Impact of P-glycoprotein and CYP3A4-interacting drugs on clinical outcomes in patients with atrial fibrillation using non-vitamin K antagonist oral anticoagulants: a nationwide cohort study

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## Abstract

**Aims**: The clinical relevance of common pharmacokinetic interactions with non-vitamin K antagonist oral anticoagulants (NOACs) often remains unclear. Therefore, the impact of P-glycoprotein (P-gp) and CYP3A4 inhibitors and inducers on clinical outcomes in NOAC-treated patients with atrial fibrillation (AF) was investigated.

**Methods and results**: AF patients were included between 2013-2019 using Belgian nationwide data. Concomitant use of P-gp/CYP3A4-interacting drugs at the time of NOAC initiation was identified. Among 193,072 NOAC-treated AF patients, 46,194 (23.9%) and 2903 (1.5%) subjects concomitantly used a P-gp/CYP3A4 inhibitor or inducer, respectively. After multivariable adjustment, concomitant use of P-gp/CYP3A4 inhibitors was associated with significantly higher major bleeding (adjusted hazard ratio (aHR) 1.24, 95% confidence interval (CI) (1.18-1.30)) and all-cause mortality risks (aHR 1.07, 95%CI (1.02-1.11)), but not with thromboembolism in NOAC-treated AF patients. A significantly increased risk of major bleeding was observed with amiodarone (aHR 1.27, 95%CI (1.21-1.34)), diltiazem (aHR 1.28, 95%CI (1.13-1.46)), verapamil (aHR 1.36, 95%CI (1.03-1.80)), ticagrelor (aHR 1.50, 95%CI (1.20-1.87)), and clarithromycin (aHR 1.55, 95%CI (1.14-2.11)); and in edoxaban (aHR 1.24, 95%CI (1.06-1.45)), rivaroxaban (aHR 1.25, 95%CI (1.16-1.34)) and apixaban users (aHR 1.27, 95%CI (1.16-1.39)), but not in dabigatran users (aHR 1.07, 95%CI (0.94-1.23)). Concomitant use of P-gp/CYP3A4 inducers (e.g., antiepileptic drugs like levetiracetam) was associated with a significantly higher stroke risk (aHR 1.31, 95%CI (1.03-1.68)), but not with bleeding or all-cause mortality.

**Conclusion**: Concomitant use of P-gp/CYP3A4 inhibitors was associated with higher bleeding and all-cause mortality risks in NOAC users, whereas the use of P-gp/CYP3A4 inducers was associated with higher stroke risks.

**Keywords:** Atrial fibrillation, NOAC, drug interaction, thromboembolism, bleeding, mortality

## Introduction

Non-vitamin K antagonist oral anticoagulants (NOACs) are the most frequently used option for stroke prevention in atrial fibrillation (AF), thanks to their ease of use, fixed dosing regimen, lower intracranial bleeding risk and fewer drug and food interactions than vitamin K antagonists (VKAs).1 All NOACs are excreted by P-glycoprotein (P-gp) efflux transporters, while apixaban and rivaroxaban are also hepatically metabolised by cytochrome P450 (CYP) enzyme CYP3A4.1,2 P-gp and CYP3A4 inhibitors may therefore increase NOAC plasma concentrations and potentially increase the risk of bleeding, while P-gp and CYP3A4 inducers may reduce NOAC plasma levels and potentially increase the risk of thromboembolism.1,3 Consequently, concomitant use of strong P-gp/CYP3A4 inhibitors (e.g., ritonavir) or inducers (e.g., rifampicin) with NOACs should be avoided, as recommended by the Summary of Product Characteristics (SmPC) of NOACs4-7 and European Heart Rhythm Association (EHRA) Practical Guide on NOAC use in AF patients1.

However, the clinical relevance of mild-to-moderate P-gp/CYP3A4 inhibitors in NOAC users, including commonly used drugs in AF patients such as amiodarone, verapamil or diltiazem, remains inconclusive. Prior studies rendered conflicting results8-12, but were often limited by small sample sizes, short follow-up durations, inclusion of non-anticoagulated AF patients, and limited assessment of interacting drug use at baseline (e.g., not accounting for the initiation or discontinuation of interacting drugs during follow-up). Moreover, data on the impact of P-gp/CYP3A4 inducers are particularly scarce, which was highlighted as an important research gap.2

Therefore, we aimed to investigate the impact of P-gp/CYP3A4 inhibitors and inducers on clinical outcomes in NOAC-treated AF patients. Differences between specific P-gp/CYP3A4-interacting drug types and individual NOACs were explored.

## Methods

### Source population

Details on the study methodology have been reported before.13,14 In brief, two nationwide databases, the InterMutualistic Agency (IMA) database and Minimal Hospital Dataset (MHD), provided the source population. The IMA centralizes all claims data from Belgian health insurance funds on reimbursed ambulatory and hospital care, including demographic characteristics (e.g., age, sex, date of death), medical procedures and drug prescription claims (e.g., dispensing date, Anatomical Therapeutic Chemical (ATC) classification code, package size…), and represents all legal residents in Belgium. The MHD aggregates hospital discharge diagnoses of every hospital admission (hospitalizations, day-care stays and emergency room contacts), coded in International Classification of Diseases (ICD) codes (ICD-9 up to 2014, ICD-10 from 2015 onwards). Every case of the study population was identified in both databases. This study was approved by the Belgian Commission for the Protection of Privacy (approval code IVC/KSZG/20/344). The Reporting of Studies Conducted Using Observational Routinely Collected Health Data for Pharmacoepidemiology (RECORD-PE) guideline was followed (eTable 1).15

### Study population

Subjects ≥45 years old with ≥1 year coverage by Belgian health insurance funds were included on the first date of filling a NOAC prescription (=index date) from 1 January 2013 to 1 January 2019 (eFigure 1). Dabigatran (approved in Belgium since August 2012), rivaroxaban (approved since September 2012), apixaban (approved since September 2013) and edoxaban users (approved since October 2016) were included. Only NOAC-naïve subjects were considered, excluding patients with an oral anticoagulant prescription filled ≤1 year before the index date, to reduce healthy user bias.

Persons were excluded in case of (1) total hip or knee replacement, or diagnosis of deep vein thrombosis or pulmonary embolism ≤6 months before the index date, (2) mechanical prosthetic heart valve or moderate/severe mitral stenosis, (3) end-stage renal disease (chronic kidney disease stage V and/or dialysis), (4) ≥2 prescription claims of different NOAC types or doses on the index date, or (5) use of NOAC doses not approved for stroke prevention in AF (e.g., rivaroxaban 10 mg) (eTable 2).

### P-gp/CYP3A4-interacting drug use

P-gp/CYP3A4 inhibitors and inducers described in the SmPCs of NOACs4-7, EHRA Practical Guide1 (except for drugs classified as P-gp/CYP3A4 competitors/substrates) or Stockley’s Drug Interactions16, were identified with ATC-coded prescription claims in ambulatory and hospital care up to 6 months before the index date.1,8,12,17 Only drugs commercially available on the Belgian market were considered (e.g., not the case for dronedarone). Patients were categorized by whether or not a P-gp/CYP3A4-interacting drug was concomitantly used at the time of NOAC initiation and the type of interaction (inhibitor or inducer).8,10,18,19. Patients using both P-gp/CYP3A4 inhibitors and inducers on the index date were analysed separately due to potentially opposing interaction effects. Concomitant use was considered when interacting drugs were concurrently started on the index date or when the treatment period of an interacting drug included the index date (overlapping drug supply), in line with previous research8,17-21. The treatment period of interacting drugs was calculated based on prescription claims data and the recommended dosing regimen.13

### Outcomes

Outcomes of interest were stroke or systemic embolism (stroke/SE), stroke, all-cause mortality, major bleeding, intracranial bleeding and gastrointestinal bleeding. Major bleeding was defined as a hospitalized bleeding event in a critical area or organ (e.g., intracranial), fatal bleeding or bleeding event with a medical procedure code for blood transfusion ≤10 days after admission, which is adapted from the International Society on Thrombosis and Haemostasis definition due to a lack of data on haemoglobin levels or number of blood transfusion units.14,22 Outcomes were identified using ICD-coded hospital discharge diagnoses and medical procedure codes (eTable 3).13 The incident date of outcomes was defined as the date of hospital admission, date of registration for medical procedures or date of death, whichever occurred first.14

### Follow-up

Subjects were followed from NOAC initiation until the first occurrence of the investigated outcome, discontinuation (>60-day gap of drug supply) or switch of NOAC treatment, death, emigration or end of the study period (1 January 2019), whichever came first (on-treatment analysis).13 To account for changes in interacting drug use after NOAC initiation, patients who used P-gp/CYP3A4-interacting drugs at baseline and subsequently discontinued treatment, or patients who were not treated with interacting drugs at baseline but initiated one during follow-up, were censored on those dates, as done before8,10,18,20,21,23.

### Covariates

Baseline characteristics were assessed on the index date and included age, sex, NOAC type and dose, comorbidities, medication history and clinical risk scores. Comorbidities were identified with specific ICD-coded diagnoses, medical procedure codes and/or medication prescription claims ≤1 year before the index date (eTable 2). Medication history was identified with medication prescription claims, considering recent use ≤6 months before the index date. The CHA2DS2-VASc score, modified HAS-BLED score (without the ‘labile INR’ criterion) and age-adjusted Charlson Comorbidity Index (CCI) were calculated.1

### Statistical analyses

Mean and standard deviation (SD), and counts and percentages were presented for continuous and categorical variables, respectively. Crude event rates were calculated as the total number of events per 100 person-years at risk. Outcomes were investigated between AF patients initiating NOAC treatment without concomitant use of P-gp/CYP3A4-interacting drugs (reference group) and NOAC users concomitantly treated with P-gp/CYP3A4 inhibitors, inducers, or both inhibitors and inducers in pairwise comparisons. To minimize confounding by indication and improve comparability, 3:1 propensity score matching (PSM) was performed using nearest neighbor matching without replacement and a caliper of 0.05.19,24 Propensity scores were calculated with logistic regression models including the 40 confounding covariates described in Table 1 (age, sex, NOAC type and dose, comorbidities, medication history, risk scores), stratified by calendar year. Covariate balance before and after matching was checked using standardized mean differences with a ≥0.1 threshold to indicate imbalance. Cox proportional hazard regression models were used to calculate adjusted hazard ratios (aHRs) with 95% confidence intervals (CI). The proportional hazard assumption was assessed using scaled Schoenfeld residuals. A two-sided p-value of <0.05 was considered statistically significant. All analyses were performed in R (R version 3.6.0).

### Subgroup analyses

Explorative subgroup analyses were performed in subgroups of ≥1000 patients using 3:1 PSM for each pairwise comparison. First, outcomes were investigated with specific P-gp/CYP3A4-interacting drug types (e.g., amiodarone). Second, to identify a potential additive effect of concurrently using multiple inhibitors, outcomes were examined stratified by the number of P-gp/CYP3A inhibitors used per patient (1 versus ≥2 inhibitors; not possible for inducers). Third, results were stratified by the mechanism of interaction (P-gp, CYP3A4, or combined P-gp and CYP3A4 inhibitors; not possible for inducers). Lastly, outcomes were investigated stratified by individual NOAC types (not possible for inducers) and by age groups (<65, 65-<75, 75-<85 and ≥85 years for inhibitors; <75 and ≥75 years for inducers).

### Sensitivity analyses

Several sensitivity analyses were performed to check the robustness of results. First, to take into account the impact of initiating or discontinuing P-gp/CYP3A4-interacting drugs during follow-up, concomitant use of pharmacokinetically-interacting drugs was examined as a time-varying covariate in multivariable adjusted Cox regression models.19 Second, subjects using P-gp/CYP3A4-interacting drugs that also pharmacodynamically interact with NOACs, namely ticagrelor (P-gp inhibitor) and dexamethasone (CYP3A4 inducer), were excluded, to avoid mixing effects of pharmacokinetic and –dynamic interactions on bleeding outcomes. Lastly, analyses were repeated using stabilized inverse probability of treatment weighting (IPTW).

## Results

Baseline characteristics

A total of 193,072 NOAC-treated AF patients were included, among whom 46,194 (23.9%), 2903 (1.5%) and 957 (0.5%) subjects concomitantly used a P-gp/CYP3A4 inhibitor, inducer, or combination of an inhibitor and inducer at the time of NOAC initiation, respectively (eFigure 2, Table 1). The most frequently used P-gp/CYP3A4 inhibitors were amiodarone (19.6%) and diltiazem (2.6%), while levetiracetam (0.7%) and valproic acid (0.6%) were the most concurrently used P-gp/CYP3A4 inducers (eTable 4). Before matching, subjects concomitantly using P-gp/CYP3A4 inducers were on average younger (74.0 (SD 11.1) years) than subjects without P-gp/CYP3A4-interacting drug use (76.3 (SD 10.3) years), whereas subjects using P-gp/CYP3A4 inhibitors had a more comparable age (76.5 (SD 9.5) years) (Table 1). Subjects concomitantly using P-gp/CYP3A4 inhibitors and inducers were on average 74.4 (SD 9.7) years old and had the highest clinical risk scores (e.g., mean CHA2DS2-VASc score 4.3 (SD 2.0)). After matching, covariate balance was achieved (Table 1, eFigure 3).

The unadjusted number of events and event rates among NOAC-treated AF patients without pharmacokinetically-interacting drug use (mean follow-up 1.3 (SD 1.4) years; 179,544 person-years) and with concomitant use of P-gp/CYP3A4 inhibitors (follow-up 0.9 (SD 1.1) years; 39,405 person-years), inducers (follow-up 0.8 (SD 1.1) years; 2339 person-years), or inhibitors and inducers (follow-up 0.9 (SD 1.1) years; 828 person-years) are summarized in eTable 5.

### P-gp/CYP3A4 inhibitors

After multivariable adjustment, concomitant use of P-gp/CYP3A4 inhibitors was associated with significantly higher risks of major bleeding (aHR 1.24, 95%CI (1.18-1.30)), intracranial bleeding (aHR 1.15, 95%CI (1.04-1.27)), gastrointestinal bleeding (aHR 1.20, 95%CI (1.12-1.28)) and all-cause mortality (aHR 1.07, 95%CI (1.02-1.11)) compared to NOAC-treated AF patients without pharmacokinetically-interacting drug use, while the risks of stroke/SE (aHR 0.97, 95%CI (0.90-1.05)) or stroke (aHR 0.96, 95%CI (0.88-1.05)) were not significantly different (Figure 1, eTable 6).

### P-gp/CYP3A4 inducers

Concomitant use of P-gp/CYP3A4 inducers was associated with a significantly higher risk of stroke (aHR 1.31, 95%CI (1.03-1.68)) compared to NOAC-treated AF patients without pharmacokinetically-interacting drug use after multivariable adjustment, while the risks of stroke/SE (aHR 1.23, 95%CI (0.97-1.56)), all-cause mortality (aHR 0.96, 95%CI (0.82-1.11)), major bleeding (aHR 1.09, 95%CI (0.90-1.32)), intracranial bleeding (aHR 1.08, 95%CI (0.76-1.54)) or gastrointestinal bleeding (aHR 1.24, 95%CI (0.95-1.61)) were not significantly different.

### P-gp/CYP3A4 inhibitors and inducers

Among subjects concurrently using P-gp/CYP3A4 inhibitors and inducers, the risk of all-cause mortality (aHR 1.49, 95%CI (1.21-1.84)) was significantly higher compared to NOAC-treated AF patients without pharmacokinetically-interacting drug use after multivariable adjustment, whereas the risks of stroke/SE (aHR 1.32, 95%CI (0.90-1.95)), stroke (aHR 1.37, 95%CI (0.91-2.04)), major bleeding (aHR 1.28, 95%CI (0.96-1.70)), intracranial bleeding (aHR 1.14, 95%CI (0.66-1.97)) or gastrointestinal bleeding (aHR 1.11, 95%CI (0.73-1.68)) were non-significantly higher.

### Subgroup analyses

Results stratified by specific P-gp/CYP3A4-inhibiting drug types were consistent, as significantly higher major bleeding risks were observed with concomitant use of amiodarone (aHR 1.27, 95%CI (1.21-1.34)), diltiazem (aHR 1.28, 95%CI (1.13-1.46)), verapamil (aHR 1.36, 95%CI (1.03-1.80)), ticagrelor (aHR 1.50, 95%CI (1.20-1.87)) and clarithromycin (aHR 1.55, 95%CI (1.14-2.11)) compared to patients without pharmacokinetically-interacting drug use (Figure 2, eTable 7). All-cause mortality risks were significantly higher with amiodarone (aHR 1.10, 95%CI (1.05-1.15)), but not with other P-gp/CYP3A4-inhibiting drug types. Significantly higher stroke risks were observed in NOAC users concurrently treated with levetiracetam (aHR 1.45, 95%CI (1.02-2.07)) compared to subjects without pharmacokinetically-interacting drug use.

Moreover, subjects concomitantly using ≥2 P-gp/CYP3A4 inhibitors (n = 2913) had higher risk estimates of major bleeding (aHR 1.49, 95%CI (1.27-1.74)) than those using 1 inhibitor (n = 43,281; aHR 1.23, 95%CI (1.17-1.30)) compared to subjects without pharmacokinetically-interacting drug use (eTable 8).

After stratifying results by the mechanism of interaction, significantly higher major bleeding risks were observed with concomitant use of P-gp inhibitors (n = 35,261; aHR 1.25, 95%CI (1.18-1.32)), and combined P-gp and CYP3A4 inhibitors (n = 8774; aHR 1.24, 95%CI (1.12-1.37)), but not with CYP3A4 inhibitors (n = 992; aHR 0.79, 95%CI (0.57-1.09)) (eTable 9).

Furthermore, concomitant use of P-gp/CYP3A4 inhibitors was associated with a significantly higher major bleeding risk among edoxaban (aHR 1.24, 95%CI (1.06-1.45)), rivaroxaban (aHR 1.25, 95%CI (1.16-1.34)) and apixaban users (aHR 1.27, 95%CI (1.16-1.39)), but not among dabigatran users (aHR 1.07, 95%CI (0.94-1.23)) compared to patients without pharmacokinetically-interacting drug use. Conversely, the risk of all-cause mortality was only significantly higher among dabigatran users (aHR 1.20, 95%CI (1.07-1.35)) (eTable 10). Importantly, after 3:1 PSM, a lower NOAC dose was used by 58.0% of dabigatran users concomitantly treated with P-gp/CYP3A4 inhibitors compared to 40.7%, 31.8% and 34.3% of rivaroxaban, apixaban and edoxaban users, respectively.

Lastly, comparable trends were observed after stratifying by age groups (eTable 11). However, the risk of major bleeding was not significantly different among subjects <65 years old (n = 5229) concomitantly using P-gp/CYP3A4 inhibitors (aHR 1.03, 95%CI (0.84-1.27)), and higher risk estimates of stroke were observed among subjects <75 years old (n = 1432) concomitantly using P-gp/CYP3A4 inducers (aHR 1.93 (1.29-2.90)) than those ≥75 years old (n = 1479; aHR 1.45 (1.04-2.03)).

### Sensitivity analyses

When investigating P-gp/CYP3A4-interacting drug use as a time-varying covariate, concomitant use of P-gp/CYP3A4 inhibitors was also associated with significantly higher risks of major bleeding (aHR 1.43, 95%CI (1.38-1.49)), and concomitant use of P-gp/CYP3A4 inducers with an increased risk of stroke (aHR 1.68, 95%CI (1.44-1.96)) (eTable 12, eFigure 4). Likewise, results were consistent when only considering P-gp/CYP3A4-interacting drugs without pharmacodynamic interactions (n = 47,644; aHR 1.25, 95%CI (1.18-1.31) and aHR 1.41, 95%CI (1.09-1.83), respectively) (eTable 13, eFigure 5), or when using IPTW (aHR 1.28, 95%CI (1.21-1.34) and aHR 1.59, 95%CI (1.19-2.11), respectively) (eTable 14, eFigure 6). In addition, concomitant use of P-gp/CYP3A4 inducers was also associated with significantly higher risks of stroke/SE when investigated as a time-varying covariate (aHR 1.63, 95%CI (1.40-1.89)) or using IPTW (aHR 1.45, 95%CI (1.10-1.91)), and with higher risks of major bleeding (aHR 1.43, 95%CI (1.27-1.61)) and all-cause mortality (aHR 1.26, 95%CI (1.14-1.40)) when examined as a time-varying covariate.

## Discussion

In this nationwide cohort study including more than 190,000 NOAC-treated AF patients during 222,116 person-years of on-treatment follow-up, we have demonstrated that concomitant use of P-gp and combined P-gp/CYP3A4 inhibitors was associated with increased risks of major bleeding, that concomitant use of amiodarone was associated with a higher all-cause mortality risk, and that concurrent use of P-gp/CYP3A4 inducers increased the risk of stroke, compared to subjects not using pharmacokinetically-interacting drugs. NOAC users concomitantly treated with P-gp/CYP3A4 inhibitors and inducers represented a particularly vulnerable subgroup, as illustrated by the significantly higher risk of all-cause mortality and trend towards increased but non-significantly different risks of thromboembolism and bleeding.

Two major pharmacokinetic interaction mechanisms are present with NOACs. First, NOACs are excreted by P-gp efflux transporters, mostly present in the gastrointestinal tract, but also in the liver and renal proximal tubules.1,2,25,26 Second, apixaban and rivaroxaban are in part hepatically metabolized, mostly by CYP3A4 (not involved for dabigatran and only minimally (<4%) for edoxaban).1,2,25 Consequently, P-gp/CYP3A4 inhibitors may increase NOAC plasma levels due to a decreased excretion and/or hepatic metabolism. Indeed, we observed a 24% increased risk of major bleeding and 7% higher risk of death with P-gp/CYP3A4 inhibitors compared to subjects not using pharmacokinetically-interacting drugs, in line with previous observations2,8-11. On the contrary, P-gp/CYP3A4 inducers such as antiepileptic drugs (e.g., levetiracetam, carbamazepine) may decrease NOAC plasma levels3,27, which was associated with a 31% significantly higher risk of stroke in this study. Our findings address an important research gap, since, to the best of our knowledge, only two studies8,9 explored the impact of P-gp/CYP3A4 inducers on thromboembolic outcomes, rendering conflicting results.

In addition to the pharmacokinetic effect of P-gp/CYP3A4 inhibitors and inducers on NOAC plasma levels, other potential hypotheses on why increased risks of adverse outcomes were observed among NOAC-treated AF patients using pharmacokinetically-interacting drugs include the impact of underlying comorbidities for which the P-gp/CYP3A4-interacting drug was prescribed (e.g., levetiracetam for post-stroke epilepsy, clarithromycin for H. pylori eradication in case of a gastroduodenal ulcer…), direct drug effects (e.g., increased risk of bleeding with ticagrelor, although results were consistent when excluding ticagrelor), polypharmacy, reduced drug adherence, and inappropriate drug dosing (including off-label NOAC dosing).2,25 While results were robust throughout various methods used and adjustments made, any influence of unmeasured confounding and selective prescribing of pharmacokinetically-interacting drugs to more vulnerable, sicker AF patients cannot be ruled out completely. Our exploratory results should therefore be interpreted with caution and considered as hypothesis-generating.

In line with the expected increase in NOAC plasma levels due to P-gp/CYP3A4 inhibition, higher bleeding risks were consistently observed with amiodarone, diltiazem, verapamil, ticagrelor and clarithromycin, corroborating recommendations of the EHRA Practical Guide1 and SmPCs4-7 to lower the dose of NOAC (e.g., verapamil with dabigatran) or use them cautiously (e.g., amiodarone). However, while no relevant interaction with diltiazem was anticipated by guidelines1 due to its weak P-gp and moderate CYP3A4 inhibition1,4, a 28% higher major bleeding risk was observed with diltiazem, which should warrant some caution. Moreover, a significantly higher all-cause mortality risk was only observed in NOAC users concurrently treated with amiodarone, but not with other P-gp/CYP3A4-inhibiting drug types. This may be due to the considerable extracardiac side effects of amiodarone (e.g., thyroid, pulmonary, hepatic, neurologic, ophthalmologic toxicity), increasing the risk of non-cardiovascular death.2,24

Several antiepileptic drugs, used for (post-stroke) epilepsy, are P-gp/CYP3A4 inducers, and should therefore be avoided with NOACs (e.g., phenytoin) or used with caution (e.g., levetiracetam) according to the EHRA Practical Guide1. In line with the expected decrease in NOAC plasma levels3,27, a 45% higher risk of stroke was highlighted with levetiracetam in the present study. Higher thromboembolic risks with antiepileptic drugs such as levetiracetam, valproic acid and carbamazepine among NOAC users have been demonstrated before9, albeit not consistently8. Therefore, caution and close monitoring of NOAC users concurrently treated with antiepileptic drugs are warranted, and more research is urgently needed to replicate our findings and assess whether other anticoagulants (e.g., VKAs, low-molecular-weight heparins) or higher NOAC doses may be preferred in this vulnerable patient subgroup.

Concomitant use of P-gp/CYP3A4 inhibitors was associated with a significantly increased major bleeding risk among apixaban, rivaroxaban and edoxaban users, but not among dabigatran users, albeit a higher mortality risk was only observed among dabigatran users. This may in part be driven by dabigatran not being hepatically metabolised by CYP3A4.1 Consequently, CYP3A4 inhibitors without impact on P-gp activity do not affect dabigatran plasma levels, which may have contributed to the lack of a significant impact on bleeding outcomes with P-gp/CYP3A4 inhibitors in this study. However, the increased bleeding risk associated with concomitant use of P-gp/CYP3A4 inhibitors appeared to be driven by drugs inhibiting P-gp, as no significant differences in bleeding risks were observed with drugs only inhibiting CYP3A4 (e.g., fluconazole), and a similarly increased bleeding risk was seen with P-gp inhibitors (e.g., amiodarone) and combined P-gp and CYP3A4 inhibitors (e.g., diltiazem, verapamil). Moreover, dabigatran was more frequently used in a lower dose with P-gp/CYP3A4 inhibitors than other NOACs, which may have also affected bleeding and mortality risks. Prior studies investigating outcomes with individual NOACs rendered conflicting findings. Exemplary, in line with our findings, higher bleeding risk estimates with apixaban and/or rivaroxaban than with dabigatran in patients treated with P-gp/CYP3A4 inhibitors were seen in some studies8,9,19, while other studies did not observe differences in risk estimates between NOACs28 or even higher bleeding risks with dabigatran18. Therefore, our results should be interpreted with caution and no specific NOAC can yet be recommended with concomitant use of P-gp/CYP3A4 inhibitors.

Given the increased risk of adverse outcomes with pharmacokinetically-interacting drugs, caution is generally warranted, modifiable bleeding risk factors should be addressed, and the importance of high therapy adherence, especially in subjects using P-gp/CYP3A4 inducers, should be stressed.1,2,13,25 Clinicians should assess whether the P-gp/CYP3A4-interacting drug can be discontinued, especially if multiple interacting drugs are used, or should consider a non-interactive alternative or another anticoagulant with less interactions, in accordance with recommendations of the EHRA Practical Guide1 or other drug interaction management plans26.2 If not possible, NOACs should be appropriately dosed and plasma level measurement may be considered.1,3 Patients should be closely monitored by scheduling them for earlier and more frequent clinical follow-up during the concomitant use of P-gp/CYP3A4 interacting drugs, performing a regular medication review, and informing them of the increased risk of side effects.1,2

Strengths and limitations

Strengths of this nationwide cohort study include the large sample size, use of an on-treatment analysis that accounted for changes in interacting drug use during follow-up to reduce exposure misclassification, and adjustment for several confounders using PSM.

Several limitations should be acknowledged. First, coding errors and misclassification bias may be present due to the observational design using healthcare databases. However, by identifying comorbidities based on ICD, medical procedure codes and/or medication prescription claims assessed in ambulatory and hospital care, missing data and misclassification of characteristics were reduced. Second, assessment of P-gp/CYP3A4-interacting drug use was based on dispensing data, not on the patients’ actual intake and physicians’ prescriptions. Consequently, premature discontinuation of interacting drug use at the time of NOAC initiation before the calculated last day of supply could not be identified. Nevertheless, subjects were additionally censored in case of a >60-day gap of drug supply of the interacting drug during follow-up. Moreover, since interacting drug use was only considered at baseline, the impact of initiating pharmacokinetically-interacting drugs during follow-up was not assessed (as subjects were censored on those dates). Interestingly, results were generally consistent when investigating pharmacokinetically-interacting drug use as a time-varying covariate, suggesting that the potential risk of initiating an interacting drug does not wane over time. Third, use of over-the-counter (herbal) medicines interacting with P-gp/CYP3A4 (e.g., St. John’s wort)1 could not be identified. Fourth, despite thorough adjustment for 40 confounders, there is a risk of unmeasured confounding due to missing lifestyle characteristics (e.g., body weight, smoking), laboratory values (e.g., renal function) and disease severity. Likewise, (in)appropriate NOAC dosing could not be assessed. Fifth, data were only completely registered and validated up to 1 January 2019, which may reduce the generalizability to current practice. Sixth, although PSM improved comparability and reduced confounding by indication, unmatched individuals were discarded from the analysis, reducing the effective sample size and increasing the risk of selection bias.29 Nevertheless, results were consistent using IPTW, which included every individual. Lastly, due to the design and on-treatment approach, the mean follow-up was limited.

## Conclusion

In conclusion, concomitant use of P-gp/CYP3A4 inhibitors was associated with higher bleeding and all-cause mortality risks in NOAC users, largely driven by amiodarone, diltiazem, verapamil, ticagrelor and clarithromycin use. Conversely, a higher stroke risk was observed in AF patients concurrently treated with P-gp/CYP3A4 inducers, especially with antiepileptic drugs such as levetiracetam.

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## Conflicts of interests

Outside this manuscript, TDB has served as a chairperson during a lecture for Bayer and Daiichi Sankyo and participated in an expert meeting for Pfizer. Outside this manuscript, LL has been consulted as expert for AstraZeneca. Outside this manuscript, SS has given a lecture sponsored by BMS, LL a lecture sponsored by Chiesi, and SS, LL and MG lectures sponsored by IPSA vzw, a non-profit organization facilitating lifelong learning for pharmacists. Neither author has received any fees personally.

## Data Availability Statement

Requests for the data underlying this article should be directed to the administrators of the InterMutualistic Agency database or Minimal Hospital Dataset and is subject to approval.

## Author contributions

MG and LL contributed to the concept and design of the study. MG performed the statistical analysis, interpretation and writing under the supervision of LL. LC, AC, KB, EM, TDB, SS and LL revised the manuscript critically. All authors contributed to the article and approved the final manuscript.

## References

1. Steffel J, Collins R, Antz M, Cornu P, Desteghe L, Haeusler KG, et al. 2021 European Heart Rhythm Association Practical Guide on the Use of Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial Fibrillation. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology. 2021;23(10):1612-76.

2. Grymonprez M, Vanspranghe K, Capiau A, Boussery K, Steurbaut S, Lahousse L. Impact of P-glycoprotein and/or CYP3A4-interacting drugs on effectiveness and safety of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation: A meta-analysis. Br J Clin Pharmacol. 2022;88(7):3039-51.

3. Sennesael AL, Larock AS, Hainaut P, Lessire S, Hardy M, Douxfils J, et al. The impact of strong inducers on direct oral anticoagulant levels. The American journal of medicine. 2021;134(10):1295-9.

4. Eliquis. Summary of Product Characteristics. <https://www.ema.europa.eu/en/documents/product-information/eliquis-epar-product-information_en.pdf>. Accessed 10 July 2020.

5. Pradaxa. Summary of Product Characteristics. <https://www.ema.europa.eu/en/documents/product-information/pradaxa-epar-product-information_en.pdf>. Accessed 10 July 2020.

6. Lixiana. Summary of Product Characteristics. <https://www.ema.europa.eu/en/documents/product-information/lixiana-epar-product-information_en.pdf>. Accessed 10 July 2020.

7. Xarelto. Summary of Product Characteristics. <https://www.ema.europa.eu/en/documents/product-information/xarelto-epar-product-information_en.pdf>. Accessed 10 July 2020.

8. Holm J, Mannheimer B, Malmström RE, Eliasson E, Lindh JD. Bleeding and thromboembolism due to drug-drug interactions with non-vitamin K antagonist oral anticoagulants-a Swedish, register-based cohort study in atrial fibrillation outpatients. European journal of clinical pharmacology. 2021;77(3):409-19.

9. Gronich N, Stein N, Muszkat M. Association Between Use of Pharmacokinetic-Interacting Drugs and Effectiveness and Safety of Direct Acting Oral Anticoagulants: Nested Case-Control Study. Clinical pharmacology and therapeutics. 2021;110(6):1526-36.

10. Hanigan S, Das J, Pogue K, Barnes GD, Dorsch MP. The real world use of combined P-glycoprotein and moderate CYP3A4 inhibitors with rivaroxaban or apixaban increases bleeding. J Thromb Thrombolysis. 2020;49(4):636-43.

11. Bernier M, Lancrerot SL, Rocher F, Van-Obberghen EK, Olivier P, Lavrut T, et al. Major bleeding events in octagenarians associated with drug interactions between dabigatran and P-gp inhibitors. Journal of geriatric cardiology : JGC. 2019;16(11):806-11.

12. Zhang Y, Souverein PC, Gardarsdottir H, van den Ham HA, Maitland-van der Zee AH, de Boer A. Risk of major bleeding among users of direct oral anticoagulants combined with interacting drugs: A population-based nested case-control study. Br J Clin Pharmacol. 2020;86(6):1150-64.

13. Grymonprez M, Capiau A, Steurbaut S, Mehuys E, Boussery K, De Backer TL, et al. Adherence and persistence to oral anticoagulants in patients with atrial fibrillation: A Belgian nationwide cohort study. Frontiers in cardiovascular medicine. 2022;9:994085.

14. Grymonprez M, De Backer TL, Bertels X, Steurbaut S, Lahousse L. Long-term comparative effectiveness and safety of dabigatran, rivaroxaban, apixaban and edoxaban in patients with atrial fibrillation: A nationwide cohort study. Frontiers in pharmacology. 2023;14:1125576.

15. Langan SM, Schmidt SA, Wing K, Ehrenstein V, Nicholls SG, Filion KB, et al. The reporting of studies conducted using observational routinely collected health data statement for pharmacoepidemiology (RECORD-PE). BMJ (Clinical research ed). 2018;363:k3532.

16. MedicinesComplete. Stockley's drug interactions. 2023. Available from: <https://www.medicinescomplete.com/#/>. Accessed 6 March 2023.

17. Sandhu RK, Islam S, Dover DC, Andrade JG, Ezekowitz J, McAlister FA, et al. Concurrent use of P-glycoprotein or Cytochrome 3A4 drugs and non-vitamin K antagonist oral anticoagulants in non-valvular atrial fibrillation. European heart journal Quality of care & clinical outcomes. 2022;8(2):195-201.

18. Pham P, Schmidt S, Lesko L, Lip GYH, Brown JD. Association of Oral Anticoagulants and Verapamil or Diltiazem With Adverse Bleeding Events in Patients With Nonvalvular Atrial Fibrillation and Normal Kidney Function. JAMA network open. 2020;3(4):e203593.

19. Xu Y, Chang AR, Inker LA, McAdams-DeMarco M, Grams ME, Shin JI. Concomitant Use of Diltiazem With Direct Oral Anticoagulants and Bleeding Risk in Atrial Fibrillation. J Am Heart Assoc. 2022;11(14):e025723.

20. Washam JB, Hellkamp AS, Lokhnygina Y, Piccini JP, Berkowitz SD, Nessel CC, et al. Efficacy and Safety of Rivaroxaban Versus Warfarin in Patients Taking Nondihydropyridine Calcium Channel Blockers for Atrial Fibrillation (from the ROCKET AF Trial). The American journal of cardiology. 2017;120(4):588-94.

21. Bartlett JW, Renner E, Mouland E, Barnes GD, Kuo L, Ha NB. Clinical Safety Outcomes in Patients With Nonvalvular Atrial Fibrillation on Rivaroxaban and Diltiazem. The Annals of pharmacotherapy. 2019;53(1):21-7.

22. Kaatz S, Ahmad D, Spyropoulos AC, Schulman S. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis : JTH. 2015;13(11):2119-26.

23. Steinberg BA, Hellkamp AS, Lokhnygina Y, Halperin JL, Breithardt G, Passman R, et al. Use and outcomes of antiarrhythmic therapy in patients with atrial fibrillation receiving oral anticoagulation: results from the ROCKET AF trial. Heart Rhythm. 2014;11(6):925-32.

24. Saksena S, Slee A, Waldo AL, Freemantle N, Reynolds M, Rosenberg Y, et al. Cardiovascular outcomes in the AFFIRM Trial (Atrial Fibrillation Follow-Up Investigation of Rhythm Management). An assessment of individual antiarrhythmic drug therapies compared with rate control with propensity score-matched analyses. Journal of the American College of Cardiology. 2011;58(19):1975-85.

25. Grymonprez M, Steurbaut S, De Backer TL, Petrovic M, Lahousse L. Effectiveness and Safety of Oral Anticoagulants in Older Patients With Atrial Fibrillation: A Systematic Review and Meta-Analysis. Frontiers in pharmacology. 2020;11:583311.

26. Capiau A, Mehuys E, De Bolle L, Van Tongelen I, De Backer T, Boussery K. Drug-drug interactions with direct oral anticoagulants: development of a consensus list for ambulatory care. International journal of clinical pharmacy. 2023;45(2):364-74.

27. Perlman A, Goldstein R, Choshen Cohen L, Hirsh-Raccah B, Hakimian D, Matok I, et al. Effect of Enzyme-Inducing Antiseizure Medications on the Risk of Sub-Therapeutic Concentrations of Direct Oral Anticoagulants: A Retrospective Cohort Study. CNS drugs. 2021;35(3):305-16.

28. Chang SH, Chou IJ, Yeh YH, Chiou MJ, Wen MS, Kuo CT, et al. Association Between Use of Non-Vitamin K Oral Anticoagulants With and Without Concurrent Medications and Risk of Major Bleeding in Nonvalvular Atrial Fibrillation. JAMA. 2017;318(13):1250-9.

29. Allan V, Ramagopalan SV, Mardekian J, Jenkins A, Li X, Pan X, et al. Propensity score matching and inverse probability of treatment weighting to address confounding by indication in comparative effectiveness research of oral anticoagulants. Journal of comparative effectiveness research. 2020;9(9):603-14.

## Figure legends

**Figure 1:** Outcomes in AF patients concomitantly using P-gp/CYP3A4 inhibitors, inducers, or combination of an inhibitor and inducer at the time of NOAC initiation, compared to patients without pharmacokinetically-interacting drug use after 3:1 propensity score matching.

aHR: adjusted hazard ratio; CI: confidence interval; CYP: cytochrome P450; DDI: drug-drug interaction; NOAC: non-vitamin K antagonist oral anticoagulant; P-gp: P-glycoprotein; PY: person-years; SE: systemic embolism.

**Figure 2:** The risk of major bleeding with P-gp/CYP3A4 inhibitors, and of stroke with P-gp/CYP3A4 inducers compared to patients without pharmacokinetically-interacting drug use after 3:1 propensity score matching, stratified by interacting drug types, number of interacting drugs, mechanism of interaction, NOAC types and age.

aHR: adjusted hazard ratio; CI: confidence interval; CYP: cytochrome P450; DDI: drug-drug interaction; MB: major bleeding; NOAC: non-vitamin K antagonist oral anticoagulant; P-gp: P-glycoprotein; PY: person-years; SE: systemic embolism.