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Hypercoagulation in glomerulonephritis

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Abstract

The clotting values of 50 patients with glomerulonephritis were examined. Three different coagulation groups were recognised: those with normal clotting values (group 1); those with high concentrations of factor VIII but otherwise normal clotting results (group 2); and patients who showed the presence of an activator of the intrinsic coagulation pathway, indicated by the presence of a short activated partial thromboplastin time or the ability of patients' plasma to shorten control clotting time in mixing studies (group 3). Patients in group 2 either had a uniform rise in all three components of the factor VIII molecule or a disproportionately higher concentration of factor-VIII-related antigen. In contrast, the level of VIII clotting activity in patients in group 3 was always higher than concentrations of either VIII_{Ag} or VIII_{WF}. A significantly high incidence of thrombotic complications was observed in patients in group 3 but in none of the patients in either group 1 or group 2. Impaired renal function was more common in patients in groups 2 and 3, with higher mean serum creatinine concentrations in those in group 3.

Patients with glomerulonephritis who have a short partial thromboplastin time with kaolin or who shorten control clotting time form a subgroup in whom hypercoagulation could adversely affect the course of their disease. The value of antiplatelet or anticoagulant treatment in these patients needs to be explored.

Introduction

The role of the coagulation process in the pathogenesis of glomerulonephritis and its complications have become increasingly recognised. Experimental evidence and findings on pathological examination of kidneys from patients with glomerulonephritis suggest that local intravascular coagulation plays an important role in the progression of the renal disease.¹ In these patients the presence of a systemic hypercoagulable state may contribute to the deterioration of renal function and may be associated with a high incidence of thrombotic complications. In patients with established nephrotic syndrome a high incidence of thromboembolic phenomena has been reported.² The observed high concentrations of factors V and VIII³ and low concentrations of antithrombin III⁴ are thought to be important in the pathogenesis of this complication. The clotting values of other patients with glomerulonephritis have, however, received little attention in recent reports. This paper reports an investigation of the clotting values of 50 patients with glomerulonephritis and establishes the incidence of a coagulation accelerating factor recognisable in vitro and its possible influence on the clinical course of these patients.

Patients and methods

Fifty patients (24 men, 26 women) aged from 12 to 78 years (mean 37 ± 16) were examined. In all patients the diagnosis of glomerulonephritis was confirmed by renal biopsy. Two additional groups served as controls: (a) 20 healthy individuals (medical and laboratory personnel), and (b) 70 age- and sex-matched inpatients with various medical disorders. These inpatients included 20 patients with renal disease not characterised by glomerulonephritis but with a comparable degree of renal impairment as the study group and 50 with various disorders such as ischaemic heart disease, cerebrovascular accidents, chronic bronchitis, collagen disease, malignant lymphoma, and thyroid disease. None of the control patients were known to have a haemorrhagic tendency.

Blood was collected in 1/10 volume 3.8% trisodium citrate. Platelet-poor plasma was obtained by centrifugation at 2000 g for 15 minutes. Prothrombin, thrombin, and Stypven times were measured by standard methods. Partial thromboplastin time with kaolin (PTTK) was measured by incubating 0.1 ml of plasma with 0.05 ml kaolin 50 mg/ml and 0.05 ml Platelin (General Diagnostics) for 3 minutes

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and then recalcifying it with 0.1 ml, 0.025 M CaCl_2 . The presence of an activator of the clotting pathway was determined by mixing PTTK studies: the patient's plasma was added to normal fresh plasma in different ratios and PTTK was then measured on the mixed plasma sample as described above. Shortening of the control clotting time by five or more seconds was taken as evidence for the presence of an activator. Factor VIII clotting activity (VIIC) was measured on fresh platelet-poor plasma by the one-stage method,⁵ using factor VIII-deficient substrate. Factor-VIII-related antigen (VIIIaG) was assayed on frozen platelet-poor plasma by an immunoelectrophoretic technique.⁶ Von Willebrand factor activity (VIIIWF) was measured by the method of McFarlane *et al*, using formalinised washed platelets.⁷

Factor IX clotting activity (IXC) was measured on frozen platelet-poor plasma by the one-stage method, using factor IX deficient substrate. Antithrombin III (ATIII) concentration was measured on frozen platelet-poor plasma by a two-stage clotting assay (Ortho ATIII kit). Statistical analysis was calculated using the Student's *t* test for unpaired observations.

Results

There was no significant difference in the thrombin, prothrombin, and Stypven times between patients with glomerulonephritis and those in the control groups. The PTTK of healthy controls ranged from 34 to 48 s with a mean of $39 \pm (\text{SD}) 4$ s. Inpatient controls had a PTTK of 34–49 s, with a mean of 40 ± 5 s. Three different patterns of PTTK were recognised in patients with glomerulonephritis.

Eight patients (16%) had a short PTTK ranging from 26 to 32 s (mean $29 \pm \text{SD } 2$), a statistically significant shortening ($p < 0.001$). Mixing of patients' plasma with fresh control plasma resulted in shortening of control times. Table I shows that the addition of even small amounts of plasma from case 1 significantly shortened the control PTTK. This was a feature of all patients in this group which was not observed in patients with other types of renal disease. It suggested the presence of a plasma activator of the intrinsic clotting pathway.

In six patients (12%) the PTTK was within the normal range; when their plasma was mixed with fresh control plasma, however, the clotting time (> 5 s) was significantly shortened (table I; case 2). This phenomenon was not observed in any member of the control groups and again suggested the presence of a coagulation activator factor.

TABLE I—Results of mixing patients' plasma with normal plasma on partial thromboplastin time with kaolin (PTTK)

Amount of plasma (μl)		PTTK (seconds)	
Control	Patient	Case 1	Case 2
0	200	26	36
100	100	27	31
150	50	29	33
175	25	32	35
200	0	41	

Thirty-six patients (72%) had normal PTTK and their plasma caused no shortening of control times on mixing.

There was no difference between the concentration of factor IXC in patients with glomerulonephritis and that in controls, and no case of antithrombin-III deficiency was encountered. In six patients with glomerulonephritis the concentration of antithrombin III was mildly but insignificantly raised. Twenty-nine patients with glomerulonephritis (58%) and 34 inpatient controls (49%) had raised factor VIII concentrations. In healthy controls and patients with normal concentrations of factor VIIIaG, VIIC, and VIIIWF, there was a good correlation between these three functions of the VIII molecule, which was not always the case when the concentrations were raised. All 14 patients with glomerulonephritis who showed the presence of the activator of the clotting pathway had disproportionately higher VIIC concentrations than the other two factor-VIII-associated values. On the other hand, patients with high factor VIIC, VIIIaG, and VIIIWF values and normal PTTK (15 in the study group, 34 inpatient controls) either had higher VIIIaG, VIIIWF, or both, than VIIC, or an equal rise in all three components.

Patients with glomerulonephritis were classified into three groups according to the results of the clotting studies.

Group 1 comprised 21 patients with normal clotting values, four of whom had a raised serum creatinine concentration; in all the other patients it was normal (mean creatinine concentration $110 \pm 90 \mu\text{mol/l}$ (1.2 mg/100 ml)). One patient had nephrotic syndrome.

Group 2 contained patients with high concentrations of factor VIII and normal PTTK. Seven patients had raised creatinine concentrations and one subsequently required long-term haemodialysis. Four patients had slightly raised creatinine concentrations and in four they were normal. The mean serum creatinine concentration in this group was $220 \pm 140 \mu\text{mol/l}$ (2.5 mg/100 ml). No thrombotic complications were observed, and none of the patients had nephrotic syndrome.

Group 3 included patients with high levels of factor VIII whose plasma shortened control PTTK. Eight out of 14 patients in this group had a significant increase in their serum creatinine concentration; in one patient it was marginally raised and in the remaining five the concentration was normal. Five patients in this group had crescentic nephritis, and only two patients had nephrotic syndrome. The proportion of patients with raised creatinine concentrations was similar to that in group 2, but renal impairment tended to be more severe (mean serum creatinine $440 \pm 520 \mu\text{mol/l}$ (4.9 mg/100 ml)). One patient in this group died in renal failure and another three later entered the haemodialysis programme. Thrombotic complications occurred either at presentation or on follow-up in five patients (35%): pulmonary emboli in two, disseminated intravascular coagulation in another two, and renal vein thrombosis in one.

The histological diagnosis in relation to the clotting results in the

TABLE II—Renal histology and clotting results in 50 patients with glomerulonephritis

	Normal clotting results	High factor VIII with normal PTTK	High factor VIII with shortening of control PTTK
<i>Primary glomerulonephritis</i>			
Proliferative glomerulonephritis:			
Diffuse proliferative glomerulonephritis	1	2	
Mesangial proliferative	6	4	
Mesangiocapillary	2	2	2
Focal proliferative	2	1	1
Proliferative with extensive crescents		1	4
Membranous nephropathy	2	1	2
IgA nephropathy	4	3	
Focal and segmental hyalinosis	2	1	1
Minimal change			1
<i>Secondary glomerulonephritis</i>			
Wegener's (crescentic glomerulonephritis)			1
Polyarteritis nodosa:			
Focal proliferative			1
Systemic lupus erythematosus:			
Focal proliferative			1
Diffuse proliferative	1		
Subacute bacterial endocarditis:			
Focal proliferative	1		

TABLE III—Prognosis and outcome in 50 patients with glomerulonephritis as related to their coagulation profile

	Normal	High factor VIII with normal PTTK	High factor VIII with shortening of control PTTK
	Group 1 (n = 21)	Group 2 (n = 15)	Group 3 (n = 14)
Serum creatinine* ($\mu\text{mol/l}$)			
<110	17	4	5
110–140	1	4	1
>140	3	7	8
Haemodialysis		1	3
Death			1
Thrombotic complications			5

*The normal serum creatinine range is 70–110 $\mu\text{mol/l}$.

Conversion: SI to traditional units—Creatinine: 1 $\mu\text{mol/l} \approx 0.0113 \text{ mg/100 ml}$.

50 patients with glomerulonephritis is shown in table II, and a summary of the prognosis and complications observed in these patients is shown in table III.

Discussion

A short PTTK has been associated with the presence of a diffuse hypercoagulable state and an increased tendency to develop thromboembolic complications.^{8,9} In this study of 50 patients with biopsy-proved glomerulonephritis eight patients had a short PTTK. The addition of small amounts of patients' plasma to control plasma significantly shortened the control clotting time, implying the presence of a coagulation activating factor in the patients' plasma. In another six patients with normal PTTK the presence of this factor was disclosed after mixing with normal plasma, when the clotting time was significantly shortened. This phenomenon could be explained either by a deficiency of a clotting factor in the patients' plasma resulting in a relatively prolonged PTTK corrected by mixing with normal plasma or by the presence of a weak inhibitor in the patients' plasma which is diluted out by mixing. The histological diagnosis of patients who had a short PTTK was variable; but five patients with crescentic nephritis showed this feature, and only two patients had nephrotic syndrome. Fifty-eight per cent of the patients in this group had significant renal impairment, and thrombotic complications were observed in 5 out of 14 patients (35%). A similarly high incidence of thrombosis was observed by McKenna *et al* in a prospective study on patients with short PTTK.⁸

Nephrotic syndrome was observed in six patients in this study, two of whom showed coagulation accelerating factor activity. The remainder had normal PTTK with no shortening of control clotting time in mixing studies.

Raised concentrations of factor VIIIc, VIIIa, and VIIIWF were observed in 29 patients with glomerulonephritis (58%) and 34 inpatient controls (49%). In patients with glomerulonephritis the rise of the factor VIII concentration correlated well with the clinical outcome. Patients with normal concentrations mostly had good renal function (18 out of 21), whereas 20 out of 29 patients with raised concentrations had impaired renal function, with a higher mean serum creatinine concentration in patients with a short PTTK. All patients with short PTTK had high concentrations of factor VIII, which were most pronounced in the clotting activity compared with the other two functions of the factor VIII molecule. This would suggest that in these patients VIIIc exists as an activated molecule or that there is a true disproportionate rise in VIIIc over the other two VIII-related functions; assays of VIIIc-related antigen would be useful in elucidating this point. The presence of a non-specific accelerator of the clotting pathway is unlikely since the IXC assay, prothrombin, Stypven, and thrombin times were normal. It is important to note that 15 patients with glomerulonephritis and 34 inpatient controls had high concentrations of factor VIII, including VIIIc, with a normal PTTK, indicating that high concentrations of factor VIII per se do not shorten the PTTK.

The presence of an accelerator of the clotting pathway in certain diseases has long been suspected. Amundsen *et al* found that the short clotting time in patients with malignant disease and postoperative states was due to the presence of an activator which on partial purification was found to be indistinguishable from factor VIII.¹⁰ Recent work from our laboratory indicates that the factor responsible for the shortening of PTTK in patients with glomerulonephritis is in fact an active form of factor VIII (unpublished observations). In one patient with severe crescentic nephritis and short PTTK intensive plasma exchange resulted in significant reduction of factor VIII concentrations and return of PTTK to near normal, associated with temporary improvement in his renal function. In another two patients a short-lived improvement in renal function was associated with a reduction in the factor VIII concentration and a return of PTTK to near normal.

Experimental data and pathological studies indicate that local intravascular coagulation could play an important role in the pathogenesis of renal damage in glomerulonephritis.¹ The presence of a circulating accelerator of the clotting pathway would be expected to enhance this process by creating a hyper-

coagulable state. In addition, this would predispose the patients to a high incidence of thromboembolic complications. The results of the study indicate that the ability of the patient's plasma to shorten control clotting time is a simple screen for the presence of this accelerator, which should alert the clinician to the presence of an underlying prethrombotic tendency.

The occasional beneficial response to antiplatelet and anti-coagulant treatment observed in some patients with glomerulonephritis¹¹ may be limited to those with a hyper-coagulable tendency. The results of prospective trials on the value of this treatment would be more meaningful if the presence or absence of a prethrombotic tendency were taken into account.

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ONE HUNDRED YEARS AGO Thomas Carlyle and Edinburgh University. The late Thomas Carlyle has shown the interest he felt in the university, of which he had been Lord Rector, by bequeathing to it the estate of Craigenputtock, in the county of Dumfries. The estate is about eight hundred acres in extent, and of the annual value of £300. Ten bursaries are to be founded, and are to be in custody of the Senatus Academicus; they are to be called the "John Welsh Bursaries." They are to be given to students the "worthiest" after competitive examination, five on the subject of classics, and five on mathematics. We could have wished that some of them had come to the medical faculty, but we trust they may help to give a thorough arts training to some who will afterwards enter the profession. The bequest is thoroughly Carlyle-like in its wording, and finishes thus "and so may a little trace of help to the young heroic soul struggling for what is highest, spring from this poor arrangement and bequest; may it run for ever if it can, as a thread of pure water from the Scottish rocks, trickling into its little basin by the thirsty wayside, for those who it veritably belongs to. Amen." (*British Medical Journal*, 1881.)