The impact of warfarin on the rate of progression of aortic stiffness in hemodialysis patients: a longitudinal study

Fabrice Mac-Way¹,², Aurélie Poulin¹,², Mihai Silviu Utescu¹,², Sacha A. De Serres¹,², Karine Marquis¹, Pierre Douville³,4, Simon Desmeules¹,², Richard Larivièrem², Richard Larivière¹,², Marcel Lebel² and Mohsen Agharazii¹,²

¹CHU de Québec Research Center, L’Hôpital-Dieu de Québec Hospital, Québec, QC, Canada, ²Division of Nephrology, Faculty of Medicine, Université Laval, Québec, QC, Canada, ³Département de biologie moléculaire, de biochimie médicale et de pathologie, Faculty of Medicine, Université Laval, Québec, QC, Canada and ⁴Département de biologie médicale, L’Hôtel-Dieu de Québec Hospital, CHU de Québec, Québec, QC, Canada

Correspondence and offprint requests to: Mohsen Agharazii; E-mail: mohsen.agharazii@crhdq.ulaval.ca

ABSTRACT

Background. Accelerated progression of aortic stiffness in patients with advanced chronic kidney disease is not well explained by the traditional cardiovascular risk factors. We hypothesized that vitamin K deficiency may result in an accelerated progression of aortic stiffness in the pro-calcifying uremic milieu.

Received for publication: 12.2.2014; Accepted in revised form: 21.5.2014
INTRODUCTION

Cardiovascular disease is the leading cause of death in patients with advanced chronic kidney disease (CKD). The risk of cardiovascular mortality in hemodialysis (HD) patients is 10–20 times higher than in the general population, even after stratification for other cardiovascular risk factors [1]. Aortic stiffness is part of a group of non-traditional cardiovascular risk factors that has been associated with increased risk of mortality in CKD [2–5]. Among various pathways that lead to vascular stiffness, calcification plays a dominant role in the alteration of biomechanical properties of the arterial wall [6–8].

Arterial calcification is the consequence of complex imbalances between promoters and inhibitors of mineralization and vascular smooth muscle cell transdifferentiation. In CKD, hyperphosphatemia seems to play a key role in the transdifferentiation of vascular smooth muscle cells into an osteoblastic phenotype, therefore, promoting vascular calcification [9]. Matrix Gla protein (MGP) is an anti-calcifying protein, which is thought to play a significant role in inhibiting and limiting the progression of vascular calcification. MGP knockout mice develop extensive vascular calcification and die prematurely of vascular rupture [10]. However, activation of MGP requires post-translational gamma-carboxylation of Glu residues, a vitamin K-dependent reaction [11, 12]. This understanding has lead to the development of various models of vascular calcification and isolated systolic hypertension models using warfarin [13–15]. Whereas vitamin K deficiency increases international normalized ratio (INR), a more subtle degree of vitamin K deficiency can also be detected by measurements of vitamin K levels or by measurements of the under-carboxylated fractions of vitamin K-dependent proteins such as osteocalcin and prothrombin. Among these, protein induced in vitamin K absence/antagonist-factor II (PIVKA-II), which is the under-carboxylated level of prothrombin, is considered to be one of the most sensitive methods to detect vitamin K deficiency [16]. Subclinical or functional vitamin K deficiency can, therefore, be defined by high serum levels of PIVKA-II but normal INR. Based on PIVKA-II levels, the prevalence of functional vitamin K deficiency has been reported to be over 90% in patients with Stages 3–5 CKD [16].

In humans, cross-sectional studies have shown an association between vascular and valvular calcification with warfarin therapy [17–22]. However, in longitudinal studies, the association between the use of warfarin and systolic hypertension, a surrogate of vascular calcification and stiffness, has not been confirmed [23, 24]. In the context of the present study, we hypothesized that warfarin therapy and vitamin K deficiency results in enhanced progression of aortic stiffness. Therefore, we examined the impact of warfarin therapy and functional vitamin K deficiency on the rate of progression of aortic stiffness in dialysis patient, a population which is particularly vulnerable to progressive vascular calcification.

MATERIALS AND METHODS

Study design and population

This was a sub-study of a single center observational longitudinal study examining the impact of exposure to warfarin on the rate of progression of aortic stiffness that was conducted at CHU de Québec—L’Hôtel-Dieu de Québec Hospital [25]. The main study aimed to determine the rate of progression of aortic stiffness in patients on HD. The inclusion criteria for the main study were age >18 years, chronic HD (>3 months), stable dry weight, single-pool Kt/V > 1.4, the absence of any clinical conditions that would hamper hemodynamic measurements [absence of femoral pulse, systolic blood pressure (SBP) < 90 mmHg] and the absence of acute episode of illness (infection, acute heart failure and active bleeding). These 109 patients who underwent a baseline and follow-up evaluation of aortic stiffness from 2006 to 2009 were eligible for the present sub-study. The inclusion criteria for this sub-study were continuous use of warfarin (n = 20) or continuous non-use of warfarin (n = 79) during the time that the patients underwent evaluation of aortic stiffness. Exclusion criteria were, non-continuous use of warfarin during the observation period (n = 10), inability to find a proper match based on age (±2 years) and atherosclerotic cardiovascular disease. Atherosclerotic cardiovascular disease was defined by a history of
stroke, coronary revascularization, lower extremity amputation or revascularization, myocardial infarction or ischemic heart disease, as shown by either a treadmill, echo or thallium stress tests. Among these, we identified 18 patients who were continuously treated by warfarin, and matched them (1 : 3) to 54 HD patients without warfarin (control). Indications for warfarin therapy were atrial fibrillation (n = 8), maintenance of vascular access patency (n = 7), deep venous thrombosis (n = 2) and metallic aortic valve (n = 1). The median exposure time to warfarin was 29 months (25th–75th percentile: 7.5–74 months). The study was approved by the institution review board and was conducted in accordance to the Declaration of Helsinki. All patients provided informed consent.

**Outcome measures**

The primary outcome of the study was the adjusted rate of progression of aortic stiffness. The secondary outcome was survival up to transplantation or up to August 2012.

**Arterial parameters**

Hemodynamic measurements were performed after 15 min of rest in a supine position prior to the mid-week dialysis session. In the case of an arteriovenous fistula, measurements were performed on the contralateral arm. Brachial artery blood pressure (BP) was recorded using an automatic sphygmomanometer BPM-100 (BP-Tru, Coquitlam, Canada). BP was recorded six times, with a 2 min interval between each measurement, and the average of the last five measurements was used to determine the brachial systolic and diastolic BP (DBP) [26]. We determined carotid-femoral pulse wave velocity (cf-PWV) using Complior® SP (Artech Medical, Pantin—France) as described previously [27, 28]. Briefly, the sensors were positioned over carotid and femoral arteries and the transit times were measured. Three consecutive recordings were performed to determine the transit time, followed by the measurement of direct distance between the two probes to calculate cf-PWV by dividing the travel distance by transit time. To assess the impact of arterial stiffness on central pulse wave profile, radial pulse wave profile was recorded by applanation tonometry after recalibration with SBP and brachial DBP (SphygmoCor system®, AtCor Medical Pty. Ltd., Sydney, Australia). Three consecutive recordings were performed and central pulse wave profile was constructed using the generalized transfer function as previously described and validated [29]. Central SBP, DBP, mean BP (MBP), pulse pressure (PP) and heart rate adjusted central AIX (AIX = (AP/PP) × 100) were evaluated.

**Biological parameters**

Plasma PIVKA-II levels were assayed by a sandwich ELISA kit (normal < 2 ng/mL), according to the manufacturer protocol (Stago, Asnière, France). Dialysis dose, lipid profile C-reactive protein and mineral metabolism were performed by clinical biochemistry laboratory on pre-dialysis samples.

**Data analysis**

Data is expressed as means ± SD or median (25th–75th percentiles). Mann–Whitney U, Student t-test and χ² were used to compare baseline parameters between groups. Multiple linear regression model was used to assess the determinants of changes in aortic stiffness so as to take into account changes in MBP and duration of follow-up. To examine the impact of vitamin K status on the rate of progression of aortic stiffness and mortality, we defined functional vitamin K deficiency as patients in control group with a PIVKA-II level above median (>3 ng/mL). Kaplan–Meier method and log-rank test was used to examine the impact of warfarin and vitamin K status on patients’ survival. Univariate Cox proportional hazards model were used to estimate the risk of mortality related to warfarin therapy. Multivariable conditional Cox proportional hazards model was used to study the adjusted risk of mortality related to warfarin therapy after incorporation of clinically and biologically important confounding factors in the analysis. We then used a forward conditional Cox regression analysis in order to identify the determinants of mortality in the cohort. Violations of the proportional hazards assumption were examined by plots of the logarithm of the negative logarithm of the estimated survivor function versus log time. A two-tailed P-value < 0.05 was considered to be statistically significant.

**RESULTS**

**Patient population**

The baseline parameters of control and warfarin groups are summarized in Table 1. The groups were well matched for age and atherosclerotic cardiovascular disease by design. Overall, the groups were similar in terms of comorbidities. In the warfarin group, there was a non-significant lower prevalence of diabetes (P = 0.10), higher dialysis vintage (P = 0.12), but a significantly lower level of albumin. PIVKA-II levels were higher in the warfarin group, as expected. The parameters of mineral metabolism, lipid profile and pharmacological treatments were comparable in both groups.

**Warfarin and progression of aortic stiffness**

Hemodynamic parameters were similar in both groups (Table 2). There was a statistically non-significant earlier timing of wave reflection (Tr; P = 0.09) and higher AIX (P = 0.054) in the warfarin group. After a mean follow-up of 1.2 ± 0.4 years, cf-PWV increased by 0.86 ± 1.87 m/s in the control group and by 2.24 ± 2.68 m/s in the warfarin group (P = 0.024). Figure 1 shows the unadjusted and adjusted changes in cf-PWV in each group. In a multivariable analysis, warfarin therapy was a significant determinant of progression of aortic stiffness, after adjustments for MBP, time between two evaluations, age, diabetes, atherosclerotic cardiovascular disease and dialysis vintage (1.5 m/s, 95% CI: 0.30–2.83 m/s, P = 0.016). In the subgroup of patients with warfarin, we found no significant relationships between the duration of warfarin therapy and the progression of aortic stiffness.

**Vitamin K status and progression of aortic stiffness**

To further determine whether the accelerated progression of aortic stiffness is related to warfarin therapy could be the result of an indication bias, we divided the group of patients...
without warfarin into patients with PIVKA-II levels above or below the median level of 3 ng/mL. Figure 2 shows that the adjusted rate of progression of aortic stiffness follows a linear trend in response to PIVKA-II levels and warfarin therapy.

**Warfarin, vitamin K status and patient outcome**

After a mean follow-up of 29 months (95% CI: 25–34) from the second evaluation of aortic stiffness, 46 deaths occurred, 30 (56%) in the control and 16 (89%) in warfarin group. There were no deaths related to bleeding. Kaplan–Meier survival curves (Figure 3) show a lower probability of survival in warfarin-treated patients. In the univariate Cox model, warfarin treatment was associated with a 2.40-fold increase in risk of death (95% CI: 1.29–4.46, P = 0.006). To examine the impact of other potential confounding factors that could have influenced survival of patients, we constructed various adjusted models that are presented in Table 3. Even in the fully adjusted model, including age, atherosclerotic cardiovascular disease, diabetes, dialysis vintage, albumin and vascular access, as potential confounders, warfarin therapy was associated with a 2.53-fold increase in risk of death (95% CI: 1.31–4.89, P = 0.006). In Model 4, incorporation of Alx, resulted in a 2.20-fold increase in risk of death (95% CI: 1.12–4.36, P = 0.023). To examine whether the increased risk of
mortality in the warfarin group is related to aortic stiffness, we introduced follow-up cf-PWV and MBP in the Model 5 of Table 3, and indeed showed that after inclusion of aortic stiffness the risk of mortality related to warfarin therapy was no longer statistically significant (HR: 2.06, 95% CI: 0.90–4.71, P = 0.086).

Furthermore, we used a multivariable forward conditional Cox regression analysis in order to identify the determinants of mortality. As summarized in Table 4, a follow-up cf-PWV > 13.8 m/s (median), was retained in the model, whereas the use of warfarin was no longer statistically significant.

Finally, to further examine whether the enhanced mortality is related to warfarin therapy could be the result of an indication bias, we also studied the group of patients without warfarin according to the PIVKA-II levels above or below the median level of 3 ng/mL. The impact of this stratification on patient survival is shown in Figure 4.

Table 3. Risk estimate of mortality related to warfarin therapy

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>2.40 (1.29–4.46)</td>
<td>0.006</td>
</tr>
<tr>
<td>Adjusted</td>
<td>Model 1&lt;sup&gt;a&lt;/sup&gt; 2.64 (1.38–5.04)</td>
<td>0.003</td>
</tr>
<tr>
<td>Adjusted</td>
<td>Model 2&lt;sup&gt;b&lt;/sup&gt; 2.65 (1.37–5.13)</td>
<td>0.004</td>
</tr>
<tr>
<td>Adjusted</td>
<td>Model 3&lt;sup&gt;c&lt;/sup&gt; 2.53 (1.31–4.89)</td>
<td>0.006</td>
</tr>
<tr>
<td>Adjusted</td>
<td>Model 4&lt;sup&gt;d&lt;/sup&gt; 2.20 (1.12–4.36)</td>
<td>0.023</td>
</tr>
<tr>
<td>Adjusted</td>
<td>Model 5&lt;sup&gt;e&lt;/sup&gt; 2.06 (0.90–4.71)</td>
<td>0.086</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for age, atherosclerotic cardiovascular disease, diabetes and dialysis vintage.
<sup>b</sup>Adjusted for parameters in Model 1 + vascular access.
<sup>c</sup>Adjusted for parameters in Model 2 + albumin.
<sup>d</sup>Adjusted for parameters in Model 3 + AIx.
<sup>e</sup>Adjusted for parameters in Model 4 + carotid-femoral pulse wave velocity and MBP of the follow-up visit.

Table 4. Determinants of mortality

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.05 (1.02–1.09)</td>
<td>0.002</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.81 (0.73–0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AIx (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05 (1.01–1.09)</td>
<td>0.007</td>
</tr>
<tr>
<td>cf-PWV &gt; 13.8 m/s&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.11 (1.001–4.43)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Cox forward conditional analysis was used and the following variables were entered but excluded from the model: warfarin, cardiovascular disease, diabetes, dialysis vintage, MBP and vascular access.
<sup>a</sup>AIx adjusted heart rate of 75 beat/min.
<sup>b</sup>cf-PWV > the median of 13.8 at follow-up visit.
DISCUSSION

This study shows, for the first time, that the use of warfarin in HD patients is associated with an accelerated progression of aortic stiffness. In addition, patients receiving warfarin had twice the risk of all-cause mortality after adjustment for the usual confounding risk factors of death in this population. Our secondary analysis shows that patients with a more severe functional vitamin K deficiency (higher PIVKA-II levels) had an intermediate degree of progression of aortic stiffness and mortality, compared with controls with lower PIVKA-II levels and warfarin treated patients.

Warfarin, an inhibitor of vitamin K epoxide reductase, impedes vitamin K recycling and vitamin K-dependent carboxylation of a number of proteins, such as coagulation factors and MGP. MGP is considered to be an anti-calcifying protein that binds tightly to the crystal nuclei, preventing further growth, and it has also been proposed that carboxylated MGP inhibits bone morphogenic protein-2, a potent osteogenic growth factor [12, 30, 31]. Experimental studies in rats have shown that high-dose warfarin can induce arterial calcification and isolated systolic hypertension [13–15, 32]. In humans, Rennenberg et al. [17] studied femoral vascular calcification in 18 patients treated by warfarin for over 10 years as compared with 18 healthy controls. They showed that the odds ratio for femoral vascular calcification was 8.5-fold (95% CI: 2.01–35.95) higher in warfarin treated subjects. The cross-sectional association of valvular and coronary artery calcification with warfarin therapy has also been reported by other groups [18–22]. However, the association between warfarin treatment and arterial stiffness and its hemodynamic consequences in humans are still unclear.

In order to investigate the impact of warfarin on SBP and PP, Krishnan et al. [23] performed post hoc analyses of the Stroke Prevention in Non-rheumatic Atrial Fibrillation (SPINAF) trial, a randomized, double-blind, placebo-controlled trial comparing warfarin and placebo in 525 subjects with non-rheumatic atrial fibrillation. Their analysis, found no statistically significant differences between the placebo and warfarin groups in any of the BP parameters at any time point. However, stratified analyses showed a significant increase in PP in warfarin-treated subjects with a history of hypertension and those with baseline SBP ≥140 mmHg. They also found a similar tendency in diabetic subjects which, however, failed to reach the statistical level of significance. These observations suggest that warfarin may have an adverse effect on PP in patients with increased susceptibility to vascular calcification. However, Lim et al. [24] failed to show any significant changes in SBP in diabetic hypertensive patients. In our study, we did not find any significant differences in PP between groups. However, SBP and PP are influenced by other factors such as heart rate, MBP and the timing of wave reflection. Therefore, one cannot conclude from the lack of increase in SBP or PP under warfarin therapy seen in previous studies that warfarin therapy has no impact on aortic stiffness. Contrary to these indirect measurements of arterial stiffness, we studied cf-PWV, which is considered to be a gold standard and robust measurement of aortic stiffness [33].

Our patients were on warfarin therapy for a median of 29 months prior to enrollment into the study and yet had similar baseline aortic stiffness. First, this could be explained by the fact that patients in each group were match for both age and presence of cardiovascular disease, which are two of the strongest determinants of aortic stiffness. Second, given the mortality rate in this population and that the subjects had also to survive an additional year for the follow-up evaluation, there certainly may have been a selection bias of survivors [34, 35]. Third, the temporal relationship between warfarin therapy and vascular calcification may not follow a linear pattern. Indeed, in non-CKD experimental model, high doses of warfarin are required for at least >3 weeks in order to induce vascular calcification, with a plateau effect after 4–8 weeks [15, 36]. In addition, therapeutic warfarin in non-CKD rats (INR of between two and three) markedly depleted vitamin K levels within the vasculature, but it did not cause vascular calcification during the 7-week time frame of the study (unlike their CKD counterparts) [14, 32]. However, in prone to ectopic calcification DBA/2 mice, warfarin induced arterial calcification and stiffness in a time and dose-dependent manner, which was inhibited by vitamin K2 treatment [37]. These observations may suggest that the extent of the impact of warfarin on vasculature is potentially the result of complex interactions between duration and intensity of warfarin exposure, and duration and intensity of exposure to various pro-calcifying risk factors.

It is still unclear whether all the pro-calcifying effects of warfarin are explained by under carboxylation of MGP. Indeed, there are emerging studies showing that warfarin can also induce vascular calcification through transglutaminase 2-mediated activation of β-catenin pathway without alterations in carboxylated MGP levels [38, 39]. We, therefore, performed a secondary analysis based on vitamin K status, and showed that greater severity of spontaneous functional vitamin K deficiency had an intermediate impact on the rate of progression of aortic stiffness and mortality, supporting, at least partially, that functional vitamin K deficiency per se may contribute to the progression of aortic stiffness and mortality, in the pro-calcifying uremic milieu. Holden et al. [16], have shown that in CKD (Stages 3–5) subclinical vitamin K deficiency, defined as PIVKA-II level >2 ng/mL, was present in 97% of the patients. Using this cut-off, 72% of our control patients would have been considered to have subclinical vitamin K deficiency.

The strength of the study is that it evaluates aortic stiffness, rather than peripheral SBP or PP, in two groups well matched for clinical comorbidities and with similar hemodynamic, pharmacotherapy and mineral metabolism parameters. In contrast to previous cross-sectional studies, the longitudinal design of the study establishes a temporal relationship between exposure to warfarin and progression of aortic stiffness. We acknowledge that because of the observational nature of the study and the inherent indication bias for warfarin therapy, the causality link between progression of aortic stiffness and warfarin therapy cannot be formally established. Indeed, the optimal control group would have been composed of patients with indications for anticoagulation such as atrial fibrillation or thrombosis. However, the
study, even though small, is quite robust as it shows in a multivariate Cox regression analysis that adjustments for comorbidities had limited impact on the mortality risk associated with warfarin therapy (Models 1–3). Since AIX is influenced by arterial stiffness, proximal reflecting sites and ejection duration, and it has been associated with increased mortality in HD population, in Model 4 we included AIX which was slightly higher at baseline in the warfarin group and still found a significantly higher risk of death associated with warfarin therapy (unadjusted HR of 2.40 versus adjusted HR of 2.20). In Model 5, we included MBP and follow-up cf-PWV and showed that introduction of aortic stiffness into the equation attenuates the risk, suggesting that at least part of the excess risk of mortality is related to enhanced aortic stiffness. We then applied a different approach using a forward conditional method, which showed that a follow-up cf-PWV > 13.8 m/s was retained as a predictor of mortality and that in this model the use of warfarin was no longer a statistically risk for mortality. Furthermore, our analysis show that patients with a greater severity of spontaneous functional vitamin K deficiency had an intermediate degree of progression of aortic stiffness and mortality, adding support to the role of vitamin K metabolism in the progression of aortic stiffness and adding to the validity of the results in patients with warfarin therapy.

In conclusion, this observational study shows that the use of warfarin is associated with an accelerated rate of progression of aortic stiffness and mortality. The greater risk associated with increased aortic stiffness suggests that the higher mortality associated with warfarin may be mediated through enhanced aortic stiffness. These hypothesis-generating results should be confirmed in larger cohorts and the net cardiovascular benefit of warfarin in this group of patients needs to be urgently examined in prospective randomized trials. Further studies are also required to examine the potential benefits of treating vitamin K deficiency in this population.

ACKNOWLEDGEMENTS

We are grateful to the dialysis personnel for their generous contribution and kind collaboration.

FUNDING

This project was supported by the Canadian Institute of Health Research (CIHR), New Emerging Team Grant (NET-54008), the Heart & Stroke Foundation of Canada, the Kidney Foundation of Canada, and the Canadian Diabetes Association. A.P. holds a scholarship from Fonds de Recherche du Québec—Santé (FRQ-S), Dr De Serres holds a Kidney Research Scientist Core Education and National Training Program (KRESCECENT) scholarship supported by CIHR, Kidney Foundation of Canada and Canadian Society of Nephrology and from FRQ-S, M.A. holds a scholarship from FRQ-S, and a research chair in nephrology from Université Laval.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


Impact of warfarin on aortic stiffness 2119
Misdiagnosing renal amyloidosis as minimal change disease

Rabya Hussain Sayed¹,², Janet Anne Gilbertson¹, David Frederick Hutt¹, Helen Jane Lachmann¹,², Philip Nigel Hawkins¹, Paul Bass² and Julian David Gillmore¹,²

¹National Amyloidosis Centre, UCL Medical School, London, UK, ²Centre for Nephrology, Division of Medicine, Royal Free Campus, UCL Medical School, London, UK

Correspondence and offprint requests to: Julian David Gillmore; E-mail: j.gillmore@ucl.ac.uk

ABSTRACT

Background. Minimal change disease (MCD) accounts for 10–15% of all adult nephrotic syndrome cases and requires normal renal histology by light microscopy and negative immunohistology. Foot process effacement on electron microscopy (EM) is typical. Renal amyloid deposits demonstrate pathognomonic green birefringence when viewed under cross-polarized light after staining tissue with Congo red (CR) and may reveal fibrils on EM. Late diagnosis and delayed treatment of renal amyloidosis negatively impact on renal and patient survival.

Received for publication: 20.2.2014; Accepted in revised form: 27.5.2014