Risk of Renal Cell Carcinoma Associated with Calcium Channel Blockers

A Nationwide Observational Study Focusing on Confounding by Indication

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Background: We examined whether the apparent association between renal cell carcinoma (RCC) and use of dihydropyridine calcium channel blockers (CCBs) was explained by confounding by indication since hypertension, the main indication for CCBs, is a risk factor for RCC.

Methods: Using Danish health registries, we conducted a nested case-control study including 7315 RCC cases during 2000–2015. We matched each case with up to 20 controls on age and sex using risk-set sampling. We estimated odds ratios (ORs) for long-term CCB use associated with RCC using conditional logistic regression. We addressed confounding by indication by (1) adjusting for hypertension severity indicators; (2) evaluating dose-response patterns; (3) examining whether other first-line anti-hypertensives were associated with RCC; and (4) using an active comparator new user design by nesting the study in new users of CCBs or angiotensin-converting enzyme inhibitors (ACEIs).

Results: The adjusted OR for RCC associated with long-term CCB use compared to non-use was 1.76 (1.63–1.90). After we additionally adjusted for hypertension severity indicators, the OR remained elevated (OR 1.37; confidence interval [CI] 1.25, 1.49) with evidence of a dose-response pattern. Other anti-hypertensives were also associated with RCC, for example, ACEIs (OR 1.27; 95% CI = 1.16, 1.39) and thiazides (OR 1.22; 95% CI = 1.12, 1.34). In the active comparator new user design, the OR was 1.21 (95% CI = 0.95, 1.53) for use of CCBs compared with ACEIs.

Conclusions: In this population, confounding by indication appeared to explain at least part of the association between RCC and dihydropyridine CCBs.

Keywords: Anti-hypertensives; Calcium channel blockers; Case-control studies; Pharmacoepidemiology; Renal cell carcinoma

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The incidence of renal cell carcinoma (RCC) has risen markedly during the last decades.[1] RCCs account for more than 90% of adult kidney cancers, with the most common histologic subtypes being clear cell and papillary carcinomas.[2,3] The established modifiable risk factors for RCC are smoking, elevated body mass index (BMI), and hypertension.[2] In a recent drug–cancer screening study, we found an increased risk of RCC in users of dihydropyridine calcium channel blockers (CCBs) with an odds ratio (OR) of 1.80 (95% confidence interval [CI] = 1.65, 1.97).[4] Use of anti-hypertensive drugs has previously been associated with increased risk of RCC, but this has largely been attributed to confounding by indication, that is, the underlying hypertension.[5–10] CCBs have been studied less extensively than other anti-hypertensive drugs and, similar to studies on other anti-hypertensive drugs, it has been difficult to disentangle the effect of hypertension from that of its treatment.[11–15] To our knowledge, no biologic mechanisms for a carcinogenic effect of dihydropyridine CCBs in the kidneys have been identified.

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Conflicts of interest

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According to Danish law, the authors are not allowed to share individual-level data. Data access can be granted through the Danish Health Data Authority to institutions licensed to access and analyze Danish registry data. Statistical code is available upon request to the corresponding author.

All authors conceived and designed the study. K.B.K., A.P., and J.H. drafted the initial manuscript. K.B.K. conducted the statistical analyses. All authors revised the manuscript critically. All authors read and approved the final version of the manuscript.

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Given the rising incidence of RCC, the high prevalence of dihydropyridine CCB use, and the preliminary signals associating dihydropyridine CCBs with RCC, we conducted a nationwide case-control study to examine whether this association could be explained by confounding by indication.

METHODS

In this nested case-control study, we identified cases of primary RCC diagnosed in Denmark during 2000–2015 and matched each case with 20 population controls. We estimated ORs for RCC associated with use of dihydropyridines addressing confounding by indication by several approaches.

Data Sources

To identify incident cases of RCC, we used the Danish Cancer Registry, which is continuously validated and has accurate and almost complete data on incident cancers in Denmark.[16] We used the Danish Civil Registry to obtain information on date of birth, vital status, and migrations.[17] We retrieved data on exposure to drugs from the Danish National Prescription Registry with data on active ingredient, date of dispensing, dose, and package size for all filled prescriptions in Denmark since 1995.[18] Information on ambulatory and discharge diagnoses is recorded in the Danish National Patient Registry.[19] We obtained information on education from the Danish Education Registries through Statistics Denmark.[20]

Population

We identified cases during 2000–2015 and matched each case to up to 20 controls on age and sex using risk-set sampling. The controls were assigned an index date corresponding to the date of diagnosis of their matched case. Persons were eligible for sampling as controls until they potentially became cases.[21] Cases and controls were eligible for inclusion if they were aged 18–85 years at index date, had no previous cancer (except non-melanoma skin cancer) at index date, and no conditions strongly predisposing to RCC (von Hippel–Lindau syndrome, polycystic kidney disease, or tuberous sclerosis). Further, we required that participants had resided continuously in Denmark for 10 years before the index date.

Exposure

The main exposure was long-term use of dihydropyridines, arbitrarily defined as a cumulative dose of more than 1000 defined daily doses (DDDs). For example, the DDD for amlodipine is 5 mg and long-term use is defined as a cumulative dose exceeding 5000 mg.[22] We defined ever use as having filled at least one dihydropyridine prescription. In the main analyses, we pooled DDDs for all dihydropyridines. In addition, we repeated the analyses with each of the individual dihydropyridines available in Denmark during the study period. We applied a lag time of 24 months (i.e., we disregarded use of dihydropyridines in the 2 years preceding the index date) to allow for a reasonable induction period of RCC and to account for protopathic bias (hypertension caused by yet undiagnosed RCC) and surveillance bias.[23] In sensitivity analyses, we varied the length of the lag time from 0 to 60 months. The dihydropyridines are the most common class of CCBs used in Denmark and accounted for 77% of all sales of CCBs in 2000 and 94% of all sales in 2010.[24] The available non-dihydropyridine CCBs in Denmark were verapamil and diltiazem. Use of these drugs was not considered in the main analyses, however, associations for these drugs with RCC were examined in supplementary analyses.

Covariates

We adjusted for the following potential confounders: (1) use of drugs (defined as two or more filled prescriptions) known or suspected to influence risk of RCC including low-dose aspirin, non-steroidal anti-inflammatory drugs, paracetamol, and lithium; (2) a history of conditions that may influence risk of RCC including diabetes mellitus type 1 and 2, chronic obstructive pulmonary disease as a proxy for heavy smoking, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education as a measure of socioeconomic status. To address confounding by indication, we further adjusted for hypertension severity as described below. We applied a 2-year lag-time for the above covariates except education. Low-dose aspirin is available over-the-counter in Denmark; however, only prescription drugs are eligible for reimbursement and the proportion of total low-dose aspirin sales dispensed by prescription, and thus recorded in the Danish Prescription Registry, is high.[25]

Analyses to Address Confounding by Indication

We estimated ORs for RCC associated with use of dihydropyridines compared to never-use with conditional logistic regression and repeated all analyses stratifying by histologic subtype (clear cell RCC, papillary RCC, and other RCC). We performed several analyses to evaluate the potential for confounding by indication:

(1) We adjusted for indicators of hypertension severity by including the following covariates: (1) number of antihypertensive drug classes used (0, 1, 2, 3, ≥4) up to 2 years before index date; (2) a discharge or ambulatory diagnosis of hypertension, that is, hypertension treated outside the primary care sector indicating more severe or treatment-refractory hypertension; (3) a history of hypertensive complications including retinopathy, hypertensive encephalopathy, peripheral artery disease, ischemic heart disease, congestive heart failure, and transient ischemic attack or stroke; and (4) use of each of the following anti-hypertensive drugs or diuretics defined as two or more filled prescriptions up to 2 years before index date: angiotensin-converting enzyme inhibitors (ACEIs), aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, furosemide, and thiazides. The threshold of two or more filled prescriptions was chosen to increase

the likelihood that these drugs were used to treat chronic conditions such as hypertension.

(2) We evaluated dose-response patterns by including cumulative dose as an ordinal variable (0–149, 150–499, 500–999, 1000–1999, 2000–3999, ≥4000 DDDs) and as a continuous variable using restricted cubic splines with four knots located at the 5th, 35th, 65th, and 95th percentile.[26]

(3) We evaluated the association between other first-line anti-hypertensive drugs (ACE-inhibitors, angiotensin receptor blockers, beta-blockers, and thiazides) and RCC risk. In these analyses, we adjusted for the covariates outlined above as well as use of dihydropyridines.

(4) We redid the study using an active comparator new user design. We identified all patients initiating dihydropyridines or ACE inhibitors from 2000 and onwards and used this cohort as the source population for identification of cases and sampling of controls. To include new users only, we excluded patients filling a prescription of dihydropyridines or ACE inhibitors during 1995–1999. We identified persons with incident RCC from 2000 to 2015 (cases) and matched each case with up to 20 controls on age, sex, and time of first prescription for ACE inhibitors or dihydropyridines (±183 days). Conditional logistic regression was used to estimate ORs for RCC associated with dihydropyridine use compared to ACE inhibitor use. Because of the smaller sample size, we stratified cumulative dose into three categories to evaluate dose-response associations (1–499, 500–1999, and ≥2000 DDDs). For each exposure category of dihydropyridine use (e.g., long-term use), we used the corresponding exposure category of ACE inhibitor use as reference. We disregarded individuals who switched between dihydropyridine and ACE inhibitor use in the main analyses. In sensitivity analyses, we allowed for low to moderate use (defined as <500 DDDs) of ACE inhibitors in the CCB user category and for low to moderate use of dihydropyridines in the ACEI user category.

In the new-user active comparator study, we measured covariates before entry into the source population (i.e., before initiation of dihydropyridines or ACE inhibitors) to avoid adjusting for on-treatment covariates. Other than the difference in covariate assessment windows as detailed above, the included covariates were the same as in the previous analyses also applying a lag time of 24 months.

We chose ACEIs as active comparator since this drug class is mainly used to treat hypertension and, like dihydropyridine therapy, was an established anti-hypertensive treatment option in Denmark at the beginning of the study period. To assess the robustness of our choice of active comparator, we repeated the study with angiotensin receptor blockers and thiazides as active comparators. That is, we conducted two separate studies identifying new users of angiotensin receptor blockers or dihydropyridines and new users of thiazides or dihydropyridines, respectively.

We used a nested case-control design because of the long follow-up and cumulative time-varying exposure definition where the nested case-control study with risk-set sampling is more computationally efficient. Nested case-control studies with an active comparator new user methodology have been carried out previously.[27,28]

Supplementary Analyses

To examine how clinical-stage influenced the findings, we repeated the main analyses while stratifying cases by clinical stage (localized disease defined as T1-2 stage tumors without nodal or metastatic spread; advanced disease defined as T3-4 stage tumors or tumors of any T-stage with nodal or metastatic spread; or unknown tumor stage).

Other

Statistical analyses were conducted using Stata 15.2 (StataCorp., College Station, TX). The study was approved by the Danish Data Protection Agency. According to Danish law, studies based solely on register data do not require approval from an ethics review board. The codes used to define outcomes, exposure, and covariates are shown in eAppendix 1; http://links.lww.com/EDE/B724.

RESULTS

During the study period, 9987 persons were diagnosed with RCC, of whom 7315 (73%) were eligible for inclusion (Figure 1). Characteristics of cases and controls at the index date are shown in eTable 1; http://links.lww.com/EDE/B724. The mean age of cases was 64 years, 66% were male, and 90% were diagnosed with clear cell RCC, 4% with papillary RCC, and 6% with other types of RCC.

Association Between Dihydropyridines and Renal Cell Carcinoma

The prevalence of long-term dihydropyridine use was 14% in cases and 8% in controls yielding a crude OR of 2.07 (95% CI = 1.92, 2.23) (Table 1). The association was attenuated when adjusting for potential confounders (OR 1.76, 95% CI = 1.63, 1.90) and was further attenuated when additionally adjusting for indicators of hypertension severity (OR 1.37, 95% CI = 1.25, 1.49). Adjusting for use of other anti-hypertensive drugs, a discharge diagnosis of hypertension, and number of different anti-hypertensive drug classes used attenuated the association the most, while adjusting for other covariates had less influence on the effect estimates (Table 2). We observed a dose-response pattern with increasing ORs for RCC with increasing cumulative dose in models with cumulative dose as an ordinal variable (Table 1) and as a continuous variable using restricted cubic splines (Figure 2).

The association with long-term use of dihydropyridines was similar for clear cell adenocarcinomas (OR 1.39, 95% CI = 1.27, 1.53) and papillary adenocarcinomas (OR 1.46,

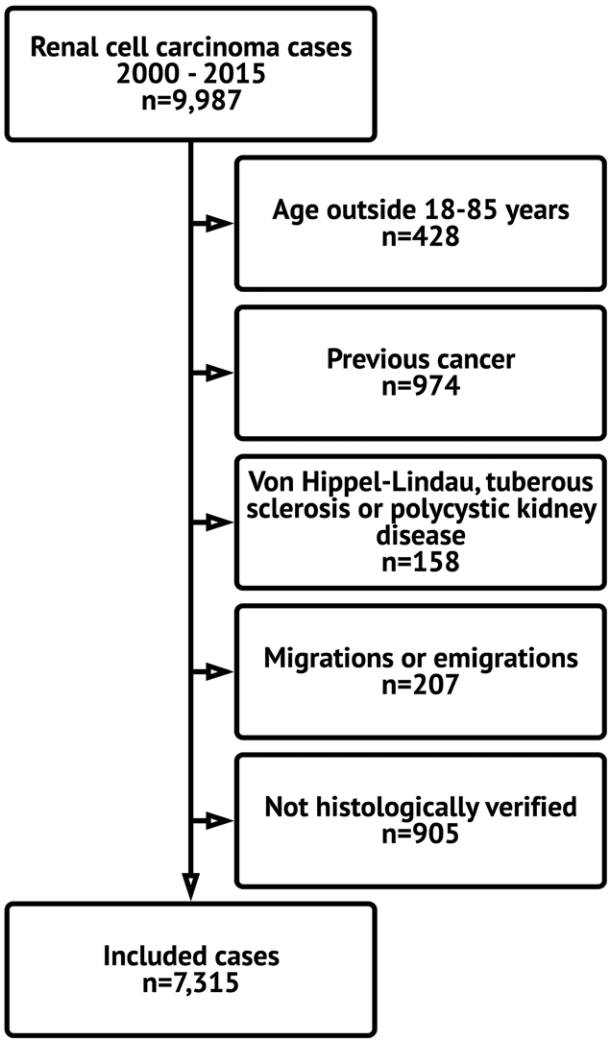

FIGURE 1. Selection of cases.

95% CI = 1.00, 2.15), whereas the OR was close to unity for other RCC (OR 0.91, 95% CI = 0.63, 1.32) (Table 1).

When stratifying by clinical stage (data not tabulated), the OR for long-term use of dihydropyridines associated with RCC was 1.62 (95% CI = 1.42, 1.84) for localized disease, 1.23 (95% CI = 1.06, 1.42) for advanced disease, and 1.09 (95% CI, 0.89 to 1.34) for unknown clinical stage. For ever-use of dihydropyridines, the OR was 1.53 (95% CI = 1.38, 1.70) for localized disease, 1.15 (95% CI = 1.02, 1.29) for advanced disease, and 1.04 (95% CI = 0.88, 1.21) for unknown clinical stage.

Effect estimates were largely similar between individual dihydropyridines; however, statistical precision varied and we observed the strongest associations for amlodipine (OR 1.33, 95% CI = 1.21, 1.45) and lercanidipine (OR 1.69, 95% CI = 1.20, 2.37) (eTable 2; http://links.lww.com/EDE/B724).

The non-dihydropyridine CCB verapamil was associated with RCC with an OR of 1.31 (95% CI = 1.06, 1.61) while diltiazem was not associated with RCC (OR 0.90, 95% CI = 0.70, 1.17) (eTable 3; http://links.lww.com/EDE/B724).

Results were similar across analyses using different lag periods (eTable 4; http://links.lww.com/EDE/B724).

Association Between Other First-line Anti-hypertensive Drugs and Renal Cell Carcinoma

Long-term use of ACE inhibitors, angiotensin receptor blockers, beta-blockers, and thiazides were all associated with increased ORs for RCC in unadjusted as well as adjusted analyses (Figure 3). In the fully adjusted analyses, use of ACE inhibitors showed the strongest association with an OR of 1.27 (95% CI = 1.16, 1.39) followed by thiazides (OR 1.22, 95% CI = 1.12, 1.34), angiotensin receptor blockers (OR 1.14, 95% CI = 1.04, 1.26), and beta-blockers (OR 1.11, 95% CI = 1.00, 1.22). For all drugs, the ORs increased from the lowest to the highest dose category; however, ORs for categories in between did not increase consistently (eTable 5; http://links.lww.com/EDE/B724).

Active Comparator New User Design

We included 2074 RCC cases when nesting the study in new users of dihydropyridines or ACE inhibitors (eFigure 1; http://links.lww.com/EDE/B724). The mean age at cohort entry (i.e., at the first prescription of dihydropyridines or ACE inhibitors) was 62 years and the mean follow-up duration from cohort entry to index date was 4.9 years (Table 3). Among controls, patient characteristics did not differ markedly between users of dihydropyridines and ACEIs besides a higher prevalence of diabetes and congestive heart failure in ACEI users (Table 3). The OR for RCC associated with long-term use of dihydropyridines compared to long-term use of ACE inhibitors was 1.21 (95% CI = 0.95, 1.53) and with no clear dose-response pattern with ORs for 1–499 DDDs of 1.24 (95% CI = 0.97, 1.58) and for 2000+ DDDs of 1.18 (95% CI = 0.84, 1.67) (Table 4 and Figure 4). When allowing for moderate switching between ACE inhibitor and dihydropyridine use, the OR was 1.32 (95% CI = 1.10, 1.60). When stratifying by clinical stage (not tabulated), the OR for long-term use of ACEIs was 1.25 (95% CI = 0.92, 1.71) for localized disease and 1.01 (95% CI = 0.67, 1.52) for advanced disease while the OR for ever-use was 1.17 (95% CI = 0.96, 1.42) for localized disease and 1.08 (95% CI = 0.86, 1.37) for advanced disease.

With angiotensin receptor blockers as active comparator, a total of 1783 cases were included and the OR for long-term use of dihydropyridines compared to long-term use of angiotensin receptor blockers was 1.32 (95% CI = 1.05, 1.67) (Figure 4, eFigure 2; http://links.lww.com/EDE/B724, eTable 6; http://links.lww.com/EDE/B724, and eTable 7; http://links.lww.com/EDE/B724). With thiazides as an active comparator, 2009 cases were included and the resulting OR for long-term use was 1.00 (95% CI = 0.75, 1.35) (Figure 4, eFigure 3; http://links.lww.com/EDE/B724, eTable 8; http://links.lww.com/EDE/B724, and eTable 9; http://links.lww.com/EDE/B724).

TABLE 1. Risk of Renal Cell Carcinoma with Use of Dihydropyridines Compared to Never–use

Exposure Group	Cases, No.	Controls, No.	Unadjusted OR[a]	Adjusted OR[b]	Fully Adjusted OR[c]
All renal cell carcinomas					
Never use	5,589	124,501	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Ever use	1,726	21,799	1.85 (1.75–1.96)	1.63 (1.53–1.74)	1.28 (1.19–1.37)
Long-term use (1000+ DDDs)	1,006	11,425	2.07 (1.92–2.23)	1.76 (1.63–1.90)	1.37 (1.25–1.49)
Cumulative dose (DDDs)					
1–149	228	3,451	1.47 (1.28–1.69)	1.33 (1.15–1.53)	1.10 (0.95–1.27)
150–499	248	3,689	1.55 (1.36–1.77)	1.37 (1.20–1.58)	1.11 (0.96–1.28)
500–999	232	3,101	1.74 (1.51–2.00)	1.52 (1.32–1.76)	1.20 (1.04–1.39)
1000–1999	337	4,119	1.90 (1.69–2.13)	1.63 (1.45–1.84)	1.29 (1.14–1.47)
2000–3999	347	4,128	1.96 (1.74–2.20)	1.68 (1.49–1.89)	1.34 (1.18–1.52)
4000+	334	3,311	2.37 (2.10–2.68)	1.94 (1.71–2.20)	1.51 (1.32–1.74)
Clear cell adenocarcinomas					
Never use	5,045	112,444	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Ever use	1,537	19,196	1.87 (1.76–1.99)	1.65 (1.55–1.77)	1.31 (1.21–1.41)
Long-term use (1000+ DDDs)	884	10,012	2.08 (1.92–2.25)	1.78 (1.64–1.93)	1.39 (1.27–1.53)
Cumulative dose (DDDs)					
1–149	210	3,051	1.54 (1.33–1.78)	1.39 (1.20–1.61)	1.15 (0.99–1.34)
150–499	223	3,275	1.56 (1.35–1.79)	1.38 (1.19–1.59)	1.12 (0.97–1.30)
500–999	209	2,743	1.76 (1.52–2.03)	1.54 (1.33–1.79)	1.22 (1.04–1.42)
1000–1999	301	3,642	1.91 (1.69–2.17)	1.64 (1.45–1.87)	1.32 (1.15–1.51)
2000–3999	316	3,604	2.05 (1.82–2.32)	1.76 (1.55–1.99)	1.42 (1.24–1.62)
4000+	278	2,881	2.28 (2.00–2.60)	1.87 (1.63–2.15)	1.48 (1.27–1.71)
Papillary adenocarcinomas					
Never use	173	4,343	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Ever use	101	1,137	2.40 (1.83–3.13)	1.90 (1.42–2.53)	1.18 (0.85–1.65)
Long-term use (1000+ DDDs)	72	627	2.99 (2.21–4.04)	2.25 (1.61–3.14)	1.46 (1.00–2.15)
Cumulative dose (DDDs)					
1–149	8	161	1.10 (0.53–2.31)	0.93 (0.44–1.97)	0.70 (0.32–1.54)
150–499	9	185	1.34 (0.67–2.69)	1.20 (0.59–2.45)	0.81 (0.38–1.72)
500–999	12	155	2.13 (1.14–4.00)	1.78 (0.93–3.39)	1.37 (0.68–2.73)
1000–1999	21	211	2.63 (1.61–4.29)	2.08 (1.25–3.49)	1.52 (0.87–2.66)
2000–3999	16	224	1.65 (0.96–2.85)	1.38 (0.77–2.44)	1.00 (0.54–1.86)
4000+	35	201	4.51 (2.96–6.88)	3.14 (1.95–5.04)	2.12 (1.24–3.62)
Other					
Never use	371	7,714	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Ever use	88	1,466	1.27 (0.99–1.63)	1.19 (0.91–1.55)	0.96 (0.71–1.29)
Long-term use (1000+ DDDs)	50	786	1.37 (1.00–1.87)	1.23 (0.88–1.72)	0.91 (0.63–1.32)
Cumulative dose (DDDs)					
1–149	10	239	0.87 (0.45–1.67)	0.80 (0.42–1.55)	0.65 (0.33–1.28)
150–499	16	229	1.61 (0.95–2.71)	1.51 (0.88–2.57)	1.21 (0.69–2.13)
500–999	11	203	1.26 (0.67–2.35)	1.12 (0.59–2.13)	0.95 (0.48–1.85)
1000–1999	15	266	1.21 (0.71–2.09)	1.12 (0.64–1.95)	0.85 (0.48–1.52)
2000–3999	15	300	1.10 (0.64–1.88)	0.98 (0.56–1.70)	0.67 (0.37–1.21)
4000+	21	229	1.99 (1.23–3.21)	1.74 (1.05–2.86)	1.43 (0.84–2.44)

[a]Adjusted for age, sex, and calendar time (by design).

[b]Adjusted for (1) use of low-dose aspirin, non-steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education.

[c]Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (2) ambulatory/discharge diagnosis of hypertension; (3) hypertensive complications; and (4) use of each of the following anti-hypertensive drugs or diuretics: ACE inhibitors, aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, furosemide, and thiazides.

TABLE 2. Effect of Adjusting for Individual Covariates on the OR Associating Long-term Use of Dihydropyridines with Renal Cell Carcinoma, Prevalence of the Covariate in Controls, and ORs for Each Covariate's Association with Exposure and Outcome

Covariates Included in the Model	Adjusted OR Associating Long-term Use of Dihydropyridines with Renal Cell Carcinoma	Prevalence of Covariate in Controls (%)	OR Associating Covariate with Renal Cell Carcinoma	OR Associating Covariate with Use of Dihydropyridines
ORs from the main analysis				
Unadjusted OR[a]	2.07 (1.92–2.23)	—	—	—
Adjusted OR[b]	1.76 (1.63–1.90)	—	—	—
Fully adjusted OR[c]	1.37 (1.25–1.49)	—	—	—
Effect of adjustment for specific covariates				
Anti-hypertensive drugs				
All anti-hypertensive drugs listed below included as covariates	1.41 (1.29–1.53)	—	—	—
Beta-blockers	1.86 (1.72–2.01)	14.5	1.58 (1.48–1.69)	5.38 (5.16–5.61)
Angiotensin receptor blockers	1.84 (1.70–1.99)	7.7	1.81 (1.67–1.96)	7.06 (6.72–7.41)
ACE-inhibitors	1.71 (1.58–1.85)	14.0	1.85 (1.74–1.98)	7.14 (6.83–7.45)
Thiazides	1.67 (1.54–1.81)	18.3	1.81 (1.71–1.92)	9.58 (9.16–10.02)
Furosemide	1.95 (1.81–2.10)	6.0	1.76 (1.61–1.92)	3.47 (3.28–3.67)
Alpha-blockers	2.04 (1.89–2.20)	0.9	1.78 (1.45–2.17)	4.97 (4.39–5.63)
Aldosterone antagonists	2.04 (1.89–2.20)	1.5	1.63 (1.38–1.92)	3.19 (2.88–3.52)
Amiloride	2.04 (1.90–2.20)	2.0	1.52 (1.31–1.77)	3.20 (2.91–3.52)
Markers of severity of hypertension				
All markers listed below included as covariates	1.41 (1.29–1.53)	—	—	—
Ambulatory/discharge diagnosis of hypertension	1.76 (1.63–1.92)	8.9	1.84 (1.71–1.98)	11.66 (11.12–12.23)
Any hypertensive complication	1.99 (1.85–2.14)	14.0	1.35 (1.26–1.44)	3.09 (2.95–3.23)
Number of used anti-hypertensive drug classes[d]	1.44 (1.33–1.57)	—	—	—
Other drugs				
All drugs listed below included as covariates	1.88 (1.75–2.03)	—	—	—
Low-dose aspirin	1.92 (1.78–2.07)	15.5	1.48 (1.39–1.58)	4.32 (4.13–4.51)
NSAIDs	2.02 (1.88–2.18)	47.0	1.34 (1.27–1.41)	1.44 (1.38–1.50)
Paracetamol	2.02 (1.87–2.17)	12.0	1.37 (1.28–1.48)	1.88 (1.79–1.98)
Lithium	2.07 (1.92–2.23)	0.4	0.84 (0.55–1.29)	1.03 (0.77–1.38)
Medical history				
All conditions listed below included as covariates	1.88 (1.74–2.02)	—	—	—
Diabetes	1.94 (1.80–2.09)	6.4	1.72 (1.58–1.87)	3.68 (3.49–3.89)
Chronic obstructive pulmonary disease	2.06 (1.91–2.21)	3.8	1.37 (1.22–1.54)	1.43 (1.32–1.55)
Alcohol-related conditions	2.06 (1.92–2.22)	4.3	1.26 (1.13–1.41)	1.21 (1.11–1.33)
Renal failure	1.98 (1.84–2.13)	0.8	2.86 (2.40–3.41)	8.12 (7.15–9.24)
Socioeconomic status				
Education[e]	2.05 (1.90–2.20)	—	—	—

[a]Adjusted for age, sex, and calendar time (by design).
[b]Adjusted for (1) use of low–dose aspirin, non–steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education.
[c]Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (2) ambulatory/discharge diagnosis of hypertension; (3) hypertensive complications; and (4) use of each of the following anti-hypertensive drugs or diuretics: ACE inhibitors, aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, furosemide, and thiazides.
[d]Number of different anti-hypertensive drug classes used until 2 years before index date (0, 1, 2, 3, ≥4).
[e]Highest achieved education (short, medium, long, unknown).
NSAIDs, non-steroidal anti-inflammatory drugs.

DISCUSSION

We studied the association between use of dihydropyridine CCBs and renal-cell carcinoma risk. Recognizing that hypertension, the main indication for dihydropyridine therapy, is a risk factor for RCC, we hypothesized that confounding by indication could explain the observed association. Using a conventional nested case-control design comparing dihydropyridine use to never use, we estimated an approximately 40%

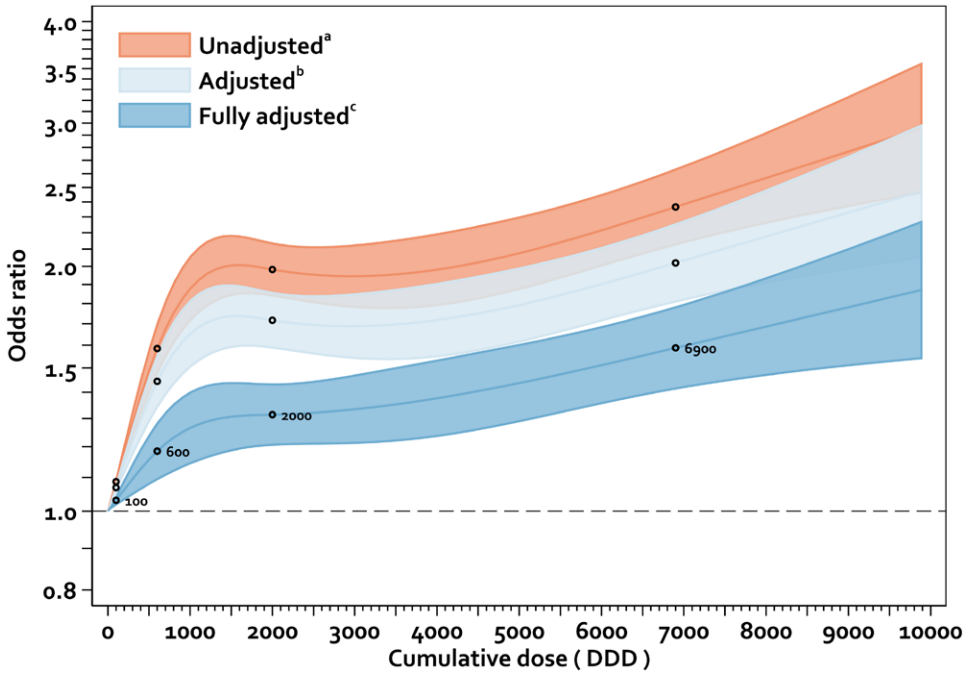

FIGURE 2. Unadjusted, adjusted and fully adjusted ORs for the association between risk of renal cell carcinoma and cumulative dose of dihydropyridines modelled using restricted cubic splines. The four-knot positions in each model are indicated by hollow circles. [a]Adjusted for age, sex, and calendar time (by design). [b]Adjusted for (1) use of low–dose aspirin, non–steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (iii) highest achieved education. [c]Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (2) ambulatory/discharge diagnosis of hypertension; (3) hypertensive complications; and (4) use of each of the following anti-hypertensive drugs or diuretics: ACE inhibitors, aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, furosemide, and thiazides.

increased risk of RCC associated with long-term use of dihydropyridines after adjusting for indicators of hypertension severity. As an indication of residual confounding of this effect estimate, we found that use of other first-line anti-hypertensives was associated with RCC and that the association was substantially attenuated when adjusting for these. Further, the active comparator new user design showed effect estimates closer to unity. Thus, the association between dihydropyridine CCBs and RCC is likely explained at least partially by confounding by indication.

Confounding by indication is often due to factors that are not measured or recorded since treatment choices are based on clinical judgement.[29] It is generally not possible to control confounding by indication entirely in observational studies. However, a systematic approach using several ways to address the issue may help to elucidate the influence of this bias on the observed association. The strategies we applied to evaluate confounding by indication are discussed below.

(1) To account directly for confounding by indication, we adjusted for severity of hypertension. As expected, the association was attenuated; however, use of dihydropyridines remained associated with an increased risk of RCC. We were able to adjust for hypertension to some extent; however, the main limitation of our approach is the lack of blood pressure measurements and the lack of data on BMI and smoking. Strongly elevated blood pressure, high BMI, and smoking are all common and associated with anti-hypertensive therapy and RCC. The prevalence of each of these potential confounders in the Danish population is approximately 20%–30%; however, the prevalence of all three combined has not been reported.[30–32] Hypertension is associated with a 1.4- to 2.5-fold increased risk of RCC.[6,9,11] Every 5 kg/m^2 increase in BMI is associated with an estimated 24% increased risk of RCC in men and 34% in women.[33] Lastly, a history of ever smoking is associated with an estimated 22% increased risk of RCC in women and 54% in men.[34] It is possible that these confounders could act together to potentially explain the observed residual association.

(2) We assessed dose-response patterns to assess the association further. We used the lowest cumulative dose category as a negative control exposure since elevated risk estimates for doses too low to plausibly affect RCC development would suggest bias, such as from residual confounding. We did not, however, observe an association

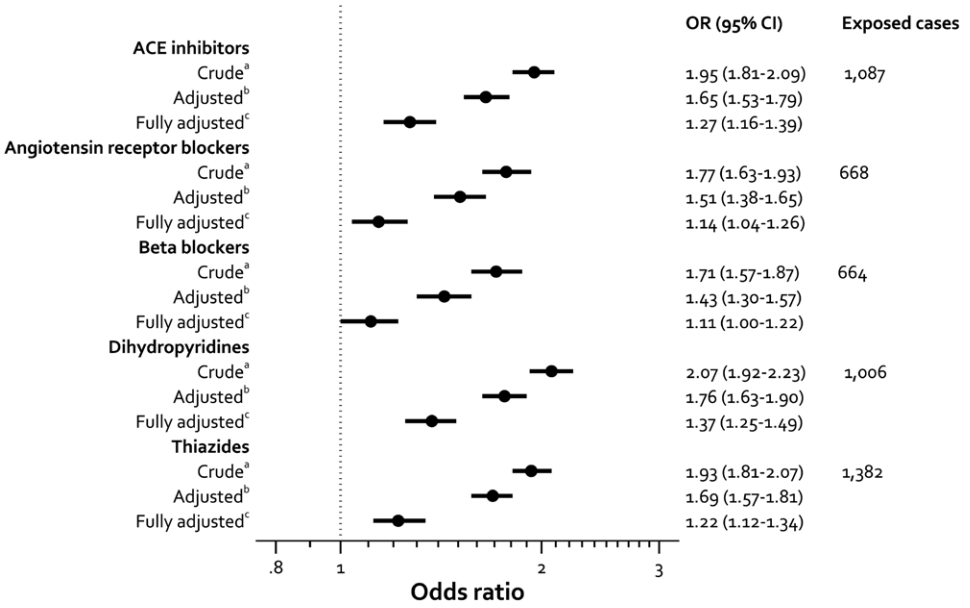

FIGURE 3. Unadjusted, adjusted and fully adjusted ORs for the association between risk of renal cell carcinoma and long-term use of dihydropyridines and other anti-hypertensive drugs. [a]Adjusted for age, sex, and calendar time (by design). [b]Adjusted for (1) use of low–dose aspirin, non-steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education. [c]Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (2) ambulatory/discharge diagnosis of hypertension; (3) hypertensive complications; and (4) use of each of the following anti-hypertensive drugs or diuretics (except if that drug constitute the exposure of interest): ACE inhibitors, aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, dihydropyridines, furosemide, and thiazides.

between low cumulative doses of dihydropyridines and RCC risk. In addition, the ORs increased with increasing cumulative dose, showing a clear dose-response relationship. It is possible, however, that unmeasured confounders related to severity or duration of hypertension could act differentially according to cumulative dose and result in a confounded dose-response pattern. This could occur if more severe hypertension is associated with higher cumulative dose, as well as RCC risk.

(3) We examined whether other first-line anti-hypertensive drugs were associated with RCC risk using these as negative control exposures. A mutual carcinogenic effect of several drugs with similar indications but entirely different mechanisms of action seems biologically implausible and would likely indicate confounding by indication. In the fully adjusted analyses, all anti-hypertensives were associated with increased RCC risk.

(4) Applying an active comparator new user approach, we sought to minimize confounding by indication by design. In these analyses, we compared long-term use of dihydropyridines to long-term use of ACE inhibitors, angiotensin receptor blockers, and thiazides. By using an active comparator, restricting the study population to incident users and aligning the start of treatment and duration of follow-up, we would expect to limit confounding by indication in addition to other potential biases, such as prevalent user bias and surveillance bias. In these analyses, we measured covariates only before cohort entry to avoid adjusting for on-treatment covariates. ACE inhibitors, angiotensin receptor blockers, and thiazides are all first-line anti-hypertensive drugs and have been available in Denmark during the entire study period. As expected, the effect estimates were closer to unity and the CIs were wider with this design, which may be interpreted as a gain in study validity (less biased effect estimates) at the expense of less precision.

Each of the strategies we applied to mitigate confounding by indication has strengths and weaknesses. The traditional approach with a nested case-control study of users compared to never-users allowed for inclusion of all verified cancer cases; however, the comparison between users and never-users of a given drug has many potential pitfalls.[35] Of note, while potential confounding by indication should always be considered, comparison to never-users is confounded only if the indication for the drug is also a risk factor for the outcome of interest. Thus, the validity of this approach relies on being able to define and measure possible confounders which is often difficult, given the elusive nature of confounding by indication.

The active comparator new user design combines many of the elements from the previous approaches (i.e., direct confounder adjustment via multivariate regression, dose-response

TABLE 3. Characteristics of Cases and Controls from a Source Population of New Users of Dihydropyridines or ACE Inhibitors

		Controls		
	All cases (n = 2,074)	**All controls (n = 39,607)**	**DHP users[a] (n = 6,712)**	**ACE-inhibitor users[b] (n = 13,422)**
Age at cohort entry, mean (SD) years	61.6 (9.6)	62.6 (8.8)	62.5 (8.8)	61.9 (8.6)
Age at index date, mean (SD) years	66.5 (9.4)	67.4 (8.5)	68.4 (8.5)	67.6 (8.4)
Follow-up duration, mean (SD) years	4.9 (3.6)	4.9 (3.5)	5.9 (3.0)	5.6 (2.8)
Male, no. (%)	1,403 (67.6%)	26,535 (67.0%)	4,171 (62.1%)	9,319 (69.4%)
Histologic subtype, no. (%)				
Clear cell RCC	1,856 (89.5%)	-	-	-
Papillary RCC	115 (5.5%)	-	-	-
Other RCC	103 (5.0%)	-	-	-
Clinical stage, no. (%)				
Localized	1,062 (51.2%)	-	-	-
Advanced	772 (37.2%)	-	-	-
Unknown	240 (11.6%)	-	-	-
Use of other anti-hypertensives before cohort entry, no. (%)				
Beta-blockers	464 (22.4%)	8,188 (20.7%)	1,681 (25.0%)	2,564 (19.1%)
Angiotensin receptor blockers	225 (10.8%)	4,177 (10.5%)	1,314 (19.6%)	877 (6.5%)
Thiazides	600 (28.9%)	10,275 (25.9%)	2,056 (30.6%)	3,119 (23.2%)
Aldosterone antagonists	17 (0.8%)	288 (0.7%)	45 (0.7%)	109 (0.8%)
Alpha-blockers	25 (1.2%)	334 (0.8%)	62 (0.9%)	114 (0.8%)
Amiloride	63 (3.0%)	1,006 (2.5%)	181 (2.7%)	321 (2.4%)
Furosemide	144 (6.9%)	2,045 (5.2%)	299 (4.5%)	765 (5.7%)
Number of used anti-hypertensive drug classes before cohort entry, no. (%)				
0	1,142 (55.1%)	22,767 (57.5%)	3,386 (50.4%)	8,123 (60.5%)
1	495 (23.9%)	9,714 (24.5%)	1,661 (24.7%)	3,319 (24.7%)
2	302 (14.6%)	5,135 (13.0%)	1,127 (16.8%)	1,487 (11.1%)
3	108 (5.2%)	1,674 (4.2%)	441 (6.6%)	407 (3.0%)
≥4	27 (1.3%)	317 (0.8%)	97 (1.4%)	86 (0.6%)
Markers of severity of hypertension before cohort entry, no. (%)				
Ambulatory/discharge diagnosis of hypertension	357 (17.2%)	6,012 (15.2%)	1,142 (17.0%)	1,687 (12.6%)
Any hypertensive complication	490 (23.6%)	9,288 (23.5%)	1,431 (21.3%)	3,509 (26.1%)
Stroke or transient ischemic attack	168 (8.1%)	2,974 (7.5%)	496 (7.4%)	986 (7.3%)
Retinopathy	9 (0.4%)	150 (0.4%)	18 (0.3%)	47 (0.4%)
Hypertensive encephalopathy	-	18 (0.0%)	(n<5)	(n<5)
Peripheral artery disease	62 (3.0%)	1,160 (2.9%)	207 (3.1%)	367 (2.7%)
Ischemic heart disease	252 (12.2%)	5,190 (13.1%)	822 (12.2%)	2,057 (15.3%)
Congestive heart failure	100 (4.8%)	1,634 (4.1%)	92 (1.4%)	857 (6.4%)
Use of other drugs before cohort entry, no. (%)				
Paracetamol	313 (15.1%)	5,129 (12.9%)	876 (13.1%)	1,634 (12.2%)
Low-dose aspirin	341 (16.4%)	6,750 (17.0%)	1,259 (18.8%)	2,261 (16.8%)
NSAIDs	1,146 (55.3%)	20,769 (52.4%)	3,509 (52.3%)	6,888 (51.3%)
Lithium	7 (0.3%)	131 (0.3%)	27 (0.4%)	32 (0.2%)
Medical history before cohort entry, no. (%)				
Diabetes	248 (12.0%)	4,252 (10.7%)	310 (4.6%)	1,825 (13.6%)
Chronic obstructive pulmonary disease	92 (4.4%)	1,761 (4.4%)	287 (4.3%)	551 (4.1%)
Alcohol related conditions	108 (5.2%)	1,848 (4.7%)	318 (4.7%)	609 (4.5%)
Moderate to severe chronic kidney disease	45 (2.2%)	401 (1.0%)	70 (1.0%)	127 (0.9%)
Education, no. (%)				
Short	809 (39.0%)	14,824 (37.4%)	2,512 (37.4%)	4,951 (36.9%)
Medium	834 (40.2%)	16,020 (40.4%)	2,682 (40.0%)	5,487 (40.9%)
Long	350 (16.9%)	7,030 (17.7%)	1,236 (18.4%)	2,399 (17.9%)
Unknown	81 (3.9%)	1,733 (4.4%)	282 (4.2%)	585 (4.4%)

[a]Ever-users of dihydropyridine CCBs with never-use of ACE-inhibitors.
[b]Ever-users of ACE-inhibitors with never-use of dihydropyridine CCBs.
DHP, dihydropyridine calcium channel blockers; NSAIDs, non-steroidal anti-inflammatory drugs.

TABLE 4. Risk of Renal Cell Carcinoma with Use of Dihydropyridines Compared to Use of ACEIs in New Users of Dihydropyridines or ACEIs

Exposure Category	Cases Exposed to Dihydropyridines/ACEIs	Controls Exposed to Dihydropyridines/ACEIs	Unadjusted OR[a]	Adjusted OR[b]	Fully Adjusted OR[c]
No switching between dihydropyridines or ACE inhibitors allowed[d]					
Ever use	358/631	6,712/13,422	1.13 (0.98–1.30)	1.14 (0.99–1.31)	1.12 (0.97–1.30)
Long-term use (1000+ DDDs)	162/261	2,873/5,597	1.30 (1.05–1.61)	1.33 (1.06–1.65)	1.21 (0.95–1.53)
Cumulative dose (DDDs)					
1–499	144/261	2,717/5,551	1.16 (0.92–1.46)	1.18 (0.93–1.49)	1.24 (0.97–1.58)
500–1999	124/224	2,454/4,764	1.10 (0.86–1.41)	1.07 (0.83–1.38)	1.01 (0.78–1.32)
+2000	90/146	1,541/3,107	1.26 (0.93–1.71)	1.31 (0.95–1.79)	1.18 (0.84–1.67)
Moderate switching between dihydropyridines and ACE inhibitors allowed[e]					
Ever use	566/794	10,271/16,734	1.17 (1.04–1.31)	1.18 (1.05–1.33)	1.17 (1.04–1.32)
Long-term use (1000+ DDDs)	258/319	4,285/6,856	1.36 (1.14–1.63)	1.39 (1.16–1.67)	1.32 (1.10–1.60)
Cumulative dose (DDDs)					
1–499	220/337	4,232/7,066	1.14 (0.94–1.39)	1.16 (0.95–1.42)	1.20 (0.97–1.47)
500–1999	204/278	3,751/5,840	1.15 (0.94–1.41)	1.13 (0.92–1.39)	1.09 (0.89–1.35)
+2000	142/179	2,288/3,828	1.37 (1.07–1.77)	1.43 (1.11–1.85)	1.37 (1.05–1.80)

[a]Adjusted for age, sex, calendar time, and year of initiation of anti-hypertensive therapy (by design).
[b]Adjusted for (1) use of low-dose aspirin, non-steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education.
[c]Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (5) a discharge diagnosis of hypertension; (6) a history of hypertensive complications; and (7) use of each of the following anti-hypertensive drugs or diuretics: Aldosterone antagonists, alpha-blockers, angiotensin receptor blockers, amiloride, beta-blockers, furosemide, and thiazides.
[d]Conditional logistic regressions were carried out for each exposure stratum comparing use of dihydropyridines to use of ACE inhibitors not allowing switching between these, for example, long term use of dihydropyridines and never use of ACE inhibitors was compared to long-term use of ACE inhibitors and never use of dihydropyridines.
[e]Conditional logistic regressions were carried out for each exposure stratum comparing use of dihydropyridines to use of ACE inhibitors allowing for switching between these, for example, long-term use of dihydropyridines and no use or less than 500 DDDs of ACE inhibitor use was compared to long-term use of ACE inhibitors and no use or less than 500 DDDs of dihydropyridine use.

assessment, use of comparators) into a single analysis. In the active comparator design, confounding by indication is mitigated by restriction to a patient population in which a decision to initiate treatment has been made for patients exposed to the drug of interest or the active comparator at start of follow-up. The effectiveness of this confounder adjustment by

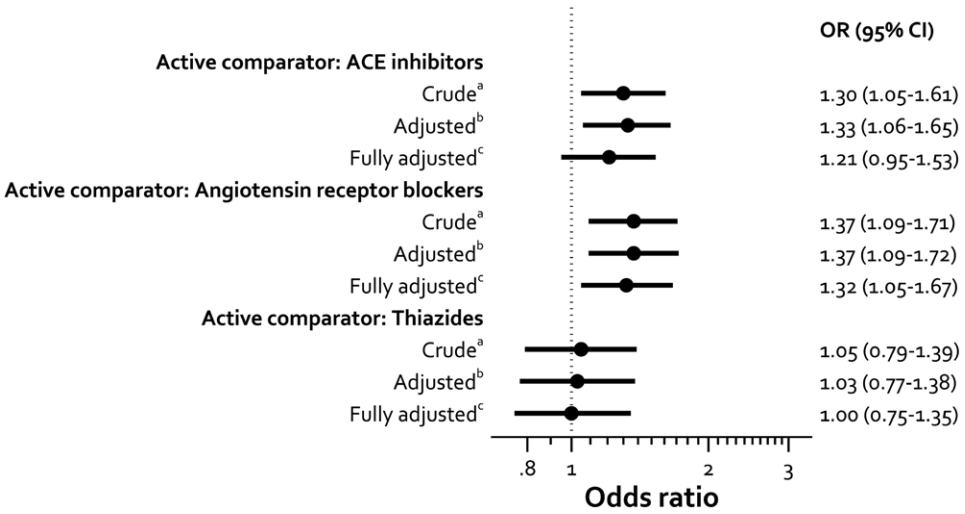

FIGURE 4. Unadjusted, adjusted and fully adjusted ORs for the association between risk of renal cell carcinoma and long-term use of dihydropyridines in a population of new users of dihydropyridines or the active comparator drug. [a]Adjusted for age, sex, calendar time, and year of initiation of anti-hypertensive therapy (by design). [b] Adjusted for (1) use of low-dose aspirin, non-steroidal anti-inflammatory drugs, paracetamol, lithium; (2) a history of diabetes mellitus, chronic obstructive pulmonary disease, alcohol-related conditions, and moderate to severe chronic kidney disease; and (3) highest achieved education. [c] Additionally adjusted for (1) number of used anti-hypertensive drug classes (0, 1, 2, 3, ≥4); (2) ambulatory/discharge diagnosis of hypertension; (3) hypertensive complications; and (4) use of anti-hypertensive drugs or diuretics.

design was shown by the similarity between unadjusted and fully adjusted point estimates in the active comparator new user design. However, it is important to remember that a suitable active comparator is not always available, which may necessitate comparisons with non-users

We conducted a nested case-control study because of the ease of handling multiple exposure and time-varying exposure definitions. However, a cohort study would be a valid alternative design choice. While a cohort study design may be preferred in some settings, the strategies outlined here can be applied to cohort studies as well. Although, we have outlined some strategies to account for ways to evaluate confounding by indication, a range of additional strategies have been proposed including use of high dimensional disease risk scores[36] and use of propensity scores with restriction to very specific indications for treatment.[37] Further, it would be of value to explore the impact of confounders that are also mediators using appropriate methods such as inverse probability weighted estimation of marginal structural models in a population where serial blood pressure measurements are available.[38]

Surveillance bias may have influenced our findings as indicated by the stronger association observed with localized compared to advanced disease. Surveillance bias may arise if users of dihydropyridines are more likely to receive diagnostic workup such as abdominal imaging compared to the reference group. Of note, in the active comparator new-user design, the difference between localized and advanced disease was less pronounced compared to the analyses with never-use as comparison indicating that this design is also helpful to mitigate other sources of bias than confounding by indication.

This is the first study to examine the association between dihydropyridine use and RCC risk specifically. Our results align well with findings from previous studies that reported an increased risk of RCC with use of anti-hypertensive drugs but attributed this to confounding by indication. A multinational nested case-control study reported an increased RCC risk with dihydropyridine and non-dihydropyridine CCB use; however, with no clear dose-response pattern with an adjusted OR of 2.4 (95% CI = 1.4, 4.6) for the lowest quartile of cumulative CCB dose and 1.7 (95% CI = 0.8, 3.4) for the highest quartile.[11] Further, similar associations were observed for other anti-hypertensive agents. A nested case-control study reported an adjusted OR of 1.8 (95% CI = 1.1, 2.7) for RCC with ever use of CCBs compared to never-use and reported similar effect estimates for beta-blockers and ACE inhibitors.[12] Another nested case-control study reported an OR of 1.7 (95% CI = 0.7, 4.2) in women and 1.4 (95% CI = 0.7, 3.2) in men for ever use of CCBs compared to never use and found similar associations for other anti-hypertensives.[13] In another study, CCB use was associated with RCC compared to never-use. However, with ever use of beta-blockers as reference, the association was close to unity.[14]

A recent case-control study reported a strong association between long-term (16+ years) use of CCBs and papillary RCC (OR 2.8, 95% CI = 1.1, 7.4).[15] We did not find a stronger association with papillary RCC compared to other histological types of RCC in this study and, to our knowledge, there is no biologic rationale to explain a carcinogenic effect of CCBs on papillary RCC specifically.

In conclusion, even though we observed an increased risk of RCC with long-term use of dihydropyridines compared to never use, the fact that adjusting for indicators of hypertension severity attenuated the association, that other first-line anti-hypertensives were similarly associated with RCC, and that an active comparator new user design yielded effect estimates close to unity suggest that the increased risk of RCC with use of CCBs is at least partially explained by confounding by indication.

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