Prevalence and clinical significance of cryofibrinogenemia in patients with renal disorders

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Abstract

Background. Cryofibrinogenemia (CryoFg) is an under-recognized cryoprotein that can be life-threatening when untreated. Symptoms are mainly thrombotic cutaneous manifestations, but other thrombotic localizations may occur. In patients with end-stage renal disease, thromboses are common. However, the implication of CryoFg was never assessed. The aim of this study is to describe the prevalence and the significance of CryoFg in patients with renal disorders.

Methods. One hundred and one consecutive patients admitted in a nephrology department for the management of renal disorders were tested for the presence of serum cryoprotein, i.e. cryoglobulinaemia and CryoFg. We analysed clinical and biological factors associated with the presence of CryoFg.

Results. Among the 101 patients, 11 patients had positive CryoFg without detectable cryoglobulin (11%). Median CryoFg level was 0.07 g/L (0.05–1.16). Main epidemiological features and causes of nephropathy, in particular vascular nephropathies, were similar between CryoFg– and CryoFg+ patients. No biological difference (haematocrit, proteinuria, creatinine level and glomerular filtration rate using Modification of Diet in Renal Disease) was found between CryoFg– and CryoFg+ patients. In contrast, CryoFg+ compared to CryoFg– patients had more frequent severe thrombotic events (36 versus 0%, P < 0.0001). Severe thrombotic events included renal artery thrombosis in two patients, recurrent arteriovenous fistula thrombosis in one and recurrent dialysis catheter thrombosis with superior vena cava obstruction in one. The presence of CryoFg was not associated with other manifestations, in particular cutaneous manifestations.

Conclusion. Cryofibrinogenemia is detected in up to 11% of patients with renal disorders. In such patients, the presence of CryoFg is associated with thrombotic events.

Keywords: cryofibrinogenemia; nephropathy; thrombosis

Introduction

Cryofibrinogenemia involves a cryoprotein originally characterized by Korst and Kratochvil [1] and is defined as the presence of cold precipitable proteins in the plasma. The cryoprecipitate is made of fibrinogen, fibrin, fibronectin,
factor VIII and smaller amounts of various plasma proteins [2]. Cryofibrinogenemia is able to precipitate in cooled plasma (4°C) and redissolve after increasing the sample temperature (37°C). The cooled temperature-induced precipitation of proteins in plasma, but not in serum, allows the distinction between cryofibrinogens and cryoglobulins.

Cryofibrinogenemia can be classified as primary (essential) or secondary to autoimmune disorders, malignancy, cardiovascular thrombosis, active sepsis and chronic infections, such as hepatitis C virus (HCV) [3–7]. In HCV-infected patients, a positive cryofibrinogen status is closely related to the presence of cryoglobulin at baseline and after antiviral therapy [5]. Essential cryofibrinogenemia is rare, but its prevalence remains unknown. An overall prevalence of 3% has been reported in hospitalized patients [8]. The skin is the most common target organ, and cutaneous manifestations are variable, including cold sensitivity, purpura, livedo reticularis, Raynaud’s phenomenon, and with less frequency, thrombotic cutaneous events, such as ulceration, gangrene and ischaemic necrosis [4, 8, 9]. Other thrombosis localizations may occur in 20% of patients, including lower limb venous thrombosis, pulmonary embolism and arterial thrombosis [7].

In patients with end-stage renal disease, in particular those on maintenance dialysis, coagulation abnormalities such as hypercoagulability and thrombosis are common and involve different mechanisms [10]. However, the implications of cryofibrinogenemia in such a thrombophilic state have never been assessed. In contrast, renal involvement has been reported in 13% of patients with cryofibrinogenemia and more frequently in patients with secondary forms than with essential cryofibrinogenemia [7]. Conversely, cryofibrinogenemia has been detected in patients with nephropathy, including IgA nephropathy, membranoproliferative glomerulonephritis and end-stage renal disease on renal replacement therapy [11–14].

This study analyses the prevalence and the clinical significance of cryofibrinogenemia in patients with renal disorders.

Patients and methods

Patients

One hundred and six consecutive patients admitted between 07 September 2009 and 30 October 2009 in the Nephrology Department of a single university hospital for the management of renal disorders were analysed for the detection of cryoglobulin and cryofibrinogen. Inclusion criteria for the study were as follows: (i) hospitalization for the management of renal disorders and (ii) informed consent obtained from each patient. Exclusion criteria were the presence of known chronic HCV infection and/or positive cryoglobulinemia. Among the 106 patients, 5 patients were excluded because of unknown positive HCV infection (RNA+) (n = 1) or detectable cryoglobulinemia (n = 4), therefore 101 patients (mean age 58 ± 18 years, female/male 35/66) were included. For each patient, the following data were collected: gender, age, characteristics of renal disorder, extra-renal involvement (cutaneous, neurologic and/or rheumatologic involvement), blood cell count, serum inflammatory parameters, serum creatinine level, hematuria and proteinuria. The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease simplified formula.

Analysis of cryoproteins and plasma fibrinogen

By using previously warmed equipment, blood was collected into anticoagulant-free tubes for cryoglobulin detection or citrated tubes for cryofibrinogenemia detection. Both sera (cryoglobulin screening) and plasma (cryofibrinogen screening) were isolated after centrifugation (2000 g, 30 min, 37°C). After antiseptic addition (sodium azide, 0.1 g/L), the sera and plasma were chilled at 4°C for 8 days and finally analysed after precipitation or gel formation. Through a heating procedure at 37°C, we checked the reversible nature of the cryofibrinogenemia precipitate before proceeding to a new precipitation at 4°C and a purification step. Cryoglobulin and cryofibrinogen were collected after centrifugation (2000 g, 15 min, 4°C) and then extensively washed (NaCl 0.15 mol/L, pH 7.4). A fraction of this washed cryoglobulinaemia or cryofibrinogenaemia sample was redissolved (NaOH 0.1 mol/L), and the absorbance was determined in a spectrophotometer (λ = 280 nm). Cryoglobulin quantification was performed according to a standard curve based on various concentrations of a purified human gammaglobulin preparation supplied by the national blood transfusion center (Centre National de Transfusion Sanguine, Paris, France). Similarly, a purified fibrinogen preparation was used for a standard curve determination allowing cryofibrinogenaemia quantification [5, 7].

Statistical analysis

Quantitative variables were expressed as medians (range). The Mann–Whitney and Fisher’s exact tests were used to compare the main characteristics between patients with and without cryofibrinogenemia. All tests were two-sided at the 0.05 significance level. Analyses were carried out using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA).

Results

Patient characteristics and prevalence of cryofibrinogenemia

The main epidemiological, clinical and biological characteristics of patients included in the study are given in Table 1. Among the 101 patients, 11 (10.9%) had positive cryofibrinogenemia without detectable cryoglobulin. The median cryofibrinogenemia level in positive patients was 0.07 g/L (0.05–1.16).

Clinical significance of cryofibrinogenaemia detection

The comparison of patients with and without cryofibrinogenemia is summarized in Table 1. The main epidemiological features and personal medical histories were similar between patients with and without cryofibrinogenemia, except for a trend toward more frequent autoimmune disorders in patients with cryofibrinogenemia (27 versus 7%, P = 0.06). The three patients with cryofibrinogenemia and autoimmune disorders had rheumatoid arthritis, antiphospholipid syndrome and anti-neutrophil cytoplasmic antibodies-negative vasculitis.

No difference was found in the type of nephropathy between groups, in particular regarding glomerular and vascular nephropathy, except for a tendency to lower urine protein excretion in patients with glomerular nephropathy and positive cryofibrinogenemia compared to those without cryofibrinogenemia (urine protein/creatinine ratio of 58 versus 234 mg/mmol, P = 0.08).

Patients with cryofibrinogenemia had a greater frequency of severe thrombotic events compared to patients without cryofibrinogenemia (36 versus 0%, P < 0.0001). The presence of cryofibrinogenemia was not associated with other manifestations, in particular cutaneous manifestations. Severe thrombotic events included renal artery thrombosis in two patients, recurrent arteriovenous fistula thrombosis in one and recurrent dialysis catheter thrombosis with superior vena cava obstruction in one. The patient...
with recurrent arteriovenous fistula thrombosis also had an underlying antiphospholipid syndrome, while the patient with recurrent dialysis catheter thrombosis had tubulointerstitial nephropathy. Detailed characteristics of the four patients with severe thrombotic events are summarized in Table 2. The search for other thrombotic states was performed in three of the four patients and confirmed the presence of antiphospholipid antibodies in the patient with recurrent arteriovenous fistula thrombosis.

No differences in the biological features of renal disorders (serum creatinine and urea levels, glomerular filtration rate, haematuria and proteinuria) and in the fibrinogen and albumin levels were found between patients with and without cryofibrinogenaemia.

Finally, no association was found between replacement therapy (dialysis or transplantation) and cryofibrinogenaemia detection.

### Discussion

In this study, we analysed the prevalence and the clinical significance of cryofibrinogenaemia in patients with renal disorders. The most striking observations were the detection of cryofibrinogenaemia in 11% of patients and the strong association of cryofibrinogenaemia with severe thrombotic events.

Thrombotic events were reported in up to 40% of patients with cryofibrinogenaemia, mainly as cutaneous thrombosis [4, 7]. However, other thrombotic localizations were described, including arterial and/or venous thrombosis. The pathogenesis of cryofibrinogenaemia-related thrombosis is not well defined. Severe fibrinolysis defects were shown in essential cryofibrinogenaemia, with an increase in the plasma level of fibrinolysis inhibitors, plasminogen activator inhibitor-1 and alpha-2-macroglobulin, as...
well as a significantly delayed euglobulin lysis time [7]. This impaired fibrinolysis may lead to cryofibrinogenaemia accumulation and clotting in small and medium arteries. Besides this hypothesis, additional vascular occlusion in patients with renal disorders may be caused by the development of hypercoagulability. In patients with end-stage renal disease on maintenance dialysis, thrombosis is common and thrombotic complications include those occurring at the vascular access site and in the coronary, cerebral and retinal arteries [10]. In the present study, patients with and without cryofibrinogenaemia had similar characteristics, including types of nephropathy, stage of renal failure and replacement therapy, suggesting that the increased rate of severe thrombosis in patients with cryofibrinogenaemia was independent of the usual factors associated with thrombotic complications. The search for other thrombotic states was performed in three of the four patients with severe thrombotic events and confirmed the presence of antiphospholipid antibodies in one patient with previously diagnosed antiphospholipid syndrome.

Most of the data regarding the treatment of essential cryofibrinogenaemia have been derived from case reports or small, uncontrolled studies. In addition to cold exposure avoidance, patients with cryofibrinogenaemia can be treated with numerous drugs, including heparin, warfarin, streptokinase, anabolic steroids, immunosuppressive therapies and plasmapheresis, all with varying degrees of success [3, 15]. In the study by Saadoun et al. [7], two-thirds of symptomatic patients received anticoagulation therapy in combination with steroids, resulting in complete clinical improvement and the disappearance of plasma cryofibrinogenaemia in 76%. However, relapses occurred in >40% of cases with plasma cryofibrinogenaemia reappearance [7]. No data are available regarding the benefits of primary thrombosis prevention in patients with asymptomatic cryofibrinogenaemia. Our findings suggest that such a strategy could be beneficial in patients with renal disorders, in particular those on replacement therapy, in order to prevent thrombosis at the vascular access site.

In conclusion, cryofibrinogenaemia was detected in up to 11% of patients with renal disorders. In such patients, the presence of cryofibrinogenaemia was associated with severe thrombotic events. Thus, the detection of cryofibrinogenaemia in patients with renal disorders could be a marker of a thrombophilia and thus raises the question about the benefits of aspirin and/or anticoagulation therapy for the primary prevention of thrombotic events in cryofibrinogen-positive patients.

Conflict of interest statement. None declared.

References

High urinary excretion of kidney injury molecule-1 is an independent predictor of end-stage renal disease in patients with IgA nephropathy

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Abstract

Background. The variable course of immunoglobulin A nephropathy (IgAN) warrants accurate tools for the prediction of progression. Urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are markers for the detection of early tubular damage caused by various renal conditions. We evaluated the prognostic value of these markers in patients with IgAN.

Methods. We included patients (n = 65, 72% male, age 43 ± 13 years) with biopsy-proven IgAN, who were evaluated for proteinuria. Urinary KIM-1 and NGAL were measured by enzyme-linked immunosorbent assay. We analysed data using Cox regression for the outcome end-stage renal disease (ESRD).

Results. Median serum creatinine was 142 μmol/L and proteinuria 2.2 g/day. During follow-up (median 75 months), 23 patients (35%) developed ESRD. In patients with IgAN median urinary KIM-1 excretion was 1.7 ng/min and NGAL excretion was 47 ng/min, both significantly higher than in healthy controls. KIM-1 and NGAL were correlated with proteinuria (r = 0.40 and 0.34, respectively, P < 0.01) and each other (r = 0.53, P < 0.01) but not with estimated glomerular filtration rate (eGFR). Interestingly, KIM-1 was not significantly correlated with the excretion of γ1-microglobulin (γ1m) and β2-microglobulin (β2m), known markers of tubular injury. Univariate analysis showed that baseline serum creatinine and urinary excretion of total protein, γ1m, β2m, immunoglobulin G, KIM-1 and NGAL were significantly associated with ESRD. By multivariate analysis, serum creatinine and KIM-1 excretion proved to be significant independent predictors of ESRD.

Conclusion. KIM-1 and NGAL excretion are increased in patients with IgAN and correlate with proteinuria but not with eGFR. Baseline serum creatinine and urinary KIM-1, but not proteinuria, are independent predictors of ESRD.

Keywords: biomarker; end-stage renal disease; IgA nephropathy; KIM-1; NGAL.