Association Between Use of Pharmacokinetic-Interacting Drugs and Effectiveness and Safety of Direct Acting Oral Anticoagulants: Nested Case-Control Study

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Concomitant use of direct oral anticoagulants (DOACs) and medications with inhibition/induction effect on P-gp/CYP3A might increase risk of bleeding/treatment failure, respectively. We designed a nested case-control study within a Clalit cohort of patients with atrial fibrillation (AF) and a cohort of patients with venous thromboembolism, new users of a DOAC (January 1, 2010 to August 24, 2020). Propensity scores were constructed from demographic/clinical characteristics, and medications at cohort entry. Each case of: (i) serious bleeding event; (ii) stroke/systemic emboli (SE) in patients with AF; (iii) recurrent thromboembolism in patients with thromboembolism, was matched by age, sex, length of follow-up, year of cohort entry, DOAC type, and DOAC indication, to up to 20 controls. Within 89,284 patients with AF and venous thromboembolism and 126,302 patient-years of follow-up, there were 1,587 serious bleeding events. Risk of serious bleeding increased in association with concurrent prescription of P-gp/CYP3A4 inhibitors. Specifically, higher bleeding risk was associated with dabigatran-verapamil, rivaroxaban-verapamil, and rivaroxaban-amiodarone concurrent prescriptions: adjusted odds ratios (ORs) 2.29 (1.13–4.60), 2.18 (1.07–4.40), and 1.68 (1.14–2.49), respectively. There were 1,116 events of stroke/SE, in 79,302 DOAC-treated patients with AF and 118,124 patient-years of follow-up. Concomitant use of phenytoin, carbamazepine, valproic acid, or levetiracetam was associated with risk for stroke/SE: adjusted OR 2.18 (1.55–3.10). Risk of recurrent venous thromboembolism could not be assessed due to the low number of cases. Concurrent prescriptions of dabigatran or rivaroxaban with verapamil, and of rivaroxaban with amiodarone, are associated with increased risk for serious bleeding. Higher risk for stroke/SE in patients with AF is associated with concurrent prescriptions of DOACs with phenytoin, carbamazepine, valproic acid, or levetiracetam.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There is inconsistency in clinical data regarding bleeding events in direct oral anticoagulant (DOAC)-patients treated concomitantly with P-gp/CYP3A4 inhibitors. In addition, there is paucity of clinical data assessing interactions between DOACs and P-gp/CYP3A4 inducers.

WHAT QUESTIONS DID THIS STUDY ADDRESS?

☑ Is risk for bleeding in DOAC-treated patients associated with concomitant use of P-gp/CYP3A4 inhibitors? Is lower effectiveness of DOACs associated with concomitant use of P-gp/CYP3A4 inducers?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Concomitant prescription of the DOACs dabigatran and rivaroxaban with the P-gp/CYP3A4 inhibitors verapamil or amiodarone is associated with increased risk for serious bleeding. Higher risk for stroke or systemic embolism in DOAC-treated patients is associated with concurrent prescriptions of phenytoin, carbamazepine, valproic acid, or levetiracetam.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☑ Our results should alert the clinical community to these interactions, as alternative drugs can be used in these scenarios. The results should also serve to guide specific clinical studies that will include pharmacokinetic analyses of DOAC plasma levels in patients treated with the interacting drugs.

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The thrombin inhibitor dabigatran etexilate, and the factor Xa inhibitors rivaroxaban and apixaban, named as a group direct acting oral anticoagulants (DOACs), largely replaced warfarin as anticoagulants for the prevention of thromboembolic complications in nonvalvular atrial fibrillation (AF), and in the treatment and prophylaxis of deep vein thrombosis (DVT) or pulmonary embolism (PE), with a decrease in major bleeding events.[1–4] Nonetheless, DOAC-associated bleeding was still an important problem in the phase III trials.[1–3,5] Moreover, real life settings include challenges to optimal anticoagulant therapy and require additional safety data to guide therapeutic decisions and clinical practice.[6] In particular, interactions with concomitantly administered drugs that inhibit cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (P-gp) might increase risk of bleeding. The P-gp transmembrane transporter plays a protective role against many xenobiotics, including the DOACs, by limiting the absorption of these substrates into the cells and facilitating their efflux[7–10]; CYP3A4 is responsible for metabolism of many drugs, followed by their excretion. While dabigatran is independent of the CYP enzyme system,[11] apixaban and rivaroxaban heavily rely on the CYP3A4 enzyme complexes for hepatic metabolism.[12]

There is considerable overlap in drug specificity for P-gp and CYP3A4. Some examples of strong and moderate inhibitors of P-gp and CYP3A4 include commonly used drugs, such as calcium channel blockers (verapamil and diltiazem), macrolide antibiotics (erythromycin and clarithromycin), azole antifungals (ketoconazole, itraconazole, and fluconazole), antiarrhythmic (amiodarone and dronedarone), and antivirals (lopinavir, ritonavir, saquinavir, and telaprevir).[13,14] It has been shown that concurrent use of DOACs and the combined strong CYP3A4/moderate P-gp inhibitor clarithromycin was associated with greater risk of hospital admission with major hemorrhage.[15,16]

On the contrary, induction of P-gp/CYP3A4 potentially enhances excretion and metabolism of DOACs, which may lead to treatment failure. Known potent metabolizing-enzymes inducers include the antibiotic drug rifampin; the anti-epileptic agents: carbamazepine, phenytoin, phenobarbitone, and primidone; and the antidepressant Hyperici herba (St. John's Wort). Interactions between DOACs and enzyme inducers were demonstrated in healthy volunteers.[17,18] Limited data exist in the literature regarding the clinical consequences of the concomitant use of DOACs and P-gp/CYP3A4 inducers[19,20] with only case reports describing severe thromboembolic complications in DOAC-patients treated concurrently with phenytoin,[21] carbamazepine,[22] or oxcarbazepine.[23,24] The impact of other commonly used anti-epileptic medications, such as valproic acid and levetiracetam, on DOAC's pharmacokinetics is not known.

The current guidance[25] of the European Heart Rhythm Association states that DOAC use is not recommended in combination with drugs that are strong inhibitors of both P-gp and CYP3A4. In addition, combinations with strong inducers of P-gp and/or CYP3A4 should be avoided or used with great caution and surveillance in patients treated with DOACs; in particular, these guidelines recommend against the use of phenobarbital, phenytoin, and carbamazepine, and also the other anti-epileptic drugs, such as valproic acid and levetiracetam.[25]

Due to the paucity and inconsistency of clinical data[26] we aimed to evaluate comprehensively risks for bleeding and for treatment failure in DOAC users and the association with use of P-gp/CYP3A inhibitors and inducers.

METHODS

Source of data

We performed a nested case-control study in a cohort of new users of DOACs. The study is based on the computerized database of Clalit Health Services, which provides inclusive health care for more than half of the Israeli population. Health care coverage in Israel is mandatory according to the National Health Insurance Law (1995), and is provided by four groups akin to not-for-profit health maintenance organizations (HMOs). All members of the different HMOs have a similar health insurance plan and similar access to health services, including low medication's copayment. The electronic medical records of Clalit include data from multiple sources: records of primary care physicians, community specialty clinics, hospitalizations, laboratories, and pharmacies. A registry of chronic disease diagnoses is compiled from these data sources. Diagnoses are captured in the registry by diagnosis-specific algorithms, using International Classification of Diseases Ninth revision (ICD-9) code reading, text reading, laboratory test results, and disease-specific drug usage. A record is kept of the data-sources and dates used to establish the diagnosis, with the earliest recorded date from any source considered to be the defining date of diagnosis. High quality population-based studies were conducted based on data retrieved from Clalit database.[27,28] The Lady Davis Carmel review board approved the study protocol.

Study population and follow-up

We identified all adult patients age > 18 years that have been dispensed for the first time a prescription of a DOAC, between January 1, 2010, and August 24, 2020, and were carrying a diagnosis of AF, or were within 30 days of receiving a diagnosis of DVT and/or PE. A period of 30 days was allowed between DVT/PE diagnosis and DOAC initiation to provide patients with sufficient time to be discharged from the hospital and pick up their prescription from an outpatient pharmacy. The date of the first prescription defined the date of cohort entry. Patients were excluded if they had used any DOAC before cohort entry date.

We followed the patients until the first occurrence of anyone of these: outcome; death; end of the study period (August 31, 2020); switch to another anticoagulant; or censoring due to treatment discontinuation, or incomplete prescription filling (if was different than dabigatran 110/150 mg twice a day; rivaroxaban 15/20 mg twice a day for 3 weeks, then, once a day; or apixaban 2.5/5 mg twice a day) with a 30-day gap allowed between the end of supply of the last prescription and the date of collection of a new prescription.

Case-control selection

Cases were defined by a new primary discharge diagnosis code from hospitalization of any one of: (i) serious bleeding event, occurring at least 7 days from cohort entry; (ii) stroke/systemic emboli (SE) in patients with AF, occurring at least 14 days following cohort entry; (iii) recurrent DVT/PE, occurring at least 14 days from cohort entry. ICD-9 codes defining the outcome events can be found in **Table S1**. Each outcome was analyzed separately, for each of the three DOACs. Dates of bleeding, recurrent DVT/PE, or stroke/SE events defined the index dates.

Fourteen-day lag was chosen in order to reduce misclassification bias in which the diagnosis that had been the reason for DOAC initiation (such as stroke) would be regarded as an outcome event because of delay in diagnosis registration in the database. Another reason for introducing lag was to allow time for the pharmacokinetic interaction with the drugs of interest to occur, because time to steady-state serum drug concentration and thus to full effect is 3–5 half-lives of the drug. In addition, enzyme

induction is mainly a result of an activation of gene transcription, and takes days to even weeks to fully manifest.[29,30]

For each case, up to 20 controls were randomly selected using risk set sampling. Controls had to be alive at index date, have the same age ± 2 years, same sex, same length of follow-up (in days), same year of cohort entry, treated with the same DOAC, and for the same indication (AF or DVT/PE).

Exposure assessment

Exposure of interest was administration of any of the potential moderate/strong DOAC-interacting drugs. These included potential P-gp/CYP3A4 inhibitors[13,14,30]: amiodarone, dronedarone, verapamil, diltiazem, clarithromycin, ketoconazole, fluconazole, itraconazole, voriconazole, ritonavir, erythromycin; bisoprolol (a potential CYP3A4 inhibitor); and amlodipine (a P-gp inhibitor, without CYP3A4 inhibition effect).[31,32] Second, we evaluated exposure to potential P-gp/CYP3A4 inducers[14,30]: rifampicin, carbamazepine, oxcarbazepine, phenytoin, phenobarbital, primidone, Hyperici herba, and topiramate. We also evaluated the effect of exposure to valproic acid or levetiracetam on treatment efficacy, due to the controversial data regarding the effect of these medications on CYP3A4 activity and P-gp induction,[25,33,34] and the potential interest in these medications as safer alternatives to enzyme inducing medications in patients treated with DOACs who require antiseizure medications.

For cases and controls, exposure was defined as dispensing an interacting drug up to 3 months before the index date. For active comparison (i.e., negative control object), we chose drugs used for similar purposes that had not been previously associated with P-gp/CYP3A4 inhibition or induction: beta-adrenergic receptor blockers, propafenone, azithromycin, and tetracycline antibiotics as comparators of P-gp/CYP3A4 inhibitors; lamotrigine,[24] clonazepam, gabapentin, and valerianae radix as comparators for the P-gp/CYP3A4 inducers.

Adjustment for confounders

Potential confounders were assessed at cohort entry and included demographic and lifestyle variables: body mass index (BMI), ever smoking, drug abuse, socioeconomic status, ethnicity; comorbidities including diagnoses of hypertension, diabetes mellitus, ischemic heart disease, S/P cerebrovascular accident (CVA), hyperlipidemia, chronic renal failure (Cr > 1.5 mg/dL), liver disease, chronic obstructive pulmonary disease, malignancy, dementia, Parkinson's disease; platelet count to ascertain thrombocytopenia; and concurrent medications that might be related to the tendency for thrombosis or bleeding, including antiplatelet therapy, proton pump inhibitors, H2-receptor blockers, hormonal therapy, or prednisone, using assessment windows of 4 months before cohort entry for these medications.

Propensity scores (PSs) were calculated at cohort entry by logistic regression, using all demographics, clinical characteristics, laboratory, and medications data described above, for each analysis, as follows: (i) a PS to receive any P-gp/CYP3A4 inhibitor drug; (ii) a PS to receive the potential CYP3A4 inhibitor, bisoprolol; (iii) a PS to receive the P-gp inhibitor, amlodipine; (iv) a PS to receive any active comparator drug to the P-gp/CYP3A4 inhibitors; (v) a PS to receive any P-gp/CYP3A4 inducer; (vi) a PS to receive other antiepileptics of interest (valproic acid/levetiracetam); and (vii) a PS to receive any active comparator to the P-gp/CYP3A4 inducers.

Statistical analysis

Continuous variables are summarized with means and SD; categorical variables are presented as numbers and proportions. Comparisons of baseline demographic and clinical characteristics between cases and controls were performed using χ^2 test for the categorical variables and independent *t*-test for the continuous variables.

Conditional logistic regression was used to estimate the odds ratio (OR) for an incident event with current use of an interacting drug or with current use of an active comparator relative to the risk with no use. In addition to the matching variables on which our models were conditioned, we adjusted for the relevant PS.[35] Analysis of the association with interacting drugs was repeated with any drug in the group, and for each drug separately.

We performed three sensitivity analyses. In the first sensitivity analysis, we adjusted for the covariates presenting $P < 0.1$ in univariate analysis between cases and control, instead of performing PS adjustment. In the second sensitivity analysis, we adjusted for all covariates instead of PS adjustment. In the third sensitivity analysis, we included DOAC dose at cohort entry in the analysis using PS, and in the analysis using all covariates, of the association between any potential inhibior and bleeding, and between any potential inducer and CVA/SE.

The associations were expressed as ORs with 95% confidence intervals (CIs). A P value < 0.05 was considered statistically significant. Data analysis was performed using IBM SPSS statistics version 24 (IBM, Armonk, NY), and SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

There were 100,168 patients who were newly dispensed DOACs in Clalit, between January 1, 2010, and August 24, 2020, and were followed until August 31, 2020. Of these, 79,317 had a diagnosis of AF before DOAC initiation date; 9,983 had a recent diagnosis of DVT/PE. We excluded 1,592 patients with a diagnosis of AF together with a diagnosis of recent DVT/PE, and 9,276 patients for which there was neither AF nor recent DVT/PE recorded at DOAC initiation date. We also excluded 12 patients due to date inconsistencies, and 4 patients who had been prescribed 2 different DOACs on the same date. There were 89,284 patients who were included in the final cohort: 79,302 patients with AF, and 9,982 patients with recent DVT/PE. Of the 89,284 patients, 52.0% were women. There were 48,907 patients (54.8%) who had been dispensed apixaban; 27,914 (31.3%) rivaroxaban; and 12,463 (14.0%) dabigatran. Of the 89,284 patients, 1,588 (1.8%) had missing data of socioeconomic status; 501 (0.6%) had missing BMI. For computation of PS we coded the missing values with a separate code and the patients were entered into the model.

Within 89,284 patients with AF and DVT/PE, with 126,302 patient-years of follow-up of DOAC treatment, there were 1,587 serious bleeding events (1.26 cases/100 patient-years). There were 29,764 controls who were successfully matched to 1,587 bleeding cases by age, sex, DOAC type, indication for DOAC, length of follow-up, and year of DOAC initiation (**Table 1**).

There were 1,116 events of stroke or SE, in 79,302 DOAC-treated patients with AF and 118,124 patient-years of follow-up (0.94/100 patient-years). There were 21,685 controls who were successfully matched to 1,116 cases by age, sex, DOAC type, length of follow-up, and year of DOAC initiation (**Table 1**).

There were 77 cases of recurrent DVT/PE in 9,982 patients prescribed DOAC for a DVT/PE diagnosis, and 8,152 patient-years of follow-up (0.94/100 patient-years). We successfully matched 20 controls to each case only for 20 cases (26%). Ten to 19 controls were successfully matched to each of 20 cases (26%); for 29 cases (37.6%) only 1–9 controls could be matched to each case; and for 8 cases (10.4%) controls could not be matched.

Risk for serious bleeding in DOAC-treated patients, in association with use of P-gp/CYP3A4 inhibitors

Risk of serious bleeding increased in association with use of P-gp/CYP3A4 inhibitors, adjusted OR 1.32 (1.07–1.62). In an analysis

Table 1 Demographic and clinical characteristics of cases of serious bleeding and matched controls within AF and DVT/PE DOAC-treated patients; and cases with CVA/SE and matched controls within patients with AF; Clalit 2010–2020, *N*(%)

	Cases of serious bleeding and matched controls			Cases with CVA/SE and matched controls		
Characteristic	Controls *N* = 29,764	Cases *N* = 1,587	*P* value	Controls *N* = 21,685	Cases *N* = 1,116	*P* value
Age[a]	80.0 ± 7.9	80.0 ± 8.6	0.863	79.0 ± 8.6	78.9 ± 9.1	0.746
Sex[a], male	14,965 (50.3)	799 (50.3)	0.958	9,538 (44.0)	491 (44.0)	0.994
Ethnicity, Jewish	27,766 (93.3)	1,442 (90.9)	< 0.0001	19,997 (92.2)	1,012 (90.7)	0.063
Socioeconomic status						
Low	8,331 (28.0)	466 (29.4)	0.213	6,173 (29.0)	351 (32.7)	0.002
Medium	13,12 (45.4)	721 (45.4)		9,911 (46.6)	507 (47.2)	
High	7,379 (24.8)	364 (22.9)		5,171 (24.3)	216 (20.1)	
DOAC type[a]			0.176			0.757
Apixaban	14,048 (47.2)	721 (45.4)		10,406 (48.0)	526 (47.1)	
Dabigatran	5,411 (18.2)	316 (19.9)		4,359 (20.1)	234 (21.0)	
Rivaroxaban	10,305 (34.6)	550 (34.7)		6,920 (31.9)	356 (31.9)	
Comorbidities						
Hyperlipidemia	26,197 (88.0)	1,420 (89.5)	0.080	19,091 (88.0)	1,008 (90.3)	0.021
Hypertension	22,910 (77.0)	1,273 (80.2)	0.003	16,635 (76.7)	915 (82.0)	< 0.0001
Ischemic heart disease	15,324 (51.5)	909 (57.3)	< 0.0001	11,069 (51.0)	644 (57.7)	< 0.0001
Diabetes mellitus	12,969 (43.6)	785 (49.5)	< 0.0001	9,363 (43.2)	570 (51.1)	< 0.0001
Chronic renal failure	6,136 (20.6)	419 (26.4)	< .0001	4,339 (20.0)	247 (22.1)	0.084
Malignancy	6,380 (21.4)	393 (24.8)	0.002	4,457 (20.6)	226 (20.3)	0.807
Chronic lung disease	3,931 (13.2)	251 (15.8)	0.003	2,920 (13.5)	155 (13.9)	0.686
Dementia	2,803 (9.4)	196 (12.4)	< 0.0001	2,040 (9.4)	155 (13.9)	< 0.0001
Parkinson's disease	899 (3.0)	54 (3.4)	0.387	628 (2.9)	35 (3.1)	0.641
Liver disease	861 (2.9)	64 (4.0)	0.009	686 (3.2)	46 (4.1)	0.077
Obesity (BMI > 25 kg/m^2)	23,334 (78.5)	1,192 (75.3)	0.002	16,826 (77.8)	845 (76.1)	0.162
Smoking	11,849 (39.8)	618 (38.9)	0.491	8,253 (38.1)	428 (38.4)	0.844
Drug abuse	76 (0.3)	5 (0.3)	0.607	82 (0.4)	4 (0.4)	> 0.99
Relevant medications use at cohort entry						
Antiplatelet therapy	15,924 (53.5)	892 (56.2)	0.035	11,875 (54.8)	632 (56.6)	0.221
Proton pump inhibitors	14,072 (47.3)	784 (49.4)	0.099	10,254 (47.3)	544 (48.7)	0.341
H2 receptor blockers	1,941 (6.5)	125 (7.9)	0.034	1,481 (6.8)	100 (9.0)	0.006
Prednisone	1,991 (6.7)	141 (8.9)	0.001	1,515 (7.0)	85 (7.6)	0.422
Hormonal treatment	711 (2.4)	21 (1.3)	0.006	566 (2.6)	28 (2.5)	0.836
Laboratory values						
Creatinine > 1.5 mg/dL	2,190 (7.4)	185 (11.7)	< 0.0001	1,699 (7.8)	102 (9.1)	0.115
Thrombocytopenia (PLT < 100,000/mm^3)	287 (1.0)	22 (1.4)	0.098	191 (0.9)	14 (1.3)	0.197

Case-control analysis of serious bleeding included 1,526 cases and 28,958 controls within patients with AF, and 61 cases and 806 controls within patients with DVT/PE. For 1,389 patients, (87%) 20 controls were successfully matched; for 107(6.7%) patients 10–19 controls were matched; for 91 patients (5.7%) 1–9 controls were successfully matched; for other 9 bleeding cases (0.56%) controls could not be matched and were not included. Within bleeding cases 58.1% received reduced-dose DOAC at cohort entry and the rest received full dose; while within controls 53.0% received reduced-dose and the rest received full dose ($P < 0.0001$). In CVA/SE case-control analysis within AF patients, each of 1,024 cases (91.8%) was successfully matched to 20 controls; for 61 (5.5%) cases only 10–19 controls were successfully matched to each case; and for 31 (2.8%) cases only 1–9 controls could be successfully matched to each case. Within CVA/SE cases 55.7% received reduced-dose DOAC at cohort entry and the rest received full dose; while within controls 53.3% received reduced-dose DOAC, and the rest received full dose ($P = 0.11$).

AF, atrial fibrillation; BMI, body mass index; CVA/SE, cerebrovascular accident/systemic emboli; DOAC, direct oral anticoagulant; DVT, deep vein thrombosis; PE, pulmonary embolism.

[a]Matching variables, along with indication for DOAC-treatment, year of cohort entry, and length of follow up.

of specific drugs, recent use of verapamil was associated with statistically significant bleeding risk in univariate and in multivariate analysis, OR 1.61 (95% CI 1.06–2.50), 1.56 (1.02–2.39), respectively. Risk associated with amiodarone was 1.31 (1.04–1.64), 1.22 (0.98–1.54), in univariate and multivariate analyses, respectively (**Table 2**).

In stratification by DOAC type, risk associated with the P-gp/CYP3A4 inhibitors group was statistically significant in rivaroxaban users and borderline significant for dabigatran. Specifically for verapamil and amiodarone: bleeding risk associated with verapamil was apparent in dabigatran (adjusted OR 2.29, 1.13–4.60) and rivaroxaban (OR 2.18, 1.07–4.40); risk associated with amiodarone was apparent only in rivaroxaban users (OR 1.68, 1.14–2.49; **Table 2**).

Concomitant treatment with any of the active comparators, including tetracycline, azithromycin, propafenone, and beta-receptor blockers (except for bisoprolol), were not associated with bleeding in DOAC users (**Table 2**).

Risk for stroke or SE in DOAC-treated patients, in association with use of P-gp/CYP3A4 inducers

Risk of stroke/SE in patients with AF treated with DOACs increased in association with use of P-gp/CYP3A4 inducers, in all three DOACs (**Table 3**). When analyzed each inducer separately, risk was statistically significant in association with recent use of phenytoin or carbamazepine; highest with phenytoin with adjusted OR 4.46 (2.46–8.1). In stratification by DOAC, risk seemed to be higher in dabigatran users. There was no risk associated with the active comparator drugs ascertained: lamotrigine, clonazepam, gabapentin, and valerianae radix, as expected.

Use of valproic acid or of levetiracetam was associated with stroke/SE (**Table 3**). To study these interactions further, we performed additional analyses in which we stratified exposure to valproic acid only, or valproic acid administered with other anti-epileptic drugs, and compared each stratum separately to no use of any of the known/potential inducers. Thirteen of the 15 patients that were exposed to valproate within the cases were taking valproate-only and the risk for stroke/SE associated with valproate-only was high, with an OR 2.55 (1.43–4.57). We also stratified to use of levetiracetam-only (7 of the 9 cases) or levetiracetam with any of the other anti-epileptic drugs. Risk for stroke/SE associated with levetiracetam-only compared to no use was high with OR 2.56 (1.17–5.6).

Risk for recurrent DVT/PE in DOAC-treated patients, in association with use of P-gp/CYP3A4 inducers could not be evaluated due to very low number of recurrent DVT/PE cases, and few users of the interacting drugs in cases and in controls.

Sensitivity analyses

There was no change in results in the three sensitivity analyses where we adjusted for covariates from **Table 1** (not as a PS) and for DOAC dosage at cohort entry (**Tables S2–S5**).

DISCUSSION

We demonstrate in this study higher risk of bleeding in DOAC-treated patients, associated with concomitant use of verapamil or amiodarone. Risk was apparent specifically for dabigatran-verapamil, dabigatran-amiodarone, and rivaroxaban-amiodarone combinations. A slightly increased risk was associated also with bisoprolol and a trend (which was not statistically significant) for an increased risk associated with clarithromycin. In addition, we have shown, for the first time in a population-based study, significantly higher risk for CVA or systemic embolism in patients with AF, anticoagulated with DOACs that were concomitantly using phenytoin, carbamazepine, valproic acid, or levetiracetam.

The three DOACs differ in their elimination from the body. Clearance of dabigatran is only 20% by nonrenal pathways[36,37] (consisting of direct intestinal excretion and metabolism by CYP enzymes); nonrenal clearance of rivaroxaban is reported to be 65%[38]; and apixaban nonrenal metabolism reports are between < 50%[39] and 73%.[40] In rivaroxaban and apixaban, CYP3A4 contributes 18% and 25% of the hepatic elimination, respectively. Dabigatran is not metabolized by CYP3A4.[36–38,40] Efflux of all three DOACs was strongly inhibited *in vitro* in the presence of P-gp inhibitors.[8,41] Phase I studies reported pharmacokinetic drug-drug interactions mediated by P-gp alone for dabigatran; and interactions mediated by P-gp with CYP3A4 enzymes for rivaroxaban and apixaban.[37–44] Verapamil, and ketoconazole (P-gp/CYP3A4 inhibitors) increased dabigatran exposure by up to 2.5-fold, and amiodarone (mainly P-gp inhibitor) increased exposure of dabigatran 1.6-fold[44]; clarithromycin, ketoconazole (P-gp/CYP3A4 inhibitors), and ritonavir (which is mainly CYP3A4 inhibitor) increased exposure of rivaroxaban by 54%, 158%, and 153%, respectively.[42] Apixaban exposure increased by 99% with co-administration of ketoconazole, and by 40% with diltiazem (CYP3A4 inhibitor).[43]

Reports on clinical outcome of the pharmacokinetic interactions with DOACs are inconsistent. In a meta-analysis of 32,465 patients from randomized controlled trials of patients with AF, P-gp/CYP3A4-modulating drugs use was correlated with increased risk of major bleeding among rivaroxaban users, when compared with warfarin (Risk ratio, 1.37, 1.01–1.85).[45] Several real-world studies have supported the association between P-gp/CYP3A4 inhibitors use and bleeding risk in patients treated with DOACs,[46–48] whereas others did not.[49]

In a retrospective cohort study using data from the Taiwan National Health Insurance database, that included 91,330 patients with AF, it was concluded that use of the inhibitors: amiodarone, fluconazole, but also, surprisingly, of the inducers rifampin and phenytoin, were associated with significant increased incidence rates of major bleeding. Moreover, incidence rate for major bleeding was lower and not higher for use of erythromycin or clarithromycin, known to be moderate-strong P-gp/CYP3A4 inhibitors; and was not higher with some known P-gp/CYP3A4 inhibitors.[50] However, it should be noted that the annual bleeding rate in the Taiwanese study[50] was threefold higher than annual serious bleeding event rates reported previously in anticoagulated patients with AF.[51] As emergency department visits without hospitalizations comprised the outcome along with inpatient diagnoses, it might have been that less serious bleeding events were included. In addition, the patient-quarters method used precluded the ability for a new-user analysis; covariates assessed on the first day of each patient

Table 2 Association between serious bleeding events and treatment with P-gp/CYP3A4 inhibitors in DOAC-treated patients, Clalit, 2010-2020

	Controls (*N* = 29,764)	Cases with serious bleeding (*N* = 1,587)	OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
P-gp/CYP3A4 inhibitors						
Any P-gp/CYP3A4 inhibitor	1,977 (6.6)	132 (8.3)	1.41 (1.15–1.73)	0.001	1.32 (1.07–1.61)	0.008
Amiodarone	1,507 (5.1)	96 (6.0)	1.31 (1.04–1.64)	0.021	1.21 (0.96–1.52)	0.101
Verapamil	310 (1.0)	25 (1.6)	1.61(1.06–2.46)	0.026	1.56 (1.02–2.39)	0.037
Dronedarone	77 (0.3)	6 (0.4)	1.51 (0.65–3.50)	0.337	1.48 (0.64–3.43)	0.367
Diltiazem	72 (0.2)	4 (0.3)	1.1 (0.40–3.01)	0.860	1.02 (0.37–2.82)	0.964
Clarithromycin	30 (0.1)	4 (0.3)	2.67 (0.94–7.57)	0.065	2.51 (0.88–7.18)	0.086
Any P-gp/CYP3A4 inhibitor by DOAC type[b]						
Dabigatran	440 (8.1)	31 (9.8)	1.43 (0.94–2.17)	0.098	1.33 (0.87–2.04)	0.184
Rivaroxaban	576 (5.6)	47 (8.5)	1.89 (1.33–2.70)	< 0.0001	1.78 (1.24–2.54)	0.002
Apixaban	961 (6.8)	54 (7.5)	1.15 (0.84–1.56)	0.381	1.06 (0.78–1.44)	0.716
Verapamil by DOAC type[b]						
Dabigatran	87 (1.6)	10 (3.2)	2.35 (1.17–4.74)	0.017	2.29 (1.13–4.62)	0.021
Rivaroxaban	82 (0.8)	9 (1.6)	2.25 (1.11–4.55)	0.025	2.18 (1.07–4.44)	0.031
Apixaban	141 (1.0)	6 (0.8)	0.84 (0.37–1.92)	0.676	0.81 (0.36–1.86)	0.627
Amiodarone by DOAC type[b]						
Dabigatran	325 (6)	17 (5.4)	0.97 (0.57–1.65)	0.918	0.90 (0.53–1.53)	0.687
Rivaroxaban	438 (4.3)	35 (6.4)	1.78 (1.20–2.63)	0.004	1.66 (1.12–2.46)	0.012
Apixaban	744 (5.3)	44 (6.1)	1.21 (0.87–1.69)	0.259	1.11 (0.79–1.55)	0.543
A potential CYP3A4 inhibitor						
Bisoporolol	14,405 (48.4)	819 (51.6)	1.17 (1.05–1.29)	0.004	1.15 (1.04–1.28)	0.007
Bisoprolol by DOAC type:[b]						
Dabigatran	2,344 (43.3)	160 (50.6)	1.36 (1.08–1.72)	0.009	1.35 (1.07–1.71)	0.012
Rivaroxaban	5,097 (49.5)	266 (48.4)	0.99 (0.83–1.17)	0.873	0.98 (0.83–1.17)	0.735
Apixaban	6,964 (49.6)	393 (54.5)	1.24 (1.06–1.45)	0.006	1.22 (1.05–1.42)	0.011
P-gp inhibitor						
Amlodipine	1,999 (6.7)	88 (5.5)	0.82 (0.65–1.04)	0.097	0.80 (0.64–1.01)	0.064
Amlodipine by DOAC type[b]						
Dabigatran	451 (8.3)	14 (4.4)	0.52 (0.29–0.91)	0.023	0.53 (0.30–0.93)	0.027
Rivaroxaban	600 (5.8)	25 (4.5)	0.77 (0.50–1.19)	0.237	0.76 (0.49–1.18)	0.217
Apixaban	948 (6.7)	49 (6.8)	1.02 (0.74–1.39)	0.917	0.97 (0.71–1.33)	0.836

(continues)

Table 2 (Continued)

	Controls (*N* = 29,764)	Cases with serious bleeding (*N* = 1,587)	OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
Active comparators to the P-gp/CYP3A4 inhibitors						
Any active comparator to P-gp/CYP3A4 inhibitors	9,474 (31.8)	475 (29.9)	0.92 (0.82–1.03)	0.149	0.92 (0.82–1.03)	0.133
Atenolol	4,271 (14.3)	217 (13.7)	0.95 (0.82–1.10)	0.457	0.94 (0.81–1.10)	0.449
Metoprolol	1,557 (5.2)	86 (5.4)	1.04 (0.83–1.31)	0.723	1.04 (0.83–1.30)	0.725
Carvedilol	1,150 (3.9)	69 (4.3)	1.15 (0.89–1.48)	0.283	1.14 (0.89–1.47)	0.284
Propranolol	456 (1.5)	29 (1.8)	1.20 (0.82–1.76)	0.353	1.20 (0.82–1.76)	0.353
Sotalol	420 (1.4)	13 (0.8)	0.57 (0.33–1.00)	0.050	0.57 (0.32–1.00)	0.050
Propafenone	1,735 (5.8)	62 (3.9)	0.67 (0.52–0.87)	0.002	0.67 (0.52–0.87)	0.002
Tetracyclines	404 (1.4)	21 (1.3)	0.99 (0.64–1.55)	0.970	0.95 (0.61–1.48)	0.804
Azithromycin	116 (0.4)	9 (0.6)	1.48 (0.75–2.92)	0.265	1.4 (0.71–2.78)	0.332

Use of erythromycin, fluconazole, and itraconazole (0,3; 1,17; 0,2; respectively, in cases and controls); and of labetalol (1, 19) was low, and did not permit separate analysis. There was no use within cases neither controls of the strong inhibitors ketoconazole, voriconazole, ritonavir, nor of pindolol.
Within strong CYP3A4 inhibitors only clarithromycin could be evaluated and seemed to be associated with the highest risk (with large CI due to low number of cases). Verapamil, diltiazem, erythromycin, and fluconazole are known as moderate inhibitors. Amiodarone exact strength of inhibition is still "yet to-be-determined" in the published lists.[14] Bisporolol, is known to be metabolized partly by CYP3A4 (and partly by CYP2D6).[57–60] Amlodipine had been shown to affect P-gp mediated transport, and is not a CYP3A4 inhibitor.[31,32]
CI, confidence interval; DOAC, direct oral anticoagulant; OR, odds ratio.
[a]Adjusted for the propensity score to receive a drug from the group.
[b]Dabigatran: 5,411 controls, 316 cases; Rivaroxaban: 10,305 controls, 550 cases; Apixaban: 14,048 controls, 721 cases.

quarter might have been influenced by DOAC exposure. For example, anemia, adjusted for in each new quarter, might have been already the early sign of the outcome associated with the DOAC, and adjusting for this intermediate variable is susceptible to bias.[52]

In the current study, bleeding risk associated with verapamil was apparent in dabigatran and rivaroxaban users, and risk associated with amiodarone was apparent in rivaroxaban users. Verapamil and amiodarone are both strong P-gp inhibitors.[53] Our findings are consistent with the variable susceptibility of the three DOACs to P-gp mediated transport. It has been shown in cell lines that dabigatran uses predominantly P-gp-dependent transport; rivaroxaban uses P-gp as well as the breast cancer resistance protein (BCRP) for transport; and preferential BCRP-dependent transport is used by apixaban.[10] Verapamil and amiodarone do not inhibit BCRP transport.[54,55] Thus, from our results, it seems likely that the clinically important pharmacokinetic inhibition of DOACs by widely used drugs occur with P-gp inhibition. Interestingly, amlodipine, a P-gp inhibitor was not associated with bleeding risk, and was associated with lower risk for bleeding (adjusted OR 0.80 (0.64–1.01)), which might be explained by its potency in reducing blood pressure (the latter associated with reduced intracranial bleeding risk).[56] Bisoprolol, a beta-receptor blocker, used by ~ 50% of patients in our AF cohort, was not previously associated with bleeding risk. Bisoprolol does not show on the lists as P-gp or CYP3A4 inhibitor but is known to be metabolized partly by CYP3A4 (and partly by CYP2D6).[57–60]

The specific interactions of dabigatran with verapamil, and rivaroxaban with verapamil or amiodarone, are consistent with previous reports in animal models[61] and in humans.[46,62–65] Verapamil increased dabigatran exposure in real life.[62] It has been demonstrated that effect was mainly due to increased bioavailability: dabigatran exposure increased in phase I studies 2.5-fold when verapamil was administered 1 hour prior to the dabigatran intake, but not when verapamil was given 2 hours after dabigatran, explained by completed dabigatran absorption after 2 hours.[44] Concomitant use of dabigatran and verapamil has been associated with increased bleeding risk in real life.[46] Increased risk of major bleeding (hazard ratio 1.50, 1.11–2.04) was associated with use of verapamil and diltiazem in a post hoc analysis of the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF).[64] Diltiazem use was not frequent, and thus risk associated with diltiazem was not statistically significant in our cohort (adjusted OR 1.02 (0.37–2.82)). Amiodarone has been shown to cause increased rivaroxaban level[65]; increased bleeding risk associated with amiodarone was described by Chang *et al*. for dabigatran and rivaroxaban.[50]

Much less is known on the clinical outcomes of DOAC-treated patients exposed concomitantly to P-gp/CYP3A4 inducers,[24] but case reports have raised concern that these inducers might decrease DOAC activity.[34,66] Consistent with this, we have demonstrated risk of stroke/SE associated with P-gp/CYP3A4 inducers (adjusted OR 2.18 (1.55–3.06)), and specifically for the association with phenytoin and carbamazepine; and, in addition, we found an association with levetiracetam and valproic acid. Use of phenobarbital, topiramate, and rifampicin was very low in the current cohort.

Table 3 Association between new CVA/systemic embolism and treatment with P-gp/CYP3A4 inducers in DOAC-treated patients, Clalit, 2010–2020

	Controls (*N* = 21,685)	Cases with CVA/SE (*N* = 1,116)	OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
P-gp/CYP3A4 inducers						
Any P-gp/CYP3A4 inducer	344 (1.6)	39 (3.5)	2.27 (1.62–3.18)	< 0.0001	2.18 (1.55–3.06)	< 0.0001
Carbamazepine	80 (0.4)	9 (0.8)	2.19 (1.10–4.39)	0.027	2.15 (1.07–4.30)	0.031
Phenytoin	57 (0.3)	14 (1.3)	4.76 (2.64–8.61)	< 0.0001	4.46 (2.46–8.08)	< 0.0001
Phenobarbital	19 (0.1)	2 (0.2)	2.11 (0.49–9.04)	0.317	1.91 (0.44–8.22)	0.386
Primidone	75 (0.3)	6 (0.5)	1.60 (0.70–3.68)	0.269	1.51 (0.65–3.48)	0.336
Topiramate	32 (0.1)	2 (0.2)	1.21 (0.29–5.07)	0.794	1.21 (0.29–5.08)	0.792
Hyperici herba	84 (0.4)	6 (0.5)	1.43 (0.62–3.28)	0.399	1.37 (0.60–3.13)	0.463
Rifampicin	4 (0.02)	1 (0.1)	5.00 (0.56–44.73)	0.150	5.23 (0.58–46.83)	0.139
Any P-gp/CYP3A4 inducer by DOAC type[b]						
Dabigatran	99 (2.3)	14 (6.0)	2.77 (1.55–4.95)	0.001	2.59 (1.44–4.65)	0.001
Rivaroxaban	124 (1.8)	13 (3.7)	2.13 (1.19–3.82)	0.011	2.02 (1.12–3.62)	0.019
Apixaban	121 (1.2)	12 (2.3)	2.0 (1.10–3.64)	0.023	1.99 (1.10–3.63)	0.024
Other antiepileptics of interest						
Valproic acid	109 (0.5)	15 (1.3)	2.58 (1.50–4.45)	0.001	2.38 (1.37–4.12)	0.002
Levetiracetam	74 (0.3)	9 (0.8)	2.38 (1.19–4.75)	0.014	2.26 (1.13–4.54)	0.021
Active comparators to the P-gp/CYP3A4 inducers						
Any active comparator to P-gp/CYP3A4 inducers	1,238 (5.7)	75 (6.7)	1.19 (0.94–1.52)	0.152	1.15 (0.90–1.47)	0.267
Lamotrigine	109 (0.5)	8 (0.7)	1.46 (0.71–3.01)	0.305	1.39 (0.67–2.86)	0.378
Clonazepam	555 (2.6)	32 (2.9)	1.13 (0.79–1.62)	0.509	1.08 (0.75–1.55)	0.680
Gabapentin	178 (0.8)	13 (1.2)	1.39 (0.78–2.46)	0.266	1.36 (0.77–2.41)	0.294
Valerianae radix	450 (2.1)	26 (2.3)	1.14 (0.76–1.70)	0.525	1.10 (0.74–1.65)	0.640

Use of Oxcarbazepine (0,5 in cases and controls, respectively) was low, and did not permit separate analysis.
CI, confidence interval; CVA/SE, cerebrovascular accident/systemic emboli; DOAC, direct oral anticoagulant.
[a]Adjusted for the propensity score to receive a drug from the group.
[b]Dabigatran: 4,359 controls, 234 cases; Rivaroxaban: 6,920 controls, 356 cases; Apixaban: 10,406 controls, 526 cases.

In vitro, phenytoin, carbamazepine, levetiracetam, and less so phenobarbital[67,68] and valproic acid[69,70] induce P-gp activity. Known inducers of CYP3A4 activity are phenytoin, carbamazepine, phenobarbital, topiramate, and rifampin. Controversial data—inhibition as well as induction of CYP3A4—were described for valproic acid.[13,34] Levetiracetam did not induce the function of CYP450 enzymes *in vitro*[71] and in healthy volunteers repeated administration of levetiracetam did not affect the pharmacokinetics of digoxin, a P-gp substrate.[72]

Use of phenytoin, carbamazepine, oxcarbazepine, phenobarbital, or primidone was associated with risk of having DOAC concentration lower than the 5th percentile reported in phase III studies.[19] Chin *et al*. reported on a patient treated with phenytoin and phenobarbital whose serum dabigatran concentration was > 3 SDs below the cohort mean.[73] In a study using the US Food and Drug Administration (FDA) Adverse Events Reporting System database there was 1.86-higher odds for reporting thrombotic or embolic adverse event during treatment with apixaban or rivaroxaban in patients co-treated with enzyme inducing anti-epileptic medication, as compared with patients treated with noninducer anti-epileptic medication.[74] Use of inducers of P-gp and/or CYP3A4 was low in the AF Swedish cohort, and effects on stroke could not be established.[48]

It should be noted that patients receiving interacting drugs might have different characteristics than patients without concomitant interacting drugs, including differences in risk factors for the effectiveness or the safety outcomes.[75] To reduce this bias, we included the PS to receive the relevant group of interacting drugs in the multivariable analysis. In addition, we used active comparators for the P-gp/CYP3A4 inhibitors and inducers, prescribed in similar indications to the studied drugs, and showed no increased risk associated with the comparators.

This study has some limitations. First, despite the multivariate model adjustment that accounted for relevant potential confounders, some residual confounding might exist, due to the retrospective nature of the study. For example, an infectious disease that might have been the reason for prescribing clarithromycin might have had an association with bleeding, that is slightly different than an association with bleeding of other infectious diseases for which

azithromycin and tetracycline are prescribed. The second limitation is the lack of genetic data for pharmacogenetics evaluation, which might have influenced interaction analyses, and was beyond the scope of our study, such as single-nucleotide polymorphisms (SNPs) in *ABCB1*, *ABCG2*, *CES1*, and *CYP3A5* genes. Interestingly, in a randomized trial of 60 volunteers, clarithromycin co-administration led to a twofold increase in dabigatran and rivaroxaban area under the curve, irrespective of ABCB1 genotype, encoding P-gp.[76] Nonetheless, these genetic determinants are expected to be nondifferential, and are likely to bias the results toward the null. Third, we had a higher proportion of apixaban users, relative to dabigatran and rivaroxaban, reflecting real patterns of use in Israel, similar to what was described recently in Sweden.[48] This, however, might not reflect patterns of use in other countries.[46] Fourth, outcome events were not frequent. However, rates were in line with published rates of anticoagulated patients with AF in the United States or England.[51,77] In addition, use of potentially interacting drugs was not frequent, and thus limited the power of calculations for associations with some of the interacting drugs. Anti-HIV drugs, that are strong CYP3A4 inhibitors, were not prescribed to patients in our real-life cohort as well. Nevertheless, this reflects use of interacting drugs in real life.

A notable strength of this study is the population-based nature of the study with a relatively large number of DOAC users, from real-world data, using a similar design to study the three DOACs. We had data on multiple covariates, and introduced them in the multivariable models. In addition, data on prescription fillings enabled us to censor patients if the prescription was not filled for more than 30 days.

In conclusion, we demonstrate in this nested case-control study that concomitant prescription of DOACs with pharmacokinetic-interacting drugs is associated with serious bleeding (verapamil and amiodarone) and with reduced effectiveness (phenytoin, carbamazepine, valproic acid, and levetiracetam). Future clinical studies with pharmacokinetic measurements should follow our results.

SUPPORTING INFORMATION

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

N.G. and M.M. wrote the manuscript. N.G., N.S., and M.M. designed the research. N.G. and N.S. performed the research. N.G., N.S., and M.M. analyzed the data.

DISCLAIMERS

Anonymized data will be available upon request by qualifying researchers. The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained. The study was approved by the institutional review board. Patient consent was waived. No materials from other resources were used.

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