to investigate the association between bisphosphonate use and EOC (Figure 1). We assembled a cohort of Australian women aged 18 years and over enrolled for Medicare, Australia’s universal health insurance scheme,
between July 2002 and December 2013. All Australian citizens and permanent residents are eligible for Medicare, therefore essentially all Australian women are included on the Medicare Enrolments File. These records were linked to the Pharmaceutical Benefits Scheme (PBS), the Australian Cancer Database and the National Death Index (NDI). The PBS included details of all subsidized dispensed medicines (including bisphosphonates) from July 2002 onwards, and all PBS medicines after April 2012 (the PBS includes most medicines prescribed in Australia). The Australian Cancer Database included all cancer diagnoses from 1982 to December 2013 (registration of cancer diagnoses is mandatory in Australia), and the NDI included all death records from 2002-2017. Supplementary Figure 1 describes each dataset and the period covered. We excluded women who enrolled for Medicare after 1st July 2002 as adults, because the majority would have been immigrants and we may not have had complete cancer histories for them. There were 8,441,783 women eligible for the study. The study was approved by the Human Research Ethics Committees of the University of Queensland, The QIMR Berghofer Medical Research Institute, the Australian Institute of Health and Welfare (AIHW) and all other relevant governance bodies.

**Case and control definition**

We required a minimum two-year PBS history, including a six-month exclusion period before cancer diagnosis (index date for controls) to account for medicine changes related to pre-diagnosis cancer symptoms, and 18-months or more prescription dispensing history to ascertain medicine use. Cases included all those with EOC registered between 1st July 2004 and 31st December 2013. We used the International Classification of Diseases for Oncology topography codes to ascertain ovarian cancer (including fallopian tube and primary peritoneal cancer, Supplementary Table 1), and morphology codes to identify epithelial ovarian cancers. We classified EOC histotype using the criteria from the 2016 CONCORD-2 study (Supplementary Table 2)¹² but could not distinguish between low- and high-grade serous carcinoma. We excluded those with previous cancer diagnoses; those aged under 50 years at diagnosis (n=1,614, as bisphosphonate use is rare in these women); five women who used bisphosphonates prescribed for Paget’s disease or cancer (pamidronate disodium, tiludronate, ibandronate and clodronate: ATC codes M05BA02, M05BA03, M05BA05 and
M05BA06); and those without a Socio-Economic Indexes for Areas (SEIFA) score (defined below) assigned to their postcode (n=50), leaving 9,367 cases.

We used EOC diagnosis date as the index date for cases. Using risk-set sampling, we randomly selected up to five controls per case from the study population alive at the index date, with no prior diagnosis of cancer or bisphosphonate use for Paget’s disease/cancer. Controls were matched to cases by birth year (within one year), state of residence, area-level socioeconomic status, and remoteness category (defined below). We identified 46,830 matched controls. Women could be selected as a control for more than one case, and women with EOC could be selected as controls prior to their EOC diagnosis.

We used Medicare enrolment postcodes to determine women’s state of residence and to estimate area-level socioeconomic status using the SEIFA Index of Relative Socio-Economic Disadvantage. We classified participants into SEIFA quintiles ranging from the most disadvantaged (1st quintile) to the least (5th quintile). We assigned a remoteness-of-residence category based on postcodes using the Accessibility/Remoteness Index of Australia (ARIA), with categories including major cities, inner regional, outer regional, remote and very remote (combining the latter two groups due to their small populations). We used the earliest available SEIFA (2001) and ARIA (2006) indices; however, if the relevant score was missing for the postcode, the 2006 or 2011 score was used. Although Medicare enrolments dated back to 1983, and residence may have changed before 2001, the socio-economic status of the population living within each postcode are unlikely to have changed substantially during this time.

Exposure variables

We categorized medicines according to the Anatomical Therapeutic Chemical (ATC) classification system, PBS item codes and dispensing dates (Supplementary Table 3), and included nitrogen-based bisphosphonates, raloxifene and other osteoporosis medicines (non-nitrogen-based bisphosphonates, strontium and denosumab). We classified participants as a medicine user when they had at least two dispensing records for that medicine within a 12-month period from July 2002 until six-months prior to index date (12-months as a sensitivity analysis), except for the injectables,
zoledronic acid (nitrogen-based bisphosphonate) and denosumab, for which women were classified as users after one dispensed prescription. We categorized women as users of nitrogen-based bisphosphonates, raloxifene or other osteoporosis medicines only, or as users of a combination of two or all three groups.

We calculated the total defined daily dose (DDD) for each woman using the WHO Collaborating Centre for Drug Statistics Methodology\textsuperscript{16} DDD for each ATC code, the number of DDDs for the PBS item code (Supplementary Table 3), and the quantity dispensed. We calculated each woman’s use period from the date first defined as a user until the date of their last prescription plus the total daily doses dispensed at this date, excluding use in the six-months prior to index date. We defined one year or more of nitrogen-based bisphosphonate use as a use period of 12 or more months plus at least 80\%\textsuperscript{17} of 365.25 daily doses. Zoledronic acid is usually administered yearly,\textsuperscript{18} therefore we considered each injection as one year’s use. We defined women as users for three years or more once they had a use period of 36 months or more and 80\% of three years of daily doses (DDD\textgeq 877), and users for five years or more once they had a use period of 60 months or more and 80\% of five years of daily doses (DDD\textgeq 1,461). Each woman was assigned one of five duration of use categorizes (no use, <1 year, 1-<3 years, 3-<5 years, 5+ years).

\textbf{Covariates}

In addition to our matching variables, we considered several other potential confounders determined using directed acyclic graphs.\textsuperscript{19}

To adjust for potential confounding by comorbidity, we used the validated weighted Rx-Risk Comorbidity Score (Rx-Risk score),\textsuperscript{20} which has been mapped to Australian PBS item codes.\textsuperscript{21} We did not have records for all medicines included in the Score (Supplementary Table 4); however, most of those missing have low or zero weighting or are used for rare conditions unlikely to materially affect the likelihood of a woman being diagnosed with EOC. Medicines for respiratory disease and depression were the only weighted, common medicines not available in the dataset; however, these conditions are unlikely to confound the relationship between osteoporosis medicines and EOC. We used the Rx-Risk score for each woman calculated at six-months prior to index date, with a maximum
of five-years medicine history used to identify comorbidities for each woman. We excluded osteoporosis/Paget's disease from the score. As a sensitivity analysis, we restricted PBS history for the Rx-Risk score to two-years prior to the index date, to check for bias due to a shorter PBS history.

We had limited information on other potential confounders for the whole national dataset, therefore we conducted several sensitivity analyses to assess the likely effect on our results. These are described in detail in the Supplementary Methods. First, we restricted analyses to Western Australian women, for whom we had additional hospital morbidity information (Supplementary Table 5) allowing us to exclude those with a bilateral salpingo-oophorectomy (BSO) prior to the index date (therefore essentially no longer at risk of ovarian cancer), and additionally matched controls to cases by parity. Secondly, we restricted our analyses to women defined as PBS concessional beneficiaries who had information available for the whole study period on all dispensed PBS medicines. In this group we additionally adjusted for MHT use, and specifically for use of type II diabetes mellitus medicines, which have been shown in Australian data to predict obesity well (see Supplementary Methods). We defined women as MHT users if they were dispensed at least two of either estrogen-only or combined MHT (ATC codes G03C and G03F) within a 12-month period at any point up to six-months prior to the index date.

We also performed quantitative bias analyses to assess for possible confounding by obesity or early age at menopause (<45 years - as risk of osteoporosis increases and EOC risk decreases with early menopause). We used estimates of obesity prevalence amongst users and non-users of bisphosphonates and estimates of prevalence of early menopause amongst women with and without hip fracture. Finally, we assessed the potential for remaining residual confounding, by calculating the E-value for our main results, to determine the minimum strength of any unmeasured confounder required to explain away our effect estimates.

Statistical Analysis

We used conditional logistic regression to estimate ORs and 95% CIs for the association between the osteoporosis medicines and the risk of EOC overall and by serous, endometrioid, mucinous and clear cell histotype. Results were considered to be statistically significant if the 95% CI
did not include 1.00. We estimated ORs as approximation for risk ratios and adjusted the models for the weighted Rx-Risk score as a continuous variable. We assessed the association for ever-use of nitrogen-based bisphosphonates compared to no use and additionally assessed the association for nitrogen-based bisphosphonate by duration of use with no nitrogen-based bisphosphonate use as the reference. We excluded cases diagnosed with EOC before 1st July 2008 for the duration of use analysis, to allow sufficient PBS history to categorize women as users for these durations. Analyses were performed in SAS 9.4 (SAS Institute Inc., N.C.).

Results

The characteristics of the cases and controls are shown in Table 1. Table 2 shows the results of our main analyses. We found that use of nitrogen-based bisphosphonates was associated with a reduced EOC risk (OR=0.81,95% CI:0.75-0.88) compared to non-use. Reducing the period included in the Rx-Risk score calculation to two years before index date, did not alter our results (OR=0.81,95% CI:0.75-0.88), nor did using a 12-month exclusion period for medicine use prior to the index date (OR=0.80,95% CI:0.74-0.87). The association was weaker for under a year of use (OR=0.94,95% CI:0.79-1.11), compared to longer durations; however, the OR for one to three years (OR=0.81,95% CI:0.67-0.97) did not differ materially from the estimate for five years or more (OR=0.80,95% CI:0.68-0.94).

When we categorized medicine use into ever-use of nitrogen-based bisphosphonate, raloxifene or other osteoporosis medicines, or users of a combination of two or all three of these groups, the association between nitrogen-based bisphosphonates and EOC risk did not change (Table 2). The odds ratios for the association between use of raloxifene or other osteoporosis medicines and EOC were below one, however confidence intervals were wide due to the small number of users. For women who used medicines from more than one group, the results were similar to nitrogen-based bisphosphonates alone because 97.0% had also used nitrogen-based bisphosphonates.

Table 3 shows the results by EOC histotype. Due to small numbers, we categorized duration of use as more or less than one year only. There was a strong inverse association with endometrioid...
EOC (OR=0.51, 95%CI:0.33-0.79), particularly for use of at least one year (OR=0.44, 95%CI:0.25-0.76). A statistically significant reduced risk of serous EOC was also observed (ever use: OR=0.84, 95%CI:0.75-0.93). The estimates for mucinous and clear cell cancers were close to one and much less precise due to the small number of women with these histotypes.

Supplementary Table 6 shows characteristics of cases and controls included in our sensitivity analyses. Our results did not materially change when we repeated our analysis restricting to Western Australian women, additionally matching cases and controls by parity and excluding women with a BSO prior to the index date (Supplementary Table 7). Further adjustment for hysterectomy and unilateral-salpingo-oophorectomy did not change the results.

When we included only women who were concessional beneficiaries, the OR was slightly closer to one compared to our main analysis (OR=0.87, 95%CI:0.80-0.95, Supplementary Table 8, but there was no change after adjusting for MHT or diabetes medication use (as an indicator of obesity). When we looked at the combination of nitrogen-based bisphosphonate with MHT and then without MHT use, associations with EOC were similar (Supplementary Table 8). Our quantitative bias analysis accounting for obesity (Supplementary Table 9) and early menopause (Supplementary Table 10) suggested that estimates would not differ materially from our main analysis had we been able to adjust for obesity or early menopause. For completeness we also calculated E-values\textsuperscript{26,27} (Figure 2), which also suggested that result was not sensitive to confounding for this dataset, although these types of sensitivity analyses cannot be definitive.

**Discussion**

Our large population-based data linkage study showed that, among women aged over 50 years, use of nitrogen-based bisphosphonates was associated with reduced EOC risk, with an almost 50% lower risk of the endometrioid histotype, and a 16% lower risk of serous cancers. Risk reduction appeared stronger for use longer than a year compared to less but did not clearly reduce further beyond this. There was no apparent association between use of nitrogen-based bisphosphonates and clear cell or mucinous histotypes.
Our overall results were consistent with a previous meta-analysis of observational studies,\textsuperscript{10} although the results of that analysis were not statistically significant, did not consider EOC histotype and did not separate out effects of nitrogen-based bisphosphonates from other bisphosphonates. Some studies have focused on nitrogen-based bisphosphonates. A follow-up of a randomized controlled trial investigating the efficacy of zoledronic acid (a nitrogen-based bisphosphonate) for fracture prevention in women (aged >65 years), found a lower risk of gynecological cancer among those receiving zoledronic acid (HR=0.50, 95%CI: 0.10-1.99).\textsuperscript{28} However, this analysis included only nine cases, and it did not specify how many had EOC. A pooled analysis found an inverse association only for risedronate use (a nitrogen-based bisphosphonate) and EOC risk, but no association with bisphosphonate use overall,\textsuperscript{29} in keeping with our findings suggesting that the different mechanisms of action for nitrogen-based bisphosphonates compared to non-nitrogen-based bisphosphonates may be relevant for EOC incidence. Nitrogen-based bisphosphonates have been shown to inhibit the mevalonate pathways within macrophages and monocytes, thereby reducing activity of tumor-associated macrophages and activating gamma delta T cells.\textsuperscript{7} These mechanisms potentially inhibit cancer cell proliferation, including in ovarian tumor cells.\textsuperscript{8,9}

We found that raloxifene, which may be an estrogen antagonist in ovarian cancer cells,\textsuperscript{30} was associated with a non-statistically significant reduced risk of EOC. The effects of raloxifene have been studied in several randomized controlled trials of postmenopausal women, and a meta-analysis showed a non-statistically significant 50% lower risk of ovarian cancer in the raloxifene treatment group.\textsuperscript{31} Studies including larger numbers of women who have used raloxifene are required to confirm this association.

Our large study had several strengths. We included 9,367 women with ovarian cancer, and we could therefore separately investigate the associations by EOC histotype. We used objective health records to measure medicine use rather than relying on self-report. Our use of linkage to cancer and death records meant that we had complete outcomes for the Australian population, and therefore our results are generalizable to Australian women over 50 years of age.
A limitation of our study was that we did not have full population data on potential confounders such as obesity, parity, MHT and early menopause. However, our sensitivity analyses exploring the likely impact of adjusting for these variables suggested that our conclusions would not have changed. While we did not separate types of MHT, the risk of EOC has been found to not differ materially between estrogen-only and combined preparations.\textsuperscript{32} We did not have hospital records for all women, so could not exclude all those who had a BSO and were therefore no longer at risk of ovarian cancer. However, our analyses restricted to Western Australian women (approximately 10\% of the Australian population), for whom we had this information, excluding those with a BSO did not materially change the results. It is possible there is residual confounding by other unmeasured factors. However, most other established ovarian cancer risk/protective factors (family history, oral contraceptive pill use, tubal ligation) do not have strong links to bisphosphonate use,\textsuperscript{33} therefore are unlikely to be strong enough confounders to explain the association. We were unable to separate low-grade and high-grade serous carcinoma. Given that these have been shown to have different etiologies, this may have affected the magnitude of our estimate and our dose-response calculations.

Another possible explanation for our results is confounding by indication. Bisphosphonates are used mainly for osteoporosis, which is associated with relatively lower estrogen levels.\textsuperscript{34} Estrogen receptors are present in the majority of serous and endometrioid carcinomas, but only a small percentage of mucinous and clear cell carcinomas,\textsuperscript{35} and MHT use has been linked to an increased risk of serous and endometrioid EOC\textsuperscript{36} suggesting that estrogen plays a role in the development of these cancers. However, arguing against confounding by indication is that a large, prospective cohort study of 36,115 women with an average follow-up of 8.3 years, did not find an association between incidence of osteoporotic fractures and risk of EOC.\textsuperscript{37} We also found some evidence of a dose-response for nitrogen-based bisphosphonate of more than one year overall and for the endometrioid histotype, as well as a stronger association for nitrogen-based bisphosphonate use compared to other osteoporosis medications. This suggests the association found between nitrogen-based bisphosphonates and EOC is unlikely to be purely due to an inverse association between osteoporosis and EOC.
While our findings require replication in other datasets with accurate medicine data, our results have the potential to help inform medicine choice for women with osteoporosis and suggest additional avenues for exploration of mechanisms of ovarian carcinogenesis. The possible benefit of using an existing chronic disease medication, potentially just a yearly injection, to reduce the risk of ovarian cancer warrants further investigation.

**Funding**

This work was supported by a project grant from the Australian National Health and Medical Research Council (NHMRC, APP1121151). PW was supported by NHMRC Investigator Grant GNT1173346. NP’s salary was supported by a NHMRC grant (APP1185416). KT was supported by an Australian Government Research Training Program scholarship.

**Notes**

*Role of the funders:* The funders had no role in the design of the study, the collection, analysis, interpretation of the data, writing of this manuscript, or the decision to submit it for publication.

*Author disclosures:* The authors have no conflicts of interest to disclose.

*Author contributions:* Conceptualization – SJ, PW, MP, SP, MC, PD, CS, LS. Data analysis: KT, SJ, KS, NP. Writing--original draft - KT, SJ. Writing--review, and editing: all authors.

*Prior presentations:* On demand presentation at the virtual World Congress of Epidemiology 2021, 3-6 September 2021.

*Acknowledgments:* The authors wish to thank the staff from the Australian Institute of Health and Welfare (AIHW) for data linkage and custodians of the Medicare Enrolments File, Pharmaceutical Benefits Scheme, Australian Cancer Database, and National Death Index. We also wish to thank the staff at the Western Australian Data Linkage Branch and staff and custodians of the Electoral Roll, Emergency Department Data Collection, Hospital Morbidity Data Collection, Midwives Notification System, and Birth Registrations.
**Data Availability Statement**

Due to the nature of this population-based research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

**References**

1. Ferlay J EM, Lam F, Colombet M, Mery L, Pineros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer. Accessed 30/01/2019, [https://gco.iarc.fr/today](https://gco.iarc.fr/today)

2. Whiteman DC, Webb PM, Green AC, et al. Cancers in Australia in 2010 attributable to modifiable factors: summary and conclusions. *Aust N Z J Public Health*. Oct 2015;39(5):477-84. doi:10.1111/1753-6405.12471

3. Lee DK, Szabo E. Repurposing Drugs for Cancer Prevention. *Curr Top Med Chem*. 2016;16(19):2169-78. doi:10.2174/1568026616666160216154946

4. Frantzi M, Latosinska A, Mokou M, Mischak H, Vlahou A. Drug repurposing in oncology. *Lancet Oncol*. Dec 2020;21(12):e543. doi:10.1016/S1470-2045(20)30610-0

5. Mohammadian-Hafshejani A, Sherwin CMT, Heidari-Soureshjani S. Do statins play any role in reducing the incidence and mortality of ovarian cancer? A systematic review and meta-analysis. *J Prev Med Hyg*. Sep 2020;61(3):E331-E339. doi:10.15167/2421-4248/jpmh2020.61.3.1497

6. Santucci C, Gallus S, Martinetti M, La Vecchia C, Bosetti C. Aspirin and the risk of nondigestive tract cancers: An updated meta-analysis to 2019. *Int J Cancer*. Mar 15 2021;148(6):1372-1382. doi:10.1002/ijc.33311

7. Billington EO, Reid IR. Benefits of Bisphosphonate Therapy: Beyond the Skeleton. *Curr Osteoporos Rep*. Oct 2020;18(5):587-596. doi:10.1007/s11914-020-00612-4

8. Abdullah MI, Abed MN, Richardson A. Inhibition of the mevalonate pathway augments the activity of pitavastatin against ovarian cancer cells. *Sci Rep*. Aug 14 2017;7(1):8090. doi:10.1038/s41598-017-08649-9
9. Kobayashi Y, Kashima H, Rahmanto YS, et al. Drug repositioning of mevalonate pathway inhibitors as antitumor agents for ovarian cancer. *Oncotarget*. Sep 22 2017;8(42):72147-72156. doi:10.18632/oncotarget.20046

10. Zhang XS, Zhang YM, Li B, Fan B, Zhao Y, Yang SJ. Risk reduction of endometrial and ovarian cancer after bisphosphonates use: A meta-analysis. *Gynecol Oncol*. Sep 2018;150(3):509-514. doi:10.1016/j.ygyno.2018.06.012

11. Wentzensen N, Poole EM, Trabert B, et al. Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. *J Clin Oncol*. Aug 20 2016;34(24):2888-98. doi:10.1200/JCO.2016.66.8178

12. Matz M, Coleman MP, Carreira H, et al. Worldwide comparison of ovarian cancer survival: Histological group and stage at diagnosis (CONCORD-2). *Gynecol Oncol*. Feb 2017;144(2):396-404. doi:10.1016/j.ygyno.2016.11.019

13. SEIFA 2011. Australian Bureau of Statistics. Updated 19 September 2013. [http://www.abs.gov.au/websitedbs/censushome.nsf/home/seifa2011?opendocument&navpos=260](http://www.abs.gov.au/websitedbs/censushome.nsf/home/seifa2011?opendocument&navpos=260)

14. ABS Geography Publications. Australian Bureau of Statistics. Updated 21 April 2017. 2017. [http://www.abs.gov.au/websitedbs/D3310114.nsf/home/ABS+Geography+Publications](http://www.abs.gov.au/websitedbs/D3310114.nsf/home/ABS+Geography+Publications)

15. Ware V. Addressing locational disadvantage effectively (International evidence). *Australian Housing and Urban Research Institute, Melbourne*. 2010;

16. ATC/DDD Index. WHO Collaborating Centre for Drug Statistics Methodology, Norgwegian Institute of Public Health. Updated 13/12/2018. [https://www.whocc.no/atc_ddd_index/](https://www.whocc.no/atc_ddd_index/)

17. Fardellone P, Lello S, Cano A, et al. Real-world Adherence and Persistence with Bisphosphonate Therapy in Postmenopausal Women: A Systematic Review. *Clin Ther*. Aug 2019;41(8):1576-1588. doi:10.1016/j.clinthera.2019.05.001

18. Lambrinoudaki I, Vlachou S, Galapi F, Papadimitriou D, Papadias K. Once-yearly zoledronic acid in the prevention of osteoporotic bone fractures in postmenopausal women. *Clin Interv Aging*. 2008;3(3):445-51. doi:10.2147/cia.s2046
19. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol*. Dec 1 2016;45(6):1887-1894. doi:10.1093/ije/dyw341

20. Lu CY, Barratt J, Vitry A, Roughead E. Charlson and Rx-Risk comorbidity indices were predictive of mortality in the Australian health care setting. *J Clin Epidemiol*. Feb 2011;64(2):223-8. doi:10.1016/j.jclinepi.2010.02.015

21. Pratt NL, Kerr M, Barratt JD, et al. The validity of the Rx-Risk Comorbidity Index using medicines mapped to the Anatomical Therapeutic Chemical (ATC) Classification System. *BMJ Open*. Apr 13 2018;8(4):e021122. doi:10.1136/bmjopen-2017-021122

22. Ali S, Na R, Waterhouse M, et al. Predicting obesity and smoking using medication data: A machine-learning approach. *Pharmacoepidemiol Drug Saf*. Oct 5 2021;doi:10.1002/pds.5367

23. Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, Greenland S. Good practices for quantitative bias analysis. *Int J Epidemiol*. Dec 2014;43(6):1969-85. doi:10.1093/ije/dyu149

24. Olsen CM, Nagle CM, Whiteman DC, et al. Obesity and risk of ovarian cancer subtypes: evidence from the Ovarian Cancer Association Consortium. *Endocr Relat Cancer*. Apr 2013;20(2):251-62. doi:10.1530/ERC-12-0395

25. Banks E, Reeves GK, Beral V, et al. Hip fracture incidence in relation to age, menopausal status, and age at menopause: prospective analysis. *PLoS Med*. Nov 2009;6(11):e1000181. doi:10.1371/journal.pmed.1000181

26. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med*. Aug 15 2017;167(4):268-274. doi:10.7326/M16-2607

27. Mathur MB, VanderWeele TJ. Sensitivity Analysis for Unmeasured Confounding in Meta-Analyses. *J Am Stat Assoc*. 2020;115(529):163-172. doi:10.1080/01621459.2018.1529598

28. Reid IR, Horne AM, Mihov B, et al. Effects of Zoledronate on Cancer, Cardiac Events, and Mortality in Osteopenic Older Women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. Jan 2020;35(1):20-27. doi:10.1002/jbmr.3860
29. Vinogradova Y, Coupland C, Hippisley-Cox J. Exposure to bisphosphonates and risk of common non-gastrointestinal cancers: series of nested case-control studies using two primary-care databases. Br J Cancer. Aug 6 2013;109(3):795-806. doi:10.1038/bjc.2013.383

30. Song J, Fadiel A, Edusa V, et al. Estradiol-induced ezrin overexpression in ovarian cancer: a new signaling domain for estrogen. Cancer Lett. Mar 18 2005;220(1):57-65. doi:10.1016/j.canlet.2004.04.024

31. Neven P, Goldstein SR, Ciaccia AV, Zhou L, Silfen SL, Muram D. The effect of raloxifene on the incidence of ovarian cancer in postmenopausal women. Gynecol Oncol. May 2002;85(2):388-90. doi:10.1006/gyno.2001.6578

32. Beral V, Million Women Study C, Bull D, Green J, Reeves G. Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet. May 19 2007;369(9574):1703-10. doi:10.1016/S0140-6736(07)60534-0

33. Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. Am J Obstet Gynecol. Feb 2006;194(2 Suppl):S3-11. doi:10.1016/j.ajog.2005.08.047

34. Ko SS, Jordan VC. Treatment of osteoporosis and reduction in risk of invasive breast cancer in postmenopausal women with raloxifene. Expert Opin Pharmacother. Mar 2011;12(4):657-74. doi:10.1517/14656566.2011.557360

35. Lindgren PR, Cajander S, Backstrom T, Gustafsson JA, Makela S, Olofsson JI. Estrogen and progesterone receptors in ovarian epithelial tumors. Mol Cell Endocrinol. Jun 30 2004;221(1-2):97-104. doi:10.1016/j.mce.2004.02.020

36. Gapstur SM, Patel AV, Banks E, et al. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. Lancet. May 9 2015;385(9980):1835-1842. doi:10.1016/S0140-6736(14)61687-1

37. Danforth KN, Schairer C, Schatzkin A, Lacey JV. Bone fractures and incident epithelial ovarian cancer in a prospective cohort study. J Womens Health (Larchmt). Nov 2009;18(11):1777-82. doi:10.1089/jwh.2008.1341
38. Tuesley K, Webb P, Protani M, et al. Bisphosphonate use and risk of ovarian cancer, a nested case-control study using national health data. *International Journal of Epidemiology*. 2021;50(Supplement_1)doi:10.1093/ije/dyab168.667
## Tables

Table 1: Characteristics of women diagnosed with epithelial ovarian cancer (cases) and matched controls

| Characteristic                   | Cases No. (%) | Controls No. (%) |
|---------------------------------|---------------|-----------------|
| **Total**                       | 9,367         | 46,830          |
| **Age at diagnosis**            |               |                 |
| 50-59 years                     | 2,380 (25.4)  | 11,900 (25.4)   |
| 60-69 years                     | 2,894 (30.9)  | 14,470 (30.9)   |
| 70-79 years                     | 2,433 (26.0)  | 12,165 (26.0)   |
| 80-89 years                     | 1,450 (15.5)  | 7,248 (15.5)    |
| 90 years and older              | 210 (2.2)     | 1,047 (2.2)     |
| **SEIFA**                       |               |                 |
| 1 (most disadvantaged)          | 1,799 (19.2)  | 8,993 (19.2)    |
| 2                               | 1,885 (20.1)  | 9,422 (20.1)    |
| 3                               | 1,849 (19.7)  | 9,245 (19.7)    |
| 4                               | 1,899 (20.3)  | 9,495 (20.3)    |
| 5 (least disadvantaged)         | 1,935 (20.7)  | 9,675 (20.7)    |
| **Remoteness**                  |               |                 |
| Major city                      | 6,448 (68.8)  | 32,240 (68.8)   |
| Inner regional                  | 1,992 (21.3)  | 9,960 (21.3)    |
| Outer regional                  | 814 (8.7)     | 4,070 (8.7)     |
| Remote/very remote              | 113 (1.2)     | 560 (1.2)       |
| **Registered state**            |               |                 |
| New South Wales                 | 3,148 (33.6)  | 15,740 (33.6)   |
| Australian Capital Territory    | 133 (1.4)     | 665 (1.4)       |
| Victoria                        | 2,397 (25.6)  | 11,982 (25.6)   |
| Queensland                      | 1,789 (19.1)  | 8,945 (19.1)    |
| South Australia                 | 784 (8.4)     | 3,920 (8.4)     |
| Western Australia               | 832 (8.9)     | 4,160 (8.9)     |
| Tasmania                        | 255 (2.7)     | 1,273 (2.7)     |
| Northern Territory              | 29 (0.3)      | 145 (0.3)       |
| **Weighted Rx-Risk score at index date** | | |
| Mean (minimum, maximum)         | 0.8 (-4.15)   | 0.8 (-4.16)     |

*Matching variable. EOC: epithelial ovarian cancer; DDD: defined daily doses; MHT: menopausal hormone therapy; SEIFA: Socio-Economic Indexes for Areas.*
Table 2: Association between nitrogen-based bisphosphonates and epithelial ovarian cancer

| Medicine use category                                                                 | Cases No. (%) | Controls No. (%) | OR (95% CI) a |
|----------------------------------------------------------------------------------------|---------------|-----------------|---------------|
| Use of nitrogen-based bisphosphonates                                                  |               |                 |               |
| Non-user of NBB                                                                         | 8,438 (90.1)  | 41,318 (88.2)   | Reference     |
| Ever use of NBB                                                                         | 929 (9.9)     | 5,512 (11.8)    | 0.81 (0.75,0.88) |
| Use of NBB, raloxifene or other osteoporosis medicines                                   |               |                 |               |
| No use of NBB, raloxifene or other osteoporosis medicines                               | 8,322 (88.8)  | 40,663 (86.8)   | Reference     |
| NBB                                                                                   | 822 (8.8)     | 4,913 (10.5)    | 0.80 (0.74,0.87) |
| Raloxifene                                                                             | 67 (0.7)      | 382 (0.8)       | 0.85 (0.65,1.10) |
| Other                                                                                  | 48 (0.5)      | 252 (0.5)       | 0.92 (0.67,1.25) |
| Combinations b                                                                         | 108 (1.2)     | 620 (1.3)       | 0.83 (0.68,1.03) |
| Duration of nitrogen-based bisphosphonate use                                          |               |                 |               |
| Non-user of NBB c                                                                       | 4,948 (88.9)  | 24,298 (87.3)   | Reference     |
| Ever use of NBB c                                                                       | 618 (11.1)    | 3,529 (12.7)    | 0.85 (0.77,0.94) |
| NBB user <1 year c                                                                      | 173 (3.1)     | 896 (3.2)       | 0.94 (0.79,1.11) |
| NBB user 1-<3 years c                                                                    | 135 (2.4)     | 814 (2.9)       | 0.81 (0.67,0.97) |
| NBB user 3-<5 years c                                                                    | 124 (2.2)     | 700 (2.5)       | 0.86 (0.71,1.04) |
| NBB user 5+ years c                                                                     | 186 (3.3)     | 1,119 (4.0)     | 0.80 (0.68,0.94) |

a All models adjusted for weighted Rx-Risk score, and matched by age, SEIFA, remoteness, and registered state. NBB: nitrogen-based bisphosphonates; OR: odds ratio; CI: confidence interval

b Combinations includes use of NBB plus raloxifene and/or other osteoporosis medicines, or raloxifene and other osteoporosis medicines.

c Excludes cases diagnosed prior to 1st July 2008.

d Duration of use categories are mutually exclusive and are based on the following minimum use period and daily doses: <1 year: no minimum use requirements, 1-<3 years: use period of 12 months plus 272 daily doses, 3-<5 years: use period of 36 months plus 877 daily doses, 5+ years: use period of 60 months plus 1,461 daily doses. If a woman did not meet the minimum requirements of both the use period and daily doses, they were categorized in the previous duration of use group.
Table 3: Association between Nitrogen-based bisphosphonates by epithelial ovarian cancer histotype

| Medicine use category | Cases No. (%) | Controls No. (%) | OR (95% CI) b |
|-----------------------|--------------|-----------------|---------------|
| **Serous**            |              |                 |               |
| Non-user of NBB       | 4,800 (90.8) | 23,583 (89.3)   | Reference     |
| Ever User of NBB      | 485 (9.2)    | 2,840 (10.7)    | 0.84 (0.75,0.93) |
| NBB user <1 year      | 134 (2.5)    | 790 (3.0)       | 0.83 (0.69,1.01) |
| NBB user 1 year+ c    | 351 (6.6)    | 2,050 (7.8)     | 0.84 (0.74,0.94) |
| **Endometrioid**      |              |                 |               |
| Non-user of NBB       | 570 (95.8)   | 2,741 (92.1)    | Reference     |
| Ever User of NBB      | 25 (4.2)     | 234 (7.9)       | 0.51 (0.33,0.79) |
| NBB user <1 year      | 10 (1.7)     | 71 (2.4)        | 0.67 (0.34,1.33) |
| NBB user 1 year+ c    | 15 (2.5)     | 163 (5.5)       | 0.44 (0.25,0.76) |
| **Mucinous**          |              |                 |               |
| Non-user of NBB       | 424 (91.2)   | 2,127 (91.5)    | Reference     |
| Ever User of NBB      | 41 (8.8)     | 198 (8.5)       | 1.07 (0.74,1.54) |
| NBB user <1 year      | 9 (1.9)      | 65 (2.8)        | 0.71 (0.35,1.46) |
| NBB user 1 year+ c    | 32 (6.9)     | 133 (5.7)       | 1.23 (0.81,1.86) |
| **Clear Cell**        |              |                 |               |
| Non-user of NBB       | 412 (92.0)   | 2,067 (92.3)    | Reference     |
| Ever User of NBB      | 36 (8.0)     | 173 (7.7)       | 1.08 (0.73,1.60) |
| NBB user <1 year      | 16 (3.6)     | 40 (1.8)        | 2.04 (1.12,3.71) |
| NBB user 1 year+ c    | 20 (4.5)     | 133 (5.9)       | 0.78 (0.47,1.28) |

a Undifferentiated cancers and less common histotypes were excluded. NBB: nitrogen-based bisphosphonates; OR: odds ratio; CI: confidence interval

b All models adjusted for weighted Rx-Risk score, and matched by age, SEIFA, remoteness, and registered state.

c Minimum 1 year use period plus at least 80% of daily doses for 1 year or any use of zoledronic acid.
Figure Legends

Figure 1: Flowchart of the selection of cases and controls for the study. SEIFA = Socio-Economic Indexes for Areas.

Figure 2: E-values for the associations between (A) nitrogen-based bisphosphonate use and risk of epithelial ovarian cancer and (B) nitrogen-based bisphosphonate use and risk of endometrioid epithelial ovarian cancer histotype. The E-value indicates the strength of association required between the exposure and the unmeasured confounder AND the confounder and the outcome to remove the observed association. Thus, here the relative risk for the relationship between the confounder and nitrogen-based bisphosphonates, and the confounder and epithelial ovarian cancer would need to be at least 1.77 (lower 95% CI = 1.53) (or values per the curve). The equivalent value for the endometrioid histotype is 3.33 (lower 95% CI = 1.85). If one of the two parameters is smaller than the E-value, the other must be larger, as defined by the plotted curve.\textsuperscript{26,27} CI = Confidence interval.
Australian Women over 18 years and alive on 1 July 2004, with no prior cancer diagnosis, who were enrolled in Medicare on or before 1 July 2002. (n=8,672,838)

Excluded:
- Women who used Paget’s disease or cancer bisphosphonate medicines prior to July 2004. (n=1,088)

Eligible women (n=8,671,750)

Women diagnosed with epithelial ovarian cancer from 1 July 2004 to 31 December 2013. (n=11,053)

Excluded:
- Women who had prior use of Paget’s disease or cancer bisphosphonate medicines prior to diagnosis (n=5)
- Women under 50 years at diagnosis (n=1,631)
  - Missing SEIFA score (n=50)

Cases (n=9,367)

The same exclusion criteria was used for control selection with all women being eligible for selection until cancer diagnosis, use of Paget’s disease or cancer bisphosphonate medicines, or death. SEIFA scores were missing in <1% of eligible women.

Up to 5 Controls matched to each case by birth year (within 1 year), state of residence, area-level socioeconomic status, and remoteness category. (n=46,830)
