Ischemic Stroke and Systemic Embolism in Warfarin Users With Atrial Fibrillation or Heart Valve Replacement Exposed to Dicloxacillin or Flucloxacillin

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The antibiotics dicloxacillin and flucloxacillin induce cytochrome P450-dependent metabolism of warfarin. We explored the influence of these drug–drug interactions on the clinical effectiveness of warfarin therapy due to atrial fibrillation or heart valve replacement. Using the population-based Danish registers, we performed a propensity-score matched cohort study including around 50,000 episodes of dicloxacillin/flucloxacillin matched to phenoxymethylpenicillin and to no antibiotic, respectively. We estimated hazard ratios (HRs) with 95% confidence intervals (CIs) by comparing 21-day (days 7–28) risks of ischemic stroke/systemic embolism (SE) following initiation of each exposure. When compared with phenoxymethylpenicillin, dicloxacillin/flucloxacillin was associated with an HR of ischemic stroke/SE of 2.09 (95% CI 1.51–2.90; strongest for dicloxacillin (HR 2.17; 95% CI 1.56–3.02)). Use of an untreated comparator strengthened the association (HR 2.84; 95% CI 1.97–4.09). Dicloxacillin should be used with caution in patients receiving warfarin. This may also apply to flucloxacillin; however, more data on the risks associated with flucloxacillin exposure during warfarin therapy are needed.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ The antibiotics dicloxacillin and flucloxacillin induce cytochrome P450-dependent metabolism of warfarin leading to decreased values of the international normalized ratio. Whether the clinical effectiveness of warfarin therapy is also affected remains to be established.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Is exposure to dicloxacillin or flucloxacillin during chronic warfarin use due to atrial fibrillation or heart valve replacement associated with an increased risk of ischemic stroke and systemic embolism (SE)?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ In this population-based propensity-score matched cohort study of warfarin users, exposure to dicloxacillin or flucloxacillin was associated with a twofold higher short-term risk of ischemic stroke and SE when compared with unexposed periods. The risk was more pronounced for dicloxacillin than for flucloxacillin.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY?

☑ Increased awareness among clinicians and researchers on these drug–drug interactions has the potential to improve the safety of warfarin therapy during infections.

Warfarin is an effective and highly valued drug in the treatment and prophylaxis of thromboembolisms. However, warfarin has a narrow therapeutic index and even minor subtherapeutic international normalized ratio (INR) levels are associated with reduced treatment effectiveness.[1]

Several reports have suggested that beta-lactamase-resistant penicillins (i.e., oxacillin, cloxacillin, dicloxacillin, and flucloxacillin), lower the anticoagulant effect of vitamin K antagonists (VKAs), especially warfarin.[2–9] In a register-based study of INR measurements and prescription fills, we have previously demonstrated that initiation of dicloxacillin treatment led to subtherapeutic INR levels in about 60% of VKA-treated patients.[10] In a subsequent clinical drug–drug interaction study in healthy volunteers, we elucidated this further by demonstrating that dicloxacillin induces several

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cytochrome P450 enzymes, including CYP2C9.[11] CYP2C9 is the enzyme that catalyzes the metabolism of the most pharmacologically active warfarin isomer (S-warfarin). Supplementary *in vitro* studies demonstrated that the underlying molecular mechanism is activation of the pregnane X receptor, leading to increased transcription and activity of cytochrome P450 enzymes relevant to warfarin metabolism.[11] Flucloxacillin use has also been associated with decreased INR values in clinical practice,[2,8] which is supported by *in vitro* data showing a potential of flucloxacillin to induce CYP2C9, although to a lesser extent than dicloxacillin.[11] Consequently, initiation of beta-lactamase-resistant penicillin treatment in patients receiving drugs with a narrow therapeutic interval such as warfarin, cyclosporine, and some anti-epileptic drugs may result in therapeutic failure.

With this study, we assessed whether initiation of dicloxacillin or flucloxacillin was associated with an increased risk of thromboembolism in warfarin users with a condition requiring chronic warfarin therapy. Specifically, we included warfarin users with atrial fibrillation (AF) and/or heart valve replacement and assessed their risk of ischemic stroke and systemic embolism (SE) in the context of concomitant treatment with these antibiotics.

RESULTS

The sampling cohort included 271,711 episodes of warfarin therapy contributed by 111,637 unique patients receiving warfarin due to AF or mechanical heart valves during the study period (**Figure 1**). Within this cohort, we identified 53,035 episodes of dicloxacillin and 5,376 episodes of flucloxacillin use as well as 144,727 episodes of phenoxymethylpenicillin use. After trimming, 49,661 episodes of dicloxacillin/flucloxacillin use were matched 1:1 to episodes of phenoxymethylpenicillin (**Figure S1**). In the matched cohorts, covariates were well balanced between dicloxacillin/flucloxacillin users and phenoxymethylpenicillin users (**Table 1**). Warfarin users exposed to antibiotics had a median age of 76 years, were most often men (63%), and had, on average, been on warfarin therapy for 4 years, typically due to AF (94%). The level of comorbidity was high; 41% had a Charlson Comorbidity Index ≥ 3 and 87% had a CHA_2DS_2-VASc score ≥ 3.

Ischemic stroke/SE occurred more commonly during exposure to dicloxacillin/flucloxacillin than during phenoxymethylpenicillin exposure (2.3/1,000 vs. 1.1/1,000 episodes, respectively; **Figure 2**). The corresponding hazard ratio (HR) for ischemic stroke/SE was 2.09 (95% CI 1.51–2.90) for warfarin users exposed to dicloxacillin/flucloxacillin and the number needed to harm (NNH) was 842 (**Table 2**). Absolute as well as relative risks of ischemic stroke/SE were similar in patients using warfarin due to AF and heart valve replacement (**Table 2**). There was no considerable effect modification of the observed HR by patient characteristics. Stratification by type of antibiotic revealed a stronger association for use of dicloxacillin (HR 2.19; 95% CI 1.56–3.02; NNH 784) than flucloxacillin (HR 1.27; 95% CI 0.55–2.95), although the estimate for flucloxacillin was unprecise due to low use in our cohort. The increased risk of ischemic stroke/SE attenuated over time, with an HR in the 29–60-day interval after the index date of 1.40 (95% CI 1.02–1.93) and an HR of 1.43 (95% CI 1.09–1.88) for the 61–120-days interval (**Table** S1). In the analysis of the 29–60-day interval, flucloxacillin was associated with a significantly increased risk of ischemic stroke/SE (HR 2.07; 95% CI 1.12–3.83).

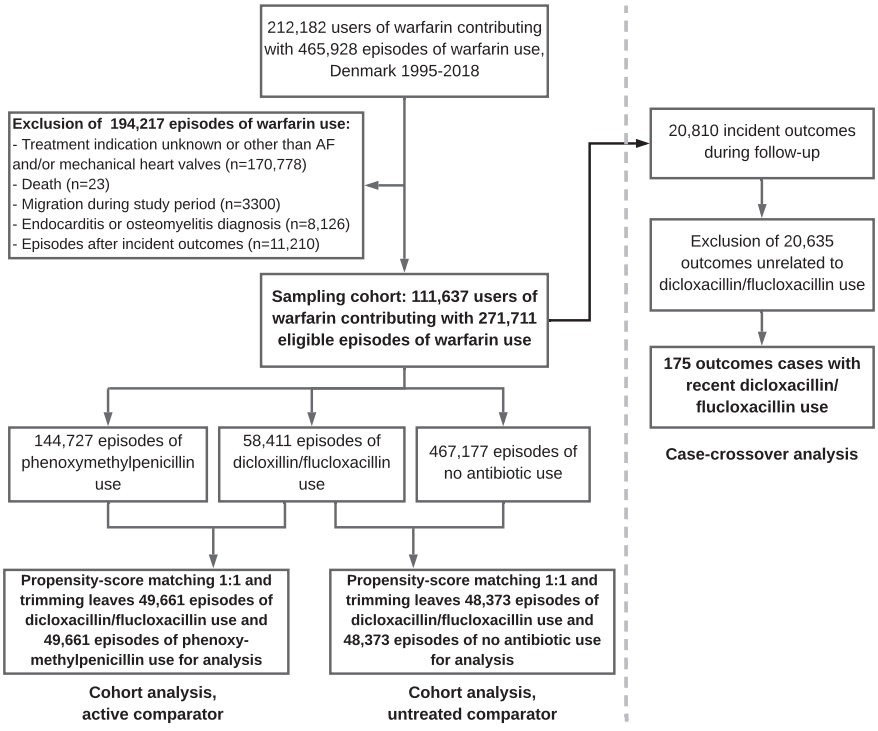

Figure 1 Flow chart describing the selection of the overall cohort of warfarin episodes, the propensity score matched cohorts, and the case-crossover population. AF, atrial fibrillation.

Table 1 Baseline characteristics of users of dicloxacillin/flucloxacillin and users of penicillin, matched on propensity scores

	Active comparator analysis			Untreated comparator analysis		
	Dicloxacillin/ flucloxacillin *n* = 49,661	Phenoxymethyl-penicillin *n* = 49,661	Standardized mean difference	Dicloxacillin/ flucloxacillin *n* = 48,373	No antibiotic *n* = 48,373	Standardized mean difference
Male sex	31,136 (62.7%)	31,285 (63.0%)	0.006	30,437 (62.9%)	30,588 (63.2%)	0.006
Age, years			0.000			0.001
Age, median (IQR)	76 (68–82)	75 (68–82)	-	76 (68–82)	75 (68–81)	-
18–69	14,574 (29.3%)	14,493 (29.2%)	-	14,159 (29.3%)	13,732 (28.4%)	-
70–79	17,952 (36.1%)	18,434 (37.1%)	-	17,381 (35.9%)	18,800 (38.9%)	-
80+	17,135 (34.5%)	16,734 (33.7%)	-	16,833 (34.8%)	15,841 (32.7%)	-
Treatment indication VKA			-			-
Atrial fibrillation/flutter	46,658 (94.0%)	46,497 (93.6%)	0.013	45,389 (93.8%)	45,304 (93.7%)	0.007
Mechanical heart valve	5,245 (10.6%)	5,245 (10.6%)	0.000	5,106 (10.6%)	5,106 (10.6%)	0.000
Time since first warfarin prescription			0.001			0.001
Mean (SD)	5.7 (4.6)	5.7 (4.5)	-	5.7 (4.6)	5.7 (4.5)	-
Median (IQR)	4 (1–8)	4 (1–8)	-	4 (1–8)	4 (2–8)	-
<3 years	17,285 (34.8%)	17,350 (34.9%)	-	16,710 (34.5%)	16,677 (34.5%)	-
3–5 years	8,257 (16.6%)	8,474 (17.1%)	-	8,025 (16.6%)	8,460 (17.5%)	-
5 + years	24,119 (48.6%)	23,837 (48.0%)	-	23,638 (48.9%)	23,236 (48.0%)	-
CHA_2DS_2-VASc score						0.002
Mean (SD)	4.3 (1.6)	4.3 (1.6)	-	4.3 (1.6)	4.3 (1.6)	-
Median (IQR)	4 (3–5)	4 (3–5)	-	4 (3–5)	4 (3–5)	-
Score < 3	6,466 (13.0%)	6,672 (13.4%)	-	6,402 (13.2%)	6,461 (13.4%)	-
Score = 3–5	32,058 (64.6%)	32,157 (64.8%)	-	31,389 (64.9%)	31,534 (65.2%)	-
Score > 5	11,137 (22.4%)	10,832 (21.8%)	-	10,582 (21.9%)	10,378 (21.5%)	-
HASBLED score			0.006			0.005
Mean (SD)	2.9 (1.0)	2.9 (1.0)	-	2.9 (1.0)	2.9 (1.0)	-
Median (IQR)	3 (2–4)	3 (2–4)	-	3 (2–3)	3 (2–3)	-
Score < 2	3,094 (6.2%)	3,207 (6.5%)	-	2,950 (6.1%)	2,911 (6.0%)	-
Score = 2–4	43,846 (88.3%)	43,799 (88.2%)	-	43,021 (88.9%)	43,049 (89.0%)	-
Score > 4	2,721 (5.5%)	2,655 (5.3%)	-	2,402 (5.0%)	2,413 (5.0%)	-
Comorbidity			-			
Congestive heart failure	19,714 (39.7%)	19,521 (39.3%)	0.008	18,830 (38.9%)	18,860 (39.0%)	0.001
Ischemic heart disease	25,881 (52.1%)	25,690 (51.7%)	0.008	24,831 (51.3%)	24,815 (51.3%)	0.001
Diabetes	14,517 (29.2%)	14,533 (29.3%)	0.001	13,802 (28.5%)	13,782 (28.5%)	0.001
Stroke	8,964 (18.1%)	8,824 (17.8%)	0.007	8,641 (17.9%)	8,534 (17.6%)	0.006

(Continued)

Table 1 (Continued)

	Active comparator analysis			Untreated comparator analysis		
	Dicloxacillin/ flucloxacillin *n* = 49,661	Phenoxymethyl-penicillin *n* = 49,661	Standardized mean difference	Dicloxacillin/ flucloxacillin *n* = 48,373	No antibiotic *n* = 48,373	Standardized mean difference
COPD	10,542 (21.2%)	10,488 (21.1%)	0.003	9,688 (20.0%)	9,632 (19.9%)	0.003
Major bleeding	15,541 (31.3%)	15,548 (31.3%)	0.000	14,927 (30.9%)	14,863 (30.7%)	0.003
Alcohol-related disease	3,260 (6.6%)	3,148 (6.3%)	0.009	3,131 (6.5%)	3,042 (6.3%)	0.008
Admission with elevated INR	1,544 (3.1%)	1,597 (3.2%)	0.006	1,474 (3.0%)	1,455 (3.0%)	0.002
Renal insufficiency	5,038 (10.1%)	4,975 (10.0%)	0.004	4,479 (9.3%)	4,497 (9.3%)	0.001
Liver failure	1,195 (2.4%)	1,162 (2.3%)	0.004	1,166 (2.4%)	1,128 (2.3%)	0.005
Thyroid disease	7,144 (14.4%)	7,191 (14.5%)	0.003	6,845 (14.2%)	6,833 (14.1%)	0.001
Peripheral artery disease	5,816 (11.7%)	5,813 (11.7%)	0.000	5,136 (10.6%)	5,136 (10.6%)	0.000
Charlson Comorbidity Index			0.029			0.044
Mean (SD)	1.8 (1.1)	1.8 (1.2)	-	1.8 (1.1)	1.8 (1.2)	-
Median (IQR)	2 (1–3)	2 (1–3)	-	2 (1–3)	2 (1–3)	-
Score = 0–1	19,909 (40.1%)	20,500 (41.3%)	-	19,696 (40.7%)	20,678 (42.7%)	-
Score = 2	9,079 (18.3%)	9,275 (18.7%)	-	9,085 (18.8%)	9,241 (19.1%)	-
Score = 3+	20,673 (41.6%)	19,886 (40.0%)	-	19,592 (40.5%)	18,454 (38.1%)	-
Comedication			-			-
Statin	23,591 (47.5%)	23,591 (47.5%)	0.000	22,841 (47.2%)	22,879 (47.3%)	0.002
NSAIDs	6,503 (13.1%)	6,334 (12.8%)	0.010	5,966 (12.3%)	5,900 (12.2%)	0.004
Platelet inhibitors	16,309 (32.8%)	16,408 (33.0%)	0.004	15,709 (32.5%)	15,717 (32.5%)	0.000
Amiodarone	3,381 (6.8%)	3,365 (6.8%)	0.001	3,164 (6.5%)	3,184 (6.6%)	0.002
Anti-epileptics	3,008 (6.1%)	3,055 (6.2%)	0.004	2,619 (5.4%)	2,615 (5.4%)	0.000
Azoles	1,132 (2.3%)	1,165 (2.3%)	0.004	924 (1.9%)	936 (1.9%)	0.002
Proton pump inhibitor	12,609 (25.4%)	12,406 (25.0%)	0.009	11,610 (24.0%)	11,714 (24.2%)	0.005
Season for first treatment			0.001			0.003
Winter	11,921 (24.0%)	12,432 (25.0%)	-	11,437 (23.6%)	11,663 (24.1%)	-
Spring	12,449 (25.1%)	12,540 (25.3%)	-	12,048 (24.9%)	11,834 (24.5%)	-
Summer	13,033 (26.2%)	11,352 (22.9%)	-	12,706 (26.3%)	12,608 (26.1%)	-
Fall	12,258 (24.7%)	13,337 (26.9%)	-	12,182 (25.2%)	12,268 (25.4%)	-

COPD, chronic obstructive pulmonary disease; INR, international normalized ratio; IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drugs; VKA, vitamin k antagonist.

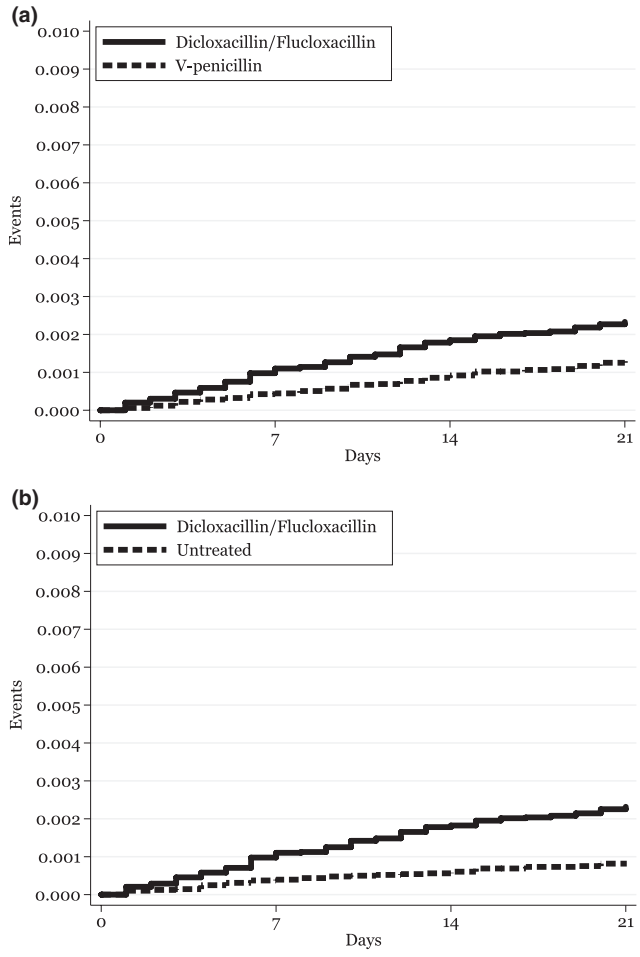

Figure 2 Kaplan–Meier plot from day 7 to day 28 with cumulative incidence of ischemic stroke/systemic embolism among warfarin users exposed to dicloxacillin/flucloxacillin and (**a**) phenoxymethylpenicillin or (**b**) no antibiotic.

In the secondary analysis, 48,373 episodes of dicloxacillin/flucloxacillin use were matched 1:1 to randomly selected episodes of no antibiotic use (**Table 1** and **Figure S2**). The rate of ischemic stroke during untreated episodes was lower (0.8/1,000 episodes) than during phenoxymethylpenicillin episodes, leading to a higher HR (2.84; 95% CI 1.97–4.09) as well as lower NNH (681) for dicloxacillin/flucloxacillin in this analysis than in the primary analysis (**Figure 2** and **Table 3**). Associations during later periods of follow-up of the secondary analysis are provided in (**Table S2**).

The case-crossover analysis included 175 cases of ischemic stroke/SE exposed to dicloxacillin/flucloxacillin during either focal or reference windows (**Figure 1** and **Figure S3**). Exposure just prior to the outcome event was more common than exposure in more distant periods, and the OR for ischemic stroke/SE during dicloxacillin/flucloxacillin exposure among warfarin users was 1.88 (95% CI 1.37–2.59) when compared with unexposed periods in the same warfarin users. A similar analysis for phenoxymethylpenicillin ($n = 348$) yielded an OR of 1.24 (95% CI 0.98–1.58).

When increasing the assigned daily warfarin dose, and thereby decreasing the assigned duration of a warfarin prescription in the study cohort, the sample sizes decreased slightly (**Table S3** and **Table S4**). Overall, the sensitivity analyses yielded results similar to the main analysis.

DISCUSSION

This population-based cohort study is the first to address the clinical impact of reduced anticoagulation inferred by the pharmacokinetic drug–drug interaction between warfarin and dicloxacillin/flucloxacillin. Our main finding was a twofold transient increase in the risk of ischemic stroke and SE upon initiation of dicloxacillin in patients receiving warfarin in the context of AF or mechanical heart valves. The estimates for flucloxacillin exposure were unprecise but overall did not support a similar increased short-term event risk when combined with warfarin.

The findings are consistent with the underlying and well-elucidated biological mechanism. As we have shown previously, dicloxacillin induces CYP2C9 *in vitro* and increases the activity of CYP2C9 in healthy volunteers.[11] Consequently, more than half of warfarin users reach subtherapeutic INR levels following initiation of dicloxacillin.[10] The latter observation was recently supported by a case series ($n = 5$) reporting that warfarin dose had to be increased by ~ 50% to maintain therapeutic INR values after dicloxacillin initiation.[12] The finding of a twofold increased risk of arterial thromboembolic complications in the present study, thus, corresponds well with the established knowledge on the drug–drug interaction.

The interpretation of our findings concerning flucloxacillin is hampered by the lack of statistical power due to the historically limited use of flucloxacillin in Denmark.[13] Nevertheless, the finding of a weaker association for flucloxacillin than dicloxacillin is compatible with previous findings concerning the individual antibiotic's potential for CYP2C9 induction and influence on the pharmacodynamic effect of warfarin as measured by the INR.[8,11] In a recent register-based study based on a large sample of warfarin users from Sweden, short-term therapy of flucloxacillin (10 days) was found to be associated with a minor, albeit significant, decrease in mean INR from 2.36 (95% CI 2.34–2.37) to 2.20 (95% CI 2.19–2.21). Long-term flucloxacillin therapy (≥ 30 days) seemed to have an additional impact on warfarin pharmacodynamics, as the mean INR decreased from 2.24 (95% CI 2.16–2.32) to 1.96 (1.89–2.02) 6 weeks after initiation in this subgroup. Importantly, the present study was designed to assess potential effects of short-term therapy with dicloxacillin/flucloxacillin on warfarin effectiveness. Clinically relevant induction of CYP2C9 by flucloxacillin in the context of long-term therapy (e.g., in endocarditis patients), can, therefore, not be ruled out by the present study. Interestingly, for the flucloxacillin-exposed patients included in our analysis, the risk of ischemic stroke/SE was higher during late than early follow-up (2.9/1,000 vs. 1.4/1,000 episodes, respectively).

The absolute risk of thromboembolic complications during a single episode of dicloxacillin treatment was low; 842 warfarin users should be treated with dicloxacillin instead of phenoxymethylpenicillin for one additional event to occur. In Denmark, beta-lactamase-resistant penicillins are the mainstay of antibiotic treatment in trivial and severe staphylococcal infection because of their narrow-spectrum characteristics, their effectiveness, and favorable safety profile. Therefore, although our

Table 2 Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared to penicillin, overall and in patient subgroups

	Outcome rate (*n*/1,000)		Dicloxacillin/flucloxacillin vs. phenoxymethylpenicillin	
	Dicloxacillin/ flucloxacillin	Phenoxymethylpenicillin	HR (95% CI)	NNH
All	2.3/1,000	1.1/1,000	2.09 (1.51–2.90)	842
Stratified analyses				
Sex				
Male	2.1/1,000	0.8/1,000	2.55 (1.61–4.05)	796
Female	2.6/1,000	1.5/1,000	1.68 (1.06–2.69)	937
Age				
< 70 years	1.8/1,000	0.9/1,000	1.98 (1.02–3.85)	1,127
70–79 years	1.9/1,000	0.7/1,000	2.97 (1.54–5.72)	770
80 + years	3.0/1,000	1.7/1,000	1.76 (1.11–2.78)	767
Treatment indication, VKA				
Heart valve	2.1/1,000	1.0/1,000	2.19 (0.76–6.31)	874
Atrial fibrillation	2.3/1,000	1.1/1,000	2.08 (1.48–2.94)	838
Type of beta-lactamase-resistant penicillin				
Dicloxacillin	2.3/1,000	1.1/1,000	2.17 (1.56–3.02)	784
Flucloxacillin	1.4/1,000	1.1/1,000	1.27 (0.55–2.95)	3,435
Restricted analyses				
No recent hospitalization	2.3/1,000	0.9/1,000	2.46 (1.72–3.53)	736
No recent antibiotic (any)	2.3/1,000	1.0/1,000	2.20 (1.50–3.25)	785
No prior exposure to dicloxacillin/flucloxacillin	2.4/1,000	1.1/1,000	2.25 (1.53–3.29)	739
No diabetes	2.4/1,000	1.0/1,000	2.44 (1.66–3.57)	694
No previous stroke	1.8/1,000	0.8/1,000	2.31 (1.52–3.52)	990

CI, confidence interval; HR, hazard ratio; NNH, number needed to harm; VKA, vitamin K antagonist.

findings indeed indicate that dicloxacillin should be used with caution in patients receiving warfarin, the increase in ischemic stroke/SE risk associated with use of dicloxacillin among warfarin users needs to be weighed against the benefits of this antibiotic. Closer INR surveillance of warfarin may mitigate the risk of experiencing insufficient anticoagulation during dicloxacillin use. Importantly, dose adjustments of warfarin in the context of short-term treatment with an interacting drug will require adjustment of warfarin dosage upon starting as well as stopping therapy. Such adjustments are complicated by the long half-life of warfarin as well as by the delay in on-set and off-set of the inductive effect of dicloxacillin. Further complicating the titration process during and after dicloxacillin therapy, is the fact that INR may be affected by infection/fever itself and by the potential changes in dietary habits during an infection. As such, the sustained, albeit attenuated, association persisting beyond dicloxacillin exposure, may reflect a long-term impact on the stability of INR control following dose adjustments in relation to a short-term treatment episode with an interacting drug. Alternative explanations include the time to normalization of CYP2C9 enzyme capacity as well as presence of residual confounding in our analysis.

Direct oral anticoagulants (DOACs) are potential treatment alternatives to warfarin in patients with AF.[14] Compared with warfarin, DOACs are overall less susceptible to drug–drug interactions, especially due to their broader therapeutic range.[15] The factor Xa inhibiting DOACs (rivaroxaban, apixaban, and edoxaban) are, however, mainly metabolized by CYP3A4,[16] which is also induced by dicloxacillin and flucloxacillin.[11] A potential drug–drug interaction between DOACs and dicloxacillin/flucloxacillin has, to our knowledge, not been studied. In general, the clinical relevance of concomitant treatment with inducers of CYP3A4 on DOAC effectiveness seems to depend on whether p-glycoprotein is also induced by the drug.[15] Interestingly, *in vitro* findings have indicated a potential of flucloxacillin to induce p-glycoprotein in addition to CYP3A4.[17]

Infection is associated with both INR changes[18] and ischemic stroke.[19] However, the decreased effect of warfarin found in the present study contrasts with the increased effect of warfarin that have been associated with fever and infection in prior studies.[20] Nevertheless, the present analysis might be affected by confounding from the underlying infection. This was mainly addressed by using propensity-score matching to an active, and thereby also infected, comparator. The fact that the association between dicloxacillin/flucloxacillin exposure and risk of ischemic stroke/SE was weaker when using an active comparator rather than an untreated comparator indicates that we have succeeded in eliminating at least part of the potentially confounding effect by the infection itself. The effect of infection *per se* is also demonstrated by the higher absolute risk of the outcome in the active than in the nontreated

Table 3 Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared with no antibiotic use, overall, and in patient subgroups

	Outcome rate (*n*/1,000)		Dicloxacillin/flucloxacillin vs. no antibiotic	
	Dicloxacillin/flucloxacillin	No antibiotic	HR (95% CI)	NNH
All	2.3/1,000	0.8/1,000	2.84 (1.97–4.09)	681
Stratified analyses				
Sex				
Male	2.0/1,000	0.9/1,000	2.28 (1.45–3.59)	892
Female	2.7/1,000	0.7/1,000	4.08 (2.17–7.67)	486
Age				
< 70 years	1.8/1,000	0.5/1,000	3.61 (1.57–8.32)	754
70–79 years	1.9/1,000	0.9/1,000	2.24 (1.24–4.08)	955
80 + years	3.0/1,000	1.0/1,000	3.02 (1.72–5.30)	495
Treatment indication, VKA				
Heart valve	2.0/1,000	0.0/1,000	-	-
Atrial fibrillation	2.3/1,000	0.9/1,000	2.58 (1.78–3.73)	709
Type of beta-lactamase-resistant penicillin				
Dicloxacillin	2.3/1,000	0.8/1,000	2.92 (2.02–4.22)	652
Flucloxacillin	1.6/1,000	0.8/1,000	2.02 (0.90–4.52)	1,239
Restricted analyses				
No recent hospitalization	2.3/1,000	0.8/1,000	2.97 (2.03–4.35)	668
No recent antibiotic (any)	2.3/1,000	0.8/1,000	2.82 (1.87–4.26)	678
No prior exposure to dicloxacillin/flucloxacillin	2.2/1,000	0.8/1,000	2.72 (1.78–4.17)	726
No diabetes	2.3/1,000	0.7/1,000	3.25 (2.09–5.04)	636
No previous stroke	1.8/1,000	0.6/1,000	3.15 (1.97–5.04)	810

CI, confidence interval; HR, hazard ratio; NNH, number needed to harm; VKA, vitamin K antagonist.

comparison group as well as by the slightly increased risks seen in the case-crossover analysis of phenoxymethylpenicillin exposure. Based on this analysis, infection in itself seems to be associated with a 25% increased risk of experiencing an ischemic stroke/SE during warfarin use.

A primary strength of our study is the use of population-based registries of high coverage and validity of exposure and outcome.[21,22] Furthermore, our findings were robust across several analyses to address potential confounding and when applying different analytical approaches. A main limitation is the lack of data on INR measurements as well as on the prescribed warfarin dose, including dose adjustments. However, the increase in ischemic stroke/SE risk found in the present study is substantiated by decreases in INR and increases in warfarin dose relative to exposure to dicloxacillin demonstrated in prior studies.[3,10] In addition, our data sources did not enable us to account for neither the severity of the infections treated nor differentiate between specific indications for antibiotic use. The overall unchanged result in the subgroup analyses excluding hospitalized patients, however, argues against severity of the underlying infection as an important modifier of the association. Furthermore, warfarin users experiencing significant decreases in INR values after receiving dicloxacillin/flucloxacillin may be more aware of INR fluctuations when using these antibiotics at a later point in time. Thus, by allowing individual warfarin users to contribute with more than one episode of dicloxacillin/flucloxacillin use, we may have underestimated the association. This concern is, however, not supported by the analysis restricted to the first treatment episode during warfarin use, which produced results similar to the main analysis. Finally, the study was performed in a primarily (> 90%) white population.[23] As such, our results may not be directly applicable to populations of other ethnic compositions, including genetic makeup important to warfarin metabolism and effect.[24]

CONCLUSION

Short-term use of dicloxacillin in warfarin users with AF or mechanical heart valves was associated with an increased short-term risk of ischemic stroke and SE. Dicloxacillin should be used with caution in patients receiving warfarin. A similar association for short-term use of flucloxacillin was not supported by our analysis. Generation of further clinical knowledge on the potential drug–drug interaction between flucloxacillin and warfarin is encouraged.

METHODS

Using the Danish population-based health registers (described in detail in the **Supplementary Methods**), we performed a cohort study examining whether dicloxacillin/flucloxacillin use was associated with short-term occurrence of ischemic stroke/SE in adult warfarin users in Denmark during the study period 1995 through 2018. Data were analyzed using the following comparators: (i) users of phenoxymethylpenicillin, (ii)

nonusers of antibiotics, and (iii) recent exposure experience of outcome cases (self-controlled design).

Setting

In Denmark, warfarin therapy is managed at hospital-based outpatient anticoagulation clinics, by general practitioners, and by patients trained to manage their own warfarin therapy (i.e., patient self-management).[25] Warfarin initiation and titration is always handled by a physician or a trained nurse. Danish guidelines recommend an initiation dose of 5 mg once daily in most patients.[26] After 5 days, the INR is measured, and from there warfarin dose is titrated to the recommended therapeutic range of INR guided by regular INR measurements. Of note, dosing instructions are not available from the Danish Prescription Register.[22] During maintenance therapy with warfarin, the interval between INR measurements are recommended to not exceed 4 weeks. Outside the hospital setting, the majority of INR measurements is performed using point-of-care coagulometers and is neither registered in laboratory nor clinical databases available for research. Pharmacogenomic testing is rarely used in the context of warfarin management in Denmark.

Sampling cohort of warfarin users

The study population for the cohort study as well as the case-crossover study was sampled from an open cohort consisting of Danish warfarin users with a treatment indication compatible with long-term warfarin use (**Figure 1**). Accordingly, patients with a registered diagnosis of AF and/or mechanical heart valves entered the sampling cohort on the date of their first warfarin prescription fill after the diagnosis. Patients with a diagnosis of venous thromboembolism during the year prior to warfarin initiation were not eligible for cohort entry. We used relevant hospital-based diagnoses (**Supplementary Methods**) as proxies for the indication for warfarin therapy, as the treatment indication is not provided in Danish prescription data. Patients remained in the sampling cohort as long as they were covered by a warfarin treatment episode, according to the definition described in the **Supplementary Methods**.

Patients were censored permanently from the sampling cohort upon (i) experience of an outcome, (ii) dispensing of > 200 capsules of dicloxacillin/flucloxacillin at one occasion or receiving an osteomyelitis or endocarditis diagnosis, as a marker of long-term dicloxacillin/flucloxacillin use, (iii) death, (iv) emigration, or (v) end of study period.

Ascertainment of antibiotic exposures and thromboembolic outcomes

Systemic infections are associated with an increased risk of ischemic stroke.[19] Thereby, comparisons of the stroke risk in infected (i.e., antibiotic recipients) and noninfected patients may be confounded. To address such potential confounding by indication, we compared the risk during dicloxacillin/flucloxacillin exposure with the risk during exposure to another antibiotic (i.e., we used an active comparator). Phenoxymethylpenicillin, an oral, narrow-spectrum penicillin, was chosen as the active comparator because it has no known effect on warfarin metabolism.[10] Exposure to antibiotic use was identified as filled outpatient prescriptions registered in The Danish Prescription Register.[22] The register does not contain information on in-hospital drug use. The date of the prescription fill was set as the index date. As a secondary analysis, exposure to dicloxacillin/flucloxacillin was compared with no antibiotic exposure.

Arterial thromboembolic outcomes were identified as registered hospital diagnoses in the Danish National Patient Register.[21] The outcome of interest was a composite of ischemic stroke and SE. Only the first occurrence of an outcome following sample cohort entry was included.

Cohort analysis

Propensity score matching. We characterized study participants' covariates (specified in the **Supplementary Methods**) at initiation of their eligible antibiotic treatment episode. We applied a look-back period of 180 days for prescription data and used all available data for diagnosis data (including prescriptions used to define existing medical conditions). We used a multivariable logistic regression model incorporating most of these covariates to calculate the probability of being treated with dicloxacillin/flucloxacillin vs. being treated with phenoxymethylpenicillin and vs. being randomly sampled as a nonuser in the primary and secondary analysis, respectively. The selection of untreated episodes is described in detail in the **Supplementary Methods**. Individual propensity score models were constructed for 3-year calendar strata, to handle possible trends in indications or clinical preferences over time. If a given individual had multiple antibiotic episodes within the 3-year period, all episodes were included in the model calculation. We applied an asymmetrical trimming (i.e., truncating the entire material at the propensity score value of the 2.5% percentile for treated subjects and 97.5% percentile for untreated subjects), thus removing tails at both ends.[27]

Based on the propensity score, we matched episodes of dicloxacillin/flucloxacillin 1:1 to episodes of phenoxymethylpenicillin and to no antibiotic use. In addition, we applied a forced match on assumed treatment indication. We matched with a caliper width of 0.02 for the absolute value of the treatment probability. Phenoxymethylpenicillin-treated and untreated episodes were censored if a dicloxacillin/flucloxacillin prescription occurred during follow-up (i.e., during days 1–28 after the index date), or if VKA treatment was discontinued.

Statistical analysis. Participants were followed from day 7 after the index date until day 28. We chose a risk window of 28 days on the basis of prior studies on the clinical pharmacological properties of the beta-lactamase-resistant penicillins and warfarin.[10,11] Specifically, the rationale behind the 7-day lag period was the latency in CYP induction[11] along with the latency in the INR decrease.[10] We calculated rates of the composite outcome of ischemic stroke/SE and 95% CIs for each exposure group using the exact Poisson distribution. We used Cox proportional hazard models to calculate HRs and 95% CI for ischemic stroke/SE associated with use of dicloxacillin/flucloxacillin vs. use of phenoxymethylpenicillin (primary analysis) and vs. no antibiotic use (secondary analysis). As a measure of the absolute risk, we calculated the NNH for one additional event to occur.

We examined whether the association was modified by patient or treatment characteristics by stratifying on age, sex, assumed indication for VKA therapy (AF or mechanical heart valves), and choice of either dicloxacillin or flucloxacillin. We performed several subgroup analyses excluding patients with potentially confounding characteristics. Specifically, we excluded patients with (i) a history of ischemic stroke, (ii) a history of diabetes, (iii) any prior use of dicloxacillin/flucloxacillin, (iv) use of other antibiotics within 30 days prior to index date, and (v) hospitalization within 10 days prior to the index date. The two latter analyses served to decrease potential misclassification of the actual time of initiation of antibiotic therapy. In addition, the analysis excluding recently hospitalized patients served to decrease the potential for confounding by the severity of the infection. Furthermore, in a sensitivity analysis, we assessed the association during later windows of follow-up; days 29–60 and days 61–120 relative to the index date. In case of a transient effect induced by a drug–drug interaction, we would expect a potentially increased risk of ischemic stroke/SE to disappear or attenuate over time. Finally, to challenge our definition of the duration of warfarin use among study cohort members, we performed two sensitivity analyses changing the assigned daily warfarin dose from 2 mg to 3.5 mg and 5 mg, respectively.

Case-crossover analysis

We also examined the proposed association through a case-crossover design; a "within-subject" study design where the same persons contribute to both exposed and unexposed follow-up time.[28] Inherent in the design is effective control of measured and unmeasured confounders that are stable over time.[29]

From the sampling cohort, we identified all incident cases of ischemic stroke/SE (**Figure 1**). We then assessed exposure during predefined time windows prior to the date of the outcome. In line with the cohort study design, we disregarded dicloxacillin/flucloxacillin initiations during the last 7 days before the outcome and set the focal window to day −7 to −28 relative to the case outcome date. In addition, we applied a washout window and four consecutive reference windows, all having a width of 21 days (**Figure S3**). We calculated ORs with 95% CIs for associations between exposure to dicloxacillin/flucloxacillin and risk of ischemic stroke/SE using conditional logistic regression by comparing the frequency of exposure in the focal vs. reference windows. No other covariates than dicloxacillin/flucloxacillin exposure were included in the conditional models. To estimate the potential effect of time-varying confounding by infection in this analysis, the case-crossover analysis was also performed with phenoxymethylpenicillin as the exposure of interest, as this would provide an estimate of the effect of infection in itself on the risk of ischemic stroke/SE in warfarin users.

Other

All analyses were performed using STATA 15.0 (StataCorp, College Station, TX). In Denmark, studies based solely on register data do not require review or approval from an ethics committee.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. Propensity score distributions. The dashed vertical lines mark the cut-offs for trimming. Primary analysis: phenoxymethylpenicillin as the comparator.
Figure S2. Propensity score distributions. The dashed vertical lines mark the cut-offs for trimming. Secondary analysis: no antibiotic use as the comparator.
Figure S3. The case-crossover design as employed in the present study.
Table S1. Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared to phenoxymethylpenicillin use during later periods of follow-up, overall, and in patient subgroups.
Table S2. Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared to no antibiotic use during later periods of follow-up, overall, and in patient subgroups.
Table S3. Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared to phenoxymethylpenicillin assigning a daily warfarin dose of 3.5 mg (47,093 included in each group) and 5 mg (39,259 included in each group), respectively, overall, and in patient subgroups.
Table S4. Association between dicloxacillin/flucloxacillin exposure during warfarin use and risk of stroke/systemic embolism compared to no antibiotic use assigning a daily warfarin dose of 3.5 mg (45,090 included in each group) and 5 mg (38,211 included in each group), respectively, overall, and in patient subgroups.
Supplementary Text S1. Supplementary Methods including code appendix and references.

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CONFLICT OF INTEREST

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1. Hylek, E.M. *et al.* Effect of intensity of oral anticoagulation on stroke severity and mortality in atrial fibrillation. *N. Engl. J. Med.* **349**, 1019–1026 (2003).
2. Martín-Pérez, M., Gaist, D., de Abajo, F.J. & Rodríguez, L.A.G. Population impact of drug interactions with warfarin: a real-world data approach. *Thromb. Haemost.* **118**, 461–470 (2018).
3. Hansen, P.W. *et al.* Identifying drug-drug interactions by data mining: a pilot study of warfarin-associated drug interactions. *Circ. Cardiovasc. Qual. Outcomes* **9**, 621–628 (2016).
4. Lacey, C.S. Interaction of dicloxacillin with warfarin. *Ann. Pharmacother.* **38**, 898 (2004).
5. Mailloux, A.T., Gidal, B.E. & Sorkness, C.A. Potential interaction between warfarin and dicloxacillin. *Ann. Pharmacother.* **30**, 1402–1407 (1996).
6. Khalili, H., Nikvarz, N., Najmeddin, F. & Dashti-Khavidaki, S. A probable clinically significant interaction between warfarin and cloxacillin: three case reports. *Eur. J. Clin. Pharmacol.* **69**, 721–724 (2013).
7. Krstenansky, P.M., Jones, W.N. & Garewal, H.S. Effect of dicloxacillin sodium on the hypoprothrombinemic response to warfarin sodium. *Clin. Pharm.* **6**, 804–806 (1987).
8. Mannheimer, B., Stage, T.B., Pottegård, A. & Lindh, J.D. The effect of flucloxacillin on warfarin anticoagulation: a Swedish Register-Based Nationwide Cohort Study. *Thromb. Haemost.* **19**, 1617–1623 (2019).
9. Halvorsen, S., Husebye, T. & Arnesen, H. Prosthetic heart valve thrombosis during dicloxacillin therapy. *Scand. Cardiovasc. J.* **33**, 366–368 (1999).
10. Pottegård, A., Henriksen, D.P., Madsen, K.G., Hellfritzsch, M., Damkier, P. & Stage, T.B. Change in international normalized ratio among patients treated with dicloxacillin and vitamin K antagonists. *JAMA* **314**, 296–297 (2015).
11. Stage, T.B. *et al.* Dicloxacillin induces CYP2C19, CYP2C9 and CYP3A4 in vivo and in vitro. *Br. J. Clin. Pharmacol.* **84**, 510–519 (2018).
12. Buhlinger, K.M. & Hollis, I.B. Effects of dicloxacillin on warfarin dose in patients with a left ventricular assist device. *J. Pharm. Pract.* (2018) doi: https://doi.org/10.1177/0897190018772978.
13. Schmidt, M., Hallas, J., Laursen, M. & Friis, S. Data resource profile: Danish online drug use statistics (MEDSTAT). *Int. J. Epidemiol.* **45**, 1401–1402g (2016).
14. Kirchhof, P. *et al.* 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur. Heart J.* **37**, 2893–2962 (2016).
15. Steffel, J. *et al.* The 2018 European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. *Eur. Heart J.* **39**, 1330–1393 (2018).
16. Mega, J.L. & Simon, T. Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. *Lancet* **386**, 281–291 (2015).
17. Huwyler, J., Wright, M.B., Gutmann, H. & Drewe, J. Induction of cytochrome P450 3A4 and P-glycoprotein by the isoxazolyl-penicillin antibiotic flucloxacillin. *Curr. Drug Metab.* **7**, 119–126 (2006).
18. Clark, N.P. *et al.* Warfarin interactions with antibiotics in the ambulatory care setting. *JAMA Intern. Med.* **174**, 409–416 (2014).
19. Fugate, J.E., Lyons, J.L., Thakur, K.T., Smith, B.R., Hedley-Whyte, E.T. & Mateen, F.J. Infectious causes of stroke. *Lancet Infect. Dis.* **14**, 869–880 (2014).
20. Self, T.H., Oliphant, C.S., Reaves, A.B., Richardson, A.M. & Sands, C.W. Fever as a risk factor for increased response to vitamin k antagonists: a review of the evidence and potential mechanisms. *Thromb. Res.* **135**, 5–8 (2015).

21. Schmidt, M., Schmidt, S.A., Sandegaard, J.L., Ehrenstein, V., Pedersen, L. & Sørensen, H.T. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin. Epidemiol.* **7**, 449–490 (2015).
22. Pottegård, A., Schmidt, S.A.J., Wallach-Kildemoes, H., Sørensen, H.T., Hallas, J. & Schmidt, M. Data resource profile: The Danish National Prescription Registry. *Int. J. Epidemiol.* **46**, 798–798f (2017).
23. Statistics Denmark <https://www.dst.dk/da> (2018). Accessed August 1, 2019.
24. Ansell, J., Hirsh, J., Hylek, E., Jacobson, A., Crowther, M. & Palareti, G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* **133**, 160S–198S (2008).
25. Christensen, T.D., Grove, E.L., Nielsen, P.B. & Larsen, T.B. Self-managed oral anticoagulant therapy: a call for implementation. *Expert Rev. Cardiovasc. Ther.* **14**, 255–257 (2016).
26. Danish Cardiology Society. National treatment guideline on anticoagulant therapy <https://www.nbv.cardio.dk/nbv> (2019). Accessed August 1, 2019.
27. Stürmer, T., Rothman, K.J., Avorn, J. & Glynn, R.J. Treatment effects in the presence of unmeasured confounding: dealing with observations in the tails of the propensity score distribution–a simulation study. *Am. J. Epidemiol.* **172**, 843–854 (2010).
28. Maclure, M. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am. J. Epidemiol.* **133**, 144–153 (1991).
29. Hallas, J. & Pottegård, A. Use of self-controlled designs in pharmacoepidemiology. *J. Intern. Med.* **275**, 581–589 (2014).