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Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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Authors' Contributions: TM originated the study concept. BSJ did the research which TM supervised. Both contributed to interpretation of data and manuscript writing.

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No additional data is available

ABSTRACT

Background

People infected with the human immunodeficiency virus (HIV) are prone to venous thrombosis. Treatment of thrombosis is primarily with warfarin. No studies have addressed the effects of HIV infection on warfarin dose. The aims of this study were to determine whether the therapeutic dose of warfarin and induction time to therapeutic dose in HIV-infected differ from that in HIV-uninfected patients.

Methods

A prospective and retrospective cohort study combined with a cross-sectional study of induction time to therapeutic warfarin dose, and similarly a retrospective study of ambulant therapeutic warfarin dose were performed. HIV-infected and HIV-uninfected patients being treated after deep venous thrombosis with or without pulmonary embolism were compared. Gender and use of antiretroviral drugs (ARVs) were also compared in the groups.

Results

Two hundred and thirty-four patients were entered into the study. The mean therapeutic dose of warfarin was higher in the HIV-infected than the HIV-uninfected group: 6.06 vs 5.72 mg/day, but this was not statistically significant ($p = 0.29$). The difference was mainly due to higher warfarin doses in HIV-infected female patients and to female patients on ARV therapy. Induction time to therapeutic warfarin dose did not differ between the two groups.

Conclusion

There appears to be little effect of HIV infection on warfarin dosing. Warfarin therapy should be administered conventionally in HIV-infected patients.

Summary

Strengths and limitations of the study.

- This is a unique study which investigates the effect of HIV infection on warfarin treatment.
- It is a clinical study of actual management of HIV-infected patients with venous thrombosis.
- For this reason the study deliberately does not investigate the effect of confounding factors affecting warfarin dosage.
- The authors did not have control over the management of the patients in the retrospective part of the study.
- While the statistical analysis is cogent, a sample size was not predetermined.

Introduction

There is a high incidence of venous thrombosis in patients infected with the human immunodeficiency virus (HIV). The risk is up to 10 times that in uninfected people. [1] The aetiology of thrombosis is multifactorial and includes HIV-mediated endothelium activation, malignancies and infections. [2] The prothrombotic state of HIV infection is also well-recognised and several laboratory coagulation parameters have been shown to be abnormal in these patients. [3, 4] While the exact mechanism of this state remains to be fully elucidated, many coagulation factors abnormalities have been reported, notably protein C, protein S and antithrombin deficiency.[3] Patients who have been treated for thrombosis may require long-term anticoagulation primarily with warfarin because the risk of thrombosis in HIV-infected thrombotic patients is not necessarily abolished by antiretroviral treatment and improvement of the CD4 count. [3,5] Antiretroviral (ARV) drugs *per se* may also contribute to the hypercoagulable state.[6] Additionally, these drugs have variable effects on warfarin blood levels and therefore may affect management of thrombosis in HIV-infected patients. [3]

While there have been many publications on the effect of ARV drugs on warfarin dose, [3,5,7] the effect of the HIV infection on warfarin dose has not been investigated. It has been our impression that HIV-infected patients require higher warfarin doses than non-infected patients to achieve therapeutic anticoagulation. Anecdotally longer therapeutic induction times have been observed and this could be due to higher warfarin requirements. We hypothesised that patients with HIV infection require higher doses of warfarin for induction and maintenance anticoagulation.

Aim

The aims of the study were:

1. To determine whether the therapeutic dose of warfarin differed between HIV-infected and HIV-uninfected patients.

2. To determine whether the induction time to therapeutic warfarin dose differed between HIV-infected and -uninfected patients.
3. Additionally, the study aimed to detect any difference in therapeutic warfarin dose between HIV-infected males and females.

The objective of the study was to compare these parameters in a group of HIV-infected and a group of HIV-uninfected patients.

Methods

Cohort and cross-sectional studies of patients with venous thrombo-embolism was undertaken. Patients aged 18 years and older who had presented with apparently spontaneous lower limb deep venous thrombosis, with or without pulmonary embolism, were included in the study. Patients with attributable causes of thrombosis such as recent surgery, cardiac failure and thrombophilias were excluded. Patients who refused HIV testing were not entered in the study. Confirmation of DVT was by Doppler ultrasound and of pulmonary embolism by CT angiography as indicated. ARV drug treatment as well as the specific drugs were also recorded. Comparisons of the mean daily warfarin dose and induction time to therapeutic warfarin dose were performed in HIV-infected and HIV-uninfected patients, in males and females, and in HIV-infected patients on and not on ARV drug therapy. The use of ARVs was recorded and analysed as a group of confounders but individual drugs were not analysed.

The study was performed in two parts.

Part 1. Prospective and retrospective study of *Induction time to stable therapeutic warfarin dosage.*

Warfarin dose data of patients treated for deep vein thrombosis (DVT) with or without pulmonary embolism whose HIV status was known were collected, either prospectively or retrospectively.

- Prospective collection (Cohort study)

  All patients admitted to hospital with acute DVT of the lower limb with or without pulmonary embolism were subjected to a consented HIV test and entered in the study. Warfarin dose data was collected during hospitalisation and at follow-up. Clinic visits were scheduled every 2-4 weeks after discharge from hospital. The cohort was observed until the attainment of therapeutic warfarin dose, and the number of days recorded.

- Retrospective collection (Cross-sectional study)

  The records of patients already attending follow-up for venous thromboembolism were studied retrospectively for therapeutic warfarin dose induction time in days. Only patients who attended clinics regularly until the therapeutic dose was achieved were included. The HIV status was determined from hospital records.

The induction time to therapeutic warfarin dose was defined as the time in days to achieve an International Normalised Ratio (INR) measurement of between 2 and 3. The number of days was calculated as the number of days from the start of warfarin therapy until the day of stable therapeutic dose (the first day of consecutive identical doses). Arithmetic means of the number of days were calculated for groups of patients.

Part 2. ***Retrospective study of ambulant therapeutic warfarin dosage in patients with known HIV status.*** **(Cross-sectional study)**

Warfarin dose data was gathered retrospectively from 2 follow-up clinics for ambulatory patients after a thrombo-embolic episode. One clinic was for general anticoagulation control (HIV-uninfected patients), the other was for general HIV-infected patients for AIDS follow-up (HIV-infected patients).

The latter patients were included irrespective of the duration of HIV infection. The last warfarin dose recorded after dosage stabilisation at the INR target range of 2 – 3 had been achieved, was recorded as the therapeutic daily dose. If the dose differed on alternate days the average of 2 days dosages was recorded.

Warfarin dosage.

The same regimen was used in both parts of the study. Warfarin was commenced at 5mg per day and followed by a modification of the slow Fennerty regimen, using dose adjustments of 2.5mg.[8] The INR was determined every 2-3 days after initiation or change of treatment dose, while maintaining anticoagulation with a low molecular weight heparin until therapeutic INR level was obtained. The target INR for ambulant warfarin therapy was 2.5, with a range of 2 to 3. Arithmetical means of warfarin dose were calculated for each of the groups described above.

The study was conducted at the Pretoria Academic Health Complex consisting of the Steve Biko Academic Hospital and Kalafong Regional Hospital, as well as the Tshwane District Hospital, during the period 2013-2015. Approval to conduct the study was granted by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria.

The study is reported in accordance with the STROBE criteria.[9]

Statistical Analysis

The 2 sample t-test of the comparison of means of warfarin dose was performed in the following groups: HIV-infected and HIV-uninfected patients, HIV-infected patients on and not on ARV drugs, and males and females in these groups. Gender and ARV drug use were analysed as possible confounders. Induction times for the groups to attain therapeutic warfarin doses were analysed by the Kruskal-Wallis test. The study was considered to be exploratory in nature. Because the

difference in warfarin dose outcomes of the study groups was expected to be small, sample size and size effect were not predetermined as a large number of patients would be required. A p-value of 0.05 was taken to be significant.

Results

Part 1: Induction time to therapeutic warfarin dose.

Induction times were analysed in 170 patients, 74 of whom were HIV-infected and 96 HIV-uninfected. The mean times were 12.87 and 11.19 days respectively, p=0.28. Thirty-six of the HIV-infected patients were on ARV drugs. Their induction times did not differ significantly from that of the HIV-infected patients not on ARV drugs or the HIV-uninfected patients (Figure 1). It must be noted that therapeutic induction times exhibited a wide range of standard deviation.

Part 2: Stable ambulant warfarin dose

One hundred and twenty-two HIV-uninfected patients (42 males and 80 females) and 112 HIV-infected patients (44 males and 68 females) with total of 234, were entered in this part of the study which included the patients in part 1 (Table 1). The table also depicts patients' gender and the CD4 cell counts of the HIV-infected patients. The mean therapeutic dose of warfarin in the whole set of patients was higher in the HIV-infected group than the HIV-uninfected group: 6.06mg/day vs 5.72mg/day (Table2a) which was, however, not statistically significant (p=0.29). Females (HIV-infected 5.84 mg/day vs HIV-uninfected 5.31mg/day, p = 0.14), were the greater contributors to this higher warfarin requirement than males (HIV-infected 6.39mg/day vs HIV-uninfected 6.49mg/day, p = 0.87).

Fifty HIV-infected patients on ARV medication were taking several ARV drugs in various combinations. The drugs and mean dosages used in this study are listed according to gender in table 3. There was no statistically significant difference in the mean therapeutic dose of warfarin between HIV-infected patients taking and not taking ARVs, 6.20 v 5.94 mg, p=0.59 (Table 2b). There was also no significant difference in warfarin dose between HIV-infected patients not taking ARVs and HIV-uninfected patients, 5.94mg/day vs 5.72mg/day, p=0.61 (Table 2c). However, HIV-infected females on ARVs required significantly more warfarin, 6.47mg/d than HIV-uninfected females, 5.31mg/d, p=0.01. The opposite was true for males, but the difference was not statistically significant, 5.86 vs 6.49, p=0.29.

The analysis of warfarin doses in males and females is shown in the tables (vertical axes). Males required slightly more warfarin than females whether they were HIV-infected or not. HIV-infected females on ARV drugs tended to require higher doses of warfarin than those not on ARV drugs, 6.47mg/day vs 5.40mg/day, p = 0.06 (Table 2b).

Discussion

We report on a comparative study of warfarin dosage in HIV-infected and -uninfected patients. This comparison has not been published previously. The assumption that HIV-infected patients require higher doses of warfarin for therapeutic effect seems to be reasonable given the acknowledged hypercoagulable state of the HIV-infected patient. Anecdotal observation in our practice suggested that this is indeed the case. Previous warfarin dosage studies in HIV-infected patients have largely addressed the effect of ARV drugs and not the HIV infection itself on warfarin dose. [3,5,7] This study attempted to determine the latter. However, no apparent increase in warfarin requirements for anticoagulation was demonstrated in HIV-infected patients compared to uninfected patients in general, except in females on ARV therapy.

There is a paucity of clinical studies on the effect of infection in general on warfarin dosage. Schelleman et al addressed this question indirectly in a review of published studies, by determining the risk of gastrointestinal bleeding in warfarin-treated patients receiving cephalexin and amoxicillin for common acute infectious conditions.[10] They found an increased risk of bleeding in the presence of these two drugs that do not produce significant interactions with warfarin. Their findings suggest that the infections *per se* may increase the risk of bleeding and, by implication, reduce the required warfarin dose. In a cohort of HIV-uninfected patients Clarke et al found that the proportion of patients on warfarin therapy with INR readings above 5 was significantly greater in those who were being treated for upper respiratory infection than the proportion of healthy control subjects, and that this effect was amplified by the use of antibiotics.[11] This study also indicated that patients with infection required less warfarin for anticoagulation. The effect of HIV infection on warfarin dosage has not been similarly studied. Infection with HIV apparently causes a propensity for thrombosis, as recovery of the CD4 count does not abolish the increased risk of thrombosis.[1, 3, 5] The current study attempted to determine indirectly the effect of the HIV infection *per se* on warfarin requirement by comparison of the dosage with that in HIV-uninfected patients. Warfarin doses were found not to differ between these two broad groups. This contrasts with the available findings quoted above, in which warfarin requirements are reduced during acute infectious episodes.

Clinical management of warfarin therapy is difficult because of inter- and intra-individual variability of dosage requirements. [12, 13] The narrow therapeutic window for warfarin dosage makes accurate dosing important. Many patient and environmental factors influence warfarin dosing. These include sex, race, height, weight and age. [12, 13] The well-known increased warfarin requirements for men are also apparent in this study. [14]

Warfarin exerts its effect on coagulation by inhibiting the vitamin K epoxide reductase (VKOR) enzyme system in the liver. [12] The latter is responsible for the essential reduction of oxidised vitamin K which is necessary for the synthesis of coagulation factors II, VII, IX and X. However, vitamin K is also necessary for the synthesis of the anticoagulation factors protein C and protein S which are depressed in HIV-infected patients. It might therefore be expected that these patients would require higher doses of warfarin to override the resultant additional tendency to coagulation due to proteins C and S deficiency. This was indeed our impression because of perceived apparent longer induction times in the HIV-infected patients. However, results from this study showed that the induction times did not in fact differ significantly between HIV-infected and -uninfected patients. Similarly, the analysis of stable warfarin doses at follow-up clinics did not reveal a statistically significant difference in warfarin requirements between the HIV-infected and HIV-uninfected groups.

Warfarin dose effects of antiretroviral drugs in HIV-infected patients have been investigated by several authors. ARV drugs have variable effects on warfarin blood levels, some causing an increase in levels and some a decrease. Jong et al demonstrated lower levels of von Willebrand factor, factor VIII, D-dimer and endogenous thrombin potential in HIV-infected patients on ARV drugs. [3] Anderson et al found that patients on efavirenz-based regimens required lower weekly warfarin doses than patients on lopinavir / ritonavir regimens. [15] The effects of the individual ARV drugs were not analysed separately in this study. Patients were taking several ARV drugs singly or in various combinations. The sample size of patients on each drug was too small to meaningfully perform separate analyses. An important comparison in this study, however, is that between the HIV-infected patients not taking ARV drugs and the HIV-uninfected patients. This comparison would discount the effects of the ARV drugs. While there was a modest difference in warfarin dosage, this did not differ significantly between these two groups (p=0.20, Table 2c).

The development of a warfarin dosing algorithm for HIV-infected persons would be difficult and complex because of the multiple influences that may be operating.[12] These include genetic factors, the hypercoaguable state, pertinent infections and malignancies, and the effect of multiple drugs on the cytochrome p450 2C9 system. These diverse effects on warfarin dosage are probably reflected in the wide range of standard deviation in this study shown in figure 1. However, the study addresses the broad group of HIV-infected patients, and does not take cognisance of the multiple factors that can affect warfarin dosing. We propose that this approach is valid because the study reflects clinical usage. Current practice in HIV-infected patients is to manage warfarin dosing as in HIV-uninfected patients, and this study would seem to support this custom. [5] Cognisance should be taken of the moderately higher doses needed for therapeutic warfarin effect in HIV-infected patients. This difference might prove to be clinically significant in a larger study, and one that includes the study of warfarin toxicity due to inaccurate dosing.

The study has some shortcomings. Retrospective data was collected from several sources over which the investigators did not have control. In addition, rates of satisfactory therapeutic anticoagulation have been reported to be low in HIV-infected patients, partly attributable to poor adherence to warfarin therapy. [15] HIV-infected patients often take ARV drugs as well as other drugs for AIDS-related illnesses such as azole antifungal agents. The drugs have variable effects on warfarin metabolism and may alter warfarin requirements. These effects were not analysed in this study. The sample size of the study was not predetermined (as explained above). While the statistical results are cogent, it is possible that a type II error occurred in analysis of the data.

Summary

This is the first study investigating the warfarin dosing effect of HIV-infection *per se*. This was done by comparison with uninfected patients. Overall there was no significant statistical difference between these groups either in induction time to therapeutic warfarin dose, or stable doses in

ambulant patients. However a subgroup of females with HIV infection on ARVs required significantly more warfarin for therapeutic effect. It is recommended that warfarin dosing and coagulation monitoring be the same in the routine management of HIV infected patients as for uninfected patients. A larger study may detect a difference in warfarin requirements of HIV-infected patients.

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Authors' Contributions: TM originated the study concept. BSJ did the research which TM supervised. Both contributed to interpretation of data and manuscript writing.

Conflict of interests: The authors declare no conflicts of interest.

Funding: No specific funding was used for this study.

No additional data is available

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1.

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Table 1. Demographics of study patients with or without HIV infection on warfarin therapy, n234

		Males	Females	CD4 cell count (mean)*		M/F	Mean age: years (range)
				<200	>200		
HIV +	n112	44	68	35	48	43/41	41.9 (20-71)
HIV -	n122	42	80	–	–	49/47	47.8 (18-83)
Total	n234	86	148				

HIV + Human immunodeficiency virus infected
HIV - Human immunodeficiency virus uninfected
* CD4 count unknown = 29
M/F = Male to female

Table 2. Mean warfarin doses (mg/day) according to patients' HIV infection and AR drug therapy status (ARVT)

2a Comparison according to HIV status and gender

	HIV-	HIV+	p value
All	(n122) 5.72	(n112) 6.06	0.29
Male	(n42) 6.49	(n44) 6.39	0.87
Female	(n80) 5.31	(n68) 5.84	0.14
p-Value (MvsF)	0.01	0.29	

2b Comparison in HIV infected patients according to ARVT status

	HIV+ ARV-	HIV+ ARV+	p value
All	(n62) 5.94	(n50) 6.20	0.59
Male	(n22) 6.92	(n22) 5.87	0.23
Female	(n40) 5.40	(n28) 6.47	0.06
p value (MvsF)	0.09	0.30	

ARVT = Antiretroviral drug therapy

2c Comparison of HIV- and HIV+ patients without ARVT

	HIV-	HIV+ ARV-	p value
All	(n122) 5.72	(n62) 5.94	0.61
Male	(n42) 6.49	(n22) 6.92	0.63
Female	(n80) 5.31	(n40) 5.40	0.84

ARVT = Antiretroviral drug therapy

Table 3. Antiretroviral drugs used by HIV-infected patients indicating number of patients on each drug (n) and mean daily warfarin dose (mg) of the groups.

Antiretroviral medication	Males Mean warfarin dose	Females Mean warfarin dose	Total Mean warfarin dose
Lamivudine (3TC)	(n12) 6.45	(n12) 6.3	(n24) 6.38
Stavudine (d4T/Zerit)	(n6) 5.42	(n5) 6.61	(n11) 5.96
Efavirenz (EFA)	(n11) 5.91	(n11) 5.55	(n22) 5.73
Abacavir (ABC)	(n0)	(n1) 5	(n1) 5
Tenofovir (TDF)	(n4) 6.25	(n5) 6.5	(n9) 6.39
Aluvia (lopinavir/ritonavir)	(n1) 12.5	(n1) 10	(n2) 11.25
Nevirapine (NVP)	(n0)	(n1) 8.25	(n1) 8.25
Zidovudine (AZT)	(n10) 5.16	(n15) 6.79	(n25) 6.14
Unknown	(n1) 7.5	(n1) 5	(n2) 6.25

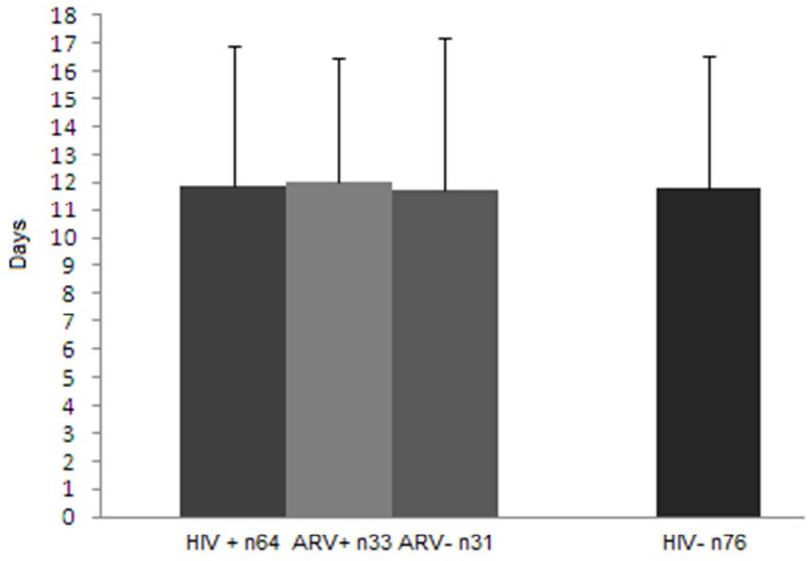

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Figure 1
38x27mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

NA: Not applicable p = page

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ Abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Methods p 3, Intro Abstract, p 3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ p 5, Intro
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ p 5–6 : Aim
Methods		
Study design	4	Present key elements of study design early in the paper ✓ p 6 : Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Location p 8, Dates p 8, Setting p7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ p 7 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants p 7–8 (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NA *Case-control study*—For matched studies, give matching criteria and the number of controls per case NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ p6-7, p6 ✓ p6 p 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ p6-7 part 1,2 → p 7 (induction time), p Warfarin dose
Bias	9	Describe any efforts to address potential sources of bias None
Study size	10	Explain how the study size was arrived at ✓ p 8 (bottom) – 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why p 8: Stats ✓ p 6 : Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding p 8 (*b*) Describe any methods used to examine subgroups and interactions NA (*c*) Explain how missing data were addressed None (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed NA *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy NA (*e*) Describe any sensitivity analyses None

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed NA
		(b) Give reasons for non-participation at each stage NA (No HIV consent p 6)
		(c) Consider use of a flow diagram NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders M/F, Age, HIV, CD4 p 6
		(b) Indicate number of participants with missing data for each variable of interest NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) NA (until ther. dose p 6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time p 9
		Case-control study—Report numbers in each exposure category, or summary measures of exposure NA
		Cross-sectional study—Report numbers of outcome events or summary measures p 9–10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included p 9–10 p 6
		(*b*) Report category boundaries when continuous variables were categorized p 6: Age ≥ 18
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses p 9–10 (gender, ARVs)
Discussion		
Key results	18	Summarise key results with reference to study objectives p 13–14: Summary
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias p 13 – para 2
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence p 13 – Summary
Generalisability	21	Discuss the generalisability (external validity) of the study results p 14 – Summary
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based p 2 (None)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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No additional data is available

ABSTRACT

Background

People infected with the human immunodeficiency virus (HIV) are prone to venous thrombosis. Treatment of thrombosis is primarily with warfarin. No studies have addressed the effects of HIV infection on warfarin dose. The aims of this study were to determine whether the therapeutic dose of warfarin and induction time to therapeutic dose in HIV-infected differ from that in HIV-uninfected patients.

Methods

A prospective and retrospective descriptive study of induction time to therapeutic warfarin dose, and of ambulant therapeutic warfarin dose were performed. HIV-infected and HIV-uninfected patients being treated after deep venous thrombosis with or without pulmonary embolism were compared. Sex and use of antiretroviral drugs (ARVs) were also compared in the groups.

Results

Two hundred and thirty-four patients were entered into the study. Induction time to therapeutic warfarin dose did not differ between the two groups. The mean therapeutic dose of warfarin was higher in the HIV-infected than the HIV-uninfected group: 6.06 vs 5.72 mg/day, but this was not statistically significant ($p = 0.29$). There was no difference in therapeutic warfarin dose between ARV-naïve groups – HIV-uninfected and HIV-infected not on ARVs.

Conclusion

There appears to be little effect of HIV infection on warfarin dosing. Warfarin therapy should be administered conventionally in HIV-infected patients.

Summary

Strengths and limitations of the study.

- This is a unique study which investigates the effect of HIV infection on warfarin treatment.
- It is a clinical study of actual management of HIV-infected patients with venous thrombosis.
- For this reason the study deliberately does not investigate the effect of confounding factors affecting warfarin dosage.
- The authors did not have control over the management of the patients in the retrospective part of the study.
- While the statistical analysis is cogent, a sample size was not predetermined.

Introduction

There is a high incidence of venous thrombosis in patients infected with the human immunodeficiency virus (HIV). The risk is up to 10 times that in uninfected people. [1, 2] The aetiology of thrombosis is multifactorial and includes HIV-mediated endothelium activation, malignancies and infections. [3] The prothrombotic state of HIV infection is also well-recognised and several laboratory coagulation parameters have been shown to be abnormal in these patients. [4, 5] While the exact mechanism of this state remains to be fully elucidated, many coagulation factor abnormalities have been reported, notably protein C, protein S and antithrombin deficiency.[4] Patients who have been treated for thrombosis may require long-term anticoagulation primarily with warfarin because the risk of thrombosis in HIV-infected thrombotic patients is not necessarily abolished by antiretroviral treatment and improvement of the CD4 count. [4,6] Antiretroviral (ARV) drugs *per se* may also contribute to the hypercoagulable state.[7] Additionally, these drugs have variable effects on CYP450 liver enzymes that metabolise warfarin and therefore may affect management of thrombosis in HIV-infected patients.[8] Warfarin has a critical therapeutic window making correct dosing important.

The effect of the HIV status of patients on warfarin dosage has not been investigated. It has been our impression that HIV-infected patients require higher warfarin doses than non-infected patients to achieve therapeutic anticoagulation. Anecdotally longer therapeutic induction times have been observed and this could be due to higher warfarin requirements. Given the possible multiple factors causing hypercoagulability in the HIV-infected patient, we hypothesised that these patients with HIV infection may require higher doses of warfarin for induction and maintenance of anticoagulation.

Aim

The aim of the study was to determine if there is an effect on therapeutic warfarin dose in patients infected with HIV.

The objectives were:

1. To determine whether the therapeutic dose of warfarin differed between groups of HIV-infected and HIV-uninfected patients hospitalized for a thromboembolic incident and followed up at a clinic for INR control.
2. To determine whether the induction time to therapeutic warfarin dose differed between groups of HIV-infected and -uninfected patients.

Motivation

An observation of apparently increased warfarin requirements by HIV-infected patients hospitalised for venous thrombosis, led the authors to investigate the induction time to therapeutic INR in these patients, and to compare this with HIV-uninfected patients. There being no anticoagulation protocol specifically for HIV-infected patients, the comparison would be done using the standard protocol in our department. The induction times to therapeutic INR were to be recorded prospectively and patients followed up at coagulation clinics to determine their ambulant therapeutic warfarin dosage. In order to augment the data, files of additional patients were studied retrospectively at two clinics.

Methods

A descriptive study of patients with venous thrombo-embolism was undertaken. Data were collected both prospectively and retrospectively. Patients aged 18 years and older who had presented with apparently spontaneous lower limb deep venous thrombosis, with or without pulmonary embolism, were included in the study. Patients with attributable causes of thrombosis such as recent surgery, cardiac failure and thrombophilias were excluded. Patients who refused HIV testing were not entered in the study. Confirmation of DVT was by Doppler ultrasound and of pulmonary embolism by CT angiography as indicated. ARV drug treatment as well as the specific drugs were also recorded. Comparisons of the mean daily warfarin dose and induction time to therapeutic warfarin dose were performed in HIV-infected and HIV-uninfected patients, in males and females, and in HIV-infected patients on and not on ARV drug therapy. The use of ARVs was

recorded and analysed as a group of possible confounders but not all individual drugs were analysed.

- Prospective data collection

  All patients admitted to hospital with acute DVT of the lower limb with or without pulmonary embolism were subjected to a consented HIV test and entered in the study. Warfarin dose data was collected during hospitalisation and at follow-up. Clinic visits were scheduled every 2-4 weeks after discharge from hospital. The patients were observed until the attainment of therapeutic warfarin dose, and the number of days recorded. Once induction time had passed the patients were followed until the therapeutic dose of each patient was attained.

- Retrospective data collection

  Warfarin dose data was gathered retrospectively from two follow-up clinics for ambulatory patients after a thrombo-embolic episode. One clinic was for general anticoagulation control (HIV-uninfected patients), the other was for HIV-infected patients for AIDS follow-up (HIV-infected patients). The latter patients were included irrespective of the duration of HIV infection. Only patients who attended clinics regularly until the therapeutic dose was achieved were included in this study. Induction time as computed and therapeutic warfarin dose recorded.

Definitions

Warfarin regimen:

The same regimen was used for all patients in the study. Sodium-warfarin (Cipla-Warfarin®, Cipla-Medpro) was commenced at 5mg per day and followed by a modification of the slow Fennerty regimen, using dose adjustments of 2.5mg.[9] The INR was determined every 2-3 days after initiation

or change of treatment dose, while maintaining anticoagulation with a low molecular weight heparin until an INR level of between 2 and 3 was obtained.

Warfarin induction time:

The induction time to therapeutic warfarin dose was defined as the time in days to achieve an International Normalised Ratio (INR) measurement of between 2 and 3. The number of days was calculated as the number of days from the start of warfarin therapy until the day of stable therapeutic dose (the first day of consecutive identical doses). Arithmetic means of the number of days were calculated for groups of patients.

Therapeutic warfarin dose:

The target INR for ambulant warfarin therapy was 2.5, with a range of 2 to 3. The last warfarin dose recorded after dosage stabilisation at the INR target range of 2 – 3 had been achieved, was recorded as the therapeutic daily dose for both the pro- and retrospectively studied patients. If the dose differed on alternate days the average of 2 days dosages was recorded. Means of ambulant warfarin dose of groups of patients were calculated for comparison. Because of the short-term goals of this study of recording only induction time and the most recent therapeutic warfarin dose, time in therapeutic range was not determined.

The study was conducted at the Pretoria Academic Health Complex consisting of the Steve Biko Academic Hospital and Kalafong Regional Hospital, as well as the Tshwane District Hospital, during the period 2013-2015. Individual consent was obtained from the prospectively studied patients. Approval to conduct the whole study was granted by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria.

The study is reported in accordance with the STROBE criteria.[10]

Statistical Analysis

The 2 sample t-test of the comparison of means of warfarin dose was performed in the following groups: HIV-infected and HIV-uninfected patients, HIV-infected patients on and not on ARV drugs, and males and females in these groups. Sex and the use of ARV drug use were analysed as possible confounders. Induction times for the groups to attain therapeutic warfarin doses were analysed by the Kruskal-Wallis test. The study was considered to be exploratory in nature. Because the difference in warfarin dose outcomes of the study groups was expected to be small, sample size and size effect were not predetermined as a large number of patients would be required. A p-value of 0.05 was taken to be significant.

Results

Patient demographics and the number of patients in each group are depicted in Table 1. The age of the patients was somewhat lower in the HIV-infected group.

Induction time to therapeutic warfarin dose.

Induction times were analysed in 170 patients. The data was collected prospectively for 93 patients and retrospectively for 77 patients. Seventy four of the patients were HIV-infected and 96 HIV-uninfected. The mean times were 12.87 and 11.19 days respectively, p=0.28. Thirty-six of the HIV-infected patients were on ARV drugs. Their induction times did not differ significantly from that of the HIV-infected patients not on ARV drugs or the HIV-uninfected patients (Figure 1). It must be noted that therapeutic induction times exhibited a wide range of standard deviation.

Stable ambulant warfarin dose

The prospectively-studied patients in the warfarin induction study were followed up for determination of their ambulant therapeutic warfarin dose. These 93 patients were augmented by data collected retrospectively from 141 patients. This gave a total of 234 patients, 122 HIV-uninfected (42 males and 80 females) and 112 HIV-infected (44 males and 68 females) (Table 2). The mean therapeutic dose of warfarin in the whole set of patients was higher in the HIV-infected group than the HIV-uninfected group: 6.06mg/day vs 5.72mg/day (Table2). This was, however, not statistically significant (p=0.29). The difference was greater in females (HIV-infected 5.84 mg/day vs HIV-uninfected 5.31mg/day, p = 0.14) than in males (HIV-infected 6.39mg/day vs HIV-uninfected 6.49mg/day, p = 0.87).

Fifty HIV-infected patients on ARV medication were taking several ARV drugs in various combinations. The drugs and mean dosages used in this study are listed in table 3. There was no statistically significant difference in the mean therapeutic dose of warfarin between HIV-infected patients taking and not taking ARVs, 6.20 v 5.94 mg/day, p=0.59. However, HIV-infected females on ARVs required significantly more warfarin, 6.47mg/day than HIV-uninfected females, 5.31mg/day, p=0.01. The opposite was true for males, but the difference was not statistically significant, 5.87mg/day vs 6.49mg/day, p=0.29.

ARV-naïve patient groups were compared. There was no significant difference in warfarin dose between HIV-infected patients not taking ARVs and HIV-uninfected patients, 5.94mg/day vs 5.72mg/day, p=0.22.

In general males required more warfarin than females as can be seen in table 3. This applied to all groups of patients but only the comparison for HIV-uninfected patients was significant: males (6.49mg) and females (5.31mg), p = 0.01.

HIV-infected females on ARV drugs tended to require higher doses of warfarin than those not on ARV drugs, 6.47mg/day vs 5.40mg/day, p = 0.06. Most of the antiretroviral drugs were taken by few patients (11 or less). In addition, drugs were taken in various different combination, making analysis of individual drugs' effects on warfarin dose unrealistic. However, two drugs were taken by substantially more patients, viz efavirenz and zidovudine. The mean warfarin dose of the 22 patients taking efavirenz (a CYP450 inhibitor) was virtually the same as that of the HIV-uninfected patients (5.73 and 5.72 respectively). Twenty five patients were taking zidovudine which is not metabolised by the CYP450 pathway and apparently does not affect warfarin blood levels.

Discussion

We report on a comparative study of warfarin dosage in HIV-infected and -uninfected patients. This comparison, using a standard warfarin treatment protocol, has not been published previously. The assumption that HIV-infected patients require higher doses of warfarin for therapeutic effect seems to be reasonable given the acknowledged hypercoagulable state of the HIV-infected patient. Anecdotal observation in our practice suggested that this is indeed the case. Many of these patients had not yet started ARV therapy. Previous warfarin dosage studies in HIV-infected patients have largely addressed the effect of ARV drugs on warfarin dose. [4, 6,7,11] This study attempted to determine the possible role of HIV infection. However, no apparent increase in warfarin requirements for anticoagulation was demonstrated in HIV-infected patients compared to uninfected patients in general, except in females on ARV therapy.

There is a paucity of clinical studies on the effect of infection in general on warfarin dosage. This has been addressed in a few studies on patients with acute infection.[12, 13] The effect of HIV infection on warfarin dosage has not been similarly studied. Infection with HIV, quite apart from the effect of concomitant infections and malignancies and various possible drug interaction, apparently causes a

propensity for thrombosis, as recovery of the CD4 count does not abolish the increased risk of thrombosis.[2, 4, 6] The current study attempted to determine indirectly the effect of the HIV infection *per se* on warfarin requirement by comparison of the dosage with that in HIV-uninfected patients. The mean warfarin dosage was found not to differ between these two broad groups.

Clinical management of warfarin therapy is difficult because of inter- and intra-individual variability of dosage requirements. [14, 15] The narrow therapeutic window for warfarin dosage makes accurate dosing important.[16] Many patient and environmental factors influence warfarin dosing. These include sex, race, height, weight and age. [14, 15] Only the known increased warfarin requirements for men was documented and is apparent in this study, in the comparison of ARV-uninfected men and women.

Warfarin dose effects of antiretroviral drugs in HIV-infected patients have been investigated by several authors. ARV drugs have variable effects on warfarin blood levels, some causing an increase in levels and some a decrease. Sekaggya et al demonstrated this in a case series of HIV-infected patients being treated for tuberculosis.[17] Jong et al demonstrated lower levels of von Willebrand factor, factor VIII, D-dimer and endogenous thrombin potential in HIV-infected patients on ARV drugs. [4] Anderson et al found that patients on efavirenz-based regimens required lower weekly warfarin doses than patients on lopinavir / ritonavir regimens. [18] This was not the case in the present study in the patients taking efavirenz. The warfarin dose in the 22 HIV-infected patients taking efavirenz did not differ from the patients not taking the drug. However, this could have been due to other influences not studied here such as the effects of other drugs. Stolbach et al caution that efavirenz and nevarapine may affect therapeutic INR levels, and that warfarin dose adjustments may be required. Little can be deduced from the ARV drug effect found in this study as the effects of most of the individual ARV drugs on warfarin requirements were not analysed statistically. Patients were taking several ARV drugs singly or in various combinations. The sample size of patients on each

drug was too small to meaningfully perform separate analyses. An important comparison in this study, however, is that between the 62 HIV-infected patients not taking ARV drugs and the 122 HIV-uninfected patients. This comparison would discount the effects of the ARV drugs. While there was a modest difference in warfarin dosage, this did not differ significantly between these two groups (p=0.20, Table 2). Once again this may be due to factors not addressed in this study.

The development of a warfarin dosing algorithm for HIV-infected persons would be difficult and complex because of the multiple influences that may be operating.[14,17] These include genetic factors, the hypercoaguable state, pertinent infections and malignancies, and the effect of multiple drugs on the cytochrome p450 2C9 system. These diverse effects on warfarin dosage are probably reflected in the wide range of standard deviation in this study shown in figure 1. However, the study addresses the broad group of HIV-infected patients, and does not take cognisance of the multiple factors that can affect warfarin dosing. We propose that this approach is valid because the study reflects clinical usage in which standard warfarin regimens are used for HIV-infected patients. Current practice in HIV-infected patients is to manage warfarin dosing as in HIV-uninfected patients, and this study would seem to support this custom. [6] While not statistically significant, cognisance should be taken of the moderately higher doses needed for therapeutic warfarin effect in HIV-infected patients. This difference might prove to be clinically significant in a larger study, and one that includes the study of warfarin toxicity due to inaccurate dosing.

The study has some shortcomings. Retrospective data was collected from several sources over which the investigators did not have control. In addition, rates of satisfactory therapeutic anticoagulation have been reported to be low in HIV-infected patients, partly attributable to poor adherence to warfarin therapy. [18] HIV-infected patients often take ARV drugs as well as other drugs for AIDS-related illnesses such as azole antifungal agents. The drugs have variable effects on warfarin metabolism and may alter warfarin requirements. These effects were not investigated in

this study. The sample size of the study was not predetermined. While the statistical results are cogent, it is possible that a type II error occurred in analysis of the data. The study nevertheless suggests the performance of a larger study.

Summary

This is the first study investigating HIV-infection status on warfarin dosing. This was done by comparison with uninfected patients using a standard warfarin administration protocol. Overall there was no significant statistical difference between these groups either in induction time to therapeutic warfarin dose, or stable doses in ambulant patients. However a subgroup of females with HIV infection on ARVs required significantly more warfarin for therapeutic effect. A larger study may detect a difference in warfarin requirements of HIV-infected patients.

Conclusion

It is recommended that warfarin dosing and coagulation monitoring be the same in the routine management of HIV infected patients as for uninfected patients.

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Authors' Contributions: TM originated the study concept. BSJ did the research which TM supervised. Both contributed to interpretation of data and manuscript writing.

Conflict of interests: The authors declare no conflicts of interest.

Funding: No specific funding was used for this study.

No additional data is available

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1.

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Table 1. Demographics of study patients with or without HIV infection on warfarin therapy, n234

		Males	Females	CD4 cell count (mean)*		M/F	Mean age years (range)
				<200	>200		
HIV +	n112	44	68	35	48	43/41	41.9 (20-71)
HIV -	n122	42	80	–	–	49/47	47.8 (18-83)
Total	n234	86	148				

HIV + Human immunodeficiency virus infected
HIV - Human immunodeficiency virus uninfected
* CD4 count unknown = 29
M/F = Males and females

Table 2. Mean warfarin doses (mg/day) according to patients' HIV infection and ARV drug therapy status (ARVT)

Patient groups (n, %)	Warfarin dose (mg/day)	p-Value
All (234, 100)		
HIV+ (112, 48)	6.06	} p = 0.29
HIV- (122, 52)	5.72*	} p = 0.61
HIV+ & ARV- (62, 55)	5.94*	} p = 0.59
HIV+ & ARV+ (50, 45)	6.20	
Male (86, 100)		
HIV+ (44, 51)	6.39 $^{\Sigma}$	} p = 0.87
HIV- (42, 49)	6.49 $^{\sim}$	} p = 0.63
HIV+ & ARVT- (22, 50)	6.92 $^{+}$	} p = 0.23
HIV+ & ARVT+ (22, 50)	5.87 ^{o}	
Female (148)		
HIV+ (68, 46)	5.84 $^{\Sigma}$	} p = 0.14
HIV- (80, 54)	5.31 $^{\sim}$	} p = 0.84
HIV+ & ARVT- (40, 58)	5.40 $^{+}$	} p = 0.06
HIV+ & ARVT+ (28, 42)	6.47 ^{o}	

ARVT = Antiretroviral drug therapy
* Comparison of ARVT-naïve groups, p = 0.22
$^{\Sigma}$ Male vs female, p =0.29
$^{\sim}$Male vs female, p =0.01
$^{+}$Male vs female, p =0.09
^{o}Male vs female, p =0.3

Table 3. Antiretroviral drugs used by HIV-infected patients indicating number of patients on each drug (n) and mean daily warfarin dose (mg).

Antiretroviral medication		Mean warfarin dose
Lamivudine (3TC)	(n24)	6.38
Stavudine (d4T/Zerit)	(n11)	5.96
Efavirenz (EFA)	(n22)	5.73
Abacavir (ABC)	(n1)	5
Tenofovir (TDF)	(n9)	6.39
Aluvia (lopinavir/ritonavir)	(n2)	11.25
Nevirapine (NVP)	(n1)	8.25
Zidovudine (AZT)	(n25)	6.14
Unknown	(n2)	6.25

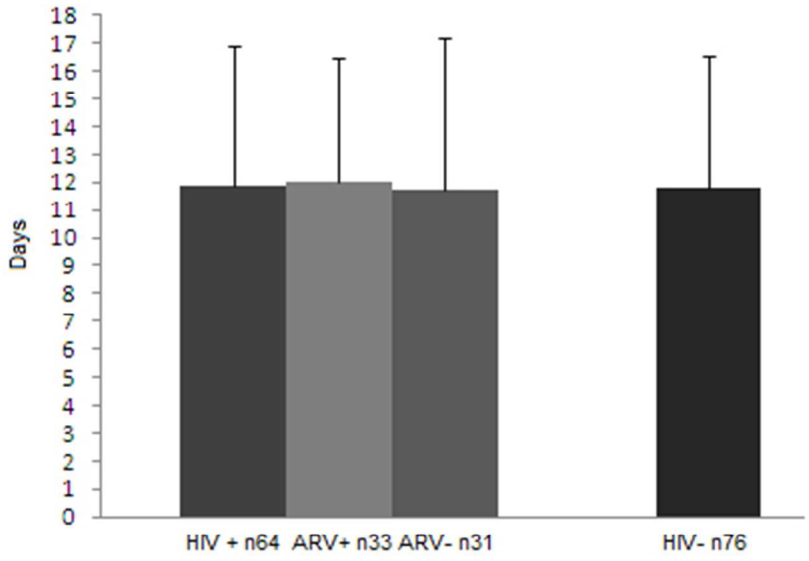

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Figure 1
38x27mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

NA: Not applicable p = page

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ Abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Methods p 3, Intro Abstract, p 3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ p 5, Intro
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ p 5–6 : Aim
Methods		
Study design	4	Present key elements of study design early in the paper ✓ p 6 : Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Location p 8, Dates p 8, Setting p7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ p 7 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants p 7–8 (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NA *Case-control study*—For matched studies, give matching criteria and the number of controls per case NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ p6-7, p6 ✓ p6 p 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ p6-7 part 1,2 → p 7 (induction time), p Warfarin dose
Bias	9	Describe any efforts to address potential sources of bias None
Study size	10	Explain how the study size was arrived at ✓ p 8 (bottom) – 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why p 8: Stats ✓ p 6 : Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding p 8 (*b*) Describe any methods used to examine subgroups and interactions NA (*c*) Explain how missing data were addressed None (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed NA *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy NA (*e*) Describe any sensitivity analyses None

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed NA
		(b) Give reasons for non-participation at each stage NA (No HIV consent p 6)
		(c) Consider use of a flow diagram NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders M/F, Age, HIV, CD4 p 6
		(b) Indicate number of participants with missing data for each variable of interest NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) NA (until ther. dose p 6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time p 9
		Case-control study—Report numbers in each exposure category, or summary measures of exposure NA
		Cross-sectional study—Report numbers of outcome events or summary measures p 9–10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included p 9–10 p 6
		(*b*) Report category boundaries when continuous variables were categorized p 6: Age ≥ 18
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses p 9–10 (gender, ARVs)
Discussion		
Key results	18	Summarise key results with reference to study objectives p 13–14: Summary
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias p 13 – para 2
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence p 13 – Summary
Generalisability	21	Discuss the generalisability (external validity) of the study results p 14 – Summary
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based p 2 (None)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice.

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ABSTRACT

Background

People infected with the human immunodeficiency virus (HIV) are prone to venous thrombosis. Treatment of thrombosis is primarily with warfarin. No studies have addressed the effects of HIV infection on warfarin dose. The aims of this study were to determine whether the therapeutic dose of warfarin and induction time to therapeutic dose in HIV-infected differ from that in HIV-uninfected patients.

Methods

A prospective and retrospective descriptive study of induction time to therapeutic warfarin dose, and of ambulant therapeutic warfarin dose were performed. HIV-infected and HIV-uninfected patients being treated after deep venous thrombosis with or without pulmonary embolism were compared. Sex and use of antiretroviral drugs (ARVs) were also compared in the groups.

Results

Two hundred and thirty-four patients were entered into the study. Induction time to therapeutic warfarin dose did not differ between the two groups. The mean therapeutic dose of warfarin was higher in the HIV-infected than the HIV-uninfected group: 6.06 vs 5.72 mg/day, but this was not statistically significant ($p = 0.29$). There was no difference in therapeutic warfarin dose between ARV-naïve groups – HIV-uninfected and HIV-infected not on ARVs.

Conclusion

There appears to be little effect of HIV infection on warfarin dosing. Warfarin therapy should be administered conventionally in HIV-infected patients.

Summary

Strengths and limitations of the study.

- This is a unique study which investigates the effect of HIV infection on warfarin treatment.
- It is a clinical study of actual management of HIV-infected patients with venous thrombosis.
- For this reason the study deliberately does not investigate the effect of confounding factors affecting warfarin dosage.
- The authors did not have control over the management of the patients in the retrospective part of the study.
- While the statistical analysis is cogent, a sample size was not predetermined.

Introduction

There is a high incidence of venous thrombosis in patients infected with the human immunodeficiency virus (HIV). The risk is up to 10 times that in uninfected people. [1, 2] The aetiology of thrombosis is multifactorial and includes HIV-mediated endothelium activation, malignancies and infections. [3] The prothrombotic state of HIV infection is also well-recognised and several laboratory coagulation parameters have been shown to be abnormal in these patients. [4, 5] While the exact mechanism of this state remains to be fully elucidated, many coagulation factor abnormalities have been reported, notably protein C, protein S and antithrombin deficiency.[4] Patients who have been treated for thrombosis may require long-term anticoagulation primarily with warfarin because the risk of thrombosis in HIV-infected thrombotic patients is not necessarily abolished by antiretroviral treatment and improvement of the CD4 count. [4,6] Antiretroviral (ARV) drugs *per se* may also contribute to the hypercoagulable state.[7] Additionally, these drugs have variable effects on CYP450 liver enzymes that metabolise warfarin and therefore may affect management of thrombosis in HIV-infected patients.[8] Warfarin has a critical therapeutic window making correct dosing important.

The effect of the HIV status of patients on warfarin dosage has not been investigated. It has been our impression that HIV-infected patients require higher warfarin doses than non-infected patients to achieve therapeutic anticoagulation. Anecdotally longer therapeutic induction times have been observed and this could be due to higher warfarin requirements. Given the possible multiple factors causing hypercoagulability in the HIV-infected patient, we hypothesised that these patients with HIV infection may require higher doses of warfarin for induction and maintenance of anticoagulation.

Aim

The aim of the study was to determine if there is an effect on therapeutic warfarin dose in patients infected with HIV.

The objectives were:

1. To determine whether the therapeutic dose of warfarin differed between groups of HIV-infected and HIV-uninfected patients hospitalized for a thromboembolic incident and followed up at a clinic for INR control.
2. To determine whether the induction time to therapeutic warfarin dose differed between groups of HIV-infected and -uninfected patients.

Motivation

An observation of apparently increased warfarin requirements by HIV-infected patients hospitalised for venous thrombosis, led the authors to investigate the induction time to therapeutic INR in these patients, and to compare this with HIV-uninfected patients. There being no anticoagulation protocol specifically for HIV-infected patients, the comparison would be done using the standard protocol in our department. The induction times to therapeutic INR were to be recorded prospectively and patients followed up at coagulation clinics to determine their ambulant therapeutic warfarin dosage. In order to augment the data, files of additional patients were studied retrospectively at two clinics.

Methods

A descriptive study of patients with venous thrombo-embolism was undertaken. Data were collected both prospectively and retrospectively. Patients aged 18 years and older who had presented with apparently spontaneous lower limb deep venous thrombosis, with or without pulmonary embolism, were included in the study. Patients with attributable causes of thrombosis such as recent surgery, cardiac failure and thrombophilias were excluded. Patients who refused HIV testing were not entered in the study. Confirmation of DVT was by Doppler ultrasound and of pulmonary embolism by CT angiography as indicated. ARV drug treatment as well as the specific drugs were also recorded. Comparisons of the mean daily warfarin dose and induction time to therapeutic warfarin dose were performed in HIV-infected and HIV-uninfected patients, in males and females, and in HIV-infected patients on and not on ARV drug therapy. The use of ARVs was

recorded and analysed as a group of possible confounders but not all individual drugs were analysed.

- Prospective data collection

  All patients admitted to hospital with acute DVT of the lower limb with or without pulmonary embolism were subjected to a consented HIV test and entered in the study. Warfarin dose data was collected during hospitalisation and at follow-up. Clinic visits were scheduled every 2-4 weeks after discharge from hospital. The patients were observed until the attainment of therapeutic warfarin dose, and the number of days recorded. Once induction time had passed the patients were followed until the therapeutic dose of each patient was attained.

- Retrospective data collection

  Warfarin dose data was gathered retrospectively from two follow-up clinics for ambulatory patients after a thrombo-embolic episode. One clinic was for general anticoagulation control (HIV-uninfected patients), the other was for HIV-infected patients for AIDS follow-up (HIV-infected patients). The latter patients were included irrespective of the duration of HIV infection. Only patients who attended clinics regularly until the therapeutic dose was achieved were included in this study. Induction time as computed and therapeutic warfarin dose recorded.

Definitions

Warfarin regimen:

The same regimen was used for all patients in the study. Sodium-warfarin (Cipla-Warfarin®, Cipla-Medpro) was commenced at 5mg per day and followed by a modification of the slow Fennerty regimen, using dose adjustments of 2.5mg.[9] The INR was determined every 2-3 days after initiation

or change of treatment dose, while maintaining anticoagulation with a low molecular weight heparin until an INR level of between 2 and 3 was obtained.

Warfarin induction time:

The induction time to therapeutic warfarin dose was defined as the time in days to achieve an International Normalised Ratio (INR) measurement of between 2 and 3. The number of days was calculated as the number of days from the start of warfarin therapy until the day of stable therapeutic dose (the first day of consecutive identical doses). Arithmetic means of the number of days were calculated for groups of patients.

Therapeutic warfarin dose:

The target INR for ambulant warfarin therapy was 2.5, with a range of 2 to 3. The last warfarin dose recorded after dosage stabilisation at the INR target range of 2 – 3 had been achieved, was recorded as the therapeutic daily dose for both the pro- and retrospectively studied patients. If the dose differed on alternate days the average of 2 days dosages was recorded. Means of ambulant warfarin dose of groups of patients were calculated for comparison. Because of the short-term goals of this study of recording only induction time and the most recent therapeutic warfarin dose, time in therapeutic range was not determined.

The study was conducted at the Pretoria Academic Health Complex consisting of the Steve Biko Academic Hospital and Kalafong Regional Hospital, as well as the Tshwane District Hospital, during the period 2013-2015. Individual consent was obtained from the prospectively studied patients. Approval to conduct the whole study was granted by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria.

The study is reported in accordance with the STROBE criteria.[10]

Statistical Analysis

The 2 sample t-test of the comparison of means of warfarin dose was performed in the following groups: HIV-infected and HIV-uninfected patients, HIV-infected patients on and not on ARV drugs, and males and females in these groups. Sex and the use of ARV drug use were analysed as possible confounders. Induction times for the groups to attain therapeutic warfarin doses were analysed by the Kruskal-Wallis test. The study was considered to be exploratory in nature. Because the difference in warfarin dose outcomes of the study groups was expected to be small, sample size and size effect were not predetermined as a large number of patients would be required. A p-value of 0.05 was taken to be significant.

Results

Patient demographics and the number of patients in each group are depicted in Table 1. The age of the patients was somewhat lower in the HIV-infected group.

Induction time to therapeutic warfarin dose.

Induction times were analysed in 170 patients. The data was collected prospectively for 93 patients and retrospectively for 77 patients. Seventy four of the patients were HIV-infected and 96 HIV-uninfected. The mean times were 12.87 and 11.19 days respectively, p=0.28. Thirty-six of the HIV-infected patients were on ARV drugs. Their induction times did not differ significantly from that of the HIV-infected patients not on ARV drugs or the HIV-uninfected patients (Figure 1). It must be noted that therapeutic induction times exhibited a wide range of standard deviation.

Stable ambulant warfarin dose

The prospectively-studied patients in the warfarin induction study were followed up for determination of their ambulant therapeutic warfarin dose. These 93 patients were augmented by data collected retrospectively from 141 patients. This gave a total of 234 patients, 122 HIV-uninfected (42 males and 80 females) and 112 HIV-infected (44 males and 68 females) (Table 2). In general, males required more warfarin than females. This applied to all groups of patients but only the comparison for HIV-uninfected patients was significant: males (6.49mg) and females (5.31mg), p = 0.01.

The mean therapeutic dose of warfarin in the whole set of patients was higher in the HIV-infected group than the HIV-uninfected group: 6.06mg/day vs 5.72mg/day (Table2). This was, however, not statistically significant (p=0.29). The difference was greater in females (HIV-infected 5.84 mg/day vs HIV-uninfected 5.31mg/day, p = 0.14) than in males (HIV-infected 6.39mg/day vs HIV-uninfected 6.49mg/day, p = 0.87).

Fifty HIV-infected patients on ARV medication were taking several ARV drugs in various combinations. The drugs used in this study and corresponding mean warfarin dosages are listed in table 3. Comparisons are depicted in table 2. There was no statistically significant difference in the mean therapeutic dose of warfarin between HIV-infected patients taking and not taking ARVs, 6.20 v 5.94 mg/day, p=0.59. HIV-infected females on ARV drugs tended to require higher doses of warfarin than those not on ARV drugs, 6.47mg/day vs 5.40mg/day, p = 0.06. However, HIV-infected females on ARVs required significantly more warfarin, 6.47mg/day than HIV-uninfected females, 5.31mg/day, p=0.01. The opposite was true for males, but the difference was not statistically significant, 5.87mg/day vs 6.49mg/day, p=0.29.

ARV-naïve patient groups were compared. There was no significant difference in warfarin dose between HIV-infected patients not taking ARVs and HIV-uninfected patients, 5.94mg/day vs 5.72mg/day, p=0.22 (Table 2).

Most of the individual antiretroviral drugs were taken by few patients. Eleven patients were taking stavidine and fewer were on several other drugs (Table 3). In addition, drugs were taken in various different combination, making analysis of individual drugs' effects on warfarin dose unrealistic. However, three drugs were taken by substantially more patients, efavirenz, lamivudine and zidovudine. The mean warfarin dose of the 22 patients taking efavirenz (a CYP450 inhibitor) was virtually the same as that of the HIV-uninfected patients (5.73 and 5.72 respectively). Twenty five and 24 patients respectively were taking zidovudine and lamivudine which are not metabolised by the CYP450 pathway, and apparently do not affect warfarin blood levels.

Discussion

We report on a comparative study of warfarin dosage in HIV-infected and -uninfected patients. This comparison, using a standard warfarin treatment protocol, has not been published previously. The assumption that HIV-infected patients require higher doses of warfarin for therapeutic effect seems to be reasonable given the acknowledged hypercoagulable state of the HIV-infected patient. Anecdotal observation in our practice suggested that this is indeed the case. Many of these patients had not yet started ARV therapy. Previous warfarin dosage studies in HIV-infected patients have largely addressed the effect of ARV drugs on warfarin dose. [4, 6,7,11] This study attempted to determine the possible role of HIV infection. However, no apparent increase in warfarin requirements for anticoagulation was demonstrated in HIV-infected patients compared to uninfected patients in general, except in females on ARV therapy.

There is a paucity of clinical studies on the effect of infection in general on warfarin dosage. This has been addressed in a few studies on patients with acute infection.[12, 13] The effect of HIV infection on warfarin dosage has not been similarly studied. Infection with HIV, quite apart from the effect of concomitant infections and malignancies and various possible drug interaction, apparently causes a propensity for thrombosis, as recovery of the CD4 count does not abolish the increased risk of thrombosis.[2, 4, 6] The current study attempted to determine indirectly the effect of the HIV infection *per se* on warfarin requirement by comparison of the dosage with that in HIV-uninfected patients. The mean warfarin dosage was found not to differ between these two broad groups.

Clinical management of warfarin therapy is difficult because of inter- and intra-individual variability of dosage requirements. [14, 15] The narrow therapeutic window for warfarin dosage makes accurate dosing important.[16] Many patient and environmental factors influence warfarin dosing. These include sex, race, height, weight and age. [14, 15] Only the known increased warfarin requirements for men was documented and is apparent in this study, in the comparison of ARV-uninfected men and women.

Warfarin dose effects of antiretroviral drugs in HIV-infected patients have been investigated by several authors. ARV drugs have variable effects on warfarin blood levels, some causing an increase in levels and some a decrease. Sekaggya et al demonstrated this in a case series of HIV-infected patients being treated for tuberculosis.[17] Jong et al demonstrated lower levels of von Willebrand factor, factor VIII, D-dimer and endogenous thrombin potential in HIV-infected patients on ARV drugs. [4] Anderson et al found that patients on efavirenz-based regimens required lower weekly warfarin doses than patients on lopinavir / ritonavir regimens. [18] This was not the case in the present study in the patients taking efavirenz. The warfarin dose in the 22 HIV-infected patients taking efavirenz did not differ from the patients not taking the drug. However, this could have been due to other influences not studied here such as the effects of other drugs. Stolbach et al caution that

efavirenz and nevarapine may affect therapeutic INR levels, and that warfarin dose adjustments may be required. Little can be deduced from the ARV drug effect found in this study as the effects of most of the individual ARV drugs on warfarin requirements were not analysed statistically. Patients were taking several ARV drugs singly or in various combinations. The sample size of patients on each drug was too small to meaningfully perform separate analyses. An important comparison in this study, however, is that between the 62 HIV-infected patients not taking ARV drugs and the 122 HIV-uninfected patients. This comparison would discount the effects of the ARV drugs. While there was a modest difference in warfarin dosage, this did not differ significantly between these two groups (p=0.20, Table 2). Once again this may be due to factors not addressed in this study.

The development of a warfarin dosing algorithm for HIV-infected persons would be difficult and complex because of the multiple influences that may be operating.[14,17] These include genetic factors, the hypercoaguable state, pertinent infections and malignancies, and the effect of multiple drugs on the cytochrome P450 2C9 system. These diverse effects on warfarin dosage are probably reflected in the wide range of standard deviation in this study shown in figure 1. However, the study addresses the broad group of HIV-infected patients, and does not take cognisance of the multiple factors that can affect warfarin dosing. We propose that this approach is valid because the study reflects clinical usage in which standard warfarin regimens are used for HIV-infected patients. Current practice in HIV-infected patients is to manage warfarin dosing as in HIV-uninfected patients, and this study would seem to support this custom. [6] While not statistically significant, cognisance should be taken of the moderately higher doses needed for therapeutic warfarin effect in HIV-infected patients. This difference might prove to be clinically significant in a larger study, and one that includes the study of warfarin toxicity due to inaccurate dosing.

The study has some shortcomings. Retrospective data was collected from several sources over which the investigators did not have control. In addition, rates of satisfactory therapeutic

anticoagulation have been reported to be low in HIV-infected patients, partly attributable to poor adherence to warfarin therapy. [18] HIV-infected patients often take ARV drugs as well as other drugs for AIDS-related illnesses such as azole antifungal agents. The drugs have variable effects on warfarin metabolism and may alter warfarin requirements. These effects were not investigated in this study. The sample size of the study was not predetermined. While the statistical results are cogent, it is possible that a type II error occurred in analysis of the data. The study nevertheless suggests the performance of a larger study.

Summary

This is the first study investigating HIV-infection status on warfarin dosing. This was done by comparison with uninfected patients using a standard warfarin administration protocol. Overall there was no significant statistical difference between these groups either in induction time to therapeutic warfarin dose, or stable doses in ambulant patients. However a subgroup of females with HIV infection on ARVs required significantly more warfarin for therapeutic effect. A larger study may detect a difference in warfarin requirements of HIV-infected patients.

Conclusion

It is recommended that warfarin dosing and coagulation monitoring be the same in the routine management of HIV infected patients as for uninfected patients.

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Authors' Contributions: TM originated the study concept. BSJ did the research which TM supervised. Both contributed to interpretation of data and manuscript writing.

Conflict of interests: The authors declare no conflicts of interest.

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No additional data is available

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1.

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Table 1. Demographics of study patients with or without HIV infection on warfarin therapy, n234

		Males	Females	CD4 cell count (mean)*		M/F⁺	Mean age years (range)
				<200	>200		
HIV +	n112	44	68	35	48	43/41	41.9 (20-71)
HIV -	n122	42	80	–	–	49/47	47.8 (18-83)
Total	n234	86	148				

HIV + Human immunodeficiency virus infected
HIV - Human immunodeficiency virus uninfected
* CD4 count unknown = 29 patients
⁺M/F = Ratio of males to females

Table 2. Mean warfarin doses (mg/day) according to patients' HIV infection and ARV drug therapy status (ARVT)

Patient groups (n, %)	Warfarin dose (mg/day)	p-Value
All (234, 100)		
HIV+ (112, 48)	6.06	} p = 0.29
HIV- (122, 52)	5.72*	} p = 0.61
HIV+ & ARVT- (62, 55)	5.94*	} p = 0.59
HIV+ & ARVT+ (50, 45)	6.20	
Male (86, 100)		
HIV+ (44, 51)	6.39 $^{\Sigma}$	} p = 0.87
HIV- (42, 49)	6.49 $^{\sim}$	} p = 0.63
HIV+ & ARVT- (22, 50)	6.92 $^{+}$	} p = 0.23
HIV+ & ARVT+ (22, 50)	5.87 ^{o}	
Female (148)		
HIV+ (68, 46)	5.84 $^{\Sigma}$	} p = 0.14
HIV- (80, 54)	5.31 $^{\sim}$	} p = 0.84
HIV+ & ARVT- (40, 58)	5.40 $^{+}$	} p = 0.06
HIV+ & ARVT+ (28, 42)	6.47 ^{o}	

ARVT = Antiretroviral drug therapy
* Comparison of ARVT-naïve groups, p = 0.22
$^{\Sigma}$ Male vs female, p =0.29
$^{\sim}$Male vs female, p =0.01
$^{+}$Male vs female, p =0.09
^{o}Male vs female, p =0.3

Table 3. Antiretroviral drugs used by HIV-infected patients indicating number of patients on each drug (n) and mean daily warfarin dose (mg).

Antiretroviral medication		Mean warfarin dose
Lamivudine (3TC)	(n24)	6.38
Stavudine (d4T/Zerit)	(n11)	5.96
Efavirenz (EFA)	(n22)	5.73
Abacavir (ABC)	(n1)	5
Tenofovir (TDF)	(n9)	6.39
Aluvia (lopinavir/ritonavir)	(n2)	11.25
Nevirapine (NVP)	(n1)	8.25
Zidovudine (AZT)	(n25)	6.14
Unknown	(n2)	6.25

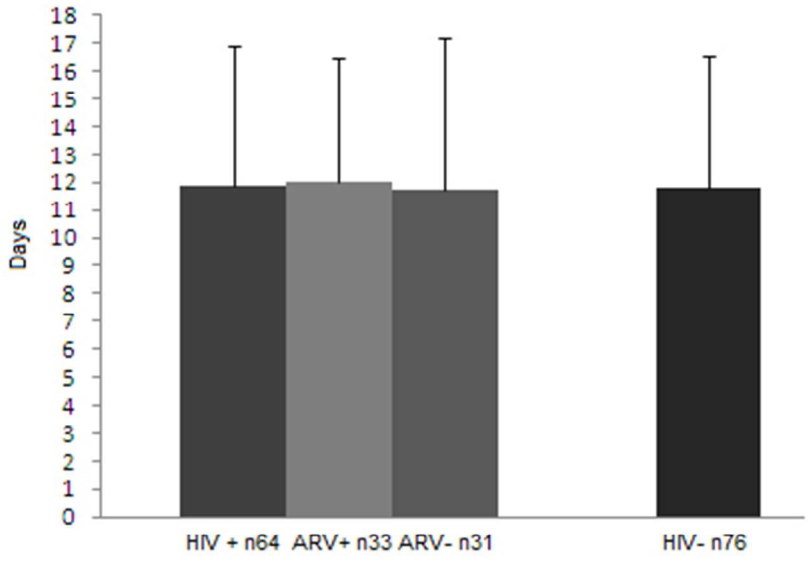

Figure 1. Induction time to therapeutic warfarin dose in days ± 1 SD in patients with (HIV+) or without HIV (HIV-) infection and on antiretroviral therapy (ARV+) or ARV therapy naïve (ARV-).

Figure 1
38x27mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

NA: Not applicable p = page

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ Abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Methods p 3, Intro Abstract, p 3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ p 5, Intro
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ p 5–6 : Aim
Methods		
Study design	4	Present key elements of study design early in the paper ✓ p 6 : Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Location p 8, Dates p 8, Setting p7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ p 7 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants p 7–8 (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NA *Case-control study*—For matched studies, give matching criteria and the number of controls per case NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ p6-7, p6 ✓ p6 p 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ p6-7 part 1,2 → p 7 (induction time), p Warfarin dose
Bias	9	Describe any efforts to address potential sources of bias None
Study size	10	Explain how the study size was arrived at ✓ p 8 (bottom) – 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why p 8: Stats ✓ p 6 : Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding p 8 (*b*) Describe any methods used to examine subgroups and interactions NA (*c*) Explain how missing data were addressed None (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed NA *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy NA (*e*) Describe any sensitivity analyses None

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed NA
		(b) Give reasons for non-participation at each stage NA (No HIV consent p 6)
		(c) Consider use of a flow diagram NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders M/F, Age, HIV, CD4 p 6
		(b) Indicate number of participants with missing data for each variable of interest NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) NA (until ther. dose p 6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time p 9
		Case-control study—Report numbers in each exposure category, or summary measures of exposure NA
		Cross-sectional study—Report numbers of outcome events or summary measures p 9–10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included p 9–10 p 6
		(*b*) Report category boundaries when continuous variables were categorized p 6: Age ≥ 18
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses p 9–10 (gender, ARVs)
Discussion		
Key results	18	Summarise key results with reference to study objectives p 13–14: Summary
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias p 13 – para 2
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence p 13 – Summary
Generalisability	21	Discuss the generalisability (external validity) of the study results p 14 – Summary
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based p 2 (None)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.