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ARTICLE

Prospective validation of the International Warfarin Pharmacogenetics Consortium algorithm in high-risk elderly people (VIALE study)

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Abstract

We assessed the predictive accuracy of the Warfarin Pharmacogenetics Consortium (IWPC) algorithm in a prospective cohort of 376 high-risk elderly patients (≥65 years) who required new treatment with warfarin for either medical (non valvular atrial fibrillation) or surgical conditions (heart valve replacement), had ≥1 comorbid conditions, and regularly used ≥2 other drugs. Follow-up visits were performed according to clinical practice and lasted for a maximum of 1 year. Two hundred and eighty-three (75%) patients achieved a stable maintenance dose. Warfarin maintenance doses were low on average (median 20.3 mg/week, interquartile range, 14.1–27.7 mg/week) and were substantially overestimated by the IWPC algorithm. Overall the percentage of patients whose predicted dose of warfarin was within 20% of the actual stable dose was equal to 37.5%, (95% CI 32.0–43.3%). IWPC algorithm explained only 31% of the actual warfarin dose variability. Modifications of the IWPC algorithm are needed in high-risk elderly people.

Introduction

After several decades, warfarin continues to be the most prescribed oral anticoagulant worldwide [1, 2], notwithstanding the increasing use of new oral anticoagulants

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(DOAC) [3, 4]. However the clinical management of warfarin may be challenging because of its narrow therapeutic window along with within- and between- subject variability of dose required.

Individual warfarin response variability has been found to be associated with clinical factors (e.g., age, gender, compliance, nutritional status, presence of comorbidity, and concomitant medications), and polymorphisms involved in the metabolic route and warfarin effector/response pathway. Therefore, a number of models, which include both clinical and genetic factors, have been developed to predict warfarin dose requirements, but their usefulness is still under debate [5].

In 2009 the International Warfarin Pharmacogenetics Consortium (IWPC) proposed an algorithm that includes both clinical and genetic data [6]. The IWPC algorithm has been externally validated in other countries, mostly in small single-center studies that included adult patients irrespective of age [7–34]. A recent meta-analysis showed that the proportion of warfarin doses that were systematically underpredicted by the IWPC algorithm in patients requiring higher than average doses was as high as 95.8% (95% confidence interval 92.4–97.9%) [35].

The predictive ability of the IWPC algorithm is particularly questionable in high-risk elderly people, who represent a highly vulnerable population because of increased risk of (i) bleeding during treatment with vitamin K antagonists, (ii) reduced metabolic clearance, and (iii) possible interactions with drugs used for comorbidities. In 2012 we planned the 'Validation of IWPC algorithm in high-risk elderly patients (VIALE)' study to validate the IWPC algorithm in a prospective cohort of high-risk elderly people (≥65 years) with at least one comorbid condition (clinicaltrials.gov identifier NCT02069132).

Materials and methods

Study design and participants

All patients aged 65 years or more, referring to six public hospitals, who first required treatment with warfarin because of either medical (non valvular atrial fibrillation) or surgical conditions (heart valve replacement), who had at least one comorbid condition, and regularly used two or more other drugs besides warfarin were prospectively recruited. Patients with systemic coagulopathies and malignancies needing chemotherapy were excluded.

Intervention

The investigators were allowed to adjust the dose of Warfarin or discontinue the treatment according to clinical practice, blind to genotype assessment. No additional interventional procedures were required.

IWPC algorithm

The IWPC pharmacogenetic algorithm [6] includes both clinical and genetic data: age, height, weight, race, VKORC1 genotype, CYP2C9 genotype, and use of drugs that are CYP2C9-inducer (rifampicin, phenytoin, and carbamazepine) or inhibitor (amiodarone). A different version of the algorithm that also included smoking and target INR was proposed in 2012 within the CoumaGen-II trial [36]. IWPC dosing algorithms 2009 and 2012 are reported in the Supplementary Table 1.

Variables

Baseline variables included demographic and clinical information, primary indication for warfarin treatment, risk factors such as smoking and drinking status, baseline and target INR, medical/surgical history, starting warfarin dose, comorbidity according to the Cumulative Index Rating Scale (CIRS) [37] and use of concomitant medications.

Follow-up (FU) visits were performed according to the clinical practice of each center and patients' need. Maximum length of FU was set equal to 1 year. At each FU visit data on warfarin dose changes, INR levels, occurrence of cardiovascular, and cerebro-vascular (CCV) events and assumed drugs were recorded.

To assess the genetic variants included in the IWPC model (CYP2C9*2 [rs1799853], CYP2C9*3 [rs1057910] and VKORC1–1639G>A [rs9923231]), peripheral blood samples (4–5 ml) were collected in BD Vacutainers, containing EDTA as anticoagulant and stored at temperature of −80 °C, or −20 °C, for a period of maximum 3 months before shipping (in dry ice, to prevent defrost) to the central laboratory in Salerno. Genomic DNA was extracted using E.Z.N.A.® Blood DNA Kit (Omega Bio-Tek). Quantification and quality analysis of DNA was performed using a NanoDrop 2000c spettrophotometer. A BeadXpress Reader using Illumina VeraCode GoldenGate Assay Kit was used for genotyping. A total of 500 ng of DNA was used per assay.

Outcomes

Consistently with the 2009 IWPC paper [6], the primary outcome was the percentage of patients whose dose of warfarin, predicted by IWPC algorithm, was within 20% of the actual stable maintenance dose. The stable dose provided by centers was used for analyses, as defined in the Section 2 of Supplementary Appendix 1 of IWPC original paper [6]. All doses are reported as weekly doses.

Clinical and laboratory secondary endpoints were also evaluated:

- overall incidence of CCV events either in the first year of warfarin treatment or in the first four weeks of warfarin treatment, defined as the occurrence of any one of death for any cause, hospitalization for CCV events, major bleeding, or thromboembolism;
- overall incidence of major bleeding in the first year of treatment, defined as the occurrence of fatal bleeding, or symptomatic bleeding in a critical area or organ, or bleeding causing a fall in hemoglobin level of ≥2 g/dL or leading to transfusion of two or more units of blood or red cells [38];
- overall incidence of thromboembolism in the first year of treatment, defined as the occurrence of cerebral infarction, or myocardial infarction, or peripheral arterial embolism;
- time to therapeutic INR, defined as the time of first achieving INR measurement within the individual's target range, providing that INR was also within the target range at the subsequent clinic visit;
- percentage time (%TIR) in the therapeutic INR range during the first 3 months of treatment;
- percentage time (%TIR) in the therapeutic INR range during the first four weeks of treatment.

As for patients lost at laboratory FU clinical information was retrieved through phone calls to patients' home or from administrative registry offices of the patients' towns of residence.

Sample size

Primary endpoint was the percentage of subjects who had a predicted dose within a range of ±20% of the stable dose [6], and sample size was calculated to achieve a predefined precision of the estimate. Initially a precision of ±3% of the 95% confidence interval (CI) was desired and 1067 subjects were required. Eventually the recruitment rate was much lower, and the actual sample size lead to a precision of CI of about ±5% of the estimate.

Statistical analysis

All statistical analyses were performed using both the 2009 [6] and the 2012 [36] version of IWPC algorithm in the whole sample and separately by primary indication (medical or surgical).

Warfarin dose distributions were graphically depicted using the box-and-whiskers plots. A scatter diagram showed the relationship between the actual and predicted warfarin therapeutic doses. Differences of percentages between groups were evaluated by means of chi-square test or Fisher's exact test if needed. Mean differences between groups were tested with Student's t test or Mann–Whitney test when normality assumption was unmet. The accuracy of the IWPC prediction algorithm was evaluated using linear regression models with the actual dose as dependent variable and the predicted dose as independent variable. Validity of the predictive algorithm was confirmed when the composite null hypothesis $\alpha = 0$ (intercept) and $\beta = 1$ (slope) was not rejected at the two-tailed α level of 0.05. The determination coefficient (R^2) measured the proportion of total variation of the actual therapeutic dose explained by the predicted dose. As measures of accuracy we also calculated the mean prediction error (MPE), defined as the average of the differences between the predicted and the actual dose, and the mean absolute error (MAE), defined as the average of the absolute value (in the mathematical sense) of the difference between the predicted and the actual doses. MAE is usually reported as a measure of predictive accuracy, but rather is a measure of variability of the difference distribution, with a similar interpretation of root mean square error [28].

The clinical usefulness of the IWPC algorithm was further quantified by the percentages of correct identification within subgroups of increasing levels of the warfarin actual dose.

Statistical analyses were performed with R software version 3.0.1 (R Foundation for Statistical Computing).

Results

Three hundred and seventy-six patients were enrolled between March 2013 and May 2016 (142 with medical and 234 with surgical indication). Baseline patients' characteristics are shown in Table 1. All patients were Caucasian. Overall, most subjects (84%) had INR target between 2 and 3. However 55 patients (14%), almost exclusively with surgical indications, had target INR between 2.5 and 3.5. As expected most patients received a warfarin starting dose of 35 mg/week, but higher starting doses were also observed in medical patients. Relevant differences between medical and surgical subjects were found for age, NYHA class, comorbidity and INR target. The allelic frequencies of the CYP2C9*2, CYP2C9*3 and VKORC1-1639G>A SNPs were similar to those reported in other Caucasian populations [39, 40]. Comorbidity distribution as assessed by the CIRS index is reported in the Supplementary Table 2.

Stable maintenance dose was achieved in 283 (75%) patients without differences according to indication: 105/142 (74%) patients with medical indication and 178/234 (76%) with surgical indication. Overall characteristics of patients who achieved or did not achieve maintenance dose

Table 1 Baseline characteristics of study patients.

		Primary indication for warfarin treatment		
	Total ($n = 376$)	Medical ($n = 142$)	Surgical ($n = 234$)	*p*-value
Age, mean (SD), years	74.8 (6.3)	77.4 (7.2)	73.2 (5.1)	<0.001
Range	62.9–96.3	62.9–96.6	64.8–84.4	
≥80 years	76 (20.2%)	53 (37.3%)	23 (9.8%)	
Male gender	177 (47.1%)	62 (43.7%)	115 (49.2%)	0.302
Height, mean (SD) m	1.62 (0.1)	1.63 (0.1)	1.61 (0.1)	0.018
Weight, mean (SD) kg	72.4 (13.9)	73.3 (16.5)	71.9 (12.0)	0.36
Caucasian Race	376 (100%)	142 (100%)	234 (100%)	–
NYHA class				<0.001
I	33 (8.8%)	12 (8.5%)	21 (9.0%)	
II	226 (60.1%)	66 (46.5%)	160 (68.4%)	
III	102 (27.1%)	51 (35.9%)	51 (21.8%)	
IV	3 (0.8%)	1 (0.7%)	2 (0.9%)	
not evaluable	12 (3.2%)	12 (8.5%)	0 (0.0%)	
Previous CVV events				0.744
previous bleeding	1 (0.3%)	0 (0%)	1 (0.4%)	
cerebral infarction	21 (5.6%)	8 (5.6%)	13 (5.6%)	
myocardial infarction	37 (9.8%)	16 (11.3%)	21 (9.0%)	
peripheral arterial thrombosis	4 (1.1%)	1 (0.7%)	3 (1.3%)	
Comorbidity				
CIRS G, mean (SD)	1.8 (0.3)	1.9 (0.4)	1.7 (0.2)	<0.001
CIRS C, mean (SD)	3.2 (1.7)	3.9 (2.3)	2.9 (0.9)	<0.001
# Drugs besides warfarin median (IQR)	5 (4–7)	6 (4–7)	5 (4–6)	0.093
Amiodarone	110 (29%)	46 (32.4%)	64 (27.4%)	0.297
Carbamazepine	2 (0.5%)	1 (0.7%)	1 (0.7%)	0.721
Phenytoin	0 (0%)	0 (0%)	0 (0%)	–
Rifampicin	0 (0%)	0 (0%)	0 (0%)	–
CYP2C9				0.005
*1/*1	214 (56.9%)	79 (55.6%)	135 (57.7%)	
*1/*2	83 (22.1%)	28 (19.7%)	55 (23.5%)	
*1/*3	46 (12.2%)	14 (9.9%)	32 (13.7%)	
*2/*2	5 (1.3%)	4 (2.8%)	1 (0.4%)	
*2/*3	18 (4.8%)	11 (7.7%)	7 (3.0%)	
*3/*3	3 (0.8%)	0 (0%)	3 (1.3%)	
Unknown	7 (1.9%)	6 (4.2%)	1 (0.4%)	
VKORC1				0.07
GG	108 (28.7%)	41 (28.9%)	67 (28.6%)	
AG	178 (47.3%)	65 (45.8%)	113 (48.3%)	
AA	83 (22.1%)	30 (21.1%)	53 (22.7%)	
Unknown	7 (1.9%)	6 (4.2%)	1 (0.4%)	
INR target				<0.001
1.5–2	1 (0%)	0 (0%)	1 (0.4%)	
1.5–2.5	1 (0%)	0 (0%)	1 (0.4%)	
2–3	314 (84%)	135 (95.1%)	179 (76.5%)	
2–3.5	5 (1%)	5 (3.5%)	0 (0%)	
2.5–3.5	55 (15%)	2 (1.4%)	53 (22.7%)	
Baseline INR mean (SD)	1.15 (0.2)	1.03 (0.2)	1.22 (0.2)	<0.001
Warfarin starting dose (mg/week)			0.001	
<35	84 (22.3%)	36 (25.3%)	48 (20.5%)	
35	273 (72.6%)	89 (62.7%)	184 (78.6%)	
>35	19 (5.1%)	17 (12.0%)	2 (0.9%)	

NYHA New York Heart Association, *CCV* cardio- and cerebro-vascular, *CIRS* Cumulative Index Rating Scale, *CIRS G* average degree of severity score (range 1–5), *CIRS C* number of severe comorbidities (range 0–13), *INR* international normalized ratio, *SD* standard deviation, *IQR* interquartile range

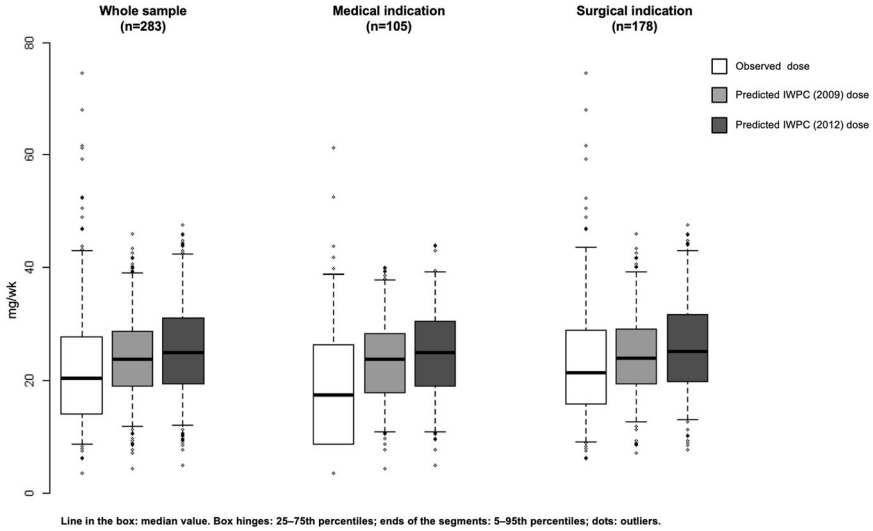

Fig. 1 Box-and-whiskers plots of the actual and IWPC predicted doses, by primary indication.

were similar (Supplementary Table 3), even though the latter ones were slightly older (76.5 vs 74.2 years on average) and had a higher prevalence of previous myocardial infarction (15.1% vs 8.1%). Twenty out of 93 (21.5%) patients, who did not achieve maintenance dose, discontinued warfarin treatment to move to DOAC or other anticoagulant, while 25/93 (26.9%) withdrew consent, possibly for the same reason. Consistently patients who did not achieve stable dose stayed less on warfarin therapy.

Distributions of warfarin maintenance doses reported by centers and predicted by the IWPC algorithm, using both the original version of 2009 [6] and the updated version of 2012 [36], are reported in Fig. 1. Median actual dose was equal to 20.3 mg/week (interquartile range, IQR, 14.1–27.7), much lower than the starting dose of 35 mg/week; median values in patients with medical and surgical indications were 17.5 mg/week (IQR 8.8–26.3) and 21.4 mg/week (IQR 15.9–28.9), respectively. On average, the IWPC 2009 predicted doses were higher than the actual therapeutic doses: overall median indeed was equal to 23.8 mg/week (IQR 19.0–28.7), and median values in patients with medical and surgical indication were 23.8 mg/week (IQR 17.8–28.2) and 23.9 mg/week (IQR 19.3–29.2), respectively. The analogous values predicted by IWPC 2012 algorithm were still higher.

The relationship between actual and predicted doses according to the 2009 (upper panel) and 2012 (lower panel) version of the algorithm is shown in Fig. 2. In the whole sample (Fig. 2, left panel) both intercept and regression coefficients were significantly far from the null values, and R^2 was as small as 0.31. In medical patients (Fig. 2, middle panel) fitted values diverged strongly from the perfect prediction with very low R^2 value (0.15 and 0.17), while a slightly better fitting was found in surgical patients (Fig. 2, right panel), with R^2 values being equal to 0.41 and 0.39. In the whole sample the MPE was equal to 1.8 and 3.3 mg/week with the 2009 and 2012 version of the algorithm, respectively, slightly lower than corresponding median values (2.3 and 3.7 mg/week). Overprediction bias was greater in medical than in surgical subjects; MPE indeed was equal to 3.4 mg/week and 0.9 mg/week in medical and surgical patients, respectively, with IWPC 2009. Overprediction increased further with IWPC 2012, MPE being equal to 4.7 and 2.5 mg/week in medical and surgical patients, respectively. The MAE was equal to 7.64 mg/week in the whole sample and to 9.31 and 6.65 mg/week in medical and surgical patients, respectively, with IWPC 2009, and increased further with IWPC 2012.

The percentage of patients whose dose of warfarin, predicted by IWPC 2009 algorithm, was within the 20% of the actual dose was equal to 37.5%, (95% CI 32.0–43.3%), better in surgical (43.3%, 95% CI 36.1–50.7%) than in medical patients (27.6%, 95% CI 19.8–37.1%) (Table 2). Overprediction was always 2–3 times higher than underprediction. Similar results were found with IWPC 2012.

A deeper investigation of the ability of the pharmacogenetic algorithm to correctly identify patients requiring lower or higher therapeutic dose is reported in Table 3. The IWPC algorithm largely overestimated the dose in patients requiring very small doses, while underestimated the dose in the few patients requiring large doses. Results did not substantially change when analyses were repeated with the 2012 version of the IWPC algorithm (data not shown).

The occurrence of clinical and laboratory secondary outcomes in all patients is reported in Table 4. Forty-two (11.2%) cardio- and cerebro-vascular (CCV) events were observed in the first year of treatment, 14 (3.7%) of which in the first 4 weeks of treatment. The risk of bleeding was higher than the risk of thromboembolism (3.7% and 2.4%, respectively). Median time to first INR in target was nearly double for medical patients (12 days) with respect to

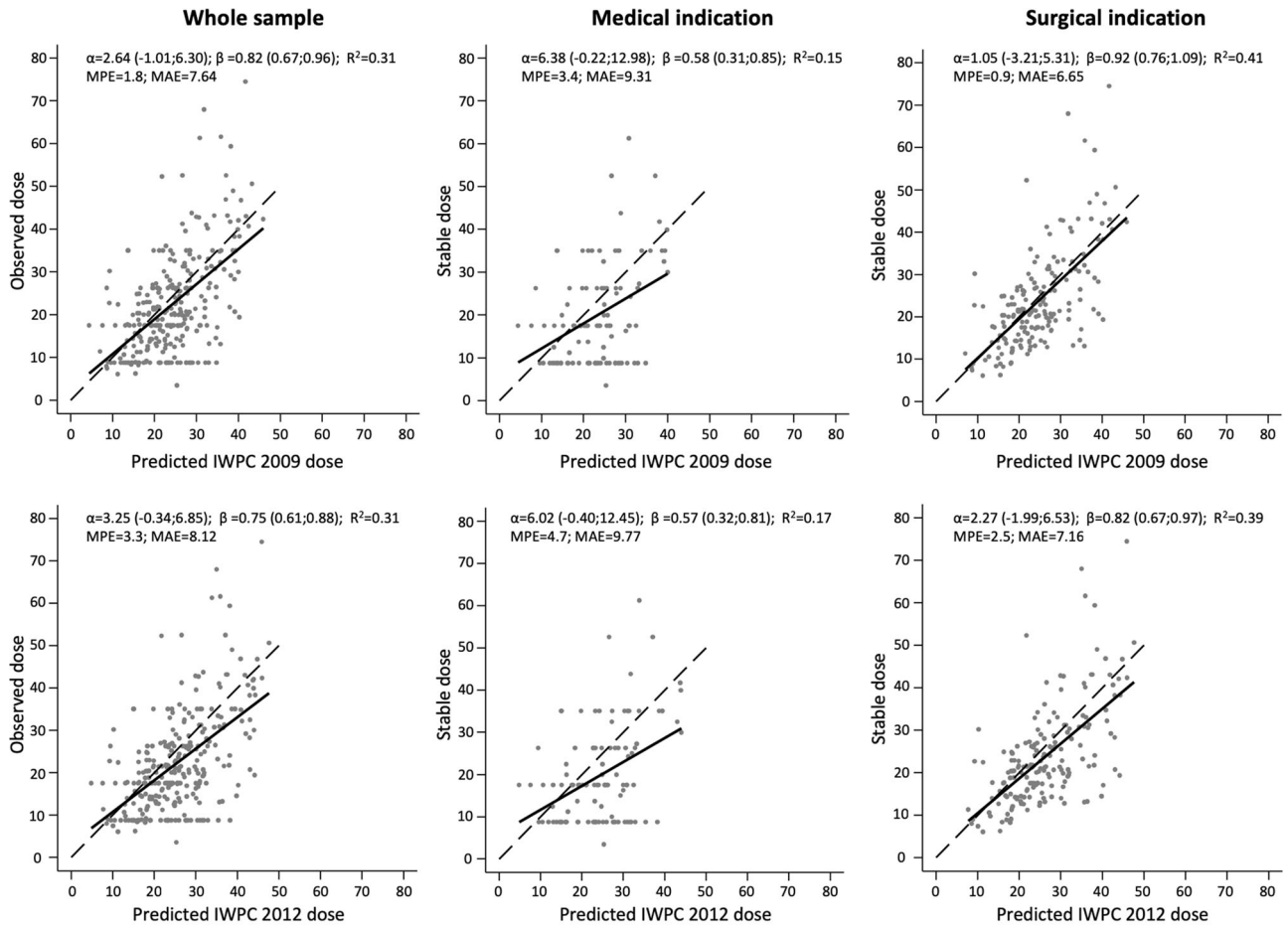

Fig. 2 Scatterplots of the actual and IWPC predicted doses, by primary indication.

Table 2 Accuracy of IWPC prediction with a relative error[a] threshold of 20%, by primary indication.

	Total (95% CI) $n = 283$	Medical indication (95% CI) $n = 105$	Surgical indication (95% CI) $n = 178$
IWPC 2009			
>20% under the actual dose	19.4% (15.2–24.5%)	21.0% (14.1–30.0%)	18.5% (13.4–25.0%)
Within 20% of the actual dose	37.5% (32.0–43.3%)	27.6% (19.8–37.1%)	43.3% (36.1–50.7%)
>20% over the actual dose	43.1% (37.4–49.0%)	51.4% (41.8–61.0%)	38.2% (31.3–45.6%)
IWPC 2012			
>20% under the actual dose	14.1% (10.5–18.7%)	16.2% (10.3–24.6%)	12.9% (8.7–18.7%)
Within 20% of the actual dose	38.9% (33.3–44.7%)	27.6% (19.9–37.0%)	45.5% (38.3–52.9%)
>20% over the actual dose	47.0% (41.2–52.9%)	56.2% (46.5–65.4%)	41.6% (34.5–49.0%)

[a]Relative error = (predicted dose − actual dose)/actual dose

surgical subjects (7 days). Overall, the percentage time in the therapeutic INR range was 52% in the first 3 months and 39% in the first 4 weeks of therapy, larger for medical than for surgical patients. About a third of subjects experienced at least one INR value ≥4.

Discussion

To our knowledge VIALE study is the first prospective study that assessed the predictive accuracy of the IWPC algorithm in a cohort of high-risk elderly people with

Table 3 Accuracy of IWPC 2009 predicted doses according to increasing levels of warfarin actual dose in the whole sample (A) and separately by medical (B) and surgical (C) indication. Absolute figures (%) are reported.

(A) Whole sample

IWPC predicted dose (mg/week)	Actual dose (mg/week)						
	(0–14) ($n = 71$)	(14–21) ($n = 79$)	(21–28) ($n = 62$)	(28–35) ($n = 28$)	(35–41) ($n = 20$)	≥ 41 ($n = 23$)	Not available ($n = 93$)
(0–14)	**16 (22.5)**	5 (6.3)	3 (4.8)	1 (3.6)	2 (10.0)	0 (0.0)	13 (14.0)
(14–21)	23 (32.4)	**30 (38.0)**	20 (32.3)	0 (0.0)	1 (5.0)	0 (0.0)	26 (28.0)
(21–28)	23 (32.4)	33 (41.8)	**28 (45.2)**	10 (35.7)	6 (30.0)	3 (13.0)	29 (31.2)
(28–35)	8 (11.3)	8 (10.1)	10 (16.1)	**10 (35.7)**	4 (20.0)	7 (30.4)	17 (18.3)
(35–41)	1 (1.4)	3 (3.8)	1 (1.6)	7 (25.0)	**6 (30.0)**	9 (39.1)	4 (4.3)
≥41	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	**4 (17.4)**	4 (4.3)

(B) Medical indication

IWPC predicted dose (mg/week)	Actual dose (mg/week)						
	(0–14) ($n = 39$)	(14–21) ($n = 28$)	(21–28) ($n = 16$)	(28–35) ($n = 3$)	(35–41) ($n = 14$)	≥41 ($n = 5$)	Not available ($n = 37$)
(0–14)	**8 (20.5)**	4 (14.3)	1 (6.3)	0 (0.0)	2 (14.3)	0 (0.0)	6 (16.2)
(14–21)	8 (20.5)	**11 (39.3)**	4 (25.0)	1 (33.3)	1 (7.1)	0 (0.0)	11 (29.7)
(21–28)	16 (41.0)	9 (32.1)	**7 (43.8)**	0 (0.0)	4 (28.6)	1 (20.0)	10 (27.0)
(28–35)	7 (18.0)	4 (14.3)	4 (25.0)	**2 (66.7)**	3 (21.4)	2 (40.0)	6 (16.2)
(35–41)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**4 (28.6)**	2 (40.0)	1 (2.7)
≥41	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**	3 (8.1)

(C) Surgical indication

IWPC predicted dose (mg/week)	Actual dose (mg/week)						
	(0–14) ($n = 32$)	(14–21) ($n = 51$)	(21–28) ($n = 46$)	(28–35) ($n = 25$)	(35–41) ($n = 6$)	≥41 ($n = 18$)	Not available ($n = 56$)
(0–14)	**8 (25.0)**	1 (2.0)	2 (4.4)	1 (4.0)	0 (0.0)	0 (0.0)	7 (12.5)
(14–21)	15 (46.9)	**19 (37.3)**	16 (34.8)	0 (0.0)	0 (0.0)	0 (0.0)	15 (26.8)
(21–28)	7 (21.9)	24 (47.1)	**21 (45.7)**	9 (36.0)	2 (33.3)	2 (11.1)	19 (33.9)
(28–35)	1 (3.1)	4 (7.8)	6 (13.0)	**10 (40.0)**	1 (16.7)	5 (27.8)	11 (19.6)
(35–41)	1 (3.1)	3 (5.9)	1 (2.2)	5 (20.0)	**2 (33.3)**	7 (38.9)	3 (5.4)
≥41	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	**4 (22.2)**	1 (1.8)

Data are reported in bold format when the level of predicted doses and actual dose are the same, that correspond to a situation of perfect agreement

Table 4 Clinical and laboratory secondary outcomes, by primary indication, in all patients.

	Total ($n = 376$)	Medical ($n = 142$)	Surgical ($n = 234$)
Cardio- and cerebro-vascular (CCV) outcomes			
CCV events in the first year of treatment	42 (11.2%)	21 (14.8%)	21 (9.0%)
Overall incidence of major bleeding	14 (3.7%)	5 (3.5%)	9 (3.8%)
Overall incidence of thromboembolism	9 (2.4%)	4 (2.8%)	5 (2.1%)
Death	20 (5.3%)	11 (7.8%)	9 (3.9%)
Hospitalization without major bleeding or thromboembolism	9 (2.4%)	6 (4.2%)	3 (1.3%)
CCV events in the first 4 weeks of treatment	14 (3.7%)	4 (2.8%)	10 (4.3%)
Overall incidence of minor bleedings	16 (4.3%)	11 (7.8%)	5 (2.1%)
Laboratory outcomes			
Time to therapeutic INR, *median (IQR), days*	10 (4–27)	12 (7–26)	7 (3–28)
% time in therapeutic INR range during the first three months of therapy, *median (IQR)*	65.0 (39.1–81.1)	73.7 (47.7–87.7)	59.2 (34.1–76.7)
% time in therapeutic INR range during the first 4 weeks of treatment, *median (IQR)*	50.0 (17.9–71.4)	46.4 (10.7–71.4)	50.0 (21.8–71.4)
Number of patients with at least one INR ≥ 4, *n* (%)	124 (33.0%)	39 (27.5%)	85 (36.3%)

comorbidity. In such a vulnerable population maintenance doses of warfarin were very low on average, particularly in medical subjects, and the IWPC algorithm substantially overestimated them. Only a third of patients had a predicted dose within a relative error of 20%. Further the pharmacogenetic algorithm only explained 30–40% of the warfarin dose variability (R^2), and this value declined to only 15% in medical patients. Results did not change substantially when the 2012 version of the IWPC algorithm was used.

Anticoagulant doses were particularly low in medical subjects possibly as a consequence of the reduced metabolic clearance in elderly people [41] and prudence of doctors in the management of high-risk elderly patients. Conversely, higher doses were found in patients with surgical indications, leading to a slightly improved prediction. In these subjects higher doses might be expected as a consequence of higher target INR, but we cannot exclude that more accurate choice of surgical patients could have induced a selection of patients with less comorbidities.

In our study a quarter of subjects were unable to achieve a stable maintenance dose without differences between medical and surgical patients. As usually in the literature we did not take them into account when evaluating the predictive accuracy of the algorithm, but, from a clinical viewpoint they should contribute to get it worse. These percentages are lower than other figures previously reported in the literature [42–44]. Chappell et al. [42, 43] found that nearly 50% of subjects were unable to achieve the defined INR target during titration to a stable warfarin dose, whereas in Roberts et al. [44] about 55% of patients were ineligible due to maintenance INR outside the acceptable range. Different definitions of stable maintenance dose, characteristics of population, and setting of recruitment could explain discrepancies, that could heavily affect the clinical usefulness of pharmacogenetic algorithms.

In the only previous study that specifically addressed the predictive accuracy of the IWPC algorithm in elderly people, the algorithm also failed to identify older patients requiring low daily doses of warfarin; however, this study included a small number of subjects and in principle was prone to selection bias because of the retrospective design [12]. Most of the studies that validated the IWPC algorithm did not have age limits, and either excluded patients with comorbidity or did not report at all this information (Table 5). Moreover, almost all studies had a retrospective design and included only patients who achieved stable dose with possible selection biases.

Yan et al. [45] also report that more than 86% of patients receiving lower doses of warfarin (<13.16 mg/week) were overpredicted by the algorithm, while Saffian et al. [35] showed a systematic underprediction in patients requiring higher than average doses. The pharmacogenetic algorithm indeed predicts quite accurately warfarin dosing requirements on average, but possibly both higher- and lower-dose patients do not benefit substantially from this information. This is elucidated by the low percentage of warfarin dose variation explained by the model that in our study was as low as 31% and declined to a very low value of 15% in medical patients. Since published pharmacogenetic algorithms mainly include variables associated to a reduction in warfarin dose possible improvements of predictive accuracy in high-risk elderly people might be attained either encompassing comorbidity information into the algorithm or evaluating more complex non-linear models.

Our clinical findings were in line with literature results. Overall incidence of major bleedings was 3.7%, similar to the value of 3.5% reported by Burmester et al. [46] in relation to standard clinical warfarin therapy management. On the other hand, our incidence of thromboembolism was slightly higher than 2% reported by Verhoef et al. [47] in elderly subjects with atrial fibrillation. In purely surgical series, the rate of heart valve prosthesis-related thromboembolism ranged between 0 and 4 per 100 patients/year, whereas the rate of hemorrhagic events ranged between 0.2 and 7.2 per 100 patients/year [48].

Our study has several strengths. To our knowledge, it is the first prospective trial that attempted to externally validate the IWPC algorithm on vulnerable elderly people (≥65 years) only. Secondly, only incident cases were prospectively recruited, and we were able to identify patients unable to achieve dose stability, who are typically excluded from retrospective studies. Thirdly, we included patients with both medical and/or surgical indications in the attempt to provide a more complete picture of warfarin use in elderly population. Lastly, to our knowledge, this is the first study that assessed generalizability of the 2012 version of the IWPC algorithm.

Our study only included Caucasian subjects, although the predicted dose should not be affected, since the IWPC algorithm entails different coefficients for non Caucasian people. A further limitation of our study is sample size, that was smaller than the planned one; accordingly the precision of our estimates was reduced from the planned ±3% to about ±5%. However our study still remains larger than most previous trials, and our estimate was quite as precise as that observed in the original paper [6]. The reduced recruitment was mainly due to the increasing use of DOAC in clinical practice, which prevented the recruitment of elderly warfarin-naive patients with medical indication and favored withdrawal from the study. Sample size was further reduced because of subjects who did not achieve the maintenance dose. About half of them withdrew from the study, likely to move from warfarin to DOAC or other anticoagulant; another possible explanation could be the clinical complexity of the study population. Unfortunately, almost none of the studies reported the percentage of unstable patients thus interpretation is difficult.

Table 5 Studies who externally validated the IWPC algorithm.

Author [Ref]	Year	Country	Study Design	Warfarin Indication	Severe comorbities	INR target	Total sample	Age, mean (sd)	Male gender (%)	Mean or *Median* Stable Dose (mg/wk)	Predictive accuracy		Clinical agreement		
											R^2	MAE or *MPE* (mg/week)	Under 20%	Within 20%	Over 20%
Roper [7]	2010	USA	R	AF, HVR, others	Excluded	02-Mar	125	70.0 (12.5)	61.6	33.4	0.65	7.1	29.00%	53.00%	18.00%
Sagreiya [8]	2010	USA	R	NA	NA	02-Mar	104	67 (53–75.3)[a]	58	*32.5*[b]	0.46	8.1	–	–	–
Shaw [9]	2010	USA	R	AF, others	NA	02-Mar	71	60.0 (15.0)	45	42.7	0.65	12.6	–	–	–
Takeuchi [10]	2010	Japan	R	AF, HVR, others	NA	1.6–2.6	200	67.8 (10.3)	68	21.4	0.28	NA	–	–	–
Cho [11]	2011	Korea	R	AF	NA	02-Mar	108	67.4 (10.1)	64	*26.6*[b]	0.48	NA	57.40%	39.80%	2.80%
Schwartz [12]	2011	USA	R	AF, HVR, others	Included	NA	69	81.4 (8.3)	46	23.1	0.5	NA	–	–	–
Zambon [13]	2011	Italy	R	AF, others	Partially excluded	02-Mar	274	73.9 (38.9–91.7)[c]	65	*28.8*[b]	0.63	7.7	–	–	–
Bazan [14]	2012	Egypt	NA	AF, HVR, others	NA	2–3.5	63	45.9 (12.5)	49.2	51.5	0.42	22.4	57.10%	25.40%	17.50%
Cini [15]	2012	Italy	R	HVR, others	Included	2–3.5	40	70.0 (16.0)	57.5	32.2	0.47	8.5	–	–	–
Marin-Leblanc [16]	2012	Canada	R	AF, HVR, others	Partially excluded	2–3.5	605	66.6 (10.7)	68.4	36.3	0.43	10.6	45.60%	39.00%	15.40%
Pathare [17]	2012	Oman	P	AF, HVR, others	Excluded	02-Mar	212	49.8 (18.1)	53.3	*33.3*[b]	0.34	NA	–	–	–
Ramirez [18]	2012	USA	R	AF, others	NA	02-Mar	1167	66.0 (35.0–87.0)[d]	55.3	*35*[b]	NA	10	–	–	–
Ramos [19]	2012	USA	R	AF, others	Included	2–3.5	55	60.7 (14.4)	36	35	0.21	11	–	–	–
Tan [20]	2012	China	P	HVR	Excluded	1.7–3.0	320	47.1 (10.9)	39.4	22.3	NA	*1.96*†	8.80%	54.40%	36.90%
Ekladious [21]	2013	Egypt	NA	AF, HVR, others	Excluded	02-Mar	35	NA	NA	NA	0.09	NA	–	–	–
Bosch [22]	2014	Puerto Rico	R	AF	Excluded	2–3.5	138	68.0 (9.2)	100	NA	NA	11.2	32.10%	26.70%	41.20%
Hernandez [23]	2014	USA	R	AF	NA	NA	149	59.2 (16.3)	52.7	46.9	0.15	12.2	–	48.80%	–
Pavani [24]	2014	India	R	AF, HVR	NA	2–3.5	125	38.4 (14.8)	52.8	NA	0.08	NA	–	–	–
Zhao [25]	2014	China	P	HVR	Excluded	1.5–2.5	122	50.3 (9.8)	31.2	17.4	0.31	3.7	3.00%	49.00%	48.00%
Karaca [26]	2015	Turkey	R	AF, HVR, others	Included	NA	97	61.5 (12.6)	48.5	31.4	0.34	11.9	–	33.00%	–
Peng [27]	2015	China	NA	HVR	NA	02-Mar	586	52.1 (11.0)	44.9	19.5	0.35	6.2	6.10%	40.60%	53.20%
Saffian [28]	2015	New Zealand	P	AF, others	NA	02-Mar	46	62.0 (29.0–87.0)[c]	41.3	*38.5*[b]	NA	*−5.0*†	–	–	–
Santos [29]	2015	Brazil	R	AF, HVR, others	Excluded	02-Mar	133	63.0 (17.0)	56.4	NA	0.51	NA	–	–	–
Xu [30]	2015	China	P	HVR	Excluded	1.8–2.5	59	NA	NA	24.2	NA	5.4	15.30%	55.90%	28.80%
Cho [31]	2016	Asia	R	AF	NA	1.5–3	101	63.6 (13.4)	63	26.2	0.37	NA	–	–	–
Duconge [32]	2016	USA	R	AF, others	Included	2–3.5	55	60.7 (14.4)	36	35	0.39	11.2	–	–	–
Lin [33]	2016	China	R	HVR	Excluded	1.5–2.5	208	NA	NA	NA	0.27	5.2	13.50%	48.60%	38.00%
Stack [34]	2016	USA	R	AF, others	NA	NA	146	72.5 (10.5)	100	*29.4*[b]	NA	6.9	–	54.80%	–
VIALE study		Italy	P	AF, HVR	Included	1.5–3.5	283	74.2 (6.2)	48.8	*22.4*[b]	0.31	7.6 *1.8*†	19.40%	37.50%	43.10%

R retrospective, *P* prospective, *AF* atrial fibrillation, *HVR* heart valve replacement, *NA* not available

[a]Data is reported as median (IQR)

[b]Median value

[c]Data is reported as median (range)

[d]Data is reported as median (5–95%)

†MPE

The IWPC algorithm overpredicted warfarin maintenance doses in high-risk elderly people, mainly in patients with medical indication. More tailored models, possibly including comorbidity information, are to be evaluated in such a vulnerable population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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