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Warfarin reduces circulating white blood cell count: Post-hoc analysis of two randomized trials

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ABSTRACT

Warfarin is a widely used vitamin K antagonist (VKA) with known pleiotropic effects beyond anticoagulation. Preclinical and case-control evidence suggests that warfarin may affect hematopoiesis, but longitudinal human evidence is lacking. To explore this potential effect, we conducted a post-hoc analysis of participants in the Hokusai-VTE and ENGAGE AF-TIMI 48 trials, which randomized patients to warfarin or the direct oral anticoagulant edoxaban with routine laboratory testing at predefined follow-up visits. We analyzed changes in total circulating white blood cells (WBC) and subpopulations (lymphocytes, monocytes, granulocytes) using linear regression and mixed-effects models, adjusting for baseline counts, age, sex, and time. Among 23,618 patients enrolled in the two phase 3 trials, warfarin use was associated with a modest but statistically significant reduction in WBC count (−2.3 %, 95 % CI −2.9 % to −1.7 %) and granulocyte count (−3.6 %, 95 % CI −4.5 % to −2.7 %) compared with edoxaban, while lymphocyte and monocyte counts did not differ. The associations remained consistent across multiple sensitivity analyses. No increase in clinically relevant granulocytopenia was observed. In the context of two large randomized trials, these findings support a subtle hematologic effect of warfarin, particularly in granulocytes, that aligns with preclinical findings and warrants further investigation into the long-term impact of VKAs on hematopoiesis.

1. Introduction

Warfarin is the most frequently used vitamin K antagonist (VKA) and is still administered to approximately 0.5–1 % of the general population globally to prevent and treat thromboembolic events [1,2]. In addition to its effect on the synthesis of coagulation factors, warfarin exerts pleiotropic effects [3]. For instance, it may harm bone health and increase the risk of fractures when administered in the long term [4]. Warfarin can also influence the expression of some endothelial proteins and promote vascular calcification and atherosclerosis [5].

A translational study showed for the first time that warfarin may adversely affect the function of hematopoietic stem cells by disrupting the bone marrow microenvironment and the periostin/integrin β3 axis in animal models [6]. The same study provided indirect evidence suggesting a similar effect in humans using a case-control analysis. The proportion of individuals using VKA was higher among patients with vs. without myelodysplastic syndromes, a group of conditions characterized by disturbances of the bone marrow function and reduction of one or more types of circulating blood cells. The use of VKA seemed to be associated with a modest reduction in white blood cells (WBCs) even in the absence of myelodysplastic syndrome.

The potential impact of VKAs on blood count parameters would improve our understanding of novel pathophysiological mechanisms and may have implications for the long-term management of patients

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treated with VKA. However, this observation necessitates confirmation through longitudinal studies assessing different exposures in a randomized fashion, namely VKA vs. other compounds, such as direct oral anticoagulants (DOAC). Because the latter inhibit coagulation through a more direct mechanism than VKA, their potential for pleiotropic effect may be lower or absent.

Our investigation aimed to determine the potential impact of warfarin on WBC count over time. To do so, we studied the course of leukocyte counts in patients randomized to receive either the VKA warfarin or the DOAC edoxaban from two distinct patient populations with an indication to anticoagulation: those diagnosed with acute venous thromboembolism (VTE) participating in the Hokusai VTE trial and those with atrial fibrillation enrolled in the Engage AF-TIMI 48 trial.

2. Methods

In the Hokusai VTE (ClinicalTrials.gov identifier: NCT00986154) and ENGAGE AF-TIMI 48 (ClinicalTrials.gov identifier: NCT00781391) randomized controlled trials, patients were randomized to receive either warfarin or edoxaban for a period of up to 12 months following a diagnosis of venous thromboembolism (Hokusai VTE) and up to 3 years for atrial fibrillation (ENGAGE AF-TIMI 48), provided they did not have a contraindication to oral anticoagulation, such as active bleeding or severe renal dysfunction [7,8]. In both trials, the study protocol had been reviewed and approved by all appropriate institutional review boards and ethics committees at each participating site, and all patients provided written informed consent. This post-hoc analysis did not require approval from an ethics committee. CA and LV analyzed the data and all authors had access to the primary clinical trial data.

In Hokusai VTE, patients were randomized in a 1:1 fashion to receive either edoxaban or warfarin. In ENGAGE AF-TIMI 48, patients were randomized in a 1:1:1 fashion to receive edoxaban at two different dosages or warfarin. In both trials, the dose was halved if any of the following characteristics were present at the time of randomization or during the study: estimated creatinine clearance of 30 to 50 ml per minute, a body weight of 60 kg or less, or the concomitant use of verapamil or quinidine. The complete list of eligibility criteria of the two studies is available as *Supplemental Material*. In both the Hokusai VTE and ENGAGE AF-TIMI 48 trials, all patients underwent routine blood tests and were seen for routine clinical visits at multiple predefined time points. For the Hokusai VTE trial, these time points included baseline (before the initiation of study treatment), 3 months, 6 months, 9 months, and 12 months. For the ENGAGE AF-TIMI 48 trial, the timepoints for data collection were baseline (before study treatment), 3 months, 6 months, 9 months, 12 months, 18 months, and 24 months.

For this analysis, we included patients from the Hokusai VTE and ENGAGE AF-TIMI 48 trials who had available baseline laboratory values and at least one follow-up test performed during active treatment with the assigned anticoagulant, without cross-over or switch to another anticoagulant. We excluded individuals who were randomized but not treated, those who did not receive the allocated drug, and patients with known active malignancy or undergoing active cancer treatment.

The primary outcomes of interest for both trials were the count of WBC and subpopulations (lymphocytes, monocytes, and granulocytes). Mean and median values, along with their standard deviations and interquartile ranges, were calculated at each timepoint for the two treatment groups. The absolute mean difference between each timepoint and the baseline throughout the follow-up period was also determined for each drug. Both drug-induced bone marrow toxicity and clinical suspicion of myelodysplastic syndrome are based on the detection of neutropenia. As information on the count of granulocyte subpopulations was not collected in ENGAGE AF-TIMI 48 and Hokusai VTE, we used overall granulocyte count with a threshold of $<1.5 \times 10^9$/L as a proxy of neutropenia with perfect specificity.

To compare cell counts in patients exposed to warfarin vs. edoxaban over time, linear models were used. In the main analysis, two regression models were applied: a linear mixed model with follow-up visits modelled as random intercepts, with no modelling of time, by assuming either equal or unequal residual variances; a linear regression model additionally adjusted for time (days) as a linear effect and for an interaction term between study drug and time. All models were additionally adjusted for sex, age ($\geq$ 75 years vs <75 years), and baseline value of the blood cell count used as outcome. To satisfy the normality assumption, the cell counts were log-transformed before models were run.

We performed four sensitivity analyses by repeating the main analysis: (a) after exclusion of patients with no major new clinical event (major or clinically relevant nonmajor bleeding or new diagnosis of cancer) during follow-up; (b) after exclusion of single laboratory measurements following a new diagnosis of cancer or in the 30 days preceding or following an infection; (c) after excluding patients with previous exposure to VKAs; (d) by introducing an interaction term of study drug with age $\geq$ 75 years at inclusion. All analyses were conducted using R version 3.0.0.

3. Results

3.1. Patient selection

We accessed the original Hokusai and ENGAGE AF-TIMI 48 datasets comprising a total of 29,345 patients. After exclusion of patients not matching our eligibility criteria, we included 23,618 patients for analysis. Of those, 11,865 were randomized to receive one of the two edoxaban dosages in the ENGAGE AF-TIMI 48 study and 2876 in the Hokusai VTE study. In contrast, 5996 and 2881 participants were randomized to receive warfarin in the respective studies. In total, the present analysis included 14,741 patients who received edoxaban and 8877 patients who received warfarin.

3.2. Patient characteristics

Table 1 and *Table S1* provide a summary of the baseline characteristics and demographics of the study population stratified per treatment group across studies. Women represented almost 40 % of the study population. The percentage of patients aged 75 years of older was 38 % in the ENGAGE AF-TIMI 48 trial and 10 % in the Hokusai VTE trial. Current VKA use (prior to randomization) was recorded in approximately 68 % of patients enrolled in the ENGAGE AF-TIMI 48 trial and in none, as per eligibility criteria, enrolled in the Hokusai VTE trial.

Overall, a total of 105,395 laboratory tests for WBCs were performed in the two groups during the first year of active follow-up on anticoagulation. At baseline, the median WBC count was 6.6 (Q1-Q3: 5.5–7.9; min-max 1.0–62.8) $\times$ 10^9/L. The median values for WBC subpopulations were 1.7 (Q1-Q3: 1.4–2.1) $\times$ 10^9/L /L for lymphocytes, 0.4 (Q1-Q3: 0.3–0.6) *10^9/L /L for monocytes, and 4.4 (Q1-Q3: 3.5–5.4) $\times$ 10^9/L /L for granulocytes.

3.3. Primary analysis

Fig. 1 shows the observed distribution of the absolute difference in cell counts, measured in 10^9 cells/L, from baseline over follow-up. The median count of overall WBCs and granulocytes was consistently lower in those treated with warfarin vs. edoxaban, despite a substantial overlap of the overall distributions. No evident differences were noticeable in the distributions of lymphocytes and monocytes.

First, we studied inferentially the course of WBC counts focusing on the two drug groups over all follow-up visits with no modelling of time and assuming equal residual variances for the exposure (drug). During anticoagulation, patients who received warfarin showed a WBC count that was 2.5 % (95 %CI: 2.1 % to 3.0 %) lower than that of patients receiving edoxaban. There was no difference in lymphocyte and monocyte counts between the groups, whereas the granulocyte count was significantly lower in the warfarin group by 3.7 % (95 %CI: 3.0 % to

Table 1
Baseline characteristics of the study population across treatment arms in the ENGAGE AF-TIMI 48 and in the Hokusai VTE randomized trials.

	ENGAGE AF-TIMI 48 (edoxaban 30 mg)	ENGAGE AF-TIMI 48 (edoxaban 60 mg)	ENGAGE AF-TIMI 48 (warfarin)	Hokusai VTE (edoxaban 60 mg)	Hokusai VTE (warfarin)
Total, n	5977	5888	5996	2876	2881
Age groups, n (%)					
< 75 years	3696 (61.8)	3653 (62.0)	3707 (61.8)	2589 (90.0)	2598 (90.2)
≥ 75 years	2281 (38.2)	2235 (38.0)	2289 (38.2)	287 (10.0)	283 (9.8)
Women, n (%)	2275 (38.1)	2174 (36.9)	2219 (37.0)	1139 (39.6)	1172 (40.7)
BMI (median [IQR])	28.7 [25.6, 32.7]	28.9 [25.6, 32.9]	28.6 [25.3, 32.5]	27.5 [24.4, 31.2]	27.7 [24.7, 31.2]
Atrial fibrillation, n (%)	5977 (100.0)	5888 (100.0)	5996 (100.0)	62 (2.2)	64 (2.2)
Venous thromboembolism, n (%)	135 (2.3)	129 (2.2)	142 (2.4)	2876 (100.0)	2881 (100.0)
Creatinine clearance, n (%)					
≤ 50 ml/min	1066 (17.8)	1070 (18.2)	1103 (18.4)	122 (4.2)	131 (4.5)
> 50 ml/min	4890 (81.8)	4796 (81.5)	4875 (81.3)	2726 (94.8)	2720 (94.4)
Not available	21 (0.4)	22 (0.4)	18 (0.3)	28 (1.0)	30 (1.0)
With criteria for edoxaban dose adjustment, n (%)	1454 (24.3)	1424 (24.2)	1479 (24.7)	445 (15.5)	447 (15.5)
Current VKA use (%)	4102 (68.6)	4003 (68.0)	4101 (68.4)	0 (0.0)	0 (0.0)
Region, n (%)					
Asia-Pacific region and South Africa	1086 (18.2)	1056 (17.9)	1106 (18.4)	995 (34.6)	987 (34.3)
Eastern Europe	2131 (35.7)	2092 (35.5)	2104 (35.1)	757 (26.3)	760 (26.4)
Latin America	790 (13.2)	786 (13.3)	790 (13.2)	96 (3.3)	112 (3.9)
North America	1200 (20.1)	1201 (20.4)	1237 (20.6)	264 (9.2)	285 (9.9)
Western Europe	770 (12.9)	753 (12.8)	759 (12.7)	764 (26.6)	737 (25.6)

IQR = interquartile range; BMI = body-mass index; VKA = vitamin K antagonists.

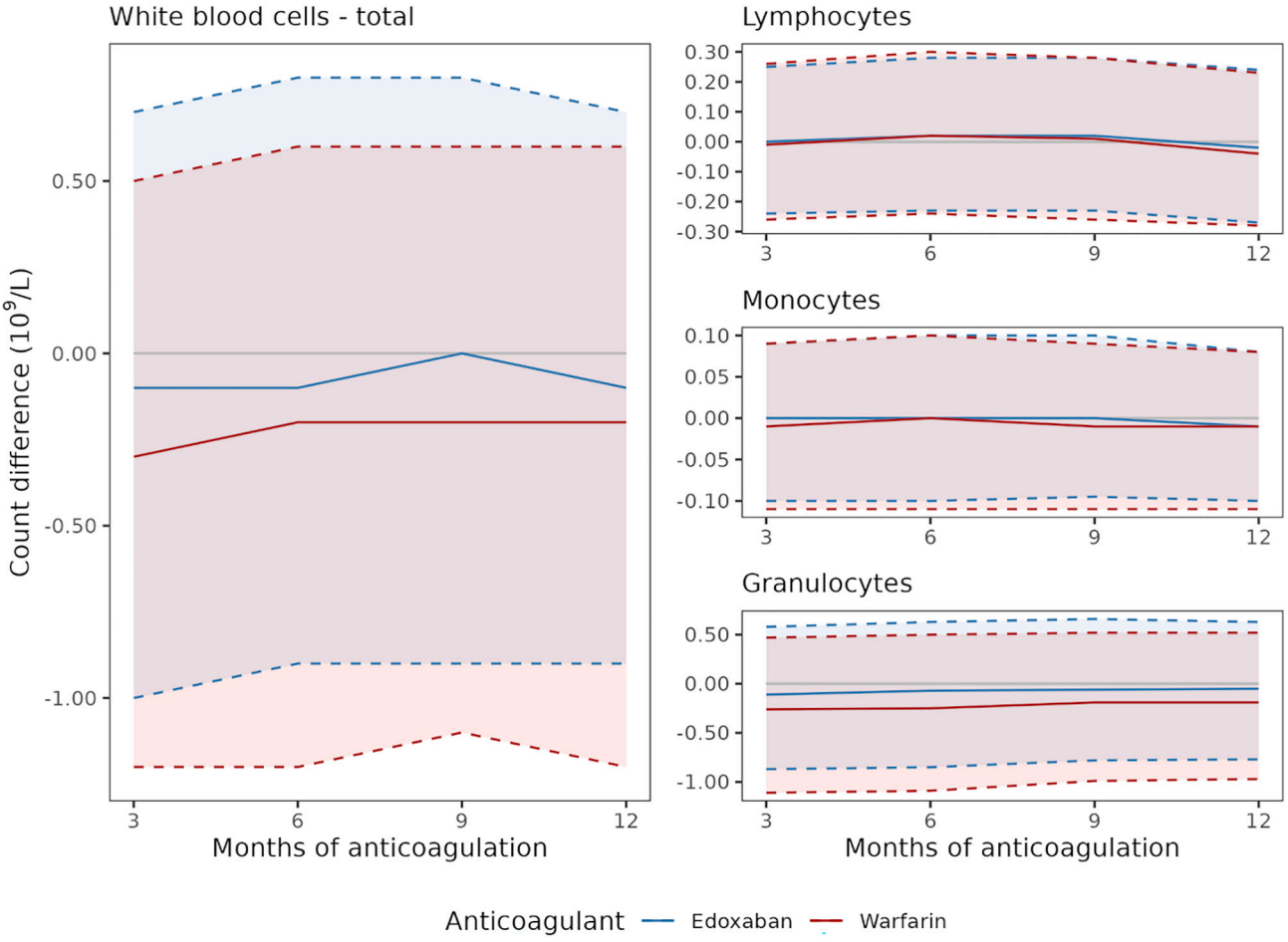

Fig. 1. Distribution of blood cell count differences vs. baseline over time. Line plots of the observed median (continuous lines) and interquartile range (dashed lines) of the absolute difference in four cell counts (expressed in cells×109/L) from baseline over follow-up visits in patients receiving the anticoagulants edoxaban (blue) and warfarin (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.3 %). The same analysis with the assumption of unequal residual variances for the exposure (drug) yielded results identical to the first decimal digit.

In the regression model incorporating time as linear covariate and adding an interaction between time and drug, the time-adjusted effect of anticoagulant drug on WBCs was similar to the unadjusted effect: the WBC count was 2.3 % (2.9 % to 1.7 %) lower and the granulocyte count 3.6 % (4.5 % to 2.7 %) lower in the warfarin group than in the edoxaban group. Lymphocyte count was not associated with drug, but decreased over time showing a decrease of 0.5 % (0.4 % to 0.6 %) in both groups within 90 days, and the association with time displayed an interaction with study drug (90-day decrease of 0.6 % with warfarin vs 0.3 % for edoxaban).

3.4. Sensitivity analyses

The association of warfarin with overall WBC count and granulocyte count was consistent across all sensitivity analyses performed (a) after excluding patients who did not experience a major or clinically relevant nonmajor bleeding event or did not receive a new diagnosis of cancer during follow-up, (b) after excluding measurements performed after a new diagnosis of cancer during follow up or less than 30 days before or after an infection, (c) focusing on patients who were VKA-naïve (N = 10,472), and (d) including an additional interaction with age; Table 2.

In the analysis with additional interaction with age for the outcome granulocyte count, the interaction of warfarin with age < 75 was statistically significant, indicating either a stronger association of warfarin with lower granulocyte count in patients younger than 75 or a weaker association in those aged 75 or older.

3.5. Prevalence of granulocytopenia over follow-up

We explored the possibility that the association between warfarin and granulocyte count was driven by a minority of patients with clinically relevant granulocytopenia with descriptive analyses of the proportion of granulocytopenia and of the absolute counts in the lowest percentiles of the granulocyte count distribution of patients treated with either anticoagulant.

Among 23,041 patients with baseline values of granulocyte count and at least one follow-up value, the proportion of those with new granulocytopenia, defined as a total granulocyte count $<1.5 \times 10^9$/L not present at baseline, did not differ considerably between treatment groups (Table 3). Agranulocytosis ($<0.5 \times 10^9$/L) at any timepoint was recorded in one patient on edoxaban and 4 patients on warfarin; *Fig. S1*.

Fig. 2 shows the lowest observed percentiles of the distribution of the absolute granulocyte counts by drug in each study visit. No divergence between the two distributions is observed at the lowest percentiles, as would be expected if a small group of patients displayed granulocytopenia in one of the two arms only.

4. Discussion

This large post-hoc analysis of two pivotal randomized trials supports the hypothesis that warfarin reduces circulating WBCs, primarily due to lower granulocyte counts. These findings align with recent animal and case-control studies suggesting that warfarin affects the bone marrow microenvironment and hematopoiesis. Our data confirm causality, but indicate that these changes, though statistically significant, remain within physiological ranges. During short-term follow-up of 12 months, warfarin is unlikely to result in clinically meaningful leukopenia. The observed effect may represent a modest population-level shift rather than a phenomenon driven by few pathological outliers. However, one cannot exclude that warfarin intake over several years or decades, especially at higher dosages, may exert a more profound effect on bone marror microenvironment and hematopoiesis.

The potential mechanisms underlying WBC count reduction in VKA-treated patients are not fully elucidated. VKAs have pleiotropic effects. In contrast to DOACs, they affect the concentration and function of several proteins: the vitamin K-dependent coagulation factors II, VII, IX, and X, as well as proteins C, S, and Z [9]. These proteins are primarily involved in coagulation, but also influence other physiological mechanisms through several molecular pathways [10–12]. Additionally, it has been postulated in an animal study that a bone marrow microenvironment disruption may play a role, as well as the migration, adhesion, and activation of immune cells [6]. In particular, compromission of the functionality of periostin by impairment of the periostin/integrin αvβ3 axis emerged as a possible mediator of the effects of warfarin [13,14]. Subsequent preclinical evidence has confirmed a possible effect of an osteoporotic bone abnormalities may be associated with changes in

Table 2
Mixed linear regression models for the association between warfarin (vs. edoxaban) and white blood cell count and subpopulations over 1 year of follow-up in the ENGAGE AF-TIMI 48 and Hokusai VTE trials.

	White blood cell count, % difference (95 % CI)	Lymphocyte count, % difference (95 % CI)	Monocyte count, % difference (95 % CI)	Granulocyte count, % difference (95 % CI)
Main analysis				
Warfarin (vs. Edoxaban), model 1	−2.5 % (−2.1 %, −3.0 %)*	−0.9 % -0.6 %, +0.4 %	−0.3 % (−1.2 %, +0.5 %,)	−3.7 % (−4.3 %, −3.0 %)*
Warfarin (vs. Edoxaban), model 2	−2.3 % (−2.9 %, −1.7 %)*	+0.4 % (−0.3 %, +1.2 %)	−0.7 % (−2.1 %, +0.5 %)	−3.6 % (−4.5 %, −2.7 %)*
Sensitivity analysis (a): exclusion of bleedings, cancer during follow-up				
Warfarin (vs. Edoxaban), model 1	−2.7 % (−3.3 %, −2.2 %)*	+0.06 % (−0.5 %, +0.7 %)	−0.1 % (−1.1 %. +0.8 %)	−4.0 % (−4.7 %, −3.3 %)*
Warfarin (vs. Edoxaban), model 2	−2.5 % (−3.2 %, −1.7 %)*	+0.6 % (−0.3 %, +1.4 %)	−1.2 % (−2.7 %, +0.4 %)	−3.9 % (−4.9 %, −2.9 %)*
Sensitivity analysis (b): exclusion of laboratory measurements ± 30 days from infection				
Warfarin (vs. Edoxaban), model 1	−2.5 % (−3.1 %, −2.1 %)*	−0.2 % (−0.7 %, +0.4 %)	−0.3 % (−1.3 %, +0.6 %)	−3.6 % (−4.3 %, −2.9 %)*
Warfarin (vs. Edoxaban), model 2	−2.5 % (−3.2 %, −1.8 %)*	+0.5 % (−0.3 %, +1.3 %)	−1.1 % (−2.6 %, +0.4 %)	−3.8 % (−4.8 %, −2.9 %)*
Sensitivity analysis (c): VKA-naïve patients only				
Warfarin (vs. Edoxaban), model 1	−2.3 % (−3.1 %, −1.5 %)*	+0.3 % (−0.5 %, +1.2 %)	+0.2 % (−1.3 %, +1.6 %)	−3.6 % (−4.6 %, −2.5 %)*
Warfarin (vs. Edoxaban), model 2	−2.0 % (−3.0 %, −1.0 %)*	+0.6 % (−0.6 %, +1.7 %)	−0.5 % (−2.7 %, +1.6 %)	−3.5 % (−4.9 %, −2.1 %)*
Sensitivity analysis (c): interaction with age ≥ 75 in model 1				
Warfarin (vs. Edoxaban)	−2.4 % (−2.9 %, −1.9 %)*	−0.2 % (+0.3 %, −0.8 %)	−0.5 % (+0.4 %, −1.5 %)	−3.4 % (−4 %, −2.7 %)*
Age < 75 (vs ≥ 75)	+0.7 % (−0.3 %, +1.2 %)*	+5.5 % (+4.9 %, +6.2 %)*	+4.4 % (+3.5 %, +5.3 %)*	2.9 % (+2.2 %, +3.5 %)*
Warfarin×Age < 75	−0.9 % (−1.9 %, 0 %)	+0.8 % (−0.4 %, +1.9 %)	+1 % (−0.9 %, +2.8 %)	−1.4 % (−2.7 %, −0.1 %)

Reported are β coefficients from mixed linear regression models (95 % confidence intervals). Model 1: time as random effect, equal variances assumed. Model 2: time as linear covariate, and interaction drug×time. All models are additionally adjusted for sex, age (≥ 75 years vs <75 years), and baseline value of the blood cell count used as outcome. VKA = vitamin K antagonists. 95 % CI = 95 % Confidence Interval. * $p < 0.05$.

Table 3
Proportion of patients with granulocytopenia by study arm over follow-up.

Time of first documentation	Warfarin n/N, % (95 % CI)	Edoxaban n/N, % (95 % CI)
At any time among those with baseline and at least one follow-up measurement	67/8635, 0.78 % (0.61 to 0.98 %)	107/14406, 0.74 % (0.62 to 0.90 %)
At 3 months or later among those with baseline and at least one follow-up measurement	55/8635, 0.64 % (0.49 to 0.83 %)	85/14406, 0.59 % (0.48 to 0.73 %)
At 6 months or later among those with baseline and at least one measurement from 6 months	39/8075, 0.48 % (0.35 to 0.66 %)	55/13652, 0.40 % (0.31 to 0.52 %)
At 9 months or later among those with baseline and at least one measurement from 9 months	19/6924, 0.27 % (0.18 to 0.42 %)	33/12253, 0.27 % (0.19 to 0.38 %)
At 12 months among those with baseline and the 12-month measurement	11/6328, 0.17 % (0.09 to 0.30 %)	17/11365, 0.15 % (0.09 to 0.25 %)

Granulocytopenia was defined as a total granulocyte count $<1.5 \times 10^9$/L. Confidence intervals are Wilson score intervals.

circulating peripheral blood cells [15]. VKA affects bone mineral density and its use has been implicated in the genesis of osteoporosis [16]. Compared with VKA, rivaroxaban and apixaban were associated with a significantly lower risk of osteoporosis [17]. However, VKAs have also been shown to influence the production and release of cytokines regulating immune response. For example, VKAs have been associated with changes in the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and interferon-gamma (IFN-gamma), all inducing pathways in hematopoietic stem cells [18–23] Moreover, antigen-presenting cells (APCs), such as dendritic cells and macrophages may be impacted by VKAs, particularly concerning their antigen-presenting capacity [24,25].

Our results suggest that the immune system dysregulation imposed by VKAs is of moderate extent and may have little clinical impact. Although the reduction in the count of WBCs was evident already during the first three months after the first VKA exposure, numerical counts of WBCs were only moderately reduced under warfarin, approximately 3 % lower count compared to edoxaban. Furthermore, there was no evidence of subgroups of patients developing clinically significant granulocytopenia during follow-up. As the analysis was limited to merely numerical counts of WBCs, an impact on immune cell function cannot be excluded.

Verna and colleagues [6] postulated a link between warfarin and myelodysplastic syndrome based on animal experiments and evidence from observational data. We could confirm the causal association between warfarin use and WBC reduction. Although this may not have clinical relevance overt short term, we cannot exclude that WBC would further reduce over time, beyond 12-month follow-up. If VKA treatment is continued over decades, as is the case for some patient subgroups, this might be of clinical relevance, similarly to the changes of bone composition during VKA treatment and the risk of bone fractures [26,27]. The hypothesis that VKA may influence the response of WBC to specific situations, including transplantation or infections [6], remains speculative.

Our results suggest that routine monitoring of WBCs may not be necessary solely due to warfarin use in patients without additional risk factors. However, a modest reduction in granulocyte count might hold

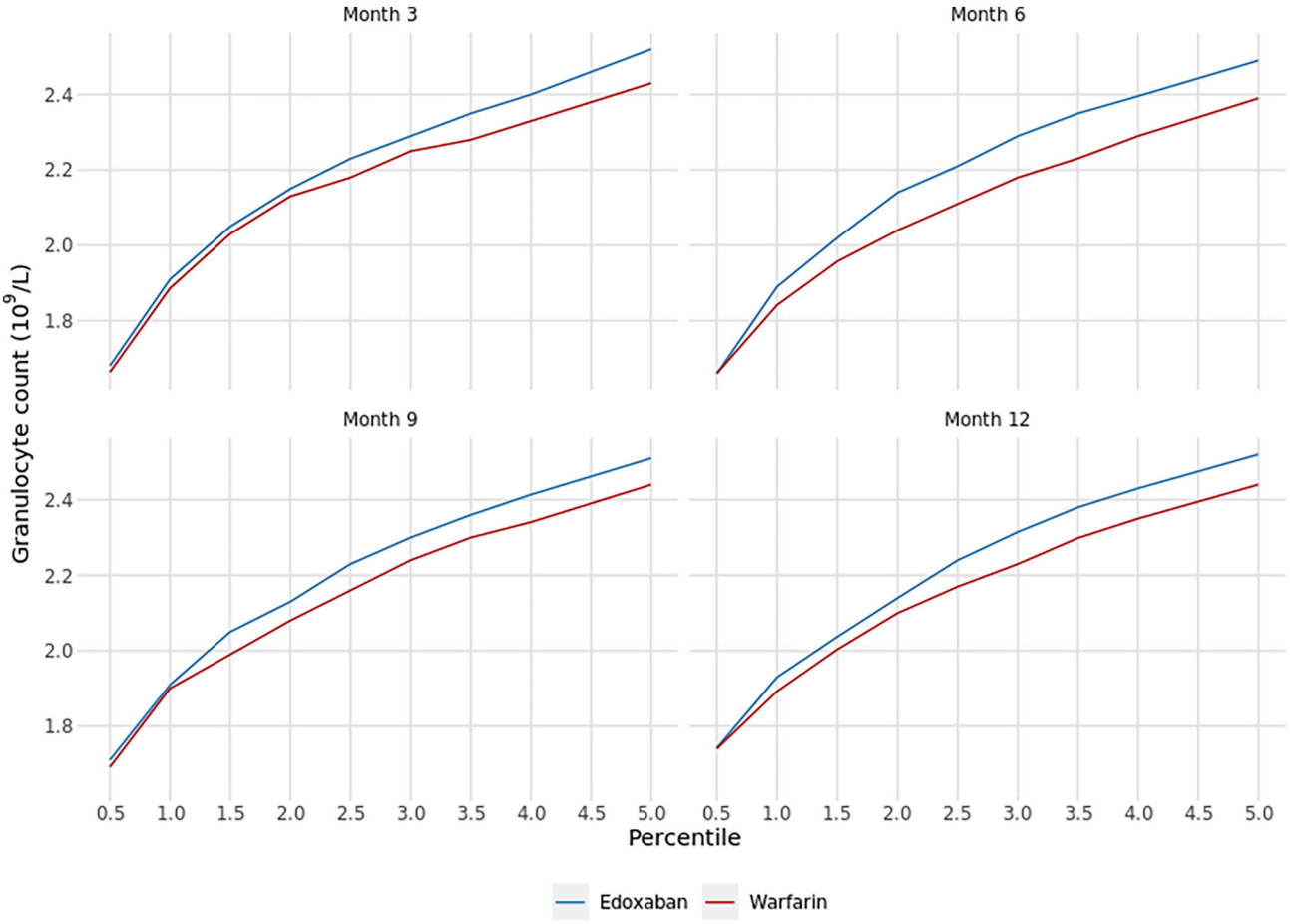

Fig. 2. Absolute granulocyte counts in the lowest percentiles by drug and by visit. Line plots of the lowest observed percentiles of the distribution of the absolute granulocyte counts by drug in each study visit.

clinical relevance in specific scenarios, such as in patients with concurrent myelosuppressive therapies, bone marrow disorders, or recurrent infections [28]. In such individuals, WBC monitoring, especially during VKA therapy carried over several years, might provide an additional layer of safety. Furthermore, clinicians may wish to factor this subtle hematologic effect into the risk-benefit assessment when choosing between VKA and DOACs for long-term anticoagulation, provided that DOACs represent the current standard of care for most patients with venous thromboembolism and atrial fibrillation. Our findings further reinforce the distinct biological profiles of VKAs and DOACs and support further comparative investigations into their systemic effects, taking into account bone and bone marrow microenvironment remodelling [16]. In contrast, clinical consequences related to VKA impact on granulocyte count are still speculative based on current data.

The strengths of this work include the analysis of longitudinal data with long follow-up from clinical trials comparing two anticoagulation strategies in a randomized fashion. All centralized laboratory analysis were performed as part of a predefined protocol at specific timepoints and not driven by clinical events. A further strength is represented by the consistency of results across several sensitivity analyses and after adjustment for multiple confounders.

The main limitations are represented by the lack of adjudicated clinical outcomes of interest for this research question (i.e. rate of bacterial infections, novel diagnoses of myelodysplastic syndromes) and the relatively short follow-up. Analyses of larger patient populations with longer follow-up and low risk of bias may help verify whether detectable laboratory changes occur with the use of VKA over a longer time. In the ENGAGE AF-TIMI 48 trial, some patients had received VKA prior to randomization: this may have slighthly confounded the results, although we have shown in a sensitivity analysis that the change in WBC count was similar after the exclusion of these patients. Furthermore, blood material was not available any longer to perform ex vivo studies of cell function, which may also affect immune function beyond cell counts. Finally, we could not test whether warfarin dosage or time in therapeutic range correlate with the degree of granulocyte count reduction [29].

In conclusion, we showed that, in a large cohort of patients from two randomized controlled trials enrolling patients with atrial fibrillation and venous thromboembolism followed for 12 months, VKA lead to a moderate, but statistically significant reduction in WBC count primarily driven by granulocyte count. Future studies should clarify the clinical relevance of these findings, particularly in patients on long-term VKA anticoagulation.

CRediT authorship contribution statement

Stefano Barco: Writing – original draft, Supervision, Methodology, Conceptualization. **Christina Abele:** Writing – original draft, Formal analysis, Data curation. **Walter Ageno:** Writing – review & editing, Validation. **Frederikus A. Klok:** Writing – review & editing. **Philip Wenzel:** Writing – review & editing, Investigation. **Alice Trinchero:** Writing – review & editing. **Luca Malcovati:** Writing – review & editing. **Stavros V. Konstantinides:** Writing – review & editing, Resources, Project administration. **Luca Valerio:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation.

Declaration of competing interest

W.A. reports participation in advisory boards for AstraZeneca, Bayer, BMS-Pfizer, Norgine, Sanofi, Viatris. FAK has received research funding from Bayer, BMS, BSCI, AstraZeneca, Angiodynamics, MSD, Leo Pharma, Actelion, Varm-X, The Netherlands Organisation for Health Research and Development, The Dutch Thrombosis Foundation, The Dutch Heart Foundation and the Horizon Europe Program, all outside this work and paid to his institution. SVK reports grants or contracts to his institution from Bayer AG, Daiichi Sankyo, Boston Scientific Corp., Penumbra Inc., and Inari Medical; and personal consulting fees from Daiichi Sankyo, Boston Scientific Corp., and Penumbra Inc., all outside the submitted work. The remaining authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2025.109452.

Data availability

The deidentified individual participant data that support the findings of this study are available on the VIVLI platform at https://vivli.org and can be accessed through a request procedure subject to approval by the Data Contributor.

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