Clinical Practice: Original Paper

Vitamin K Antagonists Predispose to Calciphylaxis in Patients with End-Stage Renal Disease

Running Title: Warfarin and Calciphylaxis Risk

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Received: September 17, 2014
Accepted after revision: December 9, 2014
Published online: ■■■

Key Words

Calciphylaxis calcific uremic arteriolopathy warfarin dialysis

Abstract

Background/Aims: Calciphylaxis is associated with a poor prognosis in dialysis patients, and its pathogenesis remains incompletely understood. Although the use of vitamin K antagonists (VKA) has been implicated, previous reports are conflicting. We aimed to determine if vitamin K antagonists conferred an increased risk of calciphylaxis in patients on dialysis. **Methods:** We performed a single-centre, retrospective cohort study of 2,234 patients receiving dialysis, and compared characteristics of those with and without calciphylaxis. **Results:** We identified 5 cases of calciphylaxis (all female) between January 2009 and December 2013. Overall, 142 patients (6.4%) were treated with VKA during the study period. Calciphylaxis was more common in the VKA group (4 of 142 patients, OR = 61, 95% CI 6.7 – 546, p=0.0001). VKA was withdrawn in all cases and treatment instituted with sodium thiosulphate, cinacalcet and supportive measures. All patients recovered, although there was one sudden cerebrovascular death during follow-up. **Conclusion:** Treatment with VKA predisposes to the development of calciphylaxis.

Background and Introduction

Calciphylaxis, also known as calcific uremic arteriolopathy (CUA), is a serious condition affecting small arterioles. It is characterised by vascular calcification, intimal hyperplasia and microthrombi, with secondary fat necrosis [1]. Clinically this gives rise to painful cutaneous purpuric plaques and nodules with ulceration [2]. Calciphylaxis carries a poor prognosis. Previous studies report a 1-year survival of less than 50%, with the majority of deaths occurring from sepsis or cardiovascular events [3,4]. Calciphylaxis occurs most commonly in end-stage renal disease (ESRD) requiring dialysis, but is also associated with primary hyperparathyroidism, malignancy, connective tissue disorders and the use of vitamin K antagonists (VKA). Other risk factors include diabetes, female gender, obesity and thrombophilia syndromes such as protein C and S deficiency [5]. However, even in the setting of ESRD, where calciphylaxis is most commonly described, it is a rare disease with an estimated prevalence of up to 5.7 per 10,000 haemodialysis patients [4,6].

The pathophysiology of calciphylaxis is incompletely understood. Extracellular fluid is supersaturated with calcium and phosphate, but precipitation and crystallization does not usually occur due to the presence of calcification inhibitors. Excessive calcium and phosphate concentrations such as commonly occur in renal failure can result in direct tissue precipitation, or may promote arteriolar calcification through osteogenic transformation of vascular smooth muscle cells [7]. Parathyroid hormone (PTH) is central to calcium and phosphate homeostasis, and perturbations in PTH concentrations may contribute to the development of calciphylaxis [8]. Other potential modulators of calcification include the NFκB pathway, the transcription factor Cbfa-1, and inhibitors of calcification including Fetuin-A and Matrix-Gla-protein (MGP) [4,9,10]. MGP has attracted particular interest given its potent inhibition of vascular calcification and requirement for vitamin K dependent gamma-carboxylation. Since MGP gamma-carboxylation status is impaired in haemodialysis patients [9,10], and VKA treatment reduces MGP gamma-carboxylation and promotes medial arterial calcification [11,12], VKA use may also predispose to the development of calciphylaxis. However, data from existing studies are conflicting [4,13-16].
We hypothesized that the use of VKAs in dialysis patients would be associated with an increased incidence of calciphylaxis. We therefore carried out a single centre cohort study to determine the predictors of calciphylaxis and the effect of treatment with VKAs.

Methods

Data

All patients who received renal replacement therapy at Addenbrooke's Hospital, Cambridge, between 1st January 2009 and 31st December 2013 were considered for the study. Patients were included if they had received haemodialysis or peritoneal dialysis during the study period. Patients were excluded if they had received acute dialysis (<90 days duration) only. Demographic and laboratory data were abstracted from the hospital's electronic database system, including age, gender, date of dialysis initiation, diabetic status, date of death, prescribing history, parathyroid hormone (PTH), albumin, calcium, phosphate, sodium, haemoglobin, and coagulation studies including INR. Biochemical and haematological data for 6 months before and after the study window were included.
Cases of calciphylaxis were identified from hospital and local histopathology records.

Albumin-corrected calcium [normal range 2.1 – 2.5 mmol/l], phosphate [normal range 0.8 – 1.4 mmol/l], and creatinine [normal range 35 - 125 μmol/l] were analysed using a Dimension® auto-analyser (SIEMENS Ltd.). Hyper- or hypophosphataemia, or -calcaemia, was defined as corrected calcium or phosphate values above or below the respective reference ranges noted above. PTH [normal range 14 – 72 ng/l] was analysed using the ADVIA® Centaur XP Immunoassay system for intact PTH (1-84) (SIEMENS Ltd.).

Analysis

Data are presented as mean (SD) or median (interquartile range) as appropriate. Comparisons for demographic data between calciphylaxis cases and controls were made by student's T-test or Mann-Whitney rank sum test for continuous variables and Fisher's exact test for frequency data. The odds ratios, point estimates and confidence intervals for developing calciphylaxis were computed by exposure to vitamin K antagonists, using Woolf's method [17]. All analyses were carried out using Stata SE v 13.1, College Station TX.

Results

Between 1 January 2009 and 31 December 2013, 2,234 patients received dialysis at our centre, representing 8,504 patient years follow-up. Of these, 289 received peritoneal dialysis during the study period. Median dialysis vintage at the end of follow-up was 3.0 (1.1 – 5.3) years. During the study period, 142 patients (6.4%) were treated with VKA, of whom 141 received warfarin, and 1 acetocoumarol. VKA-treated patients were significantly older ($p=0.02$), and had shorter follow-up times (1275, IQR 687 – 1860 days) than controls (1786, IQR 944 – 1897 days, $p<0.0001$). There were more deaths (40/142 (28%) versus 429/2092 (20%)) in the VKA-treated group ($p=0.03$).

Calciphylaxis was diagnosed in 5 patients (all female) during the study period. Characteristics of calciphylaxis cases are summarized in Table 1. Diagnosis was histologically confirmed in all cases (representative example for patient 3 shown in Figure 1). In comparison with the control population, calciphylaxis patients had

similar calcium (2.38 ± 0.25 versus 2.23 ± 0.22 mmol/l, p=0.11), phosphate (1.46 ± 0.26 versus 1.24 ± 0.49 mmol/l, p=0.3) PTH (172 (IQR 39-190) versus 115 (IQR 51 – 232) ng/l, p=0.99) and CRP concentrations (45 (34-52) versus 18 (5-58), p=0.22). In the warfarin group, 4 calciphylaxis cases occurred in 142 patients; in the control group, 1 case occurred in 2092 patients. Although the overall incidence was low (0.0005 per patient year), the incidence was significantly higher in patients receiving vitamin K antagonists (0.008 per patient year, OR = 61, 95% CI 6.7 – 546, p=0.0001).

Case Descriptions

Patient 1

A 75-year-old female haemodialysis patient developed several painful, indurated areas over the infra-umbilical anterior abdominal wall. She had received warfarin for 4 years for recurrent deep vein thrombosis. Ultrasound demonstrated nodular hyperechoic areas in subcutaneous tissue, and needle biopsy confirmed calciphylaxis. Marked secondary hyperparathyroidism was present, and had been treated for 12 months with the calcimimetic cinacalcet (180mg per day at diagnosis).
Treatment was instituted with intradialytic sodium thiosulphate (STS) along with continued cinacalcet use, and warfarin convereted to dabigatran. Subtotal parathyroidectomy was carried out 3 months later following identification of a left-sided parathyroid adenoma. The patient made a full recovery with complete resolution of symptoms, although abdominal wall lesions remain palpable.

Patient 2

A 69-year-old female type II diabetic haemodialysis patient presented with abdominal wall pain and multiple small areas of cutaneous infarction. A biopsy of one of the lesions revealed calciphylaxis. The patient had diabetic microvascular disease including retinopathy, peripheral neuropathy and nephropathy, and had received warfarin for two-and-a-half years for recurrent deep venous thrombosis (without evidence of a thrombopylia disorder).
Treatment consisted of intradialytic STS, substitution of calcium-based phosphate binders with sevelamer-HCl, cinacalcet 30mg once daily, and substitution of warfarin with dalteparin. STS was limited to 5 treatments due to severe nausea and vomiting; dalteparin was converted to dabigatran due to hair loss. The lesions healed completely

over a period of approximately 3 months, and the patient remained well for a further three months before suffering a fatal brain stem infarction secondary to basilar artery thrombosis.

Patient 3

A 45-year-old female dialysis patient received a third successful renal transplant in January 2013 after 8 years on haemodialysis, with stable primary graft function (eGFR 30ml/min/1.73m^2 after several weeks). She had developed tertiary hyperparathyroidism and received a subtotal parathyroidectomy before transplantation, but became hypercalcaemic 6 weeks after transplantation and developed painful nodular skin lesions over the right breast and both upper arms. A skin biopsy from the right deltoid region revealed features of calciphylaxis (Figure 1). She had received warfarin for 4 years up to the time of transplantation for recurrent deep venous thrombosis.

Calciphylaxis and hypercalcaemia were treated with intravenous STS, pamidronate 30mg weekly, sevelamer-HCL, and analgesia. The patient had a difficult course with severe pain and progressive lesions requiring surgical debridement, followed by poor wound healing and necrosis ultimately requiring mastectomy. Although new lesions continued to emerge up to 6 months after diagnosis, these had improved after 9 months and ultimately healed, with the patient making a full recovery.

Patient 4

A 64-year-old female haemodialysis patient presented with painful lesions over the abdominal wall and breasts, 6 years after commencing warfarin treatment for recurrent pulmonary emboli in the context of a positive lupus anticoagulant. She had presented two years previously with similar lesions but inconclusive histology and subsequent spontaneous improvement (although incomplete resolution). One year after the initial presentation, surgical debridement of abdominal lesions was undertaken. Retrospective review of surgical excision biopsies demonstrated features consistent with calciphylaxis.

Treatment included cinacalcet, substitution of calcium acetate with sevelamer, and substitution of warfarin with dabigatran. Given the severity of symptoms, several lesions were resected. The patient made a full recovery. Cinacalcet was eventually

discontinued after 9 months due to oversuppression of PTH. The patient remains asymptomatic.

Patient 5

A 73-year-old female haemodialysis patient with peripheral vascular disease and previous left above-knee amputation developed painful ulceration of the amputation stump. The ulceration did not resolve with conservative management and an empirical course of prednisolone, and a skin biopsy demonstrated calciphylaxis. Renal failure was attributed to nephrocalcinosis of unknown aetiology. Marked secondary hyperparathyroidism had resulted in hypercalcaemia at the time of diagnosis. Treatment consisted of post-dialytic STS, cinacalcet 60mg per day, and sevelamer-HCl. Lesions showed slow resolution over a period of 4 months. Subtotal parathyroidectomy was undertaken three months after presentation. The patient made a full recovery.

Patient characteristics are summarised in detail in supplementary table 1.

Discussion

Calciphylaxis is a serious complication in patients with advanced renal impairment, with a reported mortality of up to 81% and a median post-diagnosis survival of less than 3 months.[4] Here, we identified 5 cases of calciphylaxis in a large cohort of dialysis patients over a 5-year study period, 4 of whom had received treatment with vitamin K antagonists prior to diagnosis. Further, we report a good outcome in all patients (except for one sudden cerebrovascular death) after treatment with STS, calcimimetics, and the withdrawal of vitamin K antagonists.

Previous reports have implicated VKAs in the development of calciphylaxis. In addition to many anecdotal reports [13-15,18], Nigwekar *et al.* reported a strong association with warfarin use (OR = 4.3) in a case control study including 62 cases of calciphylaxis [15]. In contrast, Weenig *et al*. reported high usage of warfarin, but no association with calciphylaxis in 64 cases and 98 matched controls [4]. Similarly, Fine and Zacharias reported no association of warfarin use with calciphylaxis in a case-control study of 36 cases with calciphylaxis, although this study had methodological shortcomings [16]. Our findings support an association of calciphylaxis with warfarin use. Together with evidence that VKA use is also

associated with large vessel calcification (itself a predictor of mortality) [12], and that warfarin use increases bleeding risk and does not prevent cerebrovascular events in patients receiving dialysis [19], our findings provide a rationale for limiting exposure to VKAs in ESRD.

All cases identified were post-menopausal females. This finding is consistent with the female preponderance reported in most other studies [4,15,20], where 64 – 80% of cases are female; Mazhar and colleagues reported an odds ratio of 6.04 [2]. The mechanism underlying this excess risk is likely to be associated with oestrogen deficiency, which increases the risks of osteoporosis and vascular calcification [8] and results in reduced expression of osteoprotegerin [8]. One patient (patient 3) developed calciphylaxis post-transplantation. Although calciphylaxis is unusual in this setting, she had a high dialysis vintage (95 months) and had developed severe tertiary hyperparathyroidism pre-transplantation. Histology was consistent with a diagnosis of calciphylaxis (Figure 1).

Our study has several strengths. First, we present data from a large contemporary dialysis cohort. Second, all cases were confirmed histologically, and data on the use of VKA were recorded routinely. Third, outcome data were available on all patients. However, our findings should be considered against the limitations of our study, including the small number of cases and its retrospective nature.

Patients with ESRD often require systemic anticoagulation for atrial fibrillation or recurrent venous thrombo-embolism, and some physicians continue to use warfarin for maintenance of vascular access patency. Our data demonstrate that, where continued anticoagulation is required, the use of newer anticoagulants such as dabigatran in conjunction with STS, cincacalcet, avoidance of oral calcium intake and definitive treatment of resistant hyperparathyroidism can results in resolution of cutaneous lesions. Our findings add to a growing body of evidence that supports the avoidance of VKA treatment in patients with ESRD. Where calciphylaxis does occur, VKAs should be withdrawn.

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Fig. 1. Patient 3. Arterioles in subcutis showing mural calcification and luminal occlusion by fibrous intimal proliferation. There is surrounding fat necrosis (H&E stain).

Table 1

	Vitamin K Antagonists	**Control**	**Total**	**p-value**
n	142	2092	2234	
Age (years)	51 ± 12	47 ± 13	48 ± 14	0.02
Male (%)	89 (63)	1443 (69)	1532 (64)	0.38
Peritoneal dialysis (%)	24 (17)	265 (13)	289 (13)	0.15
Dialysis vintage (at last follow-up, days)	1111 (623 – 1826)	1106 (348 – 1943)	1107 (400 – 1933)	0.45
Follow-up time (days)	1275 (687 – 1860)	1786 (944 – 1897)	1753 (926 – 1897)	<0.0001
Deaths (%)	40 (28%)	429 (20.5)	469 (21)	0.03
Calciphylaxis (%)	4 (3)	1 (0.04)	5	0.0001

Supplementary table 1

Patient characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Case Mean/Median**
Age at Diagnosis (years)	75	69	46	64	73	66 ± 12
Gender	Female	Female	Female	Female	Female	
Dialysis Vintage (months)	22	3	95	4	47	22 (4 - 47)
Kt/V	2.06	3.8	1.72*	1.6	1.75	2.2 ± 0.9
URR (%)	73	71	79*	76	78	74.4 ± 3.8
Dialysis frequency (per week)	3	3	-	3	3	3

Primary renal disease	Htn	DN	CPN	Unknown	Unknown	-
Vascular comorbidities	-	PVD, HF	-	-	PVD, IHD	-
Radiological evidence of arterial calcification	Aorta Coronary	Aorta Iliac	Aorta Coronary Iliac	Aorta Coronary	Aorta Coronary Mesenteric Iliac	-
Diabetes (treatment)	-	Insulin	-	-	-	-
Hypertension treatment (no of drugs)	0	2	1	0	0	-
Phosphate binders	Calcium carbonate	Calcium acetate	Sevelamer	Calcium acetate	Calcium acetate	-
Anticoagulation	Warfarin	Warfarin	Warfarin	Warfarin	-	4/5
Gout	+	-	-	-	-	-
CRP	34	82	52	45	11	45 ± 26
Corrected Calcium	2.3	2.22	2.39	2.19	2.79	2.38 ± 0.24
Phosphate	1.76	1.48	1.68	1.16	1.24	1.46 ± 0.26
PTH	414	172	18	37	180	172 (37 - 190)
Post-Diagnosis Management and Outcomes						
STS	Yes	Yes	Yes	-	Yes	-
Cinacalcet	60mg/d	30mg/d	-	30mg/d	30mg/d	-
Anticoagulation	Dabigatran	Dalteparin	-	Dabigatran	-	-

Phosphate binders	Sevelamer	Sevelamer	Sevelamer	Sevelamer	Sevelamer	-
Antimicrobial treatment	-	-	Yes	No		-
Other	-	-	Pamidronate	-	PTX	-
Survival after diagnosis (months)	30	7	23	51	42	30 ± 17
Cause of death	-	Brain stem infarct	-	-	-	

Results for biochemical analyses shown represent values at diagnosis for cases, and averaged values over the observation period for control patients. PTX – parathyroidectomy. *Patient 3 presented post-transplantation. Values represent the last pre-transplantation values.