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Use of negative controls in a prescription sequence symmetry analysis to reduce time-varying bias

Scott Martin Vouri[1,2] | Xinyi Jiang[1] | Earl J. Morris[1] | Babette A. Brumback[2,3] | Almut G. Winterstein[1,2]

[1]Department of Pharmaceutical Outcomes & Policy, University of Florida—College of Pharmacy, Gainesville, Florida, USA

[2]University of Florida—Center for Drug Evaluation and Safety (CoDES), Gainesville, Florida, USA

[3]Department of Biostatistics, University of Florida—College of Public Health & Health Professions College of Medicine, Gainesville, Florida, USA

Correspondence
Scott Martin Vouri, Department of Pharmaceutical Outcomes & Policy, University of Florida—College of Pharmacy, Gainesville, FL.
Email: svouri@cop.ufl.edu

Abstract

Purpose: There is an increased use in the (prescription) sequence symmetry analysis (PSSA); however, limited studies have incorporated a negative control, and no study has formally quantified and controlled for within-patient time-varying bias using a negative control. Our aim was to develop a process to incorporate the effect of negative controls into the main analysis of a PSSA.

Methods: Using a previously assessed dihydropyridine calcium channel blocker (DH-CCB) and loop diuretic PSSA, we directly compared the adjusted sequence ratios (aSRs) of DH-CCBs to each of the two negative control index drugs (levothyroxine and angiotensin converting enzyme [ACE] inhibitor/angiotensin-2 receptor blocker [ARB]) using the ratio of the aSRs to estimate a relative aSR with a Z test. Further, we utilized the relative aSR in stratum-specific analyses and varying exposure windows.

Results: The relative aSR of DH-CCBs decreased from 1.87 to 1.72 (95% CI 1.66–1.78) using levothyroxine as a negative control index drug. ACE inhibitor/ARB negative control index drug resulted in an aSR of 1.27 thus reducing the relative aSR for DH-CBB from 1.84 to 1.45 (95% CI 1.41–1.49). When restricting the exposure window to 180 and 90 days, the relative aSR of DH-CCBs increased to 1.68 (95% CI 1.62–1.74) and 1.86 (95% CI 1.78–1.94), respectively, relative to the ACE inhibitor/ARB negative control index drug.

Conclusion: We illustrated how to incorporate negative control index drugs into a PSSA and generate relative aSRs. Stratum-specific assessments and varying the exposure windows while using negative control index drugs can yield more informative results.

KEYWORDS
pharmacovigilance, prescribing cascade, prescription sequence symmetry analysis, self-controlled

Key Points

- Interpretation of prescription sequence symmetry analyses results can be impacted by within-patient time-varying bias.
- Incorporating a negative control into the main analysis, generating a relative aSR, can reduce this potential bias.
- Negative controls can also be incorporated into stratum-specific analyses and sensitivity analyses with varying exposure windows.

1 | INTRODUCTION

A prescription sequence symmetry analysis (PSSA) is a case-only, pharmacovigilance approach that can be used to identify prescribing cascades (i.e., treatment of drug-induced adverse events with an additional medication).[1,2] The PSSA requires the identification of patients who were both initiated on a medication (or medication class) of interest (index drug) and who received a medication that can be used to treat a drug-induced adverse event (marker drug). The initiation of the marker drug after the index drug, relative to before, is then compared in exposure windows of similar duration (e.g., ± 365 days of index drug initiation).[3] If the adjusted sequence ratio (aSR) of marker drug relative to index drug is greater than 1, after considering secular trends, it suggests a positive signal for a prescribing cascade. PSSAs are very beneficial due to their simplistic design, their ability to control for time-invariant confounding, and their ability to identify significant signals in cases with few outcomes.[2]

However, one of the drawbacks of PSSA is within-patient time-varying bias such as disease progression or age-related effects that might provide alternative causes for sequential initiation of the marker drug. Two approaches have been used to decrease within-patient time-varying bias. First, reducing exposure windows (e.g., from 1 year to 6 months) may limit time-related changes, but comes at the expense of reduced sample size and may be infeasible if the time to adverse event that would trigger a prescribing cascade is longer.[4,5] The second approach is the use of a negative control index drug. A negative control index drug in a PSSA is a medication (or medication class) that is not associated with the target adverse event and subsequent prescribing cascade, but is used in a similar population and has similar healthcare follow-up as the index drug.[6] To serve as a negative control index drug, the medication (or medication class) replaces the index drug from the primary PSSA and the PSSA is then re-analyzed. If such an association is not identified and the negative control index drug is a perfect replacement of the primary index drug, then the primary PSSA may be interpreted as causal; however, if the association is significant, it must be incorporated in the analysis of the primary PSSA to avoid overestimation of the prescribing cascade.

Currently among the 44 PSSAs that were assessed in two PSSA reviews,[7,8] only eight studies[6,9-15] utilized a negative control index drug. One study identified a significant association between the negative control index drug and subsequent initiation of the marker drug, thus indicating bias. In this case, there was a stronger effect in the negative control index drugs (statins, non-steroidal anti-inflammatory drugs) relative to the marker drug (dementia medications) than seen with the index drug (proton pump inhibitor [PPI]), to which the authors concluded that the risk for dementia due to PPI's are likely overestimated without consideration of confidence intervals.[9] Another study investigating thiazolidinedione-induced edema/heart failure found a similar significant positive association in their pooled negative control analysis using metformin based on overlapping confidence interval; however, point estimates were not formally incorporated into their main analysis.[14] The other six studies that included negative control index drugs conducted separate analyses which resulted in null findings and were not addressed any further.[6,10-13,15]

Although research on the incorporation of negative controls into analyses used to mitigate bias exists for several study designs (e.g., cohort, case–control, and self-controlled case series [SCCS]),[16] there is no published methodology on how to formally mitigate the effect of within-patient time-varying bias via a negative control population beyond visual comparison and examination of confidence intervals for the PSSA. However, there are existing biostatistical techniques that can be used to incorporate the effect of the negative control PSSA into the main analysis.[17] To this end, we used data from our previously published PSSA to illustrate this approach.[18]

2 | MATERIALS AND METHODS

The graphical representation of the PSSA (Figure 1) represents the nature of confounding and how the incorporation of the effects of a negative control PSSA into the PSSA main analysis can help further reduce the effect of within-patient time-varying bias.

To formally incorporate a negative control index drug analysis into a PSSA, we employed our previous dataset assessing the dihydropyridine calcium channel blocker (DH-CCB)-induced lower extremity edema followed by the initiation of a loop diuretic prescribing cascade (DH-CCB—loop diuretic prescribing cascade).[18] Briefly, we used the IBM MarketScan Commercial and Medicare Supplemental Claims databases from January 2005 to December 2017 to identify patients aged ≥20 years with at least 720 days of continuous enrollment before and 360 days of continuous enrollment after the first DH-CCB pharmacy dispensing claim. We excluded patients coded for heart failure any time between 720 days prior to and 360 day following first DH-CCB since loop diuretics are used to treat heart failure-related fluid overload. Patients had to have a first loop diuretic claim occurring within ±360 days of the first DH-CCB claim, and we excluded patients whose first DH-CCB and loop diuretic claims occurred on the same day.

We selected two medications/medication classes to evaluate as negative control index drugs: (a) levothyroxine and (b) angiotensin converting enzyme (ACE) inhibitors or angiotension-2 receptor blockers (ARB). Levothyroxine was selected as a negative control because it does not cause edema, and like DH-CCBs, is chronically used and requires monitoring, and is thus expected to have similar health care follow-up after initiation.[6] ACE inhibitors/ARBs were selected for the same reasons as well as their similarity in indication and potential to account for hypertension progression meaning loop diuretics are likely added at similar rates following the initiation of either ACE inhibitors/ARBs or DH CCBs for the treatment of hypertension.

We applied the same inclusion and exclusion criteria as above for each of the negative control index drugs and additionally excluded patients who initiated a DH-CCB during the 360-day exposure windows. Additionally, for DH-CCB initiators, we excluded initiators for each of negative control index drug within the 360-day exposure window. These exclusions were needed to ensure DH-CCB and negative control index drug users were mutually exclusive.

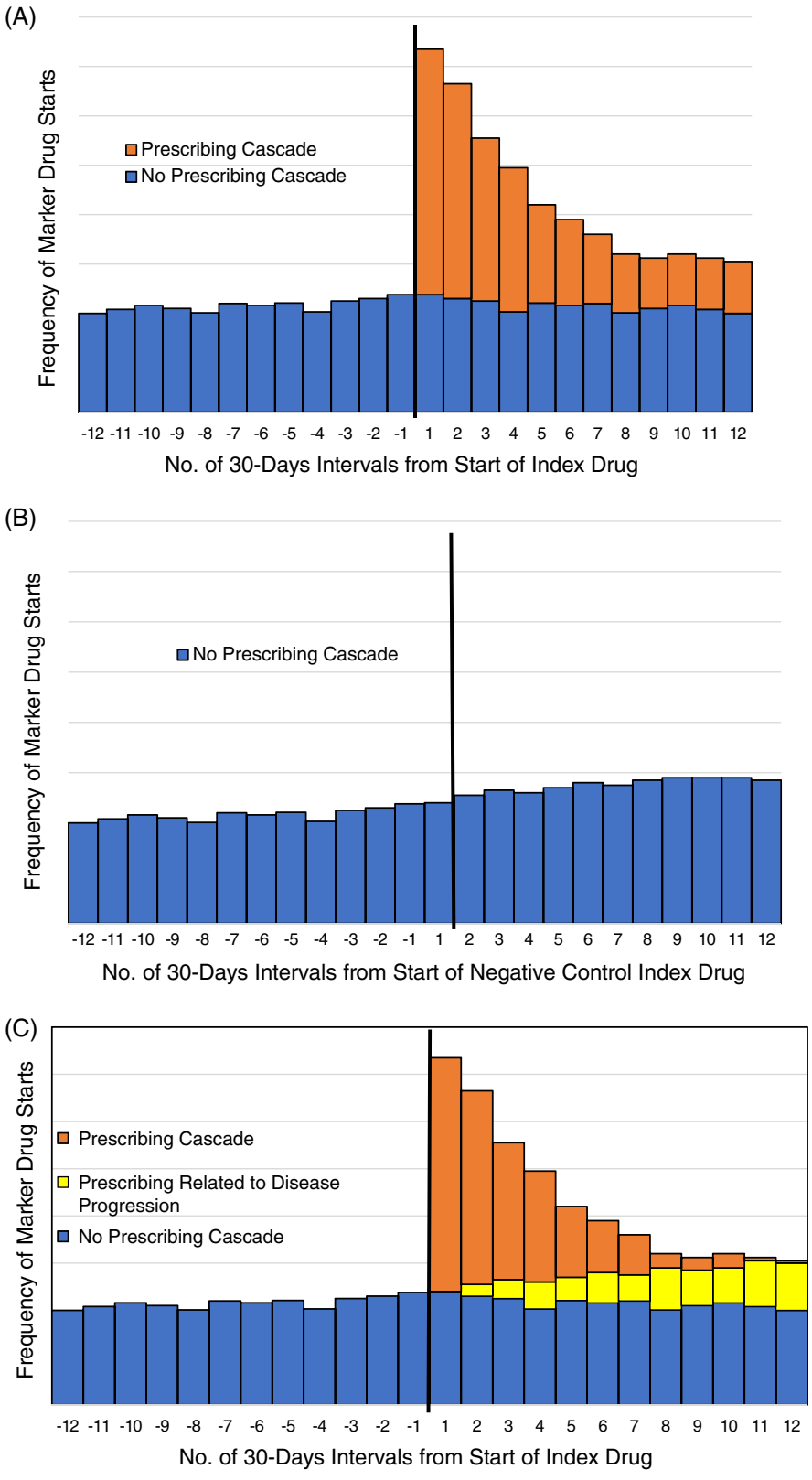

FIGURE 1 Graphical representation of the reduction in within-person time-varying bias using a negative control. A, is a representation of a hypothetical main analysis using a prescription sequence symmetry analysis which displays differences in the estimates of prescribing of a marker drug relative to index drug as prescribing cascade (orange) versus no prescribing cascade (blue). B, is a representation of a hypothetical negative control analysis using a prescription sequence symmetry analysis in which displays differences in the estimates of prescribing of a marker drug relative to a negative control index drug as no prescribing cascade (blue). C, is a representation of a hypothetical main analysis after incorporating a negative control analysis using a prescription sequence symmetry analysis which displays differences in the estimates of prescribing of a marker drug relative to index drug as prescribing cascade (orange) versus prescribing related to disease progression (yellow) and no prescribing cascade (blue)

Using the PSSA, we then calculated the crude sequence ratio (cSR) and null-effect sequence ratio, which incorporates the secular trends of medication use over the study period, in order to calculate an aSR for each DH-CCB and negative control index drug dyad independently.[19]

Next, we calculated the log of the aSR and corresponding standard errors for each DH-CCB and negative control index drug PSSA. We then calculated the difference (d) between both logs of aSR (Formula 1) as well as the standard error for this difference (SE(d)) (Formula 2).[17]

Formula 1: $d = \log(aSR_{DH-CCB}) - \log(aSR_{negative\ control})$.

Formula 2: $SE(d) = \sqrt{\left(SE_{\log(aSR_{DH-CCB})}\right)^2 + \left(SE_{\log(aSR_{negative\ control})}\right)^2}$

We used the Z value from the standard normal distribution to calculate the 95% confidence interval. Finally, we transformed back the difference between log aSRs as well as the corresponding confidence interval to the aSR scale to get the relative aSR and the accompanying confidence interval. This allowed for a direct comparison of DH-CCB aSR to each negative control aSR resulting in a relative aSR with accompanying 95% confidence intervals with the negative control as the reference category. If the relative aSR is determined to be non-significant, this suggests that within-patient time-varying bias is the likely reason for the significant primary analysis. Final coding and variable names to calculate relative aSRs using SAS were incorporated to the PSSA code by Takeuchi and colleagues (Appendix 1).[19]

Further demonstrating this methodology, we compared strata of the DH-CCB PSSA to strata of the ACE inhibitor/ARB PSSA to generate new relative aSRs for age (<65 and ≥65), sex (male and female), calendar year of the index claim (2007–2010, 2011–2013, 2014–2016), and number of other antihypertensive medications (0–1, 2–3, ≥4). We further compared the strata to assess for significant differences in relative aSR (e.g., relative aSR of males vs. relative aSR of females) using the same approach as previously noted.

To evaluate the importance of selecting an appropriate exposure window, we truncated time surrounding the index drug initiation for both the DH-CCB PSSA and the negative control index drugs to 180 and 90 days given on the expected time to the development of edema which typically occurs within weeks or months of DH-CCB initiation.[20]

3 | RESULTS

For the DH-CCB PSSA, we identified similar aSRs, 1.84 (95% CI 1.80–1.88) and 1.87 (95% CI 1.84–1.91) after removing initiators of ACE inhibitors/ARBs and levothyroxine during the exposure window, respectively. The levothyroxine PSSA showed a statistically significant aSR of 1.09 (95% 1.06–1.12); however, incorporating this marginal association into the main PSSA did not show meaningful impact with a relative aSR of 1.72 (95% CI 1.66–1.78; Table 1).

The ACE inhibitor/ARB PSSA was both significant and clinically meaningful resulting in an aSR of 1.27. After incorporating this association into the main PSSA, the relative aSR for DH-CCBs reduced to a relative aSR of 1.45 (95% CI 1.41–1.49) suggesting a clinically meaningfully influence of hypertension progression in the interpretation of the PSSA (Table 1). These relative aSRs quantify the excess initiation rate of a loop diuretic following DH-CCB initiation considering (a) the initiation rate of loop diuretics prior to DH-CCB initiation, (b) secular trends in loop diuretic initiation in the study population, and (c) disparities in loop diuretic initiation before versus after initiation of negative control index drugs to capture causes alternative to a prescribing cascade, namely increases in healthcare utilization and monitoring due to use of a chronic medication for levothyroxine and ACE inhibitors/ARB users and hypertension progression specifically for ACE inhibitor/ARB users.

Assessing the aSR across strata defined by age, gender, study year and the number of other antihypertensives that patients received during the pre-index look back period (i.e., 360 days prior to DH-CCB initiation or ACE inhibitor/ARB initiation), we found the aSR for the DH-CCB PSSA ranged from 1.27 (number of other antihypertensives, ≥4) to 2.33 (number of other antihypertensives, 0–1), and the aSR for the ACE inhibitor/ARB negative control index drug PSSA ranged from 0.80 (number of other antihypertensive, ≥4) to 1.41 (Year 2014–2016; Table 2). If the effects of the negative control index drugs are ignored, it would appear the strata of older adults (versus patients aged <65 years old), men (versus women), and recent years of the study period (2014–2016 vs. prior years) have a stronger signal for a prescribing cascade (Table 2). However, differences within strata when incorporating the aSR of the ACE inhibitor/ARB PSSA in the DH-CCB PSSA were similar across age groups (relative aSR 1.06, 95% CI 0.99–1.12 in adults aged ≥65 vs. <65), among men than women

TABLE 1 Prescribing order of initial loop diuretic in relation to initial dihydropyridine calcium channel blocker versus negative control

Index drug	Total number (*n*)	Number of patients on loop diuretic (*n*)		Null-effect ratio	Crude sequence ratio	Adjusted sequence ratio (95% CI)	Relative adjusted sequence ratio (95% CI)
		After DH-CCB	Before DH-CCB				
DH-CCB	53 074	34 821	18 253	1.03	1.91	1.87 (1.84–1.91)	1.72 (1.66–1.78)
Levothyroxine	19 759	10 387	9372	1.02	1.11	1.09 (1.06–1.12)	1.00 (reference)
DH-CCB	42 415	27 599	14 816	1.01	1.86	1.84 (1.80–1.88)	1.45 (1.41–1.49)
ACEI/ARB	44 627	25 273	19 354	1.03	1.31	1.27 (1.24–1.29)	1.00 (reference)

TABLE 2 Prescribing order of initial loop diuretic in relation to initial dihydropyridine calcium channel blocker versus initial ACEI/ARB—by strata

Strata	Index drug	Total number (*n*)	Number of patients on loop diuretic (*n*)		Null-effect ratio	Crude sequence ratio	Adjusted sequence ratio (95% CI)	Relative adjusted sequence ratio (95% CI)
			After DH-CCB	Before DH-CCB				
Age, y <65	DH-CCB	23 719	15 342	8377	1.01	1.83	1.81 (1.76–1.85)	1.43 (1.38–1.48)
	ACEI/ARB	30 980	17 622	13 358	1.04	1.32	1.27 (1.24–1.30)	1.00 (reference)
Age, y ≥65	DH-CCB	18 696	12 257	6439	1.01	1.90	1.88 (1.82–1.94)	1.51 (1.44–1.58)
	ACEI/ARB	13 647	7651	5996	1.02	1.28	1.25 (1.21–1.28)	1.00 (reference)
Sex Men	DH-CCB	17 474	11 533	5941	1.01	1.94	1.91 (1.85–1.97)	1.42 (1.36–1.49)
	ACEI/ARB	16 641	9669	6972	1.03	1.39	1.34 (1.31–1.39)	1.00 (reference)
Sex Female	DH-CCB	24 941	16 066	8875	1.01	1.81	1.79 (1.74–1.83)	1.46 (1.41–1.51)
	ACEI/ARB	27 986	15 604	12 382	1.03	1.26	1.22 (1.20–1.25)	1.00 (reference)
Year 2007–2010	DH-CCB	19 598	12 503	7095	1.04	1.76	1.69 (1.64–1.74)	1.42 (1.37–1.48)
	ACEI/ARB	21 880	12 139	9741	1.05	1.25	1.19 (1.16–1.22)	1.00 (reference)
Year 2011–2013	DH-CCB	13 228	8652	4576	1.07	1.89	1.77 (1.71–1.83	1.46 (1.39–1.54)
	ACEI/ARB	12 732	7091	5641	1.04	1.26	1.21 (1.17–1.25)	1.00 (reference)
Year 2014–2016	DH-CCB	9589	6444	3145	1.08	2.05	1.91 (1.83–1.99)	1.35 (1.28–1.43)
	ACEI/ARB	10 015	6043	3972	1.08	1.52	1.41 (1.35–1.47)	1.00 (reference)
Number of other antihypertensives 0–1	DH-CCB	11 868	8357	3511	1.02	2.38	2.33 (2.24–2.43)	1.67 (1.60–1.75)
	ACEI/ARB	32 467	19 206	13 261	1.04	1.45	1.39 (1.36–1.43)	1.00 (reference)
Number of Other Antihypertensives 2–3	DH-CCB	23 198	15 130	8068	1.01	1.88	1.85 (1.80–1.90)	1.86 (1.78–1.94)
	ACEI/ARB	11 718	5873	5845	1.01	1.00	0.99 (0.96–1.03)	1.00 (reference)
Number of other antihypertensives ≥4	DH-CCB	7349	4112	3237	1.00	1.27	1.27 (1.21–1.33)	1.59 (1.31–1.93)
	ACEI/ARB	442	194	248	0.98	0.78	0.80 (0.66–0.96)	1.00 (reference)

TABLE 3 Prescribing order of initial loop diuretic in relation to initial dihydropyridine calcium channel blocker versus negative control using a 180 or 90 day exposure window

Index drug	Exposure window	Total number (*n*)	Number of patients on loop diuretic (*n*)		Null-effect ratio	Crude sequence ratio	Adjusted sequence ratio (95% CI)	Relative adjusted sequence ratio (95% CI)
			After DH-CCB	Before DH-CCB				
DH-CCB	180 days	33 586	22 291	11 295	1.05	1.97	1.89 (1.84–1.93)	1.90 (1.82–1.98)
Levothyroxine		12 382	6359	6023	1.06	1.06	0.99 (0.96–1.03)	1.00 (reference)
DH-CCB		26 143	17 285	8858	1.03	1.95	1.89 (1.84–1.93)	1.68 (1.62–1.74)
ACEI/ARB		28 202	15 371	12 831	1.07	1.20	1.12 (1.09–1.15)	1.00 (reference)
DH-CCB	90 days	21 107	13 940	7167	1.03	1.95	1.89 (1.84–1.95)	2.09 (1.98–2.20)
Levothyroxine		7953	3881	4072	1.05	0.95	0.91 (0.87–0.95)	1.00 (reference)
DH-CCB		16 137	10 731	5406	1.02	1.99	1.95 (1.89–2.01)	1.86 (1.78–1.94)
ACEI/ARB		18 505	9668	8837	1.04	1.09	1.04 (1.01–1.07)	1.00 (reference)

(relative aSR 0.97, 95% 0.92–1.03), and in years 2011–2013 versus 2007–2010 (relative aSR 1.03, 95% CI 0.96–1.10) and 2014–2016 versus 2007–2010 (relative aSR 0.95, 95% CI 0.89–1.05). Interestingly, while the aSR decreased with use of an increasing number of antihypertensives in both the DH-CCB (aSR 2.33 [0–1], aSR 1.85 [2,3], and aSR 1.27 [≥4 other antihypertensives]) and the ACE inhibitor/ARB (aSR 1.39 [0–1], aSR 0.99 [2,3], and aSR 0.80 [≥4 other antihypertensives]) PSSAs, there was a slight upward trend in the relative aSR of 2–3 versus 0–1 other antihypertensives (1.11, 95% CI 1.05–1.19) with no difference in relative aSR between ≥4 and 0–1 other antihypertensives (0.95, 95% CI 0.78–1.16).

When reducing the assessment window to 180 or 90 days, the aSR for DH-CCB remained very similar while the aSR for ACE/ARB decreased (Table 3), likely as result of reducing the effect of disease progression. Accordingly, the relative aSR increased with smaller exposure windows, likely via enhanced focus on the prescribing cascade (i.e., onset and treatment of edema shortly after initiation of DH-CCB).

4 | DISCUSSION

Our paper further expands on PSSA methodology by illustrating how aSRs can be overestimated and how an overestimation of the aSR can be reduced while using a known prescribing cascade signal with negative control index drugs. We presented the aSR for the DH-CCB—loop diuretic prescribing cascade solely adjusted for secular trends and then showed how the aSR changed after removing the effect of within-patient time-varying bias using two negative control index drugs. Although the aSR was only slightly reduced when adjusting for aSR of levothyroxine as negative control index drugs, the use of ACE inhibitor/ARB as the negative control index drug appreciably decreased the original aSR estimate, likely as a result of within-patient time-varying bias due to hypertension progression. This finding highlights the importance of the negative control index drug(s) in capturing all alternative pathways that may explain time-varying changes in initiation of a medication that may be inappropriately attributed to a prescribing cascade.[1] While levothyroxine may have captured and quantified the probability to receive a loop diuretic due to increased monitoring after receiving a new chronic medication, this levothyroxine user population likely did not represent patients with progressing hypertension. Users of ACE inhibitors/ARBs, in contrast, captured similar risk for disease progression, which may result in therapeutically indicated initiation of loop diuretics for hypertension.

The use of a negative control index drug also helped interpret stratified analyses that have been performed in previous PSSAs. To our knowledge, stratified analyses were first assessed in the atypical antipsychotic-induced hyperlipemia prescribing cascade, with aSRs provided for two age strata.[21] Stratum-specific analyses allow for the assessment of aSRs among strata as certain populations (e.g., age, sex) may be at a differential risk for the prescribing cascade; however, alternative causes for initiation of the marker drug might also differ in certain populations. Therefore, the incorporation of stratum-specific negative control index drug analyses can provide for a better estimation of the aSR. For example, if no negative control PSSA results were considered for the analysis by number of other antihypertensives, one may conclude that patients with use of 0–1 other antihypertensives (aSR 2.33) may be at a higher risk for the prescribing cascade when compared to patients with use of 2–3 (aSR 1.85) and ≥4 (aSR 1.27) other antihypertensives. However, by incorporating ACE inhibitor/ARB negative control PSSAs, results across strata became more similar with the highest relative aSR noted among patients with 2–3 other antihypertensives (relative aSR 1.86).

Moreover, the typical timing of DH-CCB-induced edema is expected to occur soon after initiation (within 90 days as noted in a previous assessment of this prescribing cascade).[20] Knowledge of when adverse events typically present (along with how bothersome these may be to patients and the subsequent need to seek follow-up with a healthcare provider) is important to ensure an appropriate

exposure window is used as the primary analysis. As noted in our results, restricting to a 180- or 90-day exposure window found no significant aSR for levothyroxine, and a reduced aSR for ACE inhibitor/ARB as negative control index drugs without meaningfully impacting the aSR of DH-CCB. This suggests the use of exposure windows that are tailored to the expected etiology of the adverse event can further reduce within-patient time-varying bias.

Based on our findings, we recommend that future PSSA studies (a) identify and incorporate an appropriate negative control index drug, (b) generate a relative aSR that compares the probability for marker drug initiation for index drug to the negative control index drug, (c) use negative control index drugs as part of stratum-specific analyses, and (d) compare negative control index drug aSRs across varying exposure windows, depending on the nature and timing of the drug-induced adverse event and subsequent medication prescribing.

Our findings further add to the expanding literature on the utility of negative controls in observational studies, specifically when negative controls are incorporated into the primary findings in order to reduce bias.[16] Studies using PSSA, like many other studies that use other designs, has used negative controls to assess for potential bias.[6,9-13] When negative controls were found to be significant, these findings were then interpreted as indicative of an overestimation of the initial findings or authors noted that bias may explain the positive findings.

There are a few limitations to note. First, the relative aSRs comparison method can only be applied for two mutually exclusive aSRs thus resulting in a reduction in sample size, through exclusions, for both the exposure of interest and the negative control drug. Fortunately, the PSSA is well-suited for low counts.[2] Second, we focused on use of negative control index drugs to adjust aSRs. Other studies have suggested the use of negative control marker drugs, which could further advance to rigor of PSSAs. Findings using the PSSA do not support causal inference; however, use of negative controls to decrease within person, time-varying bias is an improvement on this pharmacovigilance method. Third, we used hand-selected negative controls as opposed to previous studies which used a host of negative controls (most of which are not related clinically or pharmacologically to the evaluates drug-outcome pair).[22,23] In contrast to these studies which quantified bias as well as random error arising from their data, we believe the selected negative controls closely resemble the clinical scenario at hand and are thus likely to provide a fair quantification of bias that is imposed through a single mechanism (i.e., natural progression of disease).

In conclusion, we used our previously examined PSSA of the DH-CCB—loop diuretic prescribing cascade to demonstrate how negative control index drugs can be incorporated into a PSSA to further reduce within-patient time-varying bias and avoid overestimating PSSA signals. We have illustrated the importance of generating relative aSRs, using negative control index drugs in stratum-specific analyses, and using negative control index drugs in varying exposure windows as part of the PSSA in order to focus comparisons to the likely onset of the prescribing cascade.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The study was exempted from review by the University of Florida institutional review board because of its use of deidentified data.

ORCID

Scott Martin Vouri https://orcid.org/0000-0002-0411-2160
Xinyi Jiang https://orcid.org/0000-0002-9097-2641
Earl J. Morris https://orcid.org/0000-0002-6092-8906
Almut G. Winterstein https://orcid.org/0000-0002-6518-5961

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APPENDIX 1: Final coding to compare relative adjusted sequence ratios using SAS

```
/*Calculation of adjusted sequence ratios (ASRs)*/
    data ssa_c03(keep = TOTAL DIA_Gx_DRG DIA_Lx_DRG DRG1-DRG&N DIA1-DIA&N s_DRG s_DIA s_Upper s_Diago s_Lower R_c std_R_c expcsr null_e expest estimate expll expul ll ul);
    merge ssa_c01 ssa_c02;
    DIA_Lx_DRG = TOTAL-DIA_Gx_DRG;
    /*Calculation of CSR*/
    if DIA_Gx_DRG > =0 & DIA_Lx_DRG > 0 then R_c = log (DIA_Gx_DRG/DIA_Lx_DRG); else R_c = .;
    if DIA_Gx_DRG = 0 or DIA_Lx_DRG = 0 then std_R_c = .; else std_R_c = sqrt((1/DIA_Gx_DRG) + (1/DIA_Lx_DRG));
    expcsr = exp(R_c);
    /*Calculation of NSR*/
    null_e = (s_Upper+s_Diago)/s_Lower;
    if expcsr> = 0 & null_e > 0 then expest = expcsr/null_e;else expest = .;
    estimate = log(expest);
    ll = estimate-1.96*std_R_c;
    ul = estimate+1.96*std_R_c;
    expll = exp(ll);
    expul = exp(ul);
    run;
    /**DH CCB group****/
data data1(keep = std_R_c csr nsr estimate ll ul expest expll expul);
    set ssa_c03;/**ssa_c03 is from the corresponding PSSA analysis for the DH-CCB group****/
    csr = expcsr;
    nsr = null_e;
run;
data data1 (keep = id std_R_c1 estimate1);
set data1;
rename std_R_c = std_R_c1;
rename estimate = estimate1;
id = 1;
run;
/** Negative Control Group****/
data data2(keep = std_R_c csr nsr estimate ll ul expest expll expul);
    set ssa_c03;/**ssa_c03 is from the corresponding PSSA analysis for the negative control group different from previous ssa_c03 for DH-CCB group****/
    csr = expcsr;
    nsr = null_e;
run;
data data2 (keep = id std_R_c2 estimate2);
set data2;
rename std_R_c = std_R_c2;
rename estimate = estimate2;
id = 1;
run;
proc sql;
    create table difference
    as select a.*,b.*
from data1 a left join data2 b
on a.id = b.id;
/*Calculation of relative adjusted sequence ratios (relative ASRs)*/
data result;
set difference;
diff = (estimate1-estimate2);
SE = sqrt(std_R_c2**2 + std_R_c1**2);
lldiff = diff-1.96*SE;
uldiff = diff+1.96*SE;
run;
data result1(keep = RRR explldiff expuldiff );
    set result;
    RRR = exp(diff );
    explldiff = exp(lldiff );
    expuldiff = exp(uldiff );
run;
```