Ko Humphrey H. T. (Orcid ID: 0000-0003-3107-4240)

Title: A sequence symmetry analysis of the interrelationships between statins, diabetes, and skin infections.

Running title: Statins, diabetes, and skin infections.

Authors: Humphrey H. T. Ko[1,2#], Ricky R. Lareu[1,2], Brett R. Dix[1], Jeffery D. Hughes[1], Richard W. Parsons[3]

[#]**Corresponding author:** Humphrey Ko <h.ko@curtin.edu.au>

PI statement: The authors confirm that the Principal Investigator for this paper is Humphrey Ko and that he had direct clinical responsibility for patients.

Institutional affiliations:

[1]School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University, Perth, Western Australia, Australia.

[2]Curtin Health Innovation Research Institute (CHIRI) Biosciences Research Precinct, Curtin University, Perth, Western Australia, Australia.

[3]School of Occupational Therapy, Social Work and Speech Pathology, Curtin University, Perth, Western Australia, Australia.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bcp.14077

ABSTRACT

Aims: To determine statins' impact on skin infection risk in view of conflicting literature: that statins may reduce infection risk, but are also associated with diabetes mellitus, a risk factor for skin and soft tissue infections (SSTIs).

Methods: Sequence symmetry analysis (SSA) was performed on prescription claims (2001 to 2011) from the Australian Department of Veterans' Affairs to determine the interrelationships between: [i] statins and SSTIs, [ii] statins and diabetes, and [iii] diabetes and SSTIs; as well as whether statins increased the risk of SSTIs, independent of diabetes status. Chi-square tests were performed to detect differences in Index of Relative Socio-economic Advantage and Disadvantage scores of patients within each interrelationship. Prescriptions for statins, antidiabetic medication, and antistaphylococcal antibiotics were evaluated using non-identifiable client numbers, prescription dates filled, residential electorates, and pharmaceutical codes. Adjusted sequence ratio (ASR) and confidence interval (CI) were calculated at intervals of 91, 182, and 365 days for SSA studies.

Results: Statins were associated with: [i] significant SSTI risk (ASR > 1; CI >1), [ii] significant diabetes risk, and [iii] diabetic patients had increased risk of SSTIs. Diabetic and non-diabetic statin users had significantly increased risks of SSTIs, while the influence from socio-economic status was not significant for each of the three relationships.

Conclusions: Statins are associated with increased risk of SSTIs via direct and indirect mechanisms, likely independent of diabetes or socio-economic statuses. We believe clinicians should be aware of the association between statins and SSTIs, and where appropriate, monitor blood glucose levels of statin users.

KEYWORDS

Statins, diabetes, antibiotics, pharmacoepidemiology.

STRUCTURED SUMMARY

What is already known about this subject:

- *Staphylococcus aureus* is a major bacterial cause of skin and soft tissue infections (SSTIs).
- Statins might reduce the risk of *S. aureus* infections, but being ironically associated with new-onset diabetes mellitus (a risk factor for SSTIs), it is not known if statins reduce SSTI risks.

What this study adds:

- Statin use over as little as 91 days is associated with increased risk of SSTIs and diabetes.
- The increased SSTI risk appears unbiased by diabetes or socio-economic statuses.

INTRODUCTION

Staphylococcus aureus is a major cause of bacterial skin and soft tissue infections (SSTIs), which are prevalent amongst different age groups and may range from superficial to deep-seated infections, consuming considerable hospital resources [1]. Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, widely prescribed worldwide to reduce the risk of cardiovascular diseases by lowering plasma cholesterol [2]. Additionally, statins may reduce the risk of community-acquired *S. aureus* bacteraemia and exert antibacterial effects against *S. aureus* [3]. It is thus reasonable to hypothesise that statins could lower the risk of SSTIs or evolve into promising novel treatments for SSTIs.

However, statins may also induce new-onset diabetes mellitus ("diabetes mellitus" referred as "diabetes" hereafter) [4], which is a risk factor for SSTIs [1]; and skin colonisation with *S. aureus* predisposes diabetic patients to infections [5]. Moreover, inhibition of epidermal cholesterol synthesis may compromise the skin's barrier function [6]. If statins increased the risk of SSTIs, escalated healthcare expenditure would likely ensue due to the extensive use of statins.

This study was conducted to determine which of the conflicting postulations manifest by utilising sequence symmetry analysis (SSA) on data obtained from the Australian Department of Veterans' Affairs (DVA). Thereafter, probable mechanisms based on available clinical evidence are discussed.

METHODS

The SSA was originally used as an economical and rapid means of reviewing adverse drug reactions using prescription drugs [7]. The analysis was later expounded [8], and has since

gained popularity in pharmacoepidemiology to detect adverse events [7]. Advantages of the SSA over other epidemiological study designs include controlling for confounding factors which do not vary considerably over the study period, such as age, sex, or genetics [7, 9].

To detect adverse events using SSA, the sequence of incident (first-time) prescriptions of patients taking both the drug of interest (index drug) and the drug specifically indicated for treating the adverse event (marker drug) is examined [8, 9]. Prescription sequences with intervals greater than 365 days between the index and marker drugs were not analysed to minimise potential time-varying confounders such as age. If the index drug increases the probability of an event, the number of incident index drugs prescribed first ($n_{\mathbf{index \rightarrow marker}}$) will be expected to be significantly larger than the number of incident marker drugs prescribed first ($n_{\mathbf{marker \rightarrow index}}$). The crude sequence ratio (CSR) of incident prescriptions ($n_{\mathbf{index \rightarrow marker}}$)/($n_{\mathbf{marker \rightarrow index}}$) will thus be greater than unity. The fundamental assumption for this analysis is that if there was no causal association, incident users of both the index and marker drugs follow similar incidence trends for each drug in the study population [9].

Incident prescribing trends may vary over time. Hence a null-effect sequence ratio (NSR), the expected sequence ratio in the absence of any causal relationship, is calculated to adjust for these trends (Appendix 1) [8, 9]. The adjusted sequence ratio (ASR), calculated as CSR/NSR, is the incidence rate ratio of marker drug prescribing in index drug exposed versus non-exposed person-time [8]. Since the variance of the NSR is negligible compared to the variance of the CSR (which is much larger), the confidence interval (CI) of ASR is therefore largely determined by the CI of the CSR and calculated using the binomial distribution and crude number of sequences [8].

Data source

Permission was obtained from DVA to study prescription claims made by over 228,000 veterans, war widows, and widowers from 1st January 2000 to 31st December 2012 [10]. Prescriptions filled for statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin), antidiabetic medication (insulins, insulin analogues, and oral blood glucose lowering drugs), and antistaphylococcal antibiotics (dicloxacillin and flucloxacillin) were examined using non-identifiable client numbers, dates of prescriptions filled, residential electorates, and Anatomical Therapeutic Chemical codes as defined by the World Health Organization (Appendix 2) [11].

Primary analysis

A waiting-time distribution graph of the total number of all first-time prescriptions filled was plotted from 1st January 2000 to 31st December 2012 to determine the run-in period, which was the time required to differentiate incident users (evenly distributed over the study period) from prevalent users (clustered at the initial phase of the study period) [8, 9].

Thereafter, SSA was performed on prescription data from the study period (after the run-in period) to determine if: [i] statins increased risk of SSTIs ($index_{[statins]}$; $marker_{[antistaphylococcal\ antibiotics]}$); [ii] statins increased risk of diabetes ($index_{[statins]}$; $marker_{[antidiabetic\ medication]}$); and [iii] diabetic patients were susceptible to SSTIs ($index_{[antidiabetic\ medication]}$; $marker_{[antistaphylococcal\ antibiotics]}$) (Figure 1). The SSA was performed at window intervals of 91, 182, and 365 days for each relationship to identify variations in risk over time.

Confirmatory analysis

Amongst all statin users in the study period, additional SSA was performed on diabetics (taking antidiabetic medication) and non-diabetics (not taking antidiabetic medication) to determine if statins contributed to the risk of SSTIs independently, regardless of diabetes status.

Secondary analysis

The Index of Relative Socio-economic Advantage and Disadvantage (IRSAD) provides a snapshot of the socio-economic status of inhabitants within a residential area in Australia [12]. A low or high score suggests that residents are generally disadvantaged or advantaged respectively, with the overall average score being 1006 [12]. The Chi-square test was used to detect if the proportion of ($n_{\mathbf{index \rightarrow marker}}$) patients to ($n_{\mathbf{marker \rightarrow index}}$) patients with relatively disadvantaged (IRSAD < 1006) and advantaged (IRSAD > 1006) socio-economic conditions differed significantly for each of the associations studied.

Statistical analysis

Data were analysed using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) and graphs drawn with GraphPad Prism version 7 (GraphPad Software, La Jolla, California, USA).

Ethics approval

This study was approved by the DVA Ethics Committee (E014/003).

RESULTS

Primary analysis

A run-in period of six months was required to exclude prevalent users. Our study period was hence from 1st July 2001 to 31st December 2011 inclusive, to allow the analysis of the 365 days window interval preceding the first drug prescribed, and 365 days window interval following the last drug prescribed.

Overall, statins were associated with a significant risk of SSTIs. This risk was similar over 91, 182, or 365 days (Figure 2: ASR = 1.40, 1.41, and 1.40 respectively; CI > 1), with the greatest influence from atorvastatin and simvastatin (Figure 2).

Statins were also associated with a significant risk of new-onset diabetes, with a slight decrease in risk gradually over 91, 182, and 365 days (Figure 3: ASR = 1.19, 1.14, and 1.09 respectively; CI > 1). Atorvastatin and simvastatin were also the greatest contributors to this outcome, albeit the individual results of atorvastatin and simvastatin were not statistically significant over 365 days (Figure 3).

Patients with diabetes were associated with increased risk of SSTIs at the 182 and 365 days window (Figure 4: ASR = 1.2 and 1.24 respectively, CI > 1 respectively), but the risk was nonsignificant at the 91 days window (Figure 4: ASR = 1.14; CI overlaps unity).

Confirmatory analysis

Non-diabetic statin users were found to have significant SSTI risks over 91, 182, and 365 days (Figure 5: ASR = 1.39, 1.41, and 1.37 respectively, CI > 1 respectively). Diabetic statin

users were similarly associated with significant SSTI risks over 91, 182, and 365 days (Figure 5: ASR = 1.43, 1.42, and 1.49 respectively, CI > 1 respectively).

Secondary analysis

The proportion of ($n_{\mathbf{index \rightarrow marker}}$) patients to ($n_{\mathbf{marker \rightarrow index}}$) patients with relatively disadvantaged (IRSAD < 1006) and advantaged (IRSAD > 1006) socio-economic conditions did not differ significantly for: [i] statin and antibiotic users (p = 0.716); [ii] statin and antidiabetic users (p = 0.07); and [iii] antidiabetic and antibiotic users (p = 0.94).

DISCUSSION

To our knowledge, there are currently no known clinical studies of statins distinctly associated with the risk of SSTIs. However, there are conflicting conclusions about the effect of statins on the risk of general infections, some supporting statins reducing the risk of infections [3, 13], while others refute this beneficial outcome [14, 15]. By reconciling our results with available literature that utilise non-SSA related methodologies, clinical outcomes which align with our results would support plausible mechanism(s) of action for statins in SSTIs and diabetes.

Statins and risk of SSTIs

Current clinical literature supports direction [ii] (statins being associated with diabetes) [4, 16], as well as direction [iii] (diabetes being associated as a risk factor of skin infections) [1, 17]. Our results were in alignment, showing that statins increased the risk of SSTIs (Figure 2), statin users were associated with an increased risk of diabetes (Figure 3), and diabetes was associated with an increased risk of SSTIs (Figure 4). The confirmatory analysis revealed that both non-diabetic and diabetic statin users have similar significantly increased risks of SSTIs

(Figure 5), alluding to statin use as an important contributor to SSTI risk. Viewed collectively, it may be posited that statins are associated with an increased SSTI risk, whether via direct non-diabetogenic mechanisms (Figure 1: direction [i]), or via indirect diabetogenic mechanisms (Figure 1: directions [ii] and [iii]).

The findings of this study were in contrast to those reported by Pouwels et al. [18], who reported a reduction in antibiotic use in drug-treated type 2 diabetic statin users compared to non-users. Although their research design also utilised SSA, they did not examine the effects of narrow spectrum antibiotics (such as dicloxacillin and flucloxacillin) which target mainly staphylococci, a major bacterial causative agent for SSTIs [1]. By studying all beta-lactam penicillins as a group [18, 19], the effects of broad spectrum beta-lactam antibiotics on a variety of both Gram-positive and Gram-negative bacteria may mask or confound the results specific to Gram-positive staphylococci. Hence, it is possible that our results differed despite using the same methodology.

Interestingly, although the study by Liappis et al. concluded that statins may have a potentially therapeutic role in bacteraemic infections, they noted a statistically significant increase in SSTIs among patients with bacteraemia who were receiving statins, compared to those who were not using statins [20]. The work of both Liappis et al. (not designed *a priori* to detect an association between statins and SSTIs) and our study (designed *a priori* to detect this association) suggests the association between statins and SSTIs is unlikely to be spurious.

Statins and risk of diabetes

The diabetogenic mechanisms of statins may involve increased insulin resistance and/or diminished pancreatic β-cell function [4]. Patients with diabetes have impaired immunity,

undermining the defence against pathogens such as *S. aureus*, hence increasing the risk of SSTIs [21]. Our study revealed that the sensitive period whereby statin exposure exerted the greatest risk, was within 91 days after statin commencement, especially for atorvastatin and simvastatin (Figure 3). This suggests statin-induced diabetogenic mechanisms may be completed as soon as within 91 days.

The use of statins may upregulate low-density lipoprotein (LDL) receptors to reduce plasma LDL cholesterol (LDL-C), resulting in increased intracellular LDL-C burden and diminished pancreatic β-cell function [4]. In addition, the reduction of coenzyme Q10 as a result of mevalonate pathway inhibition may disrupt mitochondrial electron transport and impair insulin secretion [4]. Clinical studies have shown that blood levels of LDL-C and coenzyme Q10 were reduced after daily doses of simvastatin (LDL-C ↓34.7%, coenzyme Q10 ↓31.2% after 28 days) and atorvastatin (LDL-C ↓51%, coenzyme Q10 ↓52% after 30 days) [22]. Reduced levels of LDL-C and/or coenzyme Q10 by statins are associated with an increased risk of diabetes [4], which could be plausible mechanisms explaining our results demonstrating increased diabetes risk induced by statins within 91 days.

Disruption of the human gut microbiome, or gut dysbiosis, has been associated with the impaired metabolism of bile acids, which may impede glucose control and diminish innate immunity [23]. Bile acids regulate glucose homeostasis through the activation of nuclear receptors such as the pregnane X receptor (PXR), and mount antimicrobial defences via activation of the vitamin D receptor [24]. Statins have been found to influence the human gut microbiome [25], and remodelling of murine gut microbiota resulted in increased risk of diabetes in mice via PXR activation [26]. The clinical implication of gut dysbiosis associated with statins is uncertain as our study could not verify this mechanism.

A decrease in vitamin D levels may raise the risk of diabetes directly (via interference with insulin receptors, signalling, and glucose transport) or indirectly (secondary to hyperparathyroidism) [27]. However, the overall effect of statins on vitamin D levels in humans is ambiguous. Statins decrease cholesterol (a precursor of vitamin D), which theoretically limits downstream vitamin D production. Yet, conflicting results revealed that statins may raise vitamin D levels (via competitive inhibition of the cytochrome P450 enzyme activity and activation of cholesterol membrane transporters to increase intestinal absorption of vitamin D) [28], as well as studies which showed that statins do not increase serum levels of vitamin D [29].

The net effects of vitamin D on infections also appear ambivalent. Vitamin D may prevent infections by boosting the innate immunity (rapid response) through augmenting chemotaxis, phagocytosis, and activation of antimicrobial peptides [30]. However, by increasing T regulatory cells (Treg), and inhibiting T helper cell type 1(Th1) and type 17 (Th17) [30], the adaptive immune system (delayed response) against pathogenic infections may be dampened. Thus, the influence of vitamin D in our study is unclear.

Statins and the immune system

The Th1 and Th17 cells are responsible for mounting the host's defence against pathogens, resulting in inflammatory responses [31]. The Treg cells on the other hand, play a role in homeostasis by suppressing T cells, exerting anti-inflammatory effects [31]. Inhibition of HMG-CoA reductase by statins reduces cholesterol and downstream isoprenoids essential for intracellular signalling, which could result in increased anti-inflammatory Treg cells and decreased pro-inflammatory Th1 and Th17 cells [32]. Clinical data show that statins inhibit the induction of Th1 and Th17 cells [32], and may increase Treg cells within 4 to 12 weeks

[32, 33], suggesting statin users may be more susceptible to pathogenic infections. This might account for our results which demonstrated that statin users were at increased risk of SSTIs via diabetes-independent mechanisms within 91 days (Figure 1: direction [i] and Figure 5).

The skin functions as a crucial permeability barrier, providing innate immunity by protecting the host from noxious agents such as bacterial pathogens. Upon acute insult, epidermal cholesterol synthesis and HMG-CoA reductase activity increases swiftly to restore the protective barrier function [6]. If epidermal cholesterol synthesis was impeded via topical application of statins, the recovery of the barrier function was delayed [6]. Clinical data also suggested that high levels of cholesterol might confer immunoprotective effects against infections [34]. By decreasing cholesterol levels, statins may theoretically attenuate the protective barrier function and immunoprotective effects, increasing susceptibility to infections. Yet, statins have been associated with decreased risks of infections [3]. As such, the net effect of lowering cholesterol on skin function remains to be elucidated.

Healthy user effect

The "healthy user effect" refers to selective bias whereby motivated patients are more inclined to undertake preventive healthcare, such as consuming healthy diets and exercising frequently, and such health-seeking attitudes correspond closely with socio-economic status [35]. Since the residential electorate is reflective of patients' socio-economic status [12], patients from electorates that are of above average IRSAD scores (> 1006) might be more likely than patients from below average IRSAD scores (< 1006) to exhibit traits such as reduced risk of infections or diabetes. However, the healthy user effect was not apparent because the role of socio-economic status was nonsignificant within the relationships examined in the secondary analysis.

Limitations of study

Due to the nature of SSA, patients were assumed to commence their medication on the day of filling their prescription and that they were compliant with medication, which might not have occurred in reality. We also assumed that all medicines were administered as a Defined Daily Dose per day (Appendix 2) [11], thus we could not determine the impact of statin dosage on clinical outcomes.

Some antibiotics used to treat SSTIs may also be prescribed for other types of infections. By narrowing our choice of marker antibiotics to dicloxacillin and flucloxacillin, we could be reasonably assured that the data generated would be specific for bacterial SSTIs, albeit this excludes signals from the other antibiotics and precludes patients with penicillin allergies.

Confounding by indication is an inherent bias in SSA [8]. Since diabetes is a risk factor for SSTIs [1, 17], an increased risk of SSTIs associated with statins could be confounded by an indication (diabetes) for taking statins. Diabetes is an important risk factor for cardiovascular diseases and statins are indicated in patients with diabetes to reduce the risk of cardiovascular diseases [36]. Hence the number of patients ($n_{\text{antidiabetics 1st}\rightarrow\text{ statins 2nd}}$) may be relatively high, creating a bias towards an underestimation of statins' effect on diabetes, favouring the reverse of direction [ii] in Figure 1 and thereby, resulting in confounding by indication.

However, recommendations for statin prescribing to manage cardiovascular disease risks target metabolic syndrome, a condition comprising three of any of the following five factors: elevated waist circumference, elevated serum triglycerides, reduced HDL-C, elevated blood pressure, and elevated fasting glucose (diabetes) [36]. As such, there are other conditions for prescribing statins which aim to control other components of metabolic syndrome but

specifically exclude diabetes [36]. In these situations, ($n_{\text{statins 1st}\rightarrow\text{ antidiabetics 2nd}}$) would be relatively larger, favouring direction [ii] in Figure 1, which our results aligned with (Figure 3). Although we were unable to categorically rule out confounding by indication, our conclusion of stains being associated with diabetes is supported by meta-analyses of randomised controlled trials [37, 38].

CONCLUSION

Our study supports the hypothesis that statin users are at increased risk of SSTIs and this risk was likely independent of diabetes status or the healthy user effect. Statins may increase SSTI risk via direct or indirect mechanisms. Clinical evidence that aligned with our results include the reduction of innate immunity via increase of Treg within 91 days and inhibition of Th1 and Th17 (direct mechanism); and reduction of LDL-C and coenzyme Q10 levels within 91 days of statin commencement, which increased the risk of diabetes, in turn a risk factor for SSTIs (indirect mechanism).

Further clinical studies are required to confirm these mechanisms, as well as to ascertain the effect of statins on gut dysbiosis, impaired bile acid metabolism, vitamin D levels, and cholesterol inhibition on skin function. Regardless of the actual mechanism(s), it would seem prudent for clinicians to monitor blood glucose levels of statin users who are predisposed to diabetes, and be mindful of possible increased SSTI risks in such patients.

ACKNOWLEDGEMENTS

We thank the Australian Department of Veterans' Affairs for granting us access to the data, and sincerely appreciate the help of Dr Nicole Pratt and her co-authors [7], who graciously shared their sequence symmetry analysis SAS program codes with us. In addition, we would

like to acknowledge the kind contributions of the Australian Government Research Training Program Scholarship, the Curtin Health Innovation Research Institute (CHIRI) Biosciences Research Precinct Core Facility, and the School of Pharmacy and Biomedical Sciences (Curtin University) in supporting this research. The funders had no role in the design and conduct of the study, preparation of the manuscript, or in the decision to publish.

AUTHOR CONTRIBUTIONS

Humphrey H. T. Ko wrote the paper, performed minor calculations of the sequence symmetry analysis, prepared the figures, reviewed drafts of the paper, selected and analysed relevant references, and contributed ideas to the manuscript.

Ricky R. Lareu and Brett R. Dix reviewed drafts of the paper, analysed the references, and contributed ideas to the manuscript.

Jeffery D. Hughes conceived the paper, reviewed drafts, analysed the references, and contributed ideas to the manuscript.

Richard W. Parsons performed major calculations and programing of the sequence symmetry analysis, reviewed drafts of the paper, analysed the references, and contributed ideas to the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study were obtained from the Australian Department of Veterans' Affairs. Restrictions apply to the availability of these data, which were used under license for this study.

REFERENCES

1. Tognetti L, Martinelli C, Berti S, Hercogova J, Lotti T, Leoncini F, Moretti S. Bacterial skin and soft tissue infections: Review of the epidemiology, microbiology, aetiopathogenesis and treatment: A collaboration between dermatologists and infectivologists. J Eur Acad Dermatol Venereol 2012; 26: 931-41.

2. Blaha MJ, Martin SS. How do statins work? Changing paradigms with implications for statin allocation. J Am Coll Cardiol 2013; 62: 2392-4.

3. Smit J, Lopez-Cortes LE, Thomsen RW, Schonheyder HC, Nielsen H, Froslev T, Rodriguez-Bano J, Sogaard M. Statin use and risk of community-acquired *Staphylococcus aureus* bacteremia: a population-based case-control study. Mayo Clin Proc 2017; 92: 1469-78.

4. Betteridge DJ, Carmena R. The diabetogenic action of statins - mechanisms and clinical implications. Nat Rev Endocrinol 2016; 12: 99-110.

5. Weintrob AC, Sexton DJ. Susceptibility to infections in persons with diabetes mellitus. In: UpToDate, edWeller PF, UpToDate, Waltham, MA. [cited 2017 Nov 02]. 2016.

6. Feingold KR. Thematic review series: skin lipids. The role of epidermal lipids in cutaneous permeability barrier homeostasis. J Lipid Res 2007; 48: 2531-46.

7. Lai EC, Pratt N, Hsieh CY, Lin SJ, Pottegard A, Roughead EE, Kao Yang YH, Hallas J. Sequence symmetry analysis in pharmacovigilance and pharmacoepidemiologic studies. Eur J Epidemiol 2017; 32: 567-82.

8. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. Epidemiology 1996; 7: 478-84.

9. Tsiropoulos I, Andersen M, Hallas J. Adverse events with use of antiepileptic drugs: a prescription and event symmetry analysis. Pharmacoepidemiol Drug Saf 2009; 18: 483-91.

10. Australian Government Department of Veterans' Affairs. Treatment population statistics. Quarterly report - December 2012. In, Canberra, Australia 2012.

11. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2018. In, Norway: Norwegian Institute of Public Health, 2018.

12. Nelson P. Socio-economic indexes for 2009 electoral divisions: 2006 Census, Research Paper No. 1 2010–11. In, Department of Parliamentary Services, Parliamentary Library, Canberra, 2010.

13. Tleyjeh IM, Kashour T, Hakim FA, Zimmerman VA, Erwin PJ, Sutton AJ, Ibrahim T. Statins for the prevention and treatment of infections: a systematic review and meta-analysis. Arch Intern Med 2009; 169: 1658-67.

14. Hauer-Jensen M, Fort C, Mehta JL, Fink LM. Influence of statins on postoperative wound complications after inguinal or ventral herniorrhaphy. Hernia 2006; 10: 48-52.

15. van den Hoek HL, Bos WJW, de Boer A, van de Garde EMW. Statins and prevention of infections: systematic review and meta-analysis of data from large randomised placebo controlled trials. BMJ 2011; 343:d7281.

16. Brault M, Ray J, Gomez Y-H, Mantzoros CS, Daskalopoulou SS. Statin treatment and new-onset diabetes: A review of proposed mechanisms. Metabolism 2014; 63: 735-45.

17. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can J Infect Dis Med Microbiol 2008; 19: 173-84.

18. Pouwels KB, Widyakusuma NN, Bos JH, Hak E. Association between statins and infections among patients with diabetes: a cohort and prescription sequence symmetry analysis. Pharmacoepidemiol Drug Saf 2016; 25: 1124-30.

19. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index: J - Antiinfectives for systemic use. In, Norway: Norwegian Institute of Public Health, 2018.

20. Liappis AP, Kan VL, Rochester CG, Simon GL. The effect of statins on mortality in patients with bacteremia. Clin Infect Dis 2001; 33: 1352-7.

21. Dryden MS. Alternative clinical indications for novel antibiotics licensed for skin and soft tissue infection? Curr Opin Infect Dis 2015; 28: 117-24.

22. Hargreaves IP, Duncan AJ, Heales SJ, Land JM. The effect of HMG-CoA reductase inhibitors on coenzyme Q10: possible biochemical/clinical implications. Drug Saf 2005; 28: 659-76.

23. Jones ML, Martoni CJ, Ganopolsky JG, Labbe A, Prakash S. The human microbiome and bile acid metabolism: dysbiosis, dysmetabolism, disease and intervention. Expert Opin Biol Ther 2014; 14: 467-82.

24. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. Nat Rev Gastroenterol Hepatol 2014; 11: 55-67.

25. Le Bastard Q, Al-Ghalith GA, Gregoire M, Chapelet G, Javaudin F, Dailly E, Batard E, Knights D, Montassier E. Systematic review: human gut dysbiosis induced by non-antibiotic prescription medications. Aliment Pharmacol Ther 2018; 47: 332-45.

26. Caparros-Martin JA, Lareu RR, Ramsay JP, Peplies J, Jerry Reen F, Headlam HA, Ward NC, Croft KD, Newsholme P, Hughes JD, O'Gara F. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. Microbiome 2017; 5: 95.

27. Mezza T, Muscogiuri G, Sorice GP, Prioletta A, Salomone E, Pontecorvi A, Giaccari A. Vitamin D deficiency: a new risk factor for type 2 diabetes? Ann Nutr Metab 2012; 61: 337-48.

28. Yavuz B, Ertugrul DT. Statins and vitamin D: a hot topic that will be discussed for a long time. Dermatoendocrinol 2012; 4: 8-9.

29. Mazidi M, Rezaie P, Vatanparast H, Kengne AP. Effect of statins on serum vitamin D concentrations: a systematic review and meta-analysis. Eur J Clin Invest 2017; 47: 93-101.

30. Iruretagoyena M, Hirigoyen D, Naves R, Burgos PI. Immune response modulation by vitamin D: role in systemic lupus erythematosus. Front Immunol 2015; 6: Article 513.

31. Nurieva RI, Chung Y. Understanding the development and function of T follicular helper cells. Cell Mol Immunol 2010; 7: 190-7.

32. Forero-Pena DA, Gutierrez FR. Statins as modulators of regulatory T-cell biology. Mediators Inflamm 2013; 2013: 167086.

33. Mausner-Fainberg K, Luboshits G, Mor A, Maysel-Auslender S, Rubinstein A, Keren G, George J. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. Atherosclerosis 2008; 197: 829-39.

34. Ravnskov U. High cholesterol may protect against infections and atherosclerosis. QJM 2003; 96: 927-34.

35. Brookhart MA, Patrick AR, Dormuth C, Avorn J, Shrank W, Cadarette SM, Solomon DH. Adherence to lipid-lowering therapy and the use of preventive health services: an investigation of the healthy user effect. Am J Epidemiol 2007; 166: 348-54.

36. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC, Sperling L, Virani SS, Yeboah J. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. Circulation 2018; 0: CIR.0000000000000625.

37. Preiss D, Seshasai SR, Welsh P, Murphy SA, Ho JE, Waters DD, DeMicco DA, Barter P, Cannon CP, Sabatine MS, Braunwald E, Kastelein JJ, de Lemos JA, Blazing MA, Pedersen TR, Tikkanen MJ, Sattar N, Ray KK. Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. JAMA 2011; 305: 2556-64.

38. Erqou S, Lee CC, Adler AI. Statins and glycaemic control in individuals with diabetes: a systematic review and meta-analysis. Diabetologia 2014; 57: 2444-52.

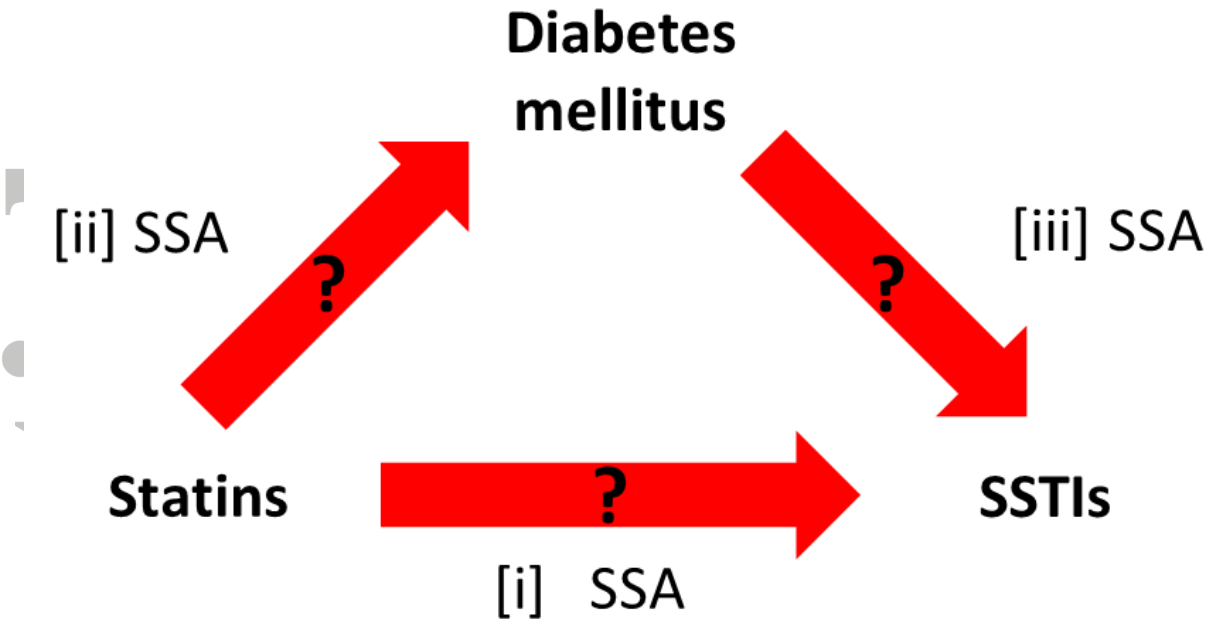

Figure 1. Using sequence symmetry analysis (SSA) to evaluate plausible interrelationships between statins, diabetes mellitus, and skin and soft tissue infections (SSTIs). [i] Between statins and SSTIs, index drug = statin, marker drug = antistaphylococcal antibiotics. [ii] Between statins and diabetes, index drug = statin, marker drug = antidiabetic medication. [iii] Between diabetes and SSTIs, index drug = antidiabetic medication, marker drug = antistaphylococcal antibiotics. Statins included atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin; antidiabetic medication included insulins, insulin analogues, and oral blood glucose lowering drugs; and antistaphylococcal antibiotics included dicloxacillin and flucloxacillin.

	Crude Sequence Ratio Statin First/Antibiotic First	Adjusted Sequence Ratio (95% confidence interval)
Any statin		
Any statin (91 days)	1457/1041	1.40 (1.29-1.52)
Any statin (182 days)	2509/1768	1.41 (1.33-1.50)
Any statin (365 days)	4540/3186	1.40 (1.34-1.47)
Atorvastatin		
Atorvastatin (91 days)	885/685	1.31 (1.19-1.45)
Atorvastatin (182 days)	1517/1163	1.31 (1.21-1.41)
Atorvastatin (365 days)	2766/2132	1.29 (1.22-1.37)
Fluvastatin		
Fluvastatin (91 days)	14/7	1.36 (0.55-3.38)
Fluvastatin (182 days)	24/12	1.75 (0.87-3.49)
Fluvastatin (365 days)	37/20	1.89 (1.10-3.26)
Pravastatin		
Pravastatin (91 days)	280/216	1.31 (1.10-1.57)
Pravastatin (182 days	467/373	1.28 (1.12-1.47)
Pravastatin (365 days)	813/698	1.17 (1.06-1.29)
Rosuvastatin		
Rosuvastatin (91 days)	225/180	1.26 (1.04-1.53)
Rosuvastatin (182 days)	384/338	1.18 (1.02-1.36)
Rosuvastatin (365 days)	715/635	1.14 (1.02-1.26)
Simvastatin		
Simvastatin (91 days)	540/390	1.38 (1.21-1.57)
Simvastatin (182 days)	953/685	1.39 (1.26-1.53)
Simvastatin (365 days)	1749/1259	1.37 (1.28-1.47)

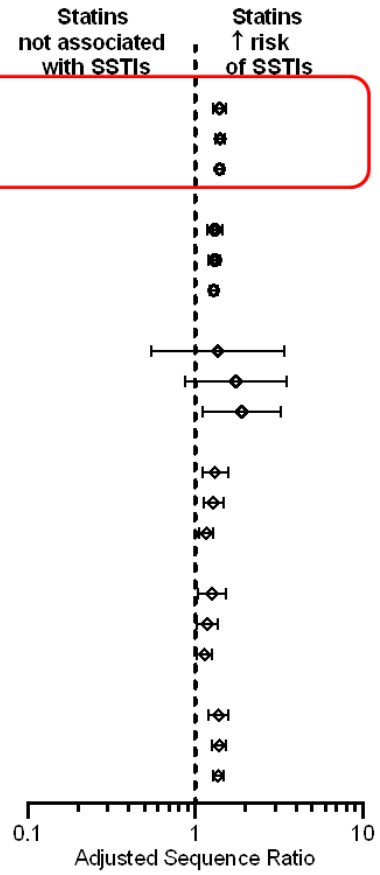

Figure 2. Sequence symmetry analysis results for the relationship between statins and skin and soft tissue infections (SSTIs). Index drugs used were statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin). Marker drugs used were antistaphylococcal antibiotics (dicloxacillin and flucloxacillin).

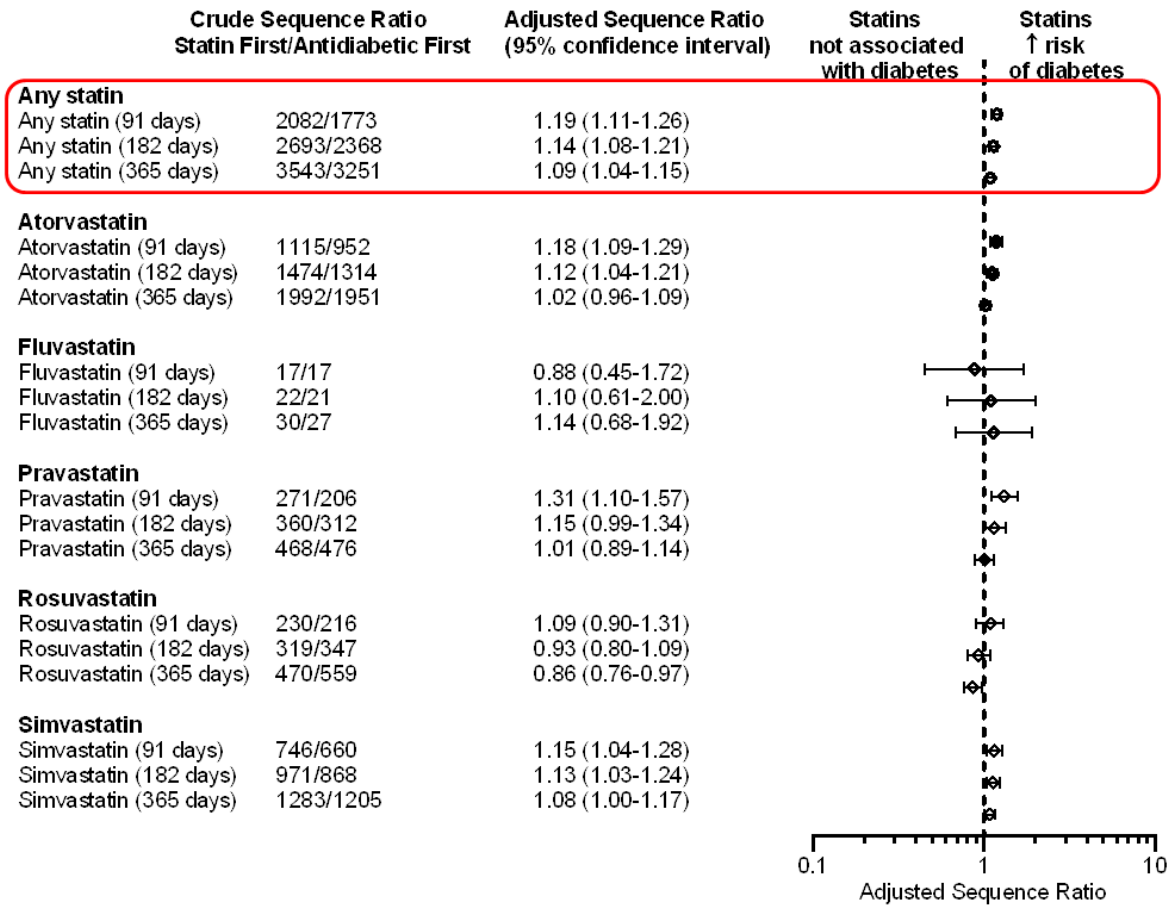

Figure 3. Sequence symmetry analysis results for the relationship between statins and diabetes mellitus. Index drugs used were statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin). Marker drugs used were antidiabetic medication (insulins, insulin analogues, and oral blood glucose lowering drugs).

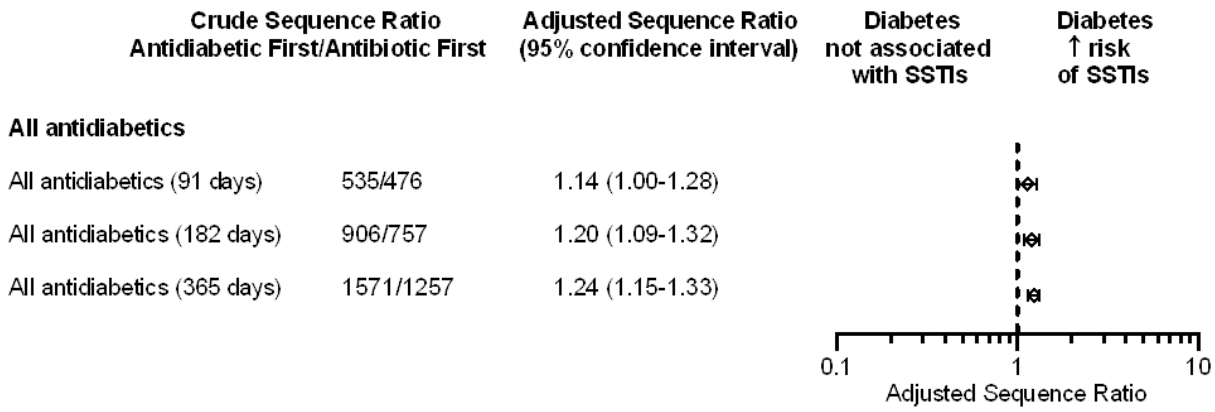

Figure 4. Sequence symmetry analysis results for the relationship between diabetes mellitus and skin and soft tissue infections (SSTIs). Index drugs used were antidiabetic medication (insulins, insulin analogues, and oral blood glucose lowering drugs). Marker drugs used were antistaphylococcal antibiotics (dicloxacillin and flucloxacillin).

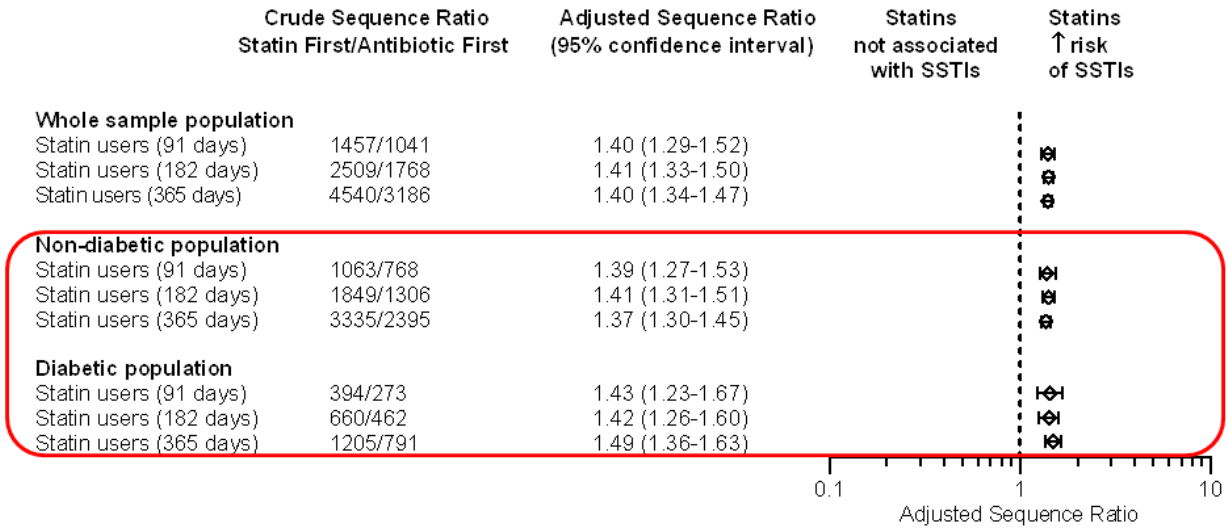

Figure 5. Confirmatory sequence symmetry analysis to determine the risk of SSTIs associated with non-diabetic statin users compared to diabetic statin users. Diabetic population was defined as patients on antidiabetic medication (insulins, insulin analogues, and oral blood glucose lowering drugs). Index drugs used were statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin). Marker drugs used were antistaphylococcal antibiotics (dicloxacillin and flucloxacillin).

APPENDICES

Please see separately attached file.

WORD COUNT

3052 words excluding the title page, structured summary, references, legends to figures, and appendices).

APPENDIX 1

Calculating null-effect sequence ratio (NSR).

The NSR is the expected sequence ratio in the absence of any causal relationship between the index and marker drugs, and it is used to adjust for incidence trend changes [8, 9]. The overall average probability (*Pa*) of an index→marker sequence may be calculated as an average for all days, weighted by the number of incident index drug users on consecutive (m) days of the study as follows [7]:

$$Pa = \frac{\sum_{m=1}^{u}\left[I_m \times \left(\sum_{n=m+1}^{m+d} M_n\right)\right]}{\sum_{m=1}^{u}\left[I_m \times \left(\sum_{n=m-d}^{m-1} M_n + \sum_{n=m+1}^{m+d} M_n\right)\right]}$$

where:

m or n = consecutive days of the study period excluding the run-in period

d = number of days for observation (window period of 91, 182, or 365 days in our study)

u = last day of the study period

I_m = number of people receiving their first index drug on the date

M_n = number of people receiving their first marker drug on the date

NSR is thus calculated as:

$$\mathrm{NSR} = \frac{Pa}{(1 - Pa)}$$

APPENDIX 2

Anatomical Therapeutic Chemical (ATC) codes and respective Daily Defined Dose (DDD) used in study.

The ATC codes of medications and respective DDD, as defined by the World Health Organization [11], used in this study included:

Drug name	**ATC code**	**DDD**
Statins		
Atorvastatin	C10AA05	20 mg
Fluvastatin	C10AA04	60 mg
Pravastatin	C10AA03	30 mg
Rosuvastatin	C10AA07	10 mg
Simvastatin	C10AA01	30 mg
Antidiabetic medication		
Acarbose	A10BF01	0.3 g
Exenatide	A10BX04 (before 2017)	0.286 mg (depot) 15 mcg
Glibenclamide	A10BB01	10 mg
Gliclazide	A10BB09	60 mg
Glimepiride	A10BB12	2 mg
Glipizide	A10BB07	10 mg
Insulin (human, fast-acting)	A10AB01	40 units
Insulin (beef, fast-acting)	A10AB02	40 units
Insulin (lispro)	A10AB04	40 units
Insulin (aspart)	A10AB05	40 units

Insulin (glulisine)	A10AB06	40 units
Insulin (human, intermediate-acting)	A10AC01	40 units
Insulin (beef, intermediate-acting)	A10AC02	40 units
Insulins and analogues for injection (intermediate or long-acting combined with fast-acting)	A10AD	-
Insulin (human, intermediate or long-acting combined with fast-acting)	A10AD01	40 units
Insulin (intermediate or long-acting combined with lispro)	A10AD04	40 units
Insulin (human, long-acting)	A10AE01	40 units
Insulin (glargine)	A10AE04	40 units
Insulin (detemir)	A10AE05	40 units
Linagliptin	A10BH05	5 mg
Metformin	A10BA02	2 g
Metformin and rosiglitazone	A10BD03	-
Metformin and sulfonylureas	A10BD02	-
Metformin and sitagliptin	A10BD07	-
Metformin and vildagliptin	A10BD08	-
Pioglitazone	A10BG03	30 mg
Rosiglitazone	A10BG02	6 mg
Saxagliptin	A10BH03	5 mg
Sitagliptin	A10BH01	0.1 g
Tolbutamide	A10BB03	1.5 g
Vildagliptin	A10BH02	0.1 g

Antistaphylococcal antibiotics		
Dicloxacillin	J01CF01	2 g
Flucloxacillin	J01CF05	2 g