JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 73, NO. 25, 2019

PUBLISHED BY ELSEVIER

Direct Oral Anticoagulants in Patients With Atrial Fibrillation and Liver Disease

So-Ryoung Lee, MD,[a,*] Hyun-Jung Lee, MD,[a,*] Eue-Keun Choi, MD, PhD,[a] Kyung-Do Han, PhD,[b] Jin-Hyung Jung, BSc,[b] Myung-Jin Cha, MD,[a] Seil Oh, MD, PhD,[a] Gregory Y.H. Lip, MD[a,c,d]

ABSTRACT

BACKGROUND Advanced liver disease is known to increase the risk for bleeding and affects the hepatic clearance and metabolism of drugs. Subjects with active liver disease were excluded from pivotal clinical trials of direct oral anticoagulants (DOACs), so the evidence regarding the efficacy and safety of DOACs in patients with liver disease is lacking.

OBJECTIVES The aim of this study was to compare DOACs with warfarin in patients with nonvalvular atrial fibrillation and liver disease.

METHODS Using the Korean National Health Insurance Service database, subjects with atrial fibrillation and active liver disease treated with oral anticoagulation were included (12,778 with warfarin and 24,575 with DOACs), and analyzed ischemic stroke, intracranial hemorrhage, gastrointestinal bleeding, major bleeding, all-cause death, and the composite outcome. Propensity score weighting was used to balance covariates between the 2 groups.

RESULTS DOACs were associated with lower risks for ischemic stroke (hazard ratio [HR]: 0.548; 95% confidence interval [CI]: 0.485 to 0.618), intracranial hemorrhage (HR: 0.479; 95% CI 0.394 to 0.581), gastrointestinal bleeding (HR: 0.819; 95% CI: 0.619 to 0.949), major bleeding (HR: 0.650; 95% CI: 0.575 to 0.736), all-cause death (HR: 0.698; 95% CI: 0.636 to 0.765), and the composite outcome (HR: 0.610; 95% CI: 0.567 to 0.656) than warfarin. Among the total study population, 13% of patients (n = 4,942) were identified as having significant active liver disease. A consistent benefit was observed in patients with significant active liver disease (HR for the composite outcome: 0.691; 95% CI: 0.577 to 0.827).

CONCLUSIONS In this large Asian population with atrial fibrillation and liver disease, DOACs showed better effectiveness and safety than warfarin, which was consistent in those with significant active liver disease. (J Am Coll Cardiol 2019;73:3295–308) © 2019 by the American College of Cardiology Foundation.

Guidelines for the treatment of nonvalvular atrial fibrillation (AF) recommend oral anticoagulation (OAC) for stroke prevention with either warfarin or direct oral anticoagulants (DOACs), with a stronger recommendation for DOACs in the general population (1–3). Advanced liver disease is known to increase the risk for bleeding and affects the hepatic clearance and metabolism of drugs. Even so, OAC is associated with a lower risk for ischemic stroke and no difference in bleeding,

Listen to this manuscript's audio summary by Editor-in-Chief Dr. Valentin Fuster on JACC.org.

From the [a]Division of Cardiology, Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea; [b]Department of Medical Statistics, College of Medicine, Catholic University of Korea, Seoul, Korea; [c]Liverpool Centre for Cardiovascular Science, University of Liverpool and Liverpool Chest & Heart Hospital, Liverpool, United Kingdom; and the [d]Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark. *Drs. S.-R. Lee and H.-J. Lee contributed equally to this work. This study was supported by the Korea National Research Foundation funded by the Ministry of Education, Science and Technology (grant 2014R1A1A2A16055218). Dr. Choi has received research grants from Daiichi-Sankyo, Bristol-Myers Squibb/Pfizer, and Biosense Webster. Dr. Lip has served as a consultant for Bayer/Janssen, Bristol-Myers Squibb/Pfizer, Medtronic, Boehringer Ingelheim, Novartis, Verseon, and Daiichi-Sankyo; and has served as a speaker for Bayer, Bristol-Myers Squibb/Pfizer, Medtronic, Boehringer Ingelheim, and Daiichi-Sankyo. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received January 22, 2019; revised manuscript received April 2, 2019, accepted April 8, 2019.

ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2019.04.052

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

ALT = alanine transaminase

ASD = absolute standardized difference

AST = aspartate transaminase

CI = confidence interval

CrCl = creatinine clearance

DOAC = direct oral anticoagulant

GI = gastrointestinal

HR = hazard ratio

ICH = intracranial hemorrhage

IPW = inverse probability weighting

OAC = oral anticoagulation

PS = propensity score(s)

including intracranial hemorrhage (ICH), in patients with AF and liver cirrhosis, and thus OAC should still be considered in this population (4,5). However, subjects with active liver disease, such as acute or chronic hepatitis and cirrhosis, or elevation of liver enzymes, were excluded from the study population of the pivotal DOAC clinical trials (6–9). Consequently, all 4 DOACs are contraindicated in severe liver disease and should be used with caution in moderate liver disease (10).

In small retrospective studies of patients with (mostly compensated) liver cirrhosis, there were no differences in thromboembolic and major bleeding risks between DOACs and warfarin (11,12). According to a recent meta-analysis, DOACs were associated with a lower risk for bleeding in patients with AF with liver cirrhosis (5). However, given the small sample sizes of these previous studies, more data are clearly needed to establish the effectiveness and safety of DOACs in patients with liver disease. Also, in patients with impaired liver function, there were limited data for the benefit of DOACs (13).

SEE PAGE 3309

In this study, we investigated the real-world effectiveness and safety of DOACs compared with warfarin in patients with AF and liver disease, particularly in the subgroup with significant active liver disease.

METHODS

DATA SOURCE. This study used the National Health Insurance Service database, which contains information on demographics, use of inpatient and outpatient health care, pharmacy dispensing claims, health examination data, and mortality data for the entire Korean population (14). The detailed information are presented in the Online Appendix. This study was exempt from review by the Seoul National University Hospital Institutional Review Board (E-1805-076-946).

In Korea, the guidelines for stroke prevention in patients with nonvalvular AF are generally in line with those from the European and American College of Cardiology, American Heart Association, and Heart Rhythm Society guidelines (1–3,15). OAC is recommended for those at high risk for stroke (e.g., CHA_2DS_2-VASc score ≥2 in men and ≥3 in women). Regarding the selection of OAC, DOACs are generally recommended in preference to warfarin in patients with nonvalvular AF. Also, all 4 DOACs have the same reimbursement criteria, so the agents were usually selected according to physicians' preference.

STUDY DESIGN. We included patients with nonvalvular AF who newly started OAC during the study period and with active liver disease diagnosed within 3 years before starting OAC. Liver diseases were defined by claims for diagnostic codes. Details of definitions of liver disease and study enrollment flow are presented in the Online Appendix, Online Table 1, and Figure 1. Ultimately, 37,353 patients who had health checkup information including liver enzymes were included in the analysis.

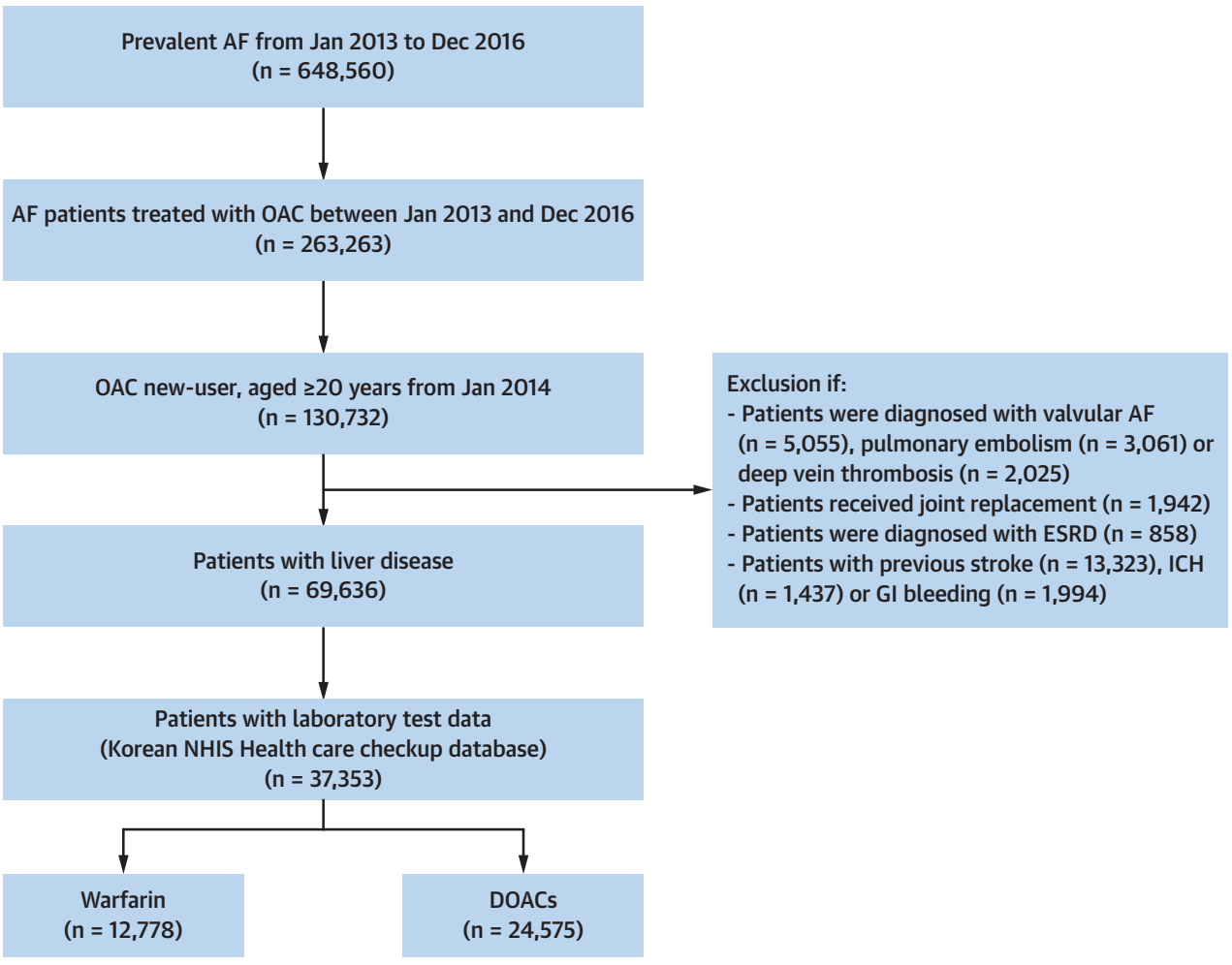
DEFINITION OF SIGNIFICANT ACTIVE LIVER DISEASE. A subpopulation of subjects with liver cirrhosis, viral hepatitis, or abnormal alanine transaminase (ALT) or aspartate transaminase (AST) more than 2 times the upper limit of normal, which largely corresponds to the criteria for liver disease excluded from pivotal DOAC trials (6–9), were defined as having significant active liver disease (Online Tables 1 and 2).

COVARIATES. Baseline covariates including age, sex, and comorbidities including hypertension, diabetes mellitus, dyslipidemia, congestive heart failure, peripheral artery disease, prior myocardial infarction, liver cirrhosis, and chronic obstructive pulmonary disease were evaluated. Definitions of comorbidities are presented in Online Table 1. CHA_2DS_2-VASc scores were also calculated (16). Health examination results such as body weight, creatinine clearance (CrCl) calculated using the Cockcroft-Gault method, serum hemoglobin level, liver function testing including serum AST, ALT, and γ-glutamyltransferase were also included as baseline covariates.

CLINICAL OUTCOMES AND FOLLOW-UP. Six clinical outcomes were used to compare the effectiveness and safety of DOACs versus warfarin: ischemic stroke, ICH, hospitalization for gastrointestinal (GI) bleeding, hospitalization for major bleeding, all-cause death, and the composite outcome (ischemic stroke + ICH + hospitalization for GI bleeding + all-cause death) as a measurement of net clinical benefit (17,18). Detailed definitions of clinical outcomes are summarized in Online Table 1. The index date was the date of initial warfarin or DOAC prescription. Patients were censored when the outcome events first occurred, or at the end of the study period (December 2016), whichever occurred first.

STATISTICAL ANALYSIS. Propensity score (PS) methods were used for the comparison between warfarin and pooled DOAC groups (19,20). Details of PS calculation are presented in the Online Appendix.

FIGURE 1 Study Enrollment Flow



AF = atrial fibrillation; DOAC = direct oral anticoagulant; ESRD = end-stage renal disease; GI = gastrointestinal; ICH = intracranial hemorrhage; NHIS = National Health Insurance Service; OAC = oral anticoagulant agent.

On the basis of calculated PS, inverse probability weighting (IPW) was used to balance covariates between 2 treatment groups regarding time-to-event analyses by using stabilized weights in both the total study population and patients with significant active liver disease (20,21). The balance of covariates between 2 treatment groups was evaluated using absolute standardized differences (ASD). An ASD ≤0.1 indicates a negligible difference between the 2 treatment groups (22).

Weighted incidence rates were calculated as the weighted number of clinical events during the follow-up period divided by 100 person-years at risk. Weighted cumulative incidence curves were presented using the Kaplan-Meier method with comparison by the log-rank test. For the outcome analysis, we performed weighted Cox proportional hazards regression model in the well-balanced cohorts. The proportional hazards assumption was tested using Schoenfeld residuals, and a significant departure from the assumption was not observed for all 6 clinical outcomes. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) of the DOAC group for 6 clinical outcomes were calculated using the warfarin group as the reference.

For a subgroup of patients with significant liver disease, 2 treatment groups were rebalanced using IPW, and the effectiveness and safety of DOAC compared with warfarin were evaluated. For a subgroup of only patients with cirrhosis from the

TABLE 1 Baseline Characteristics of Patients Using Warfarin Versus Direct Oral Anticoagulants in the Total Study Population

	Propensity Score Weighting					
	Before			After		
	Warfarin (n = 12,778)	DOACs (n = 24,575)	ASD	Warfarin (n = 12,778)	DOACs (n = 24,575)	ASD
Age, yrs	66.4 ± 11.0	70.3 ± 8.9	0.382	69.2 ± 10.5	69.0 ± 9.6	0.013
<65	41.1	23.3		31.0	27.7	
65–74	33.3	42.8		35.2	41.9	
≥75	25.6	33.9		33.8	30.4	
Men	64.8	57.0	0.159	59.1	59.6	0.009
CHA_2DS_2-VASc score	3.3 ± 1.9	3.6 ± 1.6	0.148	3.5 ± 1.9	3.5 ± 1.6	0.016
0 or 1	18.1	8.5		14.3	9.7	
2 or 3	38.7	43.1		36.6	46.6	
≥4	43.3	48.4		49.1	43.7	
Hypertension	71.6	73.9	0.054	74.0	73.4	0.014
Diabetes mellitus	24.2	26.5	0.053	26.8	26.1	0.017
Dyslipidemia	42.5	46.0	0.072	45.4	45.0	0.007
Heart failure	37.5	30.9	0.139	33.9	33.5	0.009
Prior MI	4.6	3.3	0.068	3.9	3.8	0.003
PAD	18.6	20.3	0.043	19.7	19.8	<0.001
COPD	22.9	21.1	0.045	22.0	21.8	0.005
Significant active liver disease	14.3	12.7	0.047	13.3	13.0	0.009
Liver cirrhosis	2.5	1.8	0.048	2.1	2.1	<0.001
Body weight, kg	65.8 ± 12.0	64.8 ± 11.8	0.080	65.1 ± 11.9	65.1 ± 11.9	0.003
Body weight <60 kg	31.2	33.7		33.3	32.9	
CrCl, ml/min	80.4 ± 37.1	80.8 ± 46.6	0.009	81.4 ± 59.1	80.7 ± 41.1	0.009
CrCl <50 ml/min	7.0	5.8		7.6	5.6	
AST, IU/l	29.6 ± 24.1	29.2 ± 20.1	0.021	29.3 ± 22.9	29.3 ± 19.9	<0.001
ALT, IU/l	26.8 ± 29.8	25.6 ± 18.6	0.049	26.0 ± 26.1	26.0 ± 18.9	0.001
AST or ALT ≥80 IU/l	2.8	2.4		2.5	2.5	
GGT, U/l	54.3 ± 73.6	49.7 ± 70.7	0.065	51.2 ± 71.2	51.2 ± 72.2	<0.001
GGT ≥80 U/l	16.0	13.4		14.3	14.2	
Hemoglobin, g/dl	14.0 ± 1.7	13.9 ± 1.7	0.071	13.9 ± 1.7	13.9 ± 1.7	0.005
Hemoglobin <9 g/dl	0.3	0.3		0.3	0.3	
DOAC dose						
Regular*	–	47.5		–	49.2	–
Reduced†	–	52.5		–	50.8	–

Values are mean ± SD or %, unless otherwise indicated. *Regular-dose DOACs are 20 mg rivaroxaban once daily, 150 mg dabigatran twice daily, 5 mg apixaban twice daily, and 60 mg edoxaban once daily. †Reduced-dose DOACs are 15/10 mg rivaroxaban once daily, 110 mg dabigatran once daily, 2.5 mg apixaban twice daily, and 30 mg edoxaban once daily.

ALT = alanine transaminase; ASD = absolute standardized difference; AST = aspartate transaminase; CHA_2DS_2-VASc = congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, prior stroke, transient ischemic attack, or thromboembolism, vascular disease, age 65–74 years, sex category (female); COPD = chronic obstructive pulmonary disease; CrCl = creatinine clearance; DOAC = direct oral anticoagulants; GGT = γ-glutamyltransferase; MI = myocardial infarction; PAD = peripheral artery disease.

significant liver disease population, we conducted a multivariate Cox proportional hazards regression analysis to compare outcomes according to treatment by DOAC or warfarin.

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina), and p values <0.05 were considered to indicate statistical significance.

SENSITIVITY ANALYSES. For sensitivity analyses, first, we compared outcomes using a multivariate Cox proportional hazards regression model. All variables used in PS calculation were adjusted for in the model. Second, we also performed 5% trimmed IPW by trimming those below the 5th percentile and above the 95th percentile of the stabilized weights to reduce the impact of extremely small and large weights. Third, we performed primary analyses in analogy with the intention-to-treat principle regardless of subsequent changes of treatment. We also conducted a sensitivity analysis in analogy with the on-treatment principle (18). For the on-treatment analysis, patients were also censored at the discontinuation of index treatment during the study period. Discontinuation of index treatment was defined as a 30-day gap from the last day of supply of the last prescription (18). Fourth, in considering the recent changes in the environment of OAC prescription, we performed sensitivity analysis by additionally including the year of treatment onset in the PS calculation. Fifth, when exploring the relative hazards of clinical outcomes other than death, we performed competing risk analysis considering death as a competing risk (23).

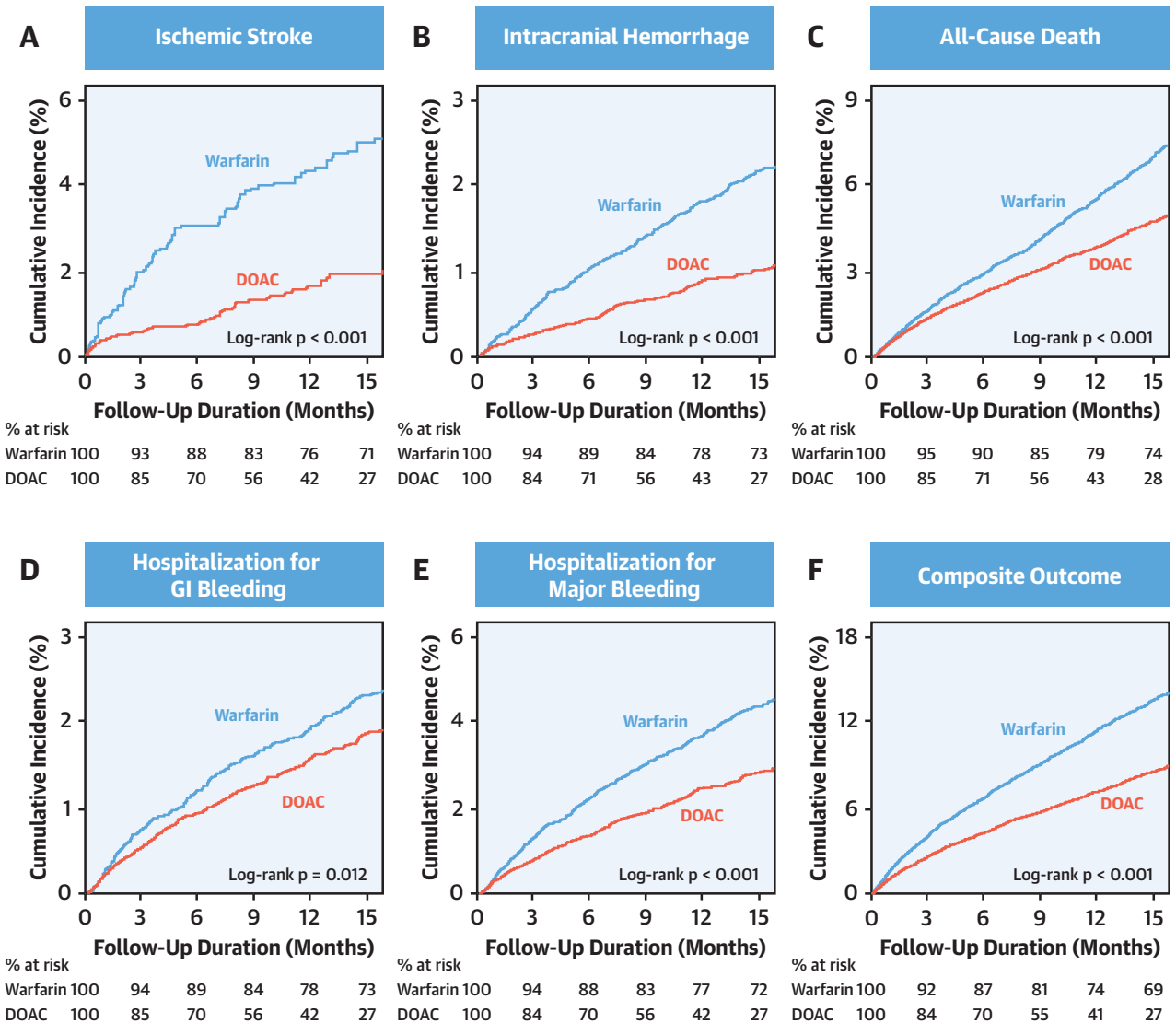
SUBGROUP ANALYSES. Comparisons between pooled DOAC and warfarin in the total study population were supplemented by stratified analyses according to DOAC types (rivaroxaban, dabigatran, apixaban, and edoxaban), and DOAC dose regimens (regular and reduced). Also, subgroup analyses were conducted for age strata (<65, 65 to 74, and ≥75 years) sex, body weight (<60 kg and ≥60 kg), liver function (AST <80 IU/l and ALT <80 IU/l, AST ≥80 IU/l or ALT ≥80 IU/l), γ-glutamyltransferase (<80 U/l and ≥80 U/l), renal function (CrCl <50 ml/min and CrCl ≥50 ml/min), and hemoglobin level (<9 g/dl and ≥9 g/dl). Subgroup analyses were performed using a multivariate Cox proportional hazards regression model. Tests for interaction using multivariable models were conducted to evaluate statistically significant ($p < 0.05$) subgroup differences in treatment.

RESULTS

BASELINE CHARACTERISTICS. A total of 37,353 patients with AF and liver disease on newly prescribed warfarin (n = 12,778) or DOACs (n = 24,575) were included. Among patients on DOACs, 42.5% of patients (n = 10,440) received rivaroxaban, 27.4% (n = 6,724) dabigatran, 22.6% (n = 5,561) apixaban, and 7.5% (n = 1,850) edoxaban. Reduced doses of DOACs were prescribed to 52.5% of patients.

Before PS weighting, DOAC users were significantly older, were less likely to be men, were less likely to have heart failure, and had higher CHA_2DS_2-VASc

FIGURE 2 Weighted Cumulative Incidence Curves of 6 Clinical Outcomes for DOAC and Warfarin Groups in the Total Study Population

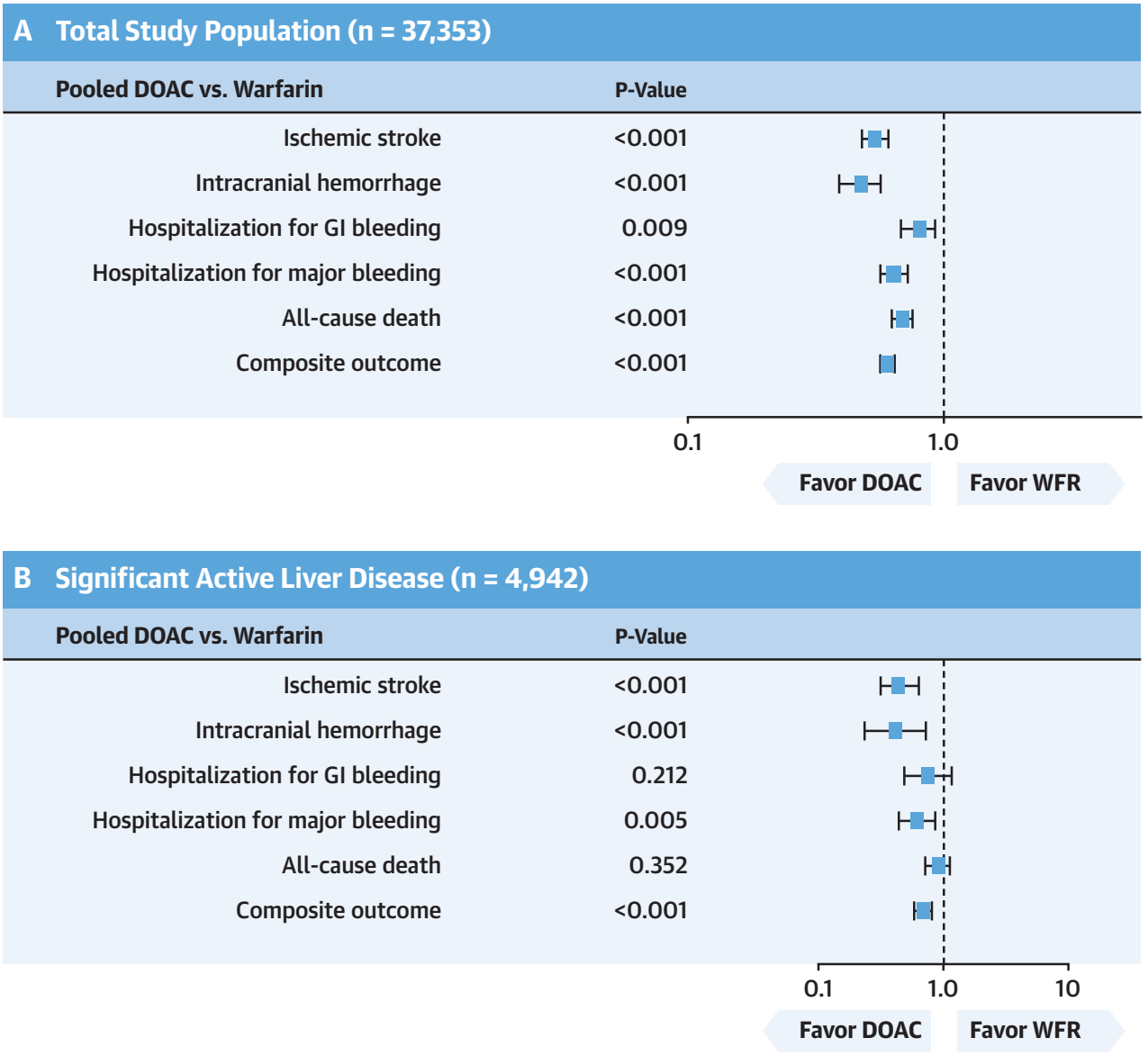


(A) Ischemic stroke, **(B)** intracranial hemorrhage, **(C)** all-cause death, **(D)** hospitalization for gastrointestinal (GI) bleeding, **(E)** hospitalization for major bleeding, and **(F)** the composite outcome. DOAC = direct oral anticoagulant.

scores compared with warfarin users (**Table 1**). The distribution of liver disease is presented in Online Table 3. After PS weighting, the 2 treatment groups were well balanced in all variables (all ASDs <0.1) (**Table 1**, Online Figure 1). The mean age was 69 years, and the mean CHA_2DS_2-VASc score was 3.5.

CLINICAL OUTCOMES IN PATIENTS WITH LIVER DISEASE. The weighted cumulative incidence curves of 6 clinical outcomes were significantly lower in the DOAC group (**Figure 2**). The weighted incidences of all clinical outcomes during a mean follow-up period of 1.2 years are shown in Online Table 4. Compared with warfarin (reference), DOAC use was associated with a 45% lower risk for ischemic stroke (HR: 0.548; 95% CI: 0.485 to 0.618) (**Central Illustration**, Online Table 5). DOAC use was associated with a 35% risk reduction in hospitalization for major bleeding compared with warfarin (HR: 0.650; 95% CI: 0.575 to 0.736), with a significant risk reduction for both ICH (HR: 0.479; 95% CI: 0.394 to 0.581) and hospitalization for GI bleeding (HR: 0.819; 95% CI: 0.691 to

CENTRAL ILLUSTRATION **6 Clinical Outcomes for Direct Oral Anticoagulants Versus Warfarin**



Lee, S.-R. et al. J Am Coll Cardiol. 2019;73(25):3295–308.

(A) In the total study population and **(B)** in the subgroup with significant active liver disease. Direct oral anticoagulants (DOACs) showed better effectiveness and safety than warfarin (WFR) in patients with active liver disease, and these benefits were consistent in patients with significant active liver disease.
GI = gastrointestinal.

0.949). DOAC use was associated with a 30% lower risk for all-cause death (HR: 0.698; 95% CI: 0.636 to 0.765) and reduced the risk for a composite outcome compared with warfarin (HR: 0.610; 95% CI: 0.567 to 0.656).

The benefits of DOAC were shown consistently in sensitivity analyses in both the multivariable Cox regression model and 5% trimmed IPW analysis (Online Figure 2). When we performed the on-treatment analysis, our results were similar to the main results for all 6 clinical outcomes (Online Figure 3). The benefit of DOACs was slightly accentuated in the on-treatment analysis. There was a difference between the 2 treatment groups in the year

of treatment onset (Online Table 6). When we additionally included the year of treatment onset in PS calculation, the results were consistent with the main results (Online Table 7). When adjusting for the competing risks for death, the results remained similar to the main results (Online Table 8).

Although the mean CrCl did not show a significant difference between the 2 treatment groups after IPW, the proportion of patients categorized by CrCl <30, 30 to 50, and >50 ml/min showed differences between 2 groups (Online Table 9). Therefore, we conducted a sensitivity analysis including CrCl as a categorical variable (Online Table 9). The HRs for 6 clinical outcomes in the total study population and patients with significant active liver disease after IPW that included CrCl as a categorical variable were similar to our main results (Online Figure 4).

CLINICAL OUTCOMES IN PATIENTS WITH SIGNIFICANT ACTIVE LIVER DISEASE. In the total study population, 13% of patients (n = 4,942) were classified as having significant active liver disease; of these, 36.9% (n = 1,827) were on warfarin and 63.0% (n = 3,115) were on DOACs. Before weighting, the pooled DOAC group was older, more likely to be female, and had higher CHA_2DS_2-VASc scores than the warfarin group (**Table 2**). After weighting, pooled DOAC and warfarin groups were well balanced in all covariates, with ASDs ≤0.1; the mean age was 68 years, and the mean CHA_2DS_2-VASc score was 3.4 (**Table 2**, Online Figure 5).

The weighted cumulative incidence curves of 6 clinical outcomes are shown in **Figure 3**, and the weighted incidence rates are presented in Online Table 4. In this subpopulation as well, DOACs showed consistently better outcomes than warfarin (reference) for ischemic stroke, ICH, and hospitalization for major bleeding (**Central Illustration**, Online Table 5). Also, DOACs showed a nonsignificant trend toward a reduced risk for hospitalization for GI bleeding and all-cause death compared with warfarin. Overall, DOACs showed better results for the composite outcome than warfarin (HR: 0.691; 95% CI: 0.577 to 0.827).

A separate analysis for patients with cirrhosis (n = 768 [2% of the study population]) was also conducted: 41.9% (n = 322) were on warfarin and 58.1% (n = 446) were on DOACs. DOAC users were older, were less likely to be male, had higher CHA_2DS_2-VASc scores, and had a higher prevalence of hypertension and dyslipidemia compared with warfarin users (Online Table 10). Despite these characteristics, DOAC users had a trend of lower risk for ischemic stroke or major bleeding and comparable risk for all-cause death compared with warfarin users (Online Figure 6), though this was statistically nonsignificant because of the small number of subjects.

TABLE 2 Baseline Characteristics of Patients Using Warfarin Versus Direct Oral Anticoagulant Agents in Patients With Significant Active Liver Disease

	Propensity Score Weighting					
	Before			After		
	Warfarin (n = 1,827)	DOACs (n = 3,115)	ASD	Warfarin (n = 1,827)	DOACs (n = 3,115)	ASD
Age, yrs	66.9 ± 11.3	69.5 ± 9.3	0.449	68.1 ± 10.8	67.9 ± 10.2	0.013
<65	47.1	26.0		35.5	32.1	
65–74	30.7	43.0		33.4	41.1	
≥75	22.2	31.0		31.1	26.8	
Men	67.7	58.9	0.183	61.2	61.8	0.013
CHA_2DS_2-VASc score	3.1 ± 1.9	3.5 ± 1.6	0.192	3.4 ± 1.9	3.4 ± 1.6	0.014
0 or 1	21.4	9.8		16.7	11.7	
2 or 3	39.4	44.4		37.6	44.7	
≥4	39.2	45.8		45.7	43.6	
Hypertension	67.9	72.2	0.093	71.1	70.5	0.011
Diabetes mellitus	24.5	27.1	0.058	27.2	26.4	0.017
Dyslipidemia	35.3	42.4	0.145	40.6	40.1	0.011
Heart failure	37.4	30.2	0.154	33.6	33.3	0.007
Prior MI	4.5	3.6	0.048	4.0	4.0	0.001
PAD	15.7	18.3	0.068	17.4	17.3	0.002
COPD	23.6	22.5	0.025	22.5	22.7	0.004
Liver cirrhosis	17.6	14.3	0.090	15.7	15.7	0.002
Body weight, kg	66.4 ± 12.3	65.0 ± 12.0	0.112	65.3 ± 12.1	65.5 ± 12.3	0.007
Body weight <60 kg	29.2	33.4		32.4	32.2	
CrCl, ml/min	80.9 ± 25.6	80.9 ± 46.3	0.002	80.1 ± 25.4	81.1 ± 39.8	0.019
CrCl <50 ml/min	7.0	5.5		7.5	5.3	
AST, IU/l	44.6 ± 56.7	42.9 ± 47.8	0.032	43.3 ± 51.9	43.3 ± 47.1	<0.001
ALT, IU/l	41.7 ± 70.9	37.8 ± 39.8	0.068	38.8 ± 57.1	38.8 ± 40.4	<0.001
AST or ALT ≥80 IU/l	19.6	18.6		18.4	19.3	
GGT, U/l	81.9 ± 125.7	74.8 ± 125.9	0.057	78.4 ± 127.5	77.7 ± 126.5	0.003
GGT ≥80 U/l	24.5	21.4		22.3	23.1	
Hemoglobin, g/dl	14.1 ± 1.8	14.0 ± 1.7	0.091	14.0 ± 1.8	14.0 ± 1.8	0.003
Hemoglobin <9 g/dl	0.4	0.5		0.3	0.5	
DOAC dose						
Regular*	–	46.6		–	48.8	–
Reduced†	–	53.4		–	51.2	–

Values are mean ± SD or %, unless otherwise indicated. *Regular-dose DOACs are 20 mg rivaroxaban once daily, 150 mg dabigatran twice daily, 5 mg apixaban twice daily, and 60 mg edoxaban once daily. †Reduced-dose DOACs are 15/10 mg rivaroxaban once daily, 110 mg dabigatran once daily, 2.5 mg apixaban twice daily, and 30 mg edoxaban once daily.

Abbreviations as in Table 1.

SUBGROUP ANALYSES. DOAC types. Baseline characteristics by DOAC type are shown in Online Table 11. The crude incidences of 6 clinical outcomes are presented in Online Table 12. All 4 DOACs were associated with risk reductions in the 6 clinical outcomes compared with warfarin (**Figure 4**), except that rivaroxaban showed comparable outcomes in hospitalization for GI bleeding compared with warfarin. Overall, the composite outcome of DOAC was consistent across all type of DOACs compared to warfarin.

DOAC doses. Among DOAC users, 52.5% of patients (n = 12,891) were prescribed reduced-dose DOACs. The results for all 6 clinical outcomes were consistent in both regular and reduced doses of DOACs (Online Figure 7). The crude incidences of 6 clinical outcomes are presented in Online Table 13.

Subgroups stratified by age, sex, body weight, liver function, renal function, and hemoglobin level. The crude incidences of 6 clinical outcomes according to treatment by pooled DOAC or warfarin in various subgroups are presented in Online Table 14. DOACs showed a trend for risk reduction across all subgroups compared with warfarin (**Figures 5 and 6**). Interaction with treatment was significant for several outcomes in age (ischemic stroke, ICH, hospitalization for major bleeding, composite outcome), sex (ischemic stroke), and body weight (hospitalization for major bleeding), but risk reduction by DOAC use in these outcomes were consistent, and the differences in HRs among the subgroups were small. When stratified by liver function, renal function, and hemoglobin level, the number of subjects in some subgroups was small, leading to wide CIs and statistical nonsignificance. DOACs generally showed clinical benefits across these subgroups compared with warfarin, and no significant interaction was found between treatment and the patients' baseline liver function, renal function, and hemoglobin levels.

DISCUSSION

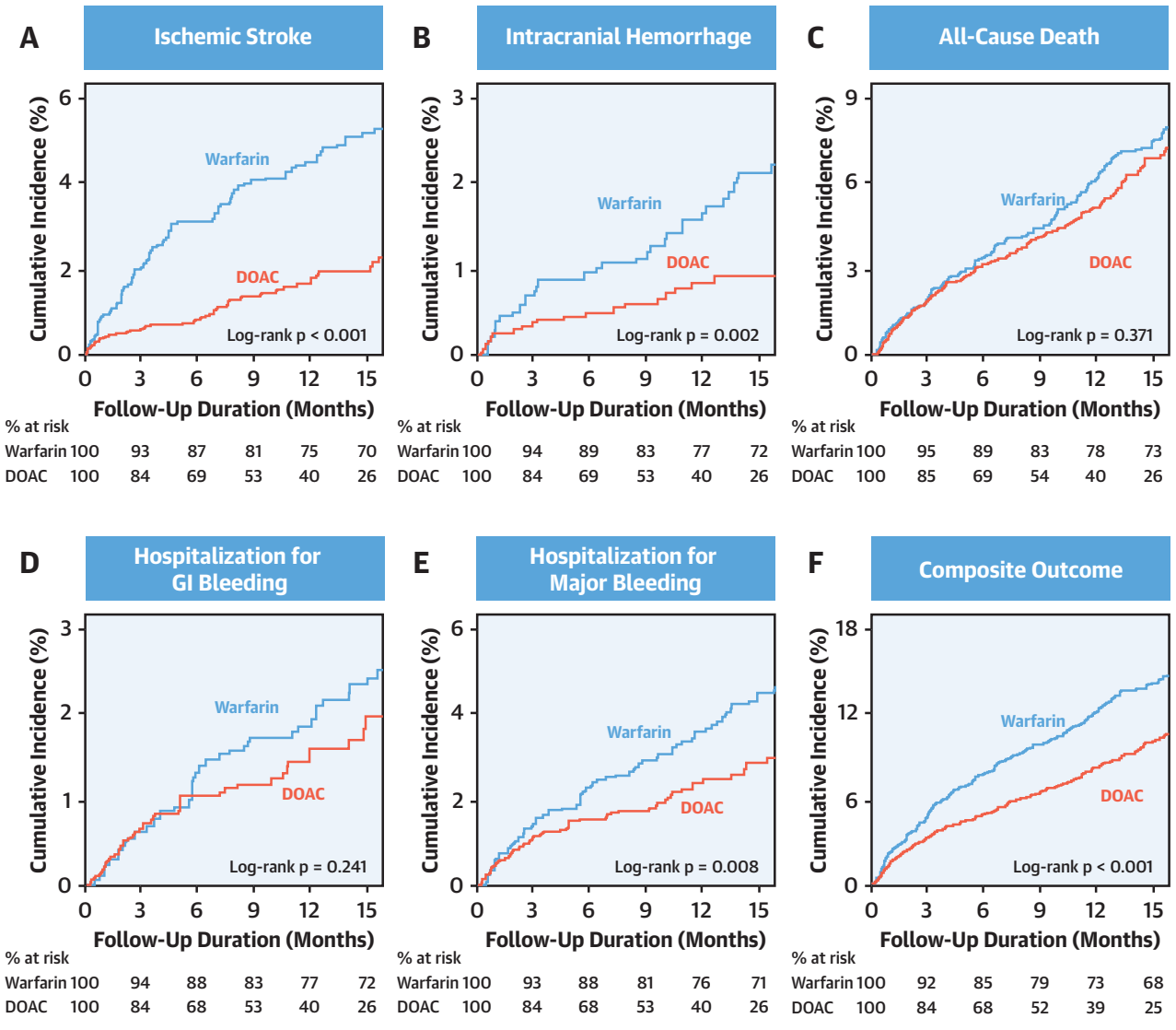
To the best of our knowledge, this is the first large study reporting the effectiveness and safety of DOACs compared with warfarin in patients with AF and liver disease, even in those with significant active liver disease excluded from pivotal clinical trials. In this study, we demonstrated that: 1) DOACs were associated with a lower risk for ischemic stroke, ICH, GI bleeding, major bleeding, and all-cause death compared with warfarin in patients with AF and liver disease; 2) DOACs showed consistently better performance than warfarin for ischemic stroke, ICH, and major bleeding in patients with AF and significant active liver disease, with a nonsignificant trend toward reduced risk for GI bleeding and all-cause death observed; 3) overall, DOACs showed better results for the composite outcome for patients with liver disease, even for those with significant active liver disease, compared with warfarin; and 4) the effectiveness and safety of DOAC were consistently observed regardless of DOAC type or dose regimen and in various high-risk subgroups.

Liver disease is a major cause of illness and death worldwide. In Asia, hepatitis carriers and patients with hepatitis-related liver cirrhosis are commonly encountered in clinical practice (24). Although viral hepatitis has declined substantially because of hepatitis B immunization and the development of treatment for hepatitis B/C, the global epidemic of obesity and metabolic diseases has fueled the increase of nonalcoholic fatty liver disease to about 25% of the population worldwide (25–27). AF and nonalcoholic fatty liver disease share common risk factors, and advanced liver disease itself is a known risk factor of incident AF; thus, AF frequently coexists in patients with liver disease (28,29). Although patients with AF and liver disease benefit from anticoagulation for stroke prevention, OAC treatment is challenging in patients with abnormal liver function because of combined intrinsic coagulopathy and increased bleeding risk (2,3,30–32).

DOAC for stroke prevention is a well-established treatment for patients with nonvalvular AF (1–3). The efficacy and safety of DOACs compared with warfarin were demonstrated from the results of landmark clinical trials. However, patients with significant active liver disease or persistent elevation of the liver enzymes or bilirubin were excluded from these pivotal DOAC trials (6–9). Also, there is a paucity of information regarding the benefit of DOACs compared with warfarin in patients with relatively mild liver disease who were not excluded from the pivotal trials. Because DOACs have a certain extent of hepatic elimination, impaired liver function may influence pharmacokinetics, leading to an increased bleeding risk (10,32,33). Recent studies have reported that DOACs show similar safety compared with warfarin in liver disease. However, sample sizes were too small to adequately answer the question (11,12,34). In a recent meta-analysis, DOACs were associated with a lower risk for bleeding compared with warfarin among patients with AF and liver cirrhosis, their effectiveness for stroke prevention was still uncertain (5). Although one previous study evaluated the effectiveness and safety of DOACs compared with warfarin in patients with AF and impaired liver function on the basis of the results of liver function tests, no differences were observed between warfarin and DOACs in both thromboembolic and bleeding outcomes, perhaps because of insufficient sample size (13).

In our analysis, we showed that the risks for ischemic stroke, ICH, GI bleeding, major bleeding, and all-cause death were lower with DOACs in patients with liver disease (mainly mild liver

FIGURE 3 Weighted Cumulative Incidence Curves of 6 Clinical Outcomes for DOAC and Warfarin Groups in Patients With Significant Active Liver Disease

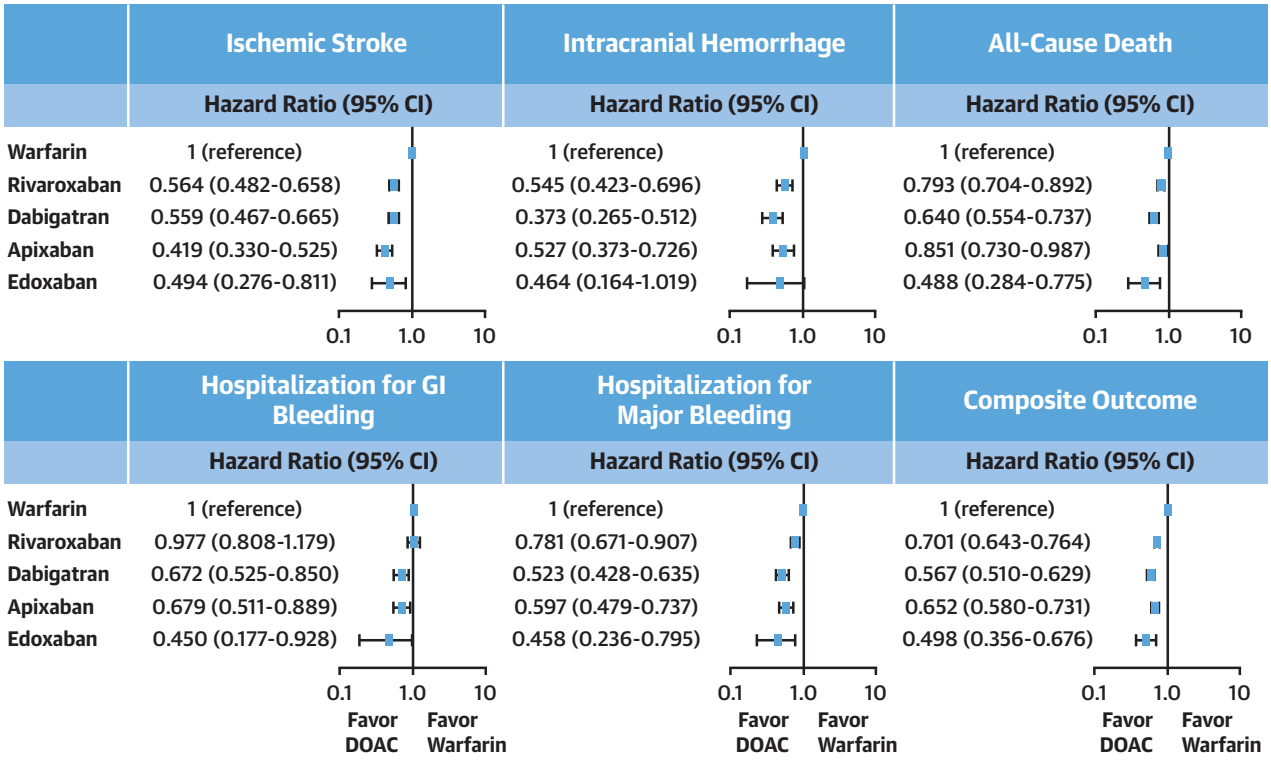


(A) Ischemic stroke, **(B)** intracranial hemorrhage, **(C)** all-cause death, **(D)** hospitalization for gastrointestinal (GI) bleeding, **(E)** hospitalization for major bleeding, and **(F)** the composite outcome. DOAC = direct oral anticoagulant.

dysfunction; 13% [n = 4,942] with significant active liver disease by our definition and 2% [n = 778] with liver cirrhosis). Interestingly, these results were consistent with those of an Asian population with nonvalvular AF but without liver disease (17,35-37). Furthermore, patients with significant active liver disease who were excluded from pivotal clinical trials of DOACs also showed a positive net clinical benefit of DOACs by significant reductions of ischemic stroke, ICH, and major bleeding. The subgroup of patients with cirrhosis also showed a trend for lower risks for ischemic stroke or major bleeding and comparable risk for all-cause death compared with warfarin users, though statistically nonsignificant. Indeed, DOACs did not cause excessive bleeding and were generally associated with greater effectiveness and net clinical benefit compared with warfarin.

Although there have been reports about the safety and net clinical benefit of DOACs in patients with AF and various degrees of renal dysfunction, the efficacy and safety of DOACs in liver dysfunction have not been well investigated. Furthermore, the few studies that evaluated the safety of DOACs in patients with liver dysfunction were very limited in subject

FIGURE 4 Hazard Ratios of 6 Clinical Outcomes for Each DOAC Compared With Warfarin



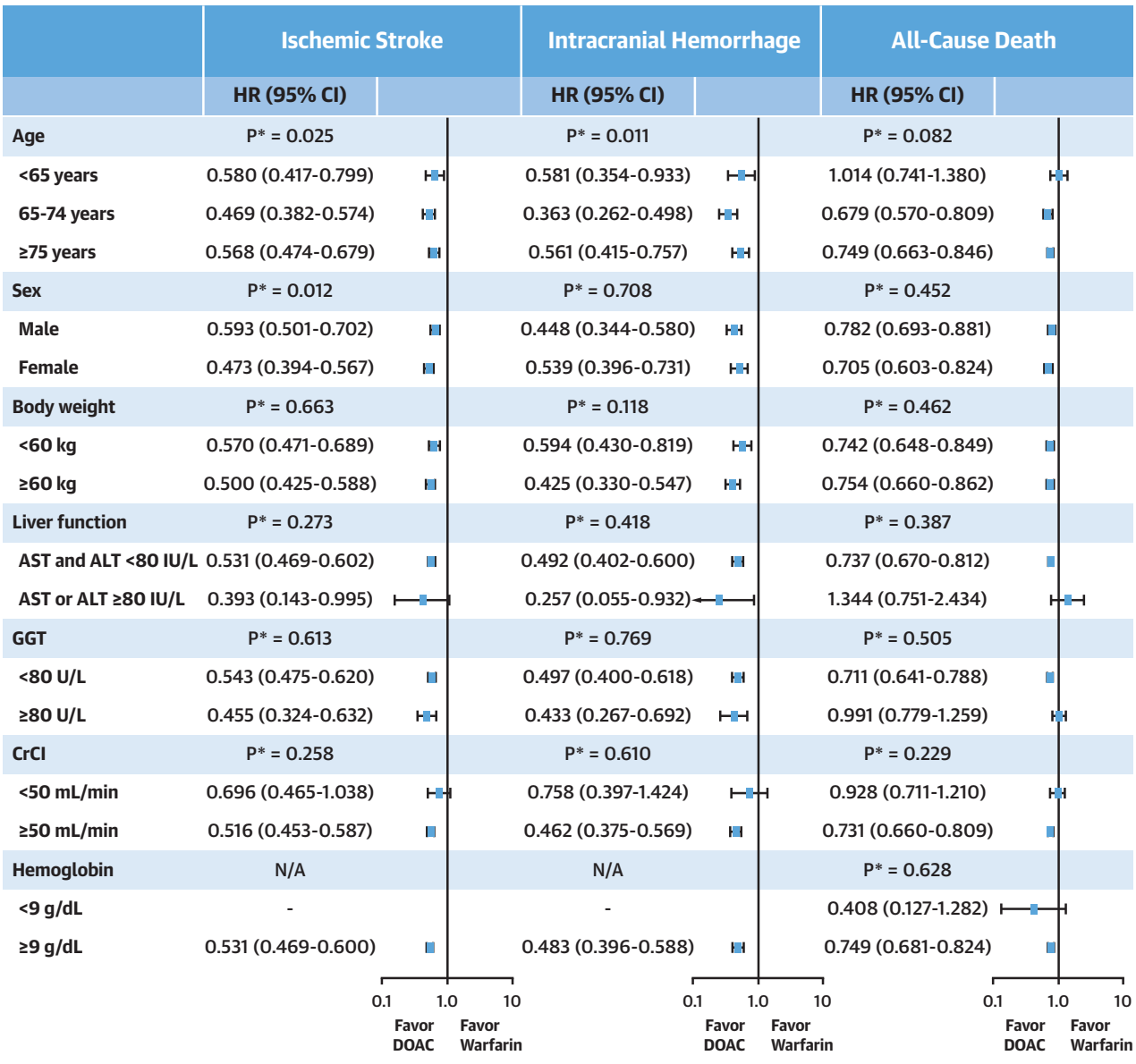
All 4 direct oral anticoagulants (DOACs) were associated with risk reduction in all 6 clinical outcomes compared with warfarin, except that rivaroxaban showed comparable outcome in hospitalization for gastrointestinal (GI) bleeding compared with warfarin. P* indicates P for interaction. CI = confidence interval.

numbers and focused only on patients with mild to moderate liver cirrhosis (11,12,34). Our study evaluated both effectiveness and safety in a large cohort and can guide optimal OAC strategy for patients with nonvalvular AF with a wide spectrum of concomitant liver diseases. However, the precise mechanism(s) are still uncertain. The liver is the site of synthesis of factors II, V, VII, IX, X, and XI. Use of warfarin, especially while maintaining optimal therapeutic levels, in these patients could be challenging. Also, liver function abnormality might affect drug metabolism, including warfarin, by human cytochrome P450, while DOACs were generally not affected by cytochrome P metabolism except for rivaroxaban (2,38).

The clinical benefit of DOACs was observed consistently with all DOAC types and dose regimens and across various subgroups. Of note, the risk of hospitalization for GI bleeding was similar between rivaroxaban and warfarin in patients with liver disease, while other DOACs clearly showed lower risk. This was consistent with prior study results that rivaroxaban had a comparable risk for GI bleeding compared with warfarin, in contrast to a significant risk reduction with other DOACs (17,37). Also, hepatic clearance for rivaroxaban is 65%, 18% of which involves cytochrome P450 metabolism (32). According to pharmacokinetic data of rivaroxaban, moderate impairment of liver function reduced rivaroxaban clearance, leading to increased exposure and pharmacodynamic effects (33). Consensus guidelines have recommended that all 4 DOACs should be contraindicated in patients with severe liver cirrhosis (Child-Turcotte-Pugh class C); additionally, rivaroxaban should also be withheld in patients with Child B liver cirrhosis (10). Although Child-Turcotte-Pugh scores were not available, our data suggest that all DOACs, including rivaroxaban, may be safely used in terms of bleeding risk in patients with liver disease.

Although all DOACs are available in patients with Child A and B liver cirrhosis (except rivaroxaban in Child B liver cirrhosis), DOAC dosing in patients with liver cirrhosis remains unclear. In the pivotal DOAC randomized controlled trials, patients who had advanced liver disease were excluded from the

FIGURE 5 Hazard Ratios of Ischemic Stroke, Intracranial Hemorrhage, and All-Cause Death for Pooled DOAC Versus Warfarin in Various Subgroups

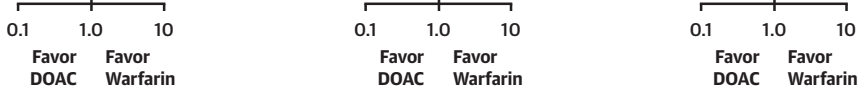


P* indicates P for interaction. ALT = alanine transaminase; AST = aspartate transaminase; CI = confidence interval; CrCl = creatinine clearance; DOAC = direct oral anticoagulant; GGT = γ-glutamyltransferase; HR = hazard ratio; N/A = not applicable.

studies. Thus, the dose reduction criteria of DOACs did not consider patients' baseline liver function. In our analysis, patients treated with reduced-dose DOACs were older, were more likely to be women, and had lower body weight and hemoglobin than those treated with regular-dose DOACs (Online Table 10). Reduced-dose users generally showed higher crude incidence rates of all 6 clinical outcomes than regular-dose users (Online Figure 8). Although there was no statistical significance because of small patient numbers, both reduced and regular dose DOACs generally showed a nonsignificant trend toward a lower risk for ischemic stroke, ICH, GI bleeding, and major bleeding compared with warfarin. However, which dose regimen might be better in patients with liver cirrhosis remains inconclusive. Further studies are needed to define the optimal DOAC dose regimen in patients with cirrhosis.

FIGURE 6 Hazard Ratios of Hospitalization for Gastrointestinal Bleeding, Hospitalization for Major Bleeding, and Composite Outcome for Pooled DOACs Versus Warfarin in Various Subgroups

	Hospitalization for GI Bleeding	Hospitalization for Major Bleeding	Composite Outcome
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age	P* = 0.212	P* = 0.008	P* = 0.017
<65 years	0.551 (0.348-0.856)	0.581 (0.416-0.804)	0.692 (0.567-0.842)
65-74 years	0.810 (0.608-1.080)	0.562 (0.454-0.695)	0.583 (0.516-0.658)
≥75 years	0.869 (0.698-1.085)	0.735 (0.614-0.881)	0.669 (0.608-0.736)
Sex	P* = 0.842	P* = 0.620	P* = 0.060
Male	0.810 (0.657-0.998)	0.638 (0.541-0.752)	0.678 (0.620-0.743)
Female	0.791 (0.617-1.017)	0.667 (0.548-0.812)	0.598 (0.537-0.667)
Body weight	P* = 0.323	P* = 0.033	P* = 0.172
<60 kg	0.827 (0.654-1.046)	0.737 (0.608-0.892)	0.662 (0.597-0.734)
≥60 kg	0.780 (0.626-0.972)	0.590 (0.499-0.697)	0.623 (0.567-0.685)
Liver function	P* = 0.986	P* = 0.619	P* = 0.696
AST and ALT <80 IU/L	0.803 (0.683-0.946)	0.655 (0.577-0.744)	0.639 (0.596-0.686)
AST or ALT ≥80 IU/L	0.790 (0.295-2.140)	0.522 (0.236-1.127)	0.769 (0.494-1.194)
GGT	P* = 0.165	P* = 0.403	P* = 0.871
<80 U/L	0.828 (0.695-0.987)	0.669 (0.583-0.767)	0.634 (0.588-0.684)
≥80 U/L	0.673 (0.451-1.002)	0.559 (0.409-0.762)	0.686 (0.574-0.820)
CrCl	P* = 0.588	P* = 0.213	P* = 0.057
<50 mL/min	0.980 (0.642-1.499)	0.926 (0.647-1.328)	0.836 (0.680-1.027)
≥50 mL/min	0.789 (0.663-0.940)	0.625 (0.547-0.715)	0.627 (0.582-0.675)
Hemoglobin	P* = 0.698	P* = 0.660	P* = 0.866
<9 g/dL	1.408 (0.392-5.530)	1.136 (0.332-4.103)	0.550 (0.231-1.307)
≥9 g/dL	0.794 (0.676-0.934)	0.646 (0.570-0.734)	0.642 (0.599-0.689)



P* indicates P for interaction. Abbreviations as in Figures 1 and 5.

STUDY LIMITATIONS. First, this study was designed as a retrospective cohort on the basis of a nationwide claims database. Although we carefully evaluated baseline covariates and balanced all covariates between the 2 treatment groups, there may have been confounding factors that were unmeasured, which may bias the comparison of treatment effects. For example, this dataset did not include information about concomitant antiplatelet agent use during the study period. This could be a potential confounder for bleeding outcomes. One of the possible remaining confounders could be patients' socioeconomic status. Although DOACs are covered by medical insurance in Korea, there is still a difference in patients' copay arrangements for DOACs and warfarin. Although reimbursement policy might affect the choice of oral

anticoagulant agents, we carefully balanced the baseline characteristics between 2 treatment groups and restricted the study patients to new oral anticoagulant agent users without previous stroke, ICH, or GI bleeding. Because there was a possibility that treatment choice might be affected by patients' socioeconomic status, we also evaluated the income levels of study patients. Low income was defined as subjects supported by the Medical Aid Program or subjects with income levels in the lower 20%. There was no significant difference in the proportion of patients with low income between the 2 treatment groups (Online Table 15). After additionally adjusting for low income, the HRs for 6 clinical outcomes were similar to the main results (Online Figure 9). Despite all these efforts, there may potentially remain some confounders from the differences in drug costs and reimbursement policy between warfarin and DOACs.

Second, we did not include patients with prior clinical events such as ischemic stroke, ICH, or GI bleeding for accurate assessment of clinical outcomes in our setting. Thus, further investigation of this high-risk population is needed.

Third, we defined significant active liver disease on the basis of the exclusion criteria of pivotal clinical trials (6-9). However, some of the laboratory values included in the exclusion criteria (i.e., serum bilirubin and alkaline phosphatase) were unavailable in our database (Online Table 2). Also, there were small discrepancies in the exclusion criteria of different trials; therefore, we made an operational definition incorporating main themes and cutoff values from these criteria. Also, we were unable to evaluate Child-Turcotte-Pugh scores as an indicator of hepatic dysfunction in cirrhosis patients because relevant clinical and laboratory information were unavailable in our database (10).

Last, the treatment quality of the warfarin group represented as time in therapeutic range was not available in the claims database.

CONCLUSIONS

In this large-scale Asian AF population with liver disease, DOACs were associated with better effectiveness and safety than warfarin. Furthermore, these benefits were generally consistent in patients with significant active liver disease who were excluded from the pivotal DOAC clinical trials. These results may rationalize careful use of DOACs for stroke prevention in patients with liver disease.

ACKNOWLEDGMENTS The authors appreciate the statistical advice from the Medical Research Collaborating Center, Seoul National University College of Medicine, Seoul National University Hospital.

ADDRESS FOR CORRESPONDENCE: Dr. Eue-Keun Choi, Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea. E-mail: choiek17@snu.ac.kr. Twitter: @SNUnow.

PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: Compared with warfarin in patients with AF and liver disease, therapy with DOACs is associated with lower risks for ischemic stroke, major bleeding, ICH, GI bleeding, and all-cause mortality.

TRANSLATIONAL OUTLOOK: These observations should be verified in adequately powered randomized trials.

REFERENCES

1. January CT, Wann LS, Calkins H, et al. 2019 AHA/ACC/HRS focused update of the 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. In press.

2. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J 2016;37:2893–962.

3. Lip GYH, Banerjee A, Boriani G, et al. Antithrombotic therapy for atrial fibrillation: CHEST guideline and expert panel report. Chest 2018;154:1121–201.

4. Kuo L, Chao TF, Liu CJ, et al. Liver cirrhosis in patients with atrial fibrillation: would oral anticoagulation have a net clinical benefit for stroke prevention? J Am Heart Assoc 2017;6:e005307.

5. Chokesuwattanaskul R, Thongprayoon C, Bathini T, et al. Efficacy and safety of anticoagulation for atrial fibrillation in patients with cirrhosis: a systematic review and meta-analysis. Dig Liver Dis 2019;51:489–95.

6. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med 2009;361:1139–51.

7. Granger CB, Alexander JH, McMurray JJ, et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2011;365:981–92.

8. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011;365:883–91.

9. Giugliano RP, Ruff CT, Braunwald E, et al. Edoxaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2013;369:2093–104.

10. Steffel J, Verhamme P, Potpara TS, 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 2018;39:1330–93.

11. Intagliata NM, Henry ZH, Maitland H, et al. Direct oral anticoagulants in cirrhosis patients pose similar risks of bleeding when compared to traditional anticoagulation. Dig Dis Sci 2016;61:1721–7.

12. Hum J, Shatzel JJ, Jou JH, Deloughery TG. The efficacy and safety of direct oral anticoagulants vs traditional anticoagulants in cirrhosis. Eur J Haematol 2017;98:393–7.

13. Wang CL, Wu VC, Kuo CF, et al. Efficacy and safety of non-vitamin K antagonist oral anticoagulants in atrial fibrillation patients with impaired liver function: a retrospective cohort study. J Am Heart Assoc 2018;7:e009263.

14. Cheol Seong S, Kim YY, Khang YH, et al. Data resource profile: the national health information database of the National Health Insurance Service in South Korea. Int J Epidemiol 2017;46:799–800.

15. Joung B, Lee JM, Lee KH, et al. 2018 Korean guideline of atrial fibrillation management. Korean Circ J 2018;48:1033–80.

16. Lip GY, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. Chest 2010;137:263–72.

17. Lee SR, Choi EK, Han KD, Jung JH, Oh S, Lip GYH. Edoxaban in Asian patients with atrial fibrillation: effectiveness and safety. J Am Coll Cardiol 2018;72:838–53.

18. Lee SR, Choi EK, Park CS, et al. Direct oral anticoagulants in patients with nonvalvular atrial fibrillation and low body weight. J Am Coll Cardiol 2019;73:919–31.

19. Austin PC. The use of propensity score methods with survival or time-to-event outcomes: reporting measures of effect similar to those used in randomized experiments. Stat Med 2014;33:1242–58.

20. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med 2015;34:3661–79.

21. Lee BK, Lessler J, Stuart EA. Weight trimming and propensity score weighting. PLoS One 2011;6:e18174.

22. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009;28:3083–107.

23. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509.

24. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–67.

25. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84.

26. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593–608.

27. Lee H, Lee H, Cho Y, Oh K, Ki M. Changes in seroprevalence of hepatitis B surface antigen and epidemiologic characteristics in the Republic of Korea 1998–2013. Epidemiol Health 2015;37:e2015055.

28. Wijarnpreecha K, Boonpheng B, Thongprayoon C, Jaruvongvanich V, Ungprasert P. The association between non-alcoholic fatty liver disease and atrial fibrillation: a meta-analysis. Clin Res Hepatol Gastroenterol 2017;41:525–32.

29. Lee H, Choi EK, Rhee TM, et al. Cirrhosis is a risk factor for atrial fibrillation: a nationwide, population-based study. Liver Int 2017;37:1660–7.

30. Pisters R, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. Chest 2010;138:1093–100.

31. Efird LM, Mishkin DS, Berlowitz DR, et al. Stratifying the risks of oral anticoagulation in patients with liver disease. Circ Cardiovasc Qual Outcomes 2014;7:461–7.

32. Qamar A, Vaduganathan M, Greenberger NJ, Giugliano RP. Oral anticoagulation in patients with liver disease. J Am Coll Cardiol 2018;71:2162–75.

33. Kubitza D, Roth A, Becka M, et al. Effect of hepatic impairment on the pharmacokinetics and pharmacodynamics of a single dose of rivaroxaban, an oral, direct Factor Xa inhibitor. Br J Clin Pharmacol 2013;76:89–98.

34. Goriacko P, Veltri KT. Safety of direct oral anticoagulants vs warfarin in patients with chronic liver disease and atrial fibrillation. Eur J Haematol 2018;100:488–93.

35. Wang KL, Lip GY, Lin SJ, Chiang CE. Non-vitamin K antagonist oral anticoagulants for stroke prevention in Asian patients with nonvalvular atrial fibrillation: meta-analysis. Stroke 2015;46:2555–61.

36. Cha MJ, Choi EK, Han KD, et al. Effectiveness and safety of non-vitamin K antagonist oral anticoagulants in Asian patients with atrial fibrillation. Stroke 2017;48:3040–8.

37. Chan YH, See LC, Tu HT, et al. Efficacy and safety of apixaban, dabigatran, rivaroxaban, and warfarin in Asians with nonvalvular atrial fibrillation. J Am Heart Assoc 2018;7:e008150.

38. Elbekai RH, Korashy HM, El-Kadi AO. The effect of liver cirrhosis on the regulation and expression of drug metabolizing enzymes. Curr Drug Metab 2004;5:157–67.

KEY WORDS atrial fibrillation, direct oral anticoagulant, liver disease, warfarin

APPENDIX For supplemental methods, tables, and figures, please see the online version of this paper.