Warfarin-related nephropathy: possible role for the warfarin pharmacogenetic profile

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Abstract
Warfarin-related nephropathy (WRN) is a renal complication of warfarin treatment associated with over-anticoagulation. We describe a case of a 73-year-old man affected by chronic kidney disease, essential hypertension and atrial fibrillation treated with warfarin. The patient presented a rapid course of kidney failure after many episodes of over-anticoagulation, and renal biopsy demonstrated WRN. Interestingly, the patient’s warfarin pharmacogenetic profile showed that he was warfarin sensitive. This is the first report describing the presence of gene polymorphisms affecting warfarin metabolism in a subject with a biopsy-proven WRN. The patient was treated with corticosteroids obtaining a partial clinical response.

Keywords: acute kidney injury; chronic kidney disease; corticosteroids; warfarin pharmacogenetics; warfarin-related nephropathy

Background
Warfarin is the most commonly prescribed oral anticoagulant worldwide. It has a narrow therapeutic range with a large inter-individual variability of dose requirements and needs cumbersome monitoring of the international normalized ratio (INR) to avoid over-anticoagulation (INR>3). Over-anticoagulation is associated with higher haemorrhagic risk, and bleeding represents the major complication of warfarin therapy [1]. Recently, the association between over-anticoagulation and acute kidney injury (AKI) has been described and named warfarin-related nephropathy (WRN) [2]. WRN is associated with glomerular haemorrhage with tubular obstruction by red blood cell (RBC) casts and affects more frequently patients with known chronic kidney disease (CKD) [3]. Since WRN represents a severe complication with a poor renal prognosis [4], the identification of patients at risk is important. In 2007 the Food and Drug Administration added pharmacogenetic information to the warfarin product label suggesting the evaluation of CYP2C9 and VKORC1 polymorphisms to establish the initial dose and reduce the risk of over-anticoagulation in warfarin-sensitive patients, although the test is costly and its applicability is still under evaluation [5–7]. So far no data are available regarding the warfarin pharmacogenetic profile in WRN affected patients. We report the case of a CKD patient with biopsy proven WRN that was a carrier of CYP2C9*3 variant, a cytochrome polymorphism associated with higher risk of over-anticoagulation and bleeding. This is the first report suggesting an association with this genetic variant and WRN. Finally, we illustrate the clinical response of WRN to steroid treatment.

Case report
A 73-year-old Caucasian man with stage 3 CKD was admitted for worsening of renal function. Some weeks before he developed dyspepsia, anorexia, weight loss, asthenia and nocturia. He was initially accepted to another hospital and discharged with the diagnosis of stage 5 CKD. During this first hospitalization he declined the surgery to create an arteriovenous fistula (AVF) for haemodialysis and refused renal replacement therapy. The medical history revealed that he had hypertension and atrial fibrillation. Medications included pantoprazole, darbepoetin, bisoprolol and warfarin. Physical examination was substantially normal, BMI was 28 kg/m2 and blood pressure was 140/75 mmHg. Renal ultrasound demonstrated normal dimensions with preserved cortical thickness and no obstruction. Chest X-ray and abdominal CT scan were normal. The admission serum creatinine (sCr) was 6.96 mg/dL (eGFR 8 mL/min/1.73 m2), while 1 year before sCr was 1.3 mg/dL (eGFR 58 mL/min/1.73 m2). Urinalysis demonstrated significant proteinuria (2.46 g/24 h) and erythrocyturia (1739 RBC/µL). Serum albumin concentration was normal (4.1 g/dL) and a small monoclonal component (IgG k < 0.1 g/dL) was found without Bence-Jones protein at urinalysis. LDL cholesterol was 210 mg/dL. A serological workup, including C3/C4, ANCA,
ANA, anti-DNA, anti-GBM, viral serology and tumour markers, was negative. INR at admission was 1.52, but the patient referred many recent episodes of over-anticoagulation, and he had discontinued the oral anticoagulant. In the next days sCr progressively increased to 8.20 mg/dL, and we performed a kidney biopsy.

Kidney histology demonstrated modest matrix expansion with focal segmental sclerosis associated with vascular hyalinosis and extensive and diffuse tubular damage; in the tubular lumina large and occlusive RBC casts were evident (Figure 1). Immunofluorescence studies were negative, and ultrastructural evaluation was compatible with nephroangiosclerosis with secondary segmental/global glomerular sclerosis and confirmed the tubular damage.

The CYP2C9*2, CYP2C9*3, VKORC1–1639G>A and VKORC1 1173C>T polymorphisms were determined by real-time PCR (Lightcycler®, Roche). Genetic analysis was consistent with warfarin sensitivity and increased haemorrhagic risk since the patient was wild-type for VKORC1 and homozygous for CYP2C9*3 allele.

The patient was treated by sodium bicarbonate for 2 weeks in the hope of obtaining cast washout, but renal function did not improve. We then started corticosteroid therapy with prednisone 1 mg/kg/die and immediately sCr began to decrease to a value of 4.44 mg/dL (eGFR = 14 mL/min/1.73 m²) after 3 months (Table 1). Thus, the need for immediate dialysis was avoided.

**Discussion**

Causes of warfarin-dependent AKI include acute interstitial nephritis, atheroembolism, direct mesangial damage and WRN, a newly recognized syndrome characterized by glomerular haemorrhage with tubular obstruction by RBC casts. Although the first description dates back only to 2000 [8], WRN has been reported as a frequent complication of warfarin treatment affecting up to 30% of CKD patients [3, 9, 10]. The present report describes the case of a CKD patient who experienced many episodes of over-anticoagulation before developing severe kidney failure, but WRN was not suspected during the first hospitalization. When he was admitted to our division pre-renal and post-renal AKI were excluded as well as atheroembolism; rapidly progressive glomerulonephritis appeared unlikely.

![Fig. 1. Kidney biopsy findings. (A) Occlusive RBC casts in tubules (PAS stain; original magnification x40). (B) Tubular obstruction by RBC casts and tubular damage also involving tubules without RBC cast obstruction (PAS stain; original magnification x60).](image)

**Table 1. Laboratory findings according to clinical phase and therapy**

| Kidney function | Data at baseline (1 year before) | Data at first hospitalization | Data at admission to our division | Data after warfarin withdrawal and hydration | Data at start of prednisone 1 mg/kg/day | Data 3 months after starting prednisone |
|-----------------|---------------------------------|------------------------------|---------------------------------|---------------------------------------------|----------------------------------------|----------------------------------------|
| Azotemia (mg/dL)| ND                              | 167                          | 148                             | 135                                         | 133                                    | 150                                    |
| Creatinine (mg/dL)| 1.3                            | 5.70                         | 6.96                            | 7.47                                        | 8.2                                    | 4.44                                   |
| eGFR MDRD (mL/min/1.73 m²)| 83                            | 10                           | 8                               | 8                                           | 7                                      | 14                                     |
| Urinalysis      |                                 |                              |                                 |                                              |                                        |                                        |
| Erythrocyturia (RBC/µL)| ND                            | 1730                         | 500                             | 154                                         | 64                                     | 70                                     |
| Proteinuria (g/24 h)| 0.5                           | 2                            | 2.46                            | ND                                          | 0.62                                   | 0.51                                   |
| Other findings  |                                 |                              |                                 |                                              |                                        |                                        |
| INR             | NA                              | 1.45                         | 1.60                            | 1.05                                        | 1.02                                   | 1.05                                   |
| Albumin (g/dL)  | ND                              | 3.7                          | 4.1                             | ND                                          | 3.4                                    | 3.30                                   |
| Leucocytes (cell/µL)| 6170                         | 3920                         | 4530                            | 4980                                        | 5700                                   | 9610                                   |
| Red blood cells (cell/µL)| 4590                        | 2690                         | 3170                            | 3400                                        | 3740                                   | 2930                                   |
| Haemoglobin (g/dL)| 13.8                          | 8.7                          | 9.9                             | 10.6                                        | 11.8                                   | 9.60                                   |
| PCR (mg/L)      | 40                              | 9.6                          | 7.3                             | ND                                          | 1.4                                    | <1                                     |
| ANA/ENA/Anti-DNA/Anti-GBM C3/C4 (mg/dL)| ND                   | 85/26                         | 81/25                            | ND                                          | ND²                                    | ND                                     |
| Warfarin pharmacogenetic profile | CYP2C9                | ND                            | ND                              | CYP2C9*1*3                                   |                                        |                                        |
| VKORC1         | ND                              | ND                            | ND                              | WT                                          |                                        |                                        |

Corticosteroids allowed a partial recovery of kidney function as demonstrated by data obtained after 3 months of treatment. ND, not determined; NA, not available; Neg, negative; RBC, red blood cell; WT, wild-type gene.
The clinical history did not mention drugs commonly associated with interstitial nephritis and was dominated by previous over-anticoagulation episodes and hypertension. Kidney biopsy definitively excluded these forms demonstrating a WRN since histological findings met the criteria previously described by Brodsky et al. [2]. We suggest that WRN should always be considered as a possible cause of AKI when episodes of over-anticoagulation are described, and kidney biopsy should be performed to confirm the diagnosis of this serious warfarin complication. Patients with CKD, hypertension, diabetes and cardiovascular disease should be considered at risk since these conditions are associated with WRN development [2, 9, 10].

The patient had significant proteinuria that is not part of WRN clinical expression. Kidney histology demonstrated a secondary focal glomerulosclerosis in a context of nephroangiosclerosis that explained this finding. This observation highlights the central role of renal biopsy which identified the cause of kidney failure and described the underlying coexisting nephropathy. Furthermore, renal biopsy could contribute to a better understanding of WRN pathogenesis. Ozcan and colleagues reproduced WRN in a 5/6 nephrectomy rat model confirming the pathogenetic role of glomerular haemorrhage and tubular obstruction. The rats developed secondary focal segmental glomerulosclerosis after nephrectomy, and CKD progression exposed them to higher risk of WRN when over-anticoagulation occurred, resembling the case we reported [11, 12]. Considering these features, the patient was treated by warfarin discontinuation, hydration and sodium bicarbonate but no clinical response was obtained.

Interestingly, haematuria and haemoglobinuria may directly induce kidney damage even without tubular obstruction. Some observational reports indicate that early steroid administration could accelerate recovery of renal function after gross haematuria [13]. Although no data are available about steroid use in WRN with microscopic haematuria, we started this therapy obtaining a good response with partial recovery of renal function. The patient remained at CKD stage 5 but avoided dialysis. To the best of our knowledge, this is the first report describing WRN clinical response to steroid treatment. Though it needs to be confirmed, we consider this a fascinating finding since it suggests a possible therapeutic strategy for this nephropathy and a less mechanical pathogenesis of the renal damage. Notably, in this case kidney function did not completely recover confirming that WRN represents a severe warfarin complication with a poor renal prognosis [4, 9].

To prevent this complication it is necessary to identify patients at risk and to individualize the correct warfarin dose. Warfarin-related haemorrhage is 2-fold more common in CKD patients with respect to the general population [14], and WRN is more frequent in patients with a pre-existing nephropathy. The study of warfarin pharmacogenetics could be useful to identify patients who require lower doses. Warfarin is metabolized by liver cytochrome P450-CYP2C9 and acts as a vitamin K antagonist (VKAs) inhibiting the vitamin K epoxide reductase enzyme complex (VKORC1). CYP2C9 and VKORC1 are, respectively, encoded by CYP2C9 and VKORC1 genes. Some polymorphisms of these genes are associated with both warfarin metabolism impairment and higher haemorrhagic risk [5]. Recently, Limdi et al. [14] demonstrated that CKD patients require lower warfarin doses, and the dose adjustments should take into account both kidney function and CYP2C9 and VKORC1 genotypes. In the case of our patient, warfarin dose had not been titrated according to the pharmacogenetic profile, and the patient experienced many episodes of over-anticoagulation. The genetic study demonstrated that he was homozygous for CYP2C9*3 variant, which is a common European ancestry allele that reduces warfarin metabolism by 80–90% exposing the carriers to the greatest risk of bleeding [15]. In contrast, −1639G>A and 1173C>T non-coding variants are associated with warfarin sensitivity and reduced dose requirements, but they are not associated with haemorrhagic events. On the basis of our observations, the CYP2C9*3 genetic variant might be associated with WRN development, suggesting for the first time a link between WRN and this polymorphism. Although it needs to be confirmed, this observation is particularly interesting given that WRN has an unfavorable prognosis and warfarin pharmacogenetics might be useful to identify patients at risk of complications. Warfarin pharmacogenetic-based algorithms could be used to establish both the initiation and maintenance dose minimizing the risk of over-anticoagulation and WRN development.

In conclusion renal biopsy should always be considered to identify WRN in patients developing AKI when over-anticoagulation is described. WRN is a serious complication with a poor renal prognosis and requires the development of successful therapeutic strategies. In our experience corticosteroids might play a role in WRN treatment. Finally, the CYP2C9*3 polymorphism could represent a genetic variant which identifies patients at risk for WRN. In general warfarin pharmacogenetics may indicate a genetic predisposition to WRN and act as a guide for dose requirements in CKD patients.

Conflict of interest statement. None declared. The results presented in this paper have not been published previously in whole or part.

References
1. Rubboli A, Becattini C, Verheugt FW. Incidence, clinical impact and risk of bleeding during oral anticoagulation therapy. *World J Cardiol* 2011; 3: 351–358
2. Brodsky SV, Satoskar A, Chen J et al. Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. *Am J Kidney Dis* 2009; 54: 1121–1126
3. Brodsky SV, Nadasdy T, Rovin BH et al. Warfarin-related nephropathy occurs in patients with and without chronic kidney disease and is associated with an increased mortality rate. *Kidney Int* 2011; 80: 181–189
4. Brodsky SV, Collins M, Park E et al. Warfarin therapy that results in an International Normalization Ratio above the therapeutic range is associated with accelerated progression of chronic kidney disease. *Nephron Clin Pract* 2010; 115: c142–c146
5. Johnson JA, Gong L, Whirl-Carrillo M et al. (Implementation Consortium Clinical Pharmacogenetics). Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther* 2011; 90: 625–629
6. Schwarz UI, Ritchie MD, Bradford V et al. Genetic determinants of response to warfarin during initial anticoagulation. *N Eng J Med* 2008; 358: 999–1008
7. Kimmel SE, French B, Kasner SE et al. A pharmacogenetic versus a clinical algorithm for warfarin dosing. *N Eng J Med* 2013; 369: 2283–2293
8. Abt AB, Carroll LE, Mohler JH. Thin basement membrane disease and acute renal failure secondary to gross hematuria and tubular necrosis. *Am J Kidney Dis* 2000; 35: 533–536
9. An JN, Ahn SY, Yoon CH et al. The occurrence of warfarin-related nephropathy and effects on renal and patient outcomes in Korean patients. *PLoS One* 2013; 8: e57661

10. Logan I, Sheerin NS. Anticoagulation and kidney injury: rare observation or common problem? *J Nephrol* 2013; 26: 603–605

11. Ozcan A, Ware K, Calomeni E et al. 5/6 nephrectomy as a validated rat model mimicking human warfarin-related nephropathy. *Am J Nephrol* 2012; 35: 356–364

12. Ware K, Brodsky P, Satoskar AA et al. Warfarin related nephropathy modeled by nephron reduction and excessive anticoagulation. *J Am Soc Nephrol* 2011; 22: 1856–1862

13. Moreno JA, Martin-Cleary C, Gutiérrez E et al. AKI associated with macroscopic glomerular hematuria: clinical and pathophysiologic consequences. *Clin J Am Soc Nephrol* 2012; 7: 175–184

14. Limdi NA, Beasley TM, Baird MF et al. Kidney function influences warfarin responsiveness and hemorrhagic complications. *J Am Soc Nephrol* 2009; 20: 912–921

15. Yang J, Chen Y, Li X et al. Influence of CYP2C9 and VKORC1 genotypes on the risk of hemorrhagic complications in warfarin-treated patients: a systematic review and meta-analysis. *Int J Cardiol* 2013; 168: 4234–4243

Received for publication: 10.8.14; Accepted in revised form: 2.10.14