Genetics and the clinical response to warfarin and edoxaban: findings from the randomised, double-blind ENGAGE AF-TIMI 48 trial

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Summary

Background Warfarin is the most widely used oral anticoagulant worldwide, but serious bleeding complications are common. We tested whether genetic variants can identify patients who are at increased risk of bleeding with warfarin and, consequently, those who would derive a greater safety benefit with a direct oral anticoagulant rather than warfarin.

Methods ENGAGE AF-TIMI 48 was a randomised, double-blind trial in which patients with atrial fibrillation were assigned to warfarin to achieve a target international normalised ratio of 2·0–3·0, or to higher-dose (60 mg) or lower-dose (30 mg) edoxaban once daily. A subgroup of patients was included in a prespecified genetic analysis and genotyped for variants in *CYP2C9* and *VKORC1*. The results were used to create three genotype functional bins (normal, sensitive, and highly sensitive responders to warfarin). This trial is registered with ClinicalTrials.gov, number NCT00781391.

Findings 14 348 patients were included in the genetic analysis. Of 4833 taking warfarin, 2982 (61·7%) were classified as normal responders, 1711 (35·4%) as sensitive responders, and 140 (2·9%) as highly sensitive responders. Compared with normal responders, sensitive and highly sensitive responders spent greater proportions of time over-anticoagulated in the first 90 days of treatment (median 2·2%, IQR 0–20·2; 8·4%, 0–25·8; and 18·3%, 0–32·6; $p_{trend}<0·0001$) and had increased risks of bleeding with warfarin (sensitive responders hazard ratio 1·31, 95% CI 1·05–1·64, p=0·0179; highly sensitive responders 2·66, 1·69–4·19, p<0·0001). Genotype added independent information beyond clinical risk scoring. During the first 90 days, when compared with warfarin, treatment with edoxaban reduced bleeding more so in sensitive and highly sensitive responders than in normal responders (higher-dose edoxaban $p_{interaction}=0·0066$; lower-dose edoxaban $p_{interaction}=0·0036$). After 90 days, the reduction in bleeding risk with edoxaban versus warfarin was similarly beneficial across genotypes.

Interpretation *CYP2C9* and *VKORC1* genotypes identify patients who are more likely to experience early bleeding with warfarin and who derive a greater early safety benefit from edoxaban compared with warfarin.

Funding Daiichi Sankyo.

Introduction

Vitamin K antagonists, such as warfarin, are the most commonly used anticoagulants to prevent and treat thromboembolic diseases worldwide. The use of warfarin, however, is hampered by several constraints, including highly variable response and the need to monitor and adjust doses. Bleeding complications due to warfarin continue to be among the leading causes of severe adverse drug events worldwide.[1–3] Polymorphisms in *CYP2C9*, which encodes an enzyme responsible for the metabolism of the potent S-warfarin isomer, and in *VKORC1*, which encodes vitamin-K epoxide reductase, the molecular target of warfarin, affect individuals' sensitivity to warfarin and account for around 40% of the variability in the response.[4] As noted in the US Food and Drug Administration (FDA) warfarin label, an individual's *CYP2C9* and *VKORC1* genotype information can assist in selecting the optimum dose.[5] European regulatory agencies comment that extra care with dosing is warranted if the genotype is known.[6] Nevertheless, the relevance of these polymorphisms for clinical outcomes continues to be debated.[7,8]

In view of the limitations of warfarin, several direct oral anticoagulants have been developed as alternatives.[9–13] Edoxaban is a factor Xa inhibitor with more predictable pharmacodynamics than warfarin. The ENGAGE AF-TIMI 48 trial was a large, worldwide, prospective, double-blind, double-dummy, randomised study that compared two dosing regimens of once-daily edoxaban and warfarin in patients with atrial fibrillation followed up for a median of 2·8 years.[14] The two edoxaban regimens were non-inferior to warfarin for prevention of stroke and systemic embolism, and were associated with significantly lower rates of bleeding and cardiovascular death. The trial included a prespecified pharmacogenetic study and, therefore, enabled comparison of clinical events by genotype. Specifically, we tested whether patients with functional genetic variants in *CYP2C9* and *VKORC1* had higher rates of bleeding than those without when treated with warfarin and, consequently, whether these genetic variants could identify patients who would derive a differential benefit from treatment with edoxaban.

Methods

Patients

ENGAGE AF-TIMI 48 enrolled 21105 patients aged 21 years or older with non-valvular atrial fibrillation within the previous 12 months, a $CHADS_2$ risk score of 2 or more, and anticoagulation planned for the duration of the trial.[14–16] Exclusion criteria included atrial fibrillation due to a reversible disorder, other indications for anticoagulation, and severe renal insufficiency.

Treatment

Patients were randomly assigned in a 1:1:1 ratio to receive warfarin with a target international normalised ratio (INR) of 2·0–3·0, or to higher-dose (60 mg) or lower-dose (30 mg) edoxaban once daily. The starting warfarin dose was determined by the local investigator on the basis of the patient's clinical profile. Investigators were encouraged to consult established practice guidelines or the online warfarin dosing algorithm. Patients attended prespecified visits for dose titration (appendix). INR was measured with an encrypted point-of-care device provided by the study organisers. To maintain masking of treatment allocation, sham INR values were generated for patients taking edoxaban. Doses of edoxaban were reduced by half at randomisation or during the course of the trial on the basis of renal function, bodyweight, and concomitant use of certain P-glycoprotein inhibitors.

A clinical events committee that was unaware of treatment allocation adjudicated all deaths and suspected cases of bleeding, stroke, systemic embolic event, or myocardial infarction. Any overt bleeding was classified as major (International Society on Thrombosis

For more on **warfarin dosing** see www.warfarindosing.org

See **Online** for appendix

		CYP2C9					
		*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
VKORC1	G/G	Normal responder 5·9 (2·2) mg, 24·0%	Normal responder 4·9 (1·9) mg, 7·0%	Sensitive responder 4·2 (1·8) mg, 4·2%	Sensitive responder 3·6 (1·4) mg, 0·6%	Sensitive responder 3·2 (1·0) mg, 0·4%	Highly sensitive responder 1·5 (0·7) mg, 0·1%
	G/A	Normal responder 4·4 (1·8) mg, 30·7%	Sensitive responder 3·7 (1·5) mg, 8·4%	Sensitive responder 3·2 (1·3) mg, 5·5%	Sensitive responder 2·7 (1·0) mg, 0·5%	Highly sensitive responder 2·0 (1·2) mg, 0·6%	Highly sensitive responder 1·2 (0·5) mg, 0·2%
	A/A	Sensitive responder 2·9 (1·3) mg, 13·4%	Sensitive responder 2·4 (0·8) mg, 2·5%	Highly sensitive responder 2·0 (0·8) mg, 1·5%	Highly sensitive responder 1·5 (0·8) mg, 0·2%	Highly sensitive responder 1·4 (0·5) mg, 0·3%	Highly sensitive responder 1·0 (0·0) mg, 0·1%

Figure 1: **Final warfarin dose and proportions of patients per category, across genotype bins**
Data are mean (SD) and proportion (%).

	Normal responder (n=2982)		Sensitive responder (n=1711)		Highly sensitive responder (n=140)		p value for trend*
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Time in INR ranges in first 28 days (%)							
<2	48·9 (35·8)	44·4 (18·5–85·2)	36·6 (32·3)	29·6 (7·4–57·1)	28·1 (28·9)	18·5 (3·7–41·2)	<0·0001
2–3	41·6 (32·5)	40·7 (11·1–66·7)	47·5 (30·9)	44·4 (22·2–70·4)	41·9 (29·3)	33·3 (18·5–63·0)	<0·0001
>3	9·5 (18·5)	0 (0–11·1)	15·9 (23·9)	0 (0–29·6)	30·1 (30·9)	23·8 (0–58·8)	<0·0001
Time in INR ranges in first 90 days (%)							
<2	37·3 (29·7)	30·3 (12·4–56·2)	30·8 (26·4)	23·8 (9·3–46·1)	27·8 (24·0)	23·0 (9·0–41·6)	<0·0001
2–3	50·5 (27·9)	51·7 (29·2–72·3)	54·0 (26·3)	53·9 (34·8–75·3)	51·3 (25·5)	51·7 (29·4–68·5)	0·0023
>3	12·1 (17·2)	2·2 (0–20·2)	15·2 (18·4)	8·4 (0–25·8)	20·9 (19·3)	18·3 (0–32·6)	<0·0001
Time in INR ranges beyond 90 days (%)							
<2	23·5 (19·1)	19·1 (11·4–29·4)	21·1 (17·1)	17·1 (10·4–26·3)	21·0 (17·2)	18·5 (9·7–26·1)	<0·0001
2–3	63·2 (19·0)	66·2 (55·1–75·8)	65·0 (17·9)	67·6 (56·1–76·7)	63·8 (18·3)	66·6 (56·6–73·4)	0·0320
>3	13·3 (11·1)	11·7 (6·4–17·7)	13·9 (11·5)	12·2 (6·6–18·9)	15·2 (12·2)	13·7 (6·9–20·7)	0·0160
Mean (SD) final average daily warfarin dose (mg)	5·1 (2·1)	..	3·3 (1·5)	..	1·8 (0·9)	..	<0·0001

INR=international normalised ratio. *p values for trend relate to the median values. When results were adjusted for ethnic origin, region, creatinine clearance, weight, and previous vitamin K antagonist use, all p values remained significant.

Table 1: **INR and dosing data among patients taking warfarin, across genotype bins**

and Haemostasis definition), clinically relevant non-major, or minor (appendix).

Genotypes

Genotypes were determined for *CYP2C9* (**2* and **3* alleles; rs1799853 and rs1057910) and *VKORC1* (–1639G→A; rs9923231) after enrolment. Genotyping was done by Integrated Laboratory Systems (Morrisville, NC, USA). The genotype acquisition rates were 99·97% for all three genotypes. The observed genotype frequencies were similar to those previously reported (appendix), and all three variants were in Hardy-Weinberg equilibrium. For analysis we grouped patients by combinations of *CYP2C9* and *VKORC1* genotypes into three genotype functional bins that corresponded to the FDA categories for response in the updated warfarin label: normal, sensitive, and highly sensitive responders (figure 1).

Statistical analysis

Baseline characteristics of patients receiving warfarin were compared between normal, sensitive, and highly sensitive responders with the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. The proportion of time in the therapeutic range in the warfarin group was calculated by linear interpolation, based on the available INR values per patient.[17] To test for differences in the proportion of time in INR ranges across the three genotype bins, we used a non-parametric Wilcoxon's test for trend. In a sensitivity analysis to adjust for clinical covariates, the values were log transformed and a generalised linear model was applied. We used Cox's proportional hazards models to compare the bleeding and efficacy outcomes between normal responders and sensitive and highly sensitive responders, and calculated hazard ratios (HRs) and 95% CIs. As well as overt bleeding, subcategories of bleeding were tested for directional consistency on the basis of the HRs and 95% CIs. Additional bivariate analyses were done to compare the risk of bleeding by genetic category and by HAS-BLED clinical bleeding score, according to the European Society of Cardiology guideline categorisation for the latter.[18,19] On the basis of previous studies, we assessed outcomes from the start of study treatment to day 90 and beyond 90 days; patients could have events in both time periods.[20–22] Sensitivity analyses were done at 28 days.

Genotyped patients who had received at least one dose of study drug were assessed. Analysis was done for the on-treatment period, defined as the time between the first study-drug dose and either the end of the planned treatment period or 3 days after the last dose of study drug if it was discontinued early. In a sensitivity analysis, data were adjusted for covariates that differed across genotype bins and previous exposure to a vitamin K antagonist. Additionally, separate analyses were done stratified by previous exposure and no previous exposure to a vitamin K antagonist. The safety and efficacy of each edoxaban regimen relative to warfarin were compared in patients stratified by genotype bin and interaction terms were generated with Cox's proportional hazards models. This trial is registered with ClinicalTrials.gov, number NCT00781391.

Role of the funding source

The funder of the study was involved in the study design and data collection, but had no role in data analysis or data interpretation, or writing of the initial report. JLM and MSS had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

14 348 patients who elected to participate in the pharmacogenetic study were included in the genetic analysis, of whom 4833 (33·7%) were assigned to warfarin. Allele frequencies for all genotypes were similar to those reported in the literature.[4] Of patients assigned warfarin, 2982 (61·7%) were normal responders, 1711 (35·4%) sensitive responders, and 140 (2·9%) highly sensitive responders. Age, sex, qualifying risk factors, $CHADS_2$ score, and type of atrial fibrillation were similar across genotype bins, whereas ethnic origin, region, creatinine clearance, and weight differed (appendix).

Sensitive and highly sensitive responders were more likely to be over-anticoagulated than normal responders, especially soon after the start of treatment (table 1). Moreover, the mean proportions of time with INR values greater than 4·0 in the first 90 days were 1·7% for normal, 2·5% for sensitive, and 6·6% for highly sensitive responders (p_{trend}<0·0001). After 90 days, the proportions were more similar across genotype bins (table 1). The mean final warfarin doses decreased going from normal to sensitive to highly sensitive responders (figure 1, table 1).

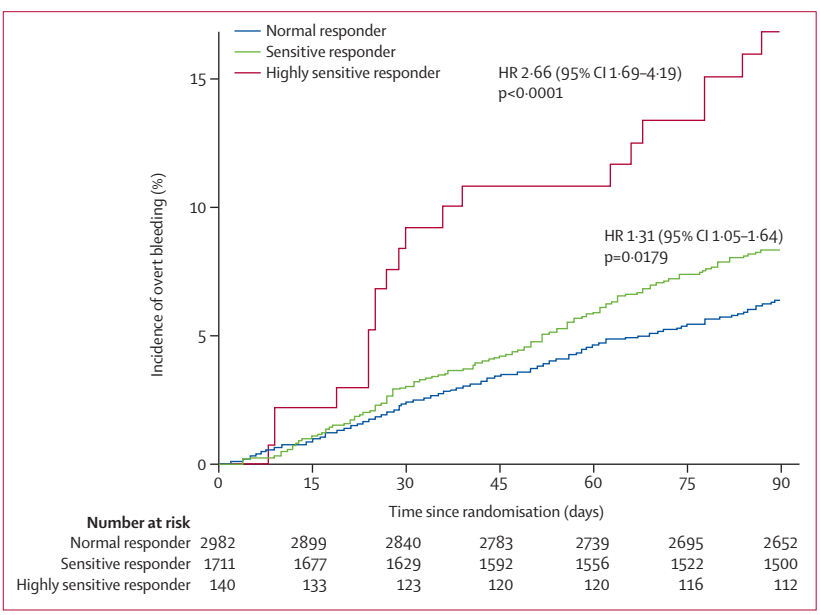

Figure 2: **Cumulative incidence of overt bleeding events in the first 90 days of treatment among patients taking warfarin, across genotype bins**
HR=hazard ratio.

	Normal responders (n=2982)		Sensitive responders (n=1711)		Sensitive *vs* normal responders HR (95% CI)	Highly sensitive responders (n=140)		Highly sensitive *vs* normal responders HR (95% CI)
	Number of events	Kaplan-Meier rates	Number of events	Kaplan-Meier rates		Number of events	Kaplan-Meier rates	
Any overt bleed	179	6·2%	134	8·0%	1·31 (1·05–1·64)	21	15·6%	2·66 (1·69–4·19)
Major or clinically relevant non-major bleed	133	4·6%	96	5·8%	1·26 (0·97–1·64)	19	14·1%	3·21 (1·99–5·18)
Major bleed	31	1·1%	23	1·4%	1·29 (0·75–2·21)	3	2·3%	2·12 (0·65–6·92)
Clinically relevant non-major bleed	109	3·8%	78	4·7%	1·25 (0·93–1·67)	18	13·4%	3·69 (2·25–6·06)

HR=hazard ratio.

Table 2: **Bleeding outcomes among patients taking warfarin in the first 90 days of treatment, across genotype bins**

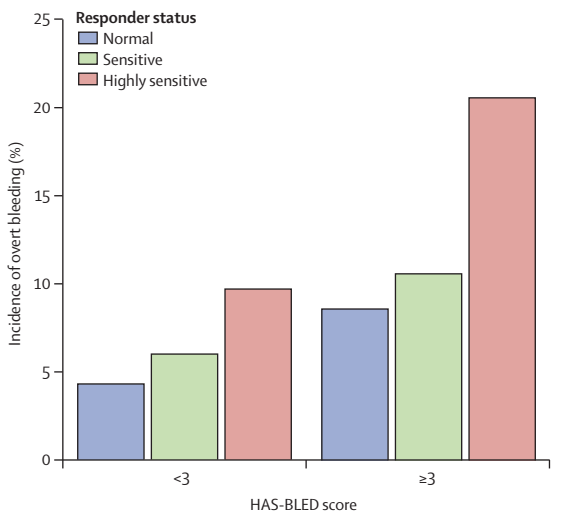

Figure 3: **Overt bleeding events in the first 90 days of treatment among patients taking warfarin, across genotype bins and HAS-BLED bleeding risk score**
Global p<0·0001.

Among the patients allocated to warfarin, 334 had an overt bleeding event in the first 90 days. Sensitive and highly sensitive responders experienced significantly higher rates of bleeding than normal responders (figure 2). The direction of results was consistent for major and clinically relevant non-major bleeding (table 2). Adjustment of these analyses for clinical covariates and outcomes in the first 28 days yielded similar findings (appendix).

Review of the final warfarin dosing data revealed that within the FDA-designated category of normal responders, wild-type individuals (*VKORC1* G/G and *CYP2C9**1/*1) were least sensitive to warfarin. By comparison, the remaining normal responders, sensitive, and highly sensitive responders were at an even more pronounced risk of bleeding (HR 1·45, 95% CI 1·05–1·99, 1·67, 1·22–2·28, and 3·39, 2·05–5·61, respectively; appendix).

When patients were stratified by genotype and HAS-BLED clinical bleeding risk score, we found a significant gradient of bleeding risk in the first 90 days for each predictor (figure 3). The lowest bleeding rates were seen in normal responders with HAS-BLED scores lower than 3 and the highest in highly sensitive responders with HAS-BLED scores of 3 or higher. Analyses stratified by previous exposure to a vitamin K antagonist suggested an increased effect in those naive to these drugs (appendix). Few cases of intracranial haemorrhage or life-threatening bleeding were seen during the first 90 days of warfarin treatment (appendix).

Beyond 90 days, genotype was not associated with an increased risk of any overt bleeding, but was associated with an increased risk of major and life-threatening bleeding in sensitive responders (table 3, appendix). A similar trend was seen for life-threatening bleeding in highly sensitive responders, albeit with a small number of events (appendix).

Among patients randomised to higher-dose and lower-dose edoxaban, genotype was not significantly associated with an increased risk of bleeding. In the entire genetic study population, the overall risk of any overt bleeding with higher-dose edoxaban compared with warfarin was HR 0·90 (95% CI 0·83–0·97) and with lower-dose edoxaban compared with warfarin was 0·70 (0·64–0·75). In the first 90 days, there was an increasing gradient of relative safety across normal, sensitive, and highly sensitive responders with higher and with lower doses of edobaxan compared with warfarin (figure 4). The direction of results was consistent for major and clinically relevant non-major bleeding (figure 4). Comparisons of intracranial haemorrhage and life-threatening bleeding across genotype bins were limited by the small number of events in the first 90 days (appendix). Beyond 90 days, the overall HRs for bleeding were 0·88 (95% CI 0·81–0·95) for higher-dose edoxaban and 0·69 (0·63–0·75) for lower-dose edoxaban compared with warfarin, and no significant interaction was seen between genotype and treatment (figure 4). These findings indicate a consistent long-term safety benefit of edoxaban over warfarin across genotypes.

Among patients taking warfarin, there were only 35 major adverse cardiovascular events in the first 90 days.

	Normal responders (n=2803)		Sensitive responders (n=1616)		Sensitive *vs* normal responders HR (95% CI)	Highly sensitive responders (n=129)		Highly sensitive *vs* normal responders HR (95% CI)
	Number of events	Percentage per year	Number of events	Percentage per year		Number of events	Percentage per year	
Any overt bleed	777	14·5%	459	14·8%	1·02 (0·91–1·15)	41	17·4%	1·20 (0·88–1·64)
Major or clinically relevant non-major bleed	637	11·4%	371	11·4%	1·00 (0·88–1·14)	28	11·2%	0·98 (0·67–1·44)
Major bleed	174	2·8%	128	3·6%	1·28 (1·02–1·61)	7	2·4%	0·86 (0·41–1·80)
Clinically relevant non-major bleed	508	8·9%	279	8·4%	0·94 (0·82–1·09)	24	9·5%	1·06 (0·70–1·62)

HR=hazard ratio.

Table 3: **Bleeding outcomes among patients taking warfarin beyond 90 days of treatment, across genotype bins**

We found no significant associations between genotype and efficacy within or after 90 days (appendix). No interactions were seen between efficacy of edoxaban versus warfarin and genotype (appendix).

Discussion

In this large, prespecified pharmacogenetic study, we found that genetic polymorphisms in *CYP2C9* and *VKORC1* affect the pharmacological and safety outcomes of warfarin therapy. Specifically, sensitive and highly sensitive responders required lower doses of warfarin to achieve INR values in the therapeutic range, and were more likely to be over-anticoagulated than normal responders, especially in the first 90 days of treatment. During this time period, the risk of overt bleeding with warfarin was increased by 1·3 times in the sensitive responders and by 2·7 times in the highly sensitive responders compared with normal responders. In the first 90 days, edoxaban compared with warfarin was associated with a greater reduction in bleeding risk in sensitive and highly sensitive responders than in normal responders.

Bleeding complications are the most important concern related to warfarin therapy because of the narrow therapeutic range and high degree of variability between individuals. Genetic variants have been suggested as a way to assist dose selection.[5,23,24] Specifically, the *CYP2C9*2* and *CYP2C9*3* variants result in reduced catalytic activity and, therefore, reduced metabolism of the highly active S-enantiomer of warfarin;[25] in patients with the *VKORC1* –1639G→A variant, a transcription factor binding site is altered, which leads to reduced levels of the molecular target of warfarin.[26]

Although these genetic variants affect the warfarin dose required, their effects on clinical outcomes, namely bleeding, remain debated, and payment for genotyping is controversial.[27] The results from clinical studies have been mixed, with some showing an association with bleeding only for variants in *CYP2C9*,[28–31] others only for variants in *VKORC1*,[32] and others for neither (panel).[33,34] Our analysis included nearly 5000 patients taking warfarin treated in centres worldwide, and prospectively followed up for a median of nearly 3 years, with events adjudicated centrally by a committee unaware of treatment allocation. In this cohort, first we validated the genetic binning provided in the FDA warfarin label.[5] Even within FDA-designated normal responders, however, we found that wild-type individuals without any risk alleles in *CYP2C9* and *VKORC1* were at the lowest bleeding risk. Second, we showed clear and significant associations between *CYP2C9* and *VKORC1* genotypes and bleeding outcomes with warfarin. Third, we showed that these genetic data offered complementary information to traditional clinical predictors.

Although the increased risk of any overt bleeding was most apparent in the first 90 days, we found a long-term excess of serious bleeding subtypes, such as major and life-threatening bleeding in sensitive and highly sensitive responders receiving warfarin. Heightened response to even small doses of warfarin might, therefore, make some individuals vulnerable to over-anticoagulation and bleeding from fluctuations in diet, drug–drug interactions, and other environmental factors at any time.

Importantly, our pharmacogenetic study was done in the context of a randomized, double-blind trial testing two dosing regimens of edoxaban compared with warfarin. This study offered the opportunity not only to assess the effects of pharmacogenetics on warfarin outcomes, but also the relative safety of a direct oral anticoagulant compared with warfarin. Overall in ENGAGE AF-TIMI 48, compared with warfarin, the two edoxaban regimens were associated with significantly reduced rates of bleeding, including fatal, life-threatening, intracranial, and major bleeding.[14] This genetic analysis revealed that during the early time period, the reduction in bleeding seen with edoxaban versus warfarin was more pronounced in sensitive and highly sensitive responders than in normal responders. After 90 days, the beneficial safety profile of edoxaban versus warfarin in terms of bleeding and cardiovascular mortality was similar across all the genetic categories.

The aggregate data from multiple clinical trials in patients with atrial fibrillation suggest that direct factor

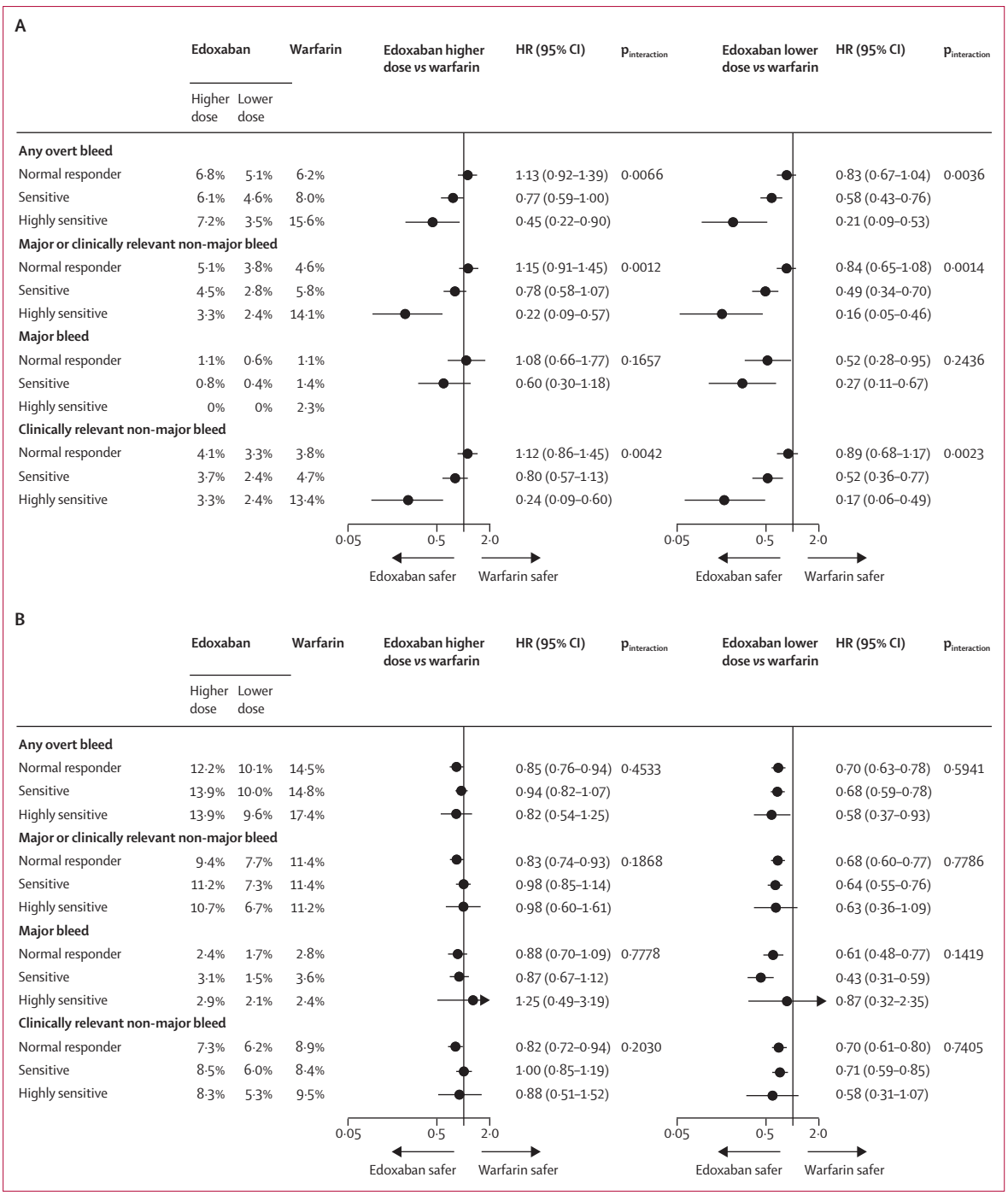

Figure 4: **Safety of edoxaban compared with warfarin, across genotype bins**
(A) Kaplan-Meier event rates for first 90 days of treatment. (B) Percentage per year beyond 90 days of treatment. HR=hazard ratio.

Xa and thrombin inhibitors are at least as efficacious as warfarin, reduce mortality, and reduce intracranial bleeding by around 50%.[13,35] The European guideline for management of patients with atrial fibrillation recommends considering direct oral anticoagulants over vitamin K antagonists in patients with CHA_2DS_2-VASc scores of 2 or higher,[19] and the US guidelines are largely neutral in terms of which type of oral anticoagulant to use.[36] As such, warfarin will continue to be used because of low cost and wide availability. In cases where there is a plan to use warfarin, genotyping could identify close to 40% of patients in whom there is an early

Panel: Research in context

Systematic review

We searched PubMed with the terms "warfarin", "genetics", "*CYP2C9*", and "*VKORC1*". Genetic variants have consistently been shown to affect warfarin dosing, but their effect on clinical outcomes, especially bleeding, remains debated. Our search showed mixed relations between genetic variants and bleeding, with some studies showing only association with variants in *CYP2C9*, others showing only associations with variants in *VKORC1*, and others associations with neither. Therefore, in the ENGAGE AF-TIMI 48 trial we aimed to test whether patients with functional genetic variants in *CYP2C9* and *VKORC1* experience increased rates of bleeding when treated with warfarin and, consequently, whether these genetic variants identify patients who would derive a differential benefit from treatment with the direct factor Xa inhibitor edoxaban instead of warfarin.

Interpretation

Patients with atrial fibrillation were randomly assigned to warfarin or higher-dose (60 mg) or lower-dose (30 mg) edoxaban once daily, and those willing were included in the prespecified genetic analysis. 14 348 patients (4833 randomised to warfarin) were genotyped for variants in *CYP2C9* and *VKORC1* and thereby classified in the genotype functional bins normal, sensitive, and highly sensitive responders. Compared with normal responders, the sensitive and highly sensitive responders required lower doses of warfarin to achieve a therapeutic INR and spent a greater proportion of time over-anticoagulated, especially in the first 90 days after the start of treatment. During this time period, sensitive and highly sensitive responders taking warfarin experienced significantly higher risks of bleeding than normal responders, with risk being highest in highly sensitive responders. As a result, edoxaban compared with warfarin reduced bleeding more so in sensitive and highly sensitive responders than in normal responders in this early period.

increased risk of over-anticoagulation and bleeding with use of standard dosing practices. Our findings, however, show that this risk could be substantially mitigated by using edoxaban, or potentially another direct factor Xa or thrombin inhibitor, instead of warfarin. If warfarin is used in patients with genotypes associated with sensitive and highly sensitive response, increased frequency of INR monitoring and precision dosing seem prudent to avoid over-anticoagulation, although we did not test this strategy. For the 60% of patients in the normal responder category, who might be functionally underdosed with warfarin initially, we found no early safety benefit with higher-dose edoxaban compared with warfarin, but a long-term benefit did emerge.

This study has some potential limitations. First, this pharmacogenetic cohort included only a small number of black patients and, therefore, further analyses among more varied populations will be important.[37] Second, previous studies have compared pharmacogenetic testing and clinical algorithms for dose selection, focusing on pharmacodynamic metrics.[20–22,38] Our focus was on clinical outcomes, and dose selection was decided by the local investigator on the basis of the clinical profiles of patients, which reflects current practice. Notably, the median proportion of time in the therapeutic range with warfarin in ENGAGE AF-TIMI 48 was 68·4%. This rate was similar to, if not better than, the standard of care in the real-world and other clinical-trial settings.[39–41] In settings where the time in therapeutic range is lower, the effect of genetic variants might be even more pronounced. Among patients receiving warfarin, a higher rate of ischaemic outcomes might have been expected among normal responders than among sensitive or highly sensitive responders, but owing to the frequency of these events, the ability to confirm or exclude a pharmacogenetic interaction was limited. Finally, we included patients with atrial fibrillation who were being treated with anticoagulation for stroke prevention. The results might differ in patients being treated for other disorders.

In conclusion, the results of this study provide strong evidence that sensitive and highly sensitive responders to warfarin, classified by *CYP2C9* and *VKORC1* genotypes, spend more time over-anticoagulated and have higher rates of bleeding than normal responders when treated with warfarin, especially in the early period after the start of treatment. For stroke prevention in such patients, a direct oral anticoagulant such as edoxaban offers a greater early safety benefit compared with warfarin.

Contributors

JLM, JRW, CTR, AGV, MFM, RPG, EMA, EB, and MSS contributed to the study design and writing and revision of the paper. All authors were involved in data analysis and data interpretation. FN, ND, SAM, and JL contributed to manuscript revisions.

Declaration of interests

JLM has been supported by grants awarded to Brigham and Women's Hospital from AstraZeneca, Bayer, BMS, Daiichi Sankyo, Eli Lilly, Janssen, and Sanofi and received consulting fees from American Genomics, AstraZeneca, Bayer, Janssen, and Portola. AGV, JL, and MFM are employees of Daiichi Sankyo. JRW was an employee of Daiichi Sankyo at the time of the study, and is now an employee of Celgene. CTR has been supported by grants awarded to Brigham and Women's Hospital from Daiichi Sankyo, and consulting fees from Boehringer Ingelheim, Bristol-Myers Squibb, and Daiichi Sankyo. RPG has been supported by grants awarded to Brigham and Women's Hospital from AstraZeneca, Daiichi Sankyo, Johnson & Johnson, Merck, and Sanofi, received consulting fees from Bristol-Myers Squibb, Daiichi Sankyo, Janssen Pharmaceuticals, Merck, Pfizer, and Portola, and lecture fees from Bristol-Myers Squibb, Daiichi Sankyo, Merck, and Sanofi. EMA has been supported by grants awarded to Brigham and Women's Hospital from Daiichi Sankyo. EB has been supported by grant awarded to Brigham and Women's Hospital from AstraZeneca, Beckman Coulter, Bristol-Myers Squibb, Daiichi Sankyo, GlaxoSmithKline, Johnson & Johnson, Merck, Pfizer, Roche Diagnostics, and Sanofi, received consulting fees from Amorcyte, Cardiorentis, Genzyme, Sanofi, and The Medicines Company, and lecture fees from Bayer HealthCare, Eli Lilly, Medscape, and Menarini. He has also done unpaid consultant work for Merck and uncompensated lectures for CVRx and Merck. MSS has been supported by grants awarded to Brigham and Women's Hospital from Abbott Laboratories, Accumetrics, Amgen, AstraZeneca, AstraZeneca/Bristol-Myers Squibb Alliance, BRAHMS, Bristol-Myers Squibb/Sanofi-Aventis Joint Venture, Critical Diagnostics, Daiichi-Sankyo, diaDexus, Eisai, Genzyme, Gilead, GlaxoSmithKline, Intarcia, Merck, Nanosphere, Ortho-Clinical Diagnostics, Roche Diagnostics, Sanofi-aventis, Singulex, and Takeda, and received consulting fees from Aegerion, Amgen, AstraZeneca, Bristol-Myers Squibb, Cubist, CVS Caremark, Daiichi-Sankyo/Eli Lilly, GlaxoSmithKline, Intarcia, Merck, MyoKardia, Pfizer, Quest Diagnostics, Sanofi-Aventis, Vertex, and Zeus Scientific. The other authors declare no competing interests.

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