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Deficiency of antithrombin III in children with hemolytic-uremic syndrome

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Abstract. In nine patients with hemolytic-uremic syndrome, the plasma activity and plasma concentrations of antithrombin III were determined on admission to the hospital and during the clinical course of the disease. Hemodialysis was necessary in six of the patients. In seven children the plasma AT III activity was moderately to markedly below the lower limit of normal at 75%, and did not rise after plasmapheresis with fresh frozen plasma. Replacement therapy with AT III concentrate was started in these patients. During the first 2 days an average dose of AT III concentrate of 2.1 U/kg in 24 h was necessary to raise plasma AT III activity by 1%. No side effects were observed. An already pre-existing procoagulant status and the administration of heparin may lead to AT III deficiency in hemolytic-uremic syndrome.

Key words: Hemolytic-uremic syndrome - Antithrombin III - Hemodialysis - Plasmapheresis

Introduction

In 1980 Brandt et al. [5, 6] were the first to demonstrate deficiency of antithrombin III (AT III) in a patient with postpartum hemolytic-uremic syndrome (HUS). After AT III had been replaced renal function promptly returned to normal. Other workers have been unable to confirm this observation [8], in particular in children with the epidemic form of HUS [22]. Notwithstanding these negative results, AT III deficiency has recently been postulated as a factor of some significance in the pathogenesis and treatment of HUS [11].

The purpose of this communication is to present data on plasma AT III activity in children with HUS.

Subjects, materials and methods

In the period from February to September 1982 we observed nine children with HUS in the University Children's Hospital. The diagnosis was made from the finding of microangiopathic hemolytic anemia in association with thrombocytopenia and impairment of renal function (Table 1). All the patients had a typical history of illness with vomiting, blood-stained diarrhea, fever and symptoms of upper respiratory tract infection. They were either oliguric with gross hematuria, or anuric. Hypertension was noted in one patient only. In no case was there a positive direct Coombs test or were there free erythrocyte Thomson-Friedenreich antigens for peanut lectin [18]. The alcohol gelation test was consistently negative. Six patients were treated by hemodialysis via a Shaldon catheter or Scribner shunt, followed by plasmapheresis with the aid of a membrane filter, the latter enabling approximately one-half to two-thirds of the plasma volume to be exchanged with fresh frozen plasma [14, 15]. In the small infants hemodialysis was carried out by the aid of special miniature equipment (Kindermonitor KM 21, Braun, Melsungen, FRG) in combination with bicarbonate dialysis (Bicarbomat BIC 50, Braun, Melsungen, FRG). Plasmapheresis was performed with the object of influencing vascular prostacyclin formation by either administering a deficient plasma factor or by removing a plasma factor which inhibits prostacyclin formation [3, 14, 15, 24]. For hemodialysis and plasmapheresis the patients were heparinized with an initial dose of 50-150 U/kg followed by heparin in amounts sufficient to keep the whole blood clotting time at 15-20 min. Other therapy was: Heparin 25-100 U/kg per day by continous intravenous infusion, dipyridamole 5 mg/kg per day, aspirin 5 mg/kg per day, furosemide up to 10 mg/kg per day i.v., and allopurinol 50-200 mg/day orally. Washed packed erythrocytes were transfused if necessary.

Plasma concentrations of AT III were measured by radial immunodiffusion ("M-Partigen", Behringwerke, Marburg, FRG); normal range 19.2 ± 4.3 mg/dl ($n = 40$, age 1-14 years). Plasma AT III activity was determined by means of a coagulation test with fibrinogen as natural substrate ("Antithrombin III Rapid Test", Behringwerke, Marburg, FRG) [27]; normal range $102.4\% \pm 19.4\%$ ($n = 40$, age 1-14 years). Regular AT III measurements were performed at daily intervals. AT III deficiency was diagnozed when the plasma activity was below 75% of normal. AT III replacement was carried out with AT III concentrate ("Kybernin", Behringwerke, Marburg, FRG) in an initial dose of 250-500 units three times daily followed by further treatment with an amount ranging from 125 units once daily to 250 units twice daily (1 unit is equivalent to the AT III activity of 1 ml pooled plasma from healthy donors).

All the parents gave their full informed written consent to the hemodialysis and plasmapheresis treatment as well as to the administration of AT III concentrate.

Results

Within 2-14 days after admission there were seven out of nine children whose plasma AT III activity was moderately below the normal lower limit of 75%, and did not rise after plasmapheresis with fresh frozen plasma (Table 2). In contrast, the concentration of immunoreactive AT III was below the normal limit in only three patients.

In these seven patients replacement therapy with AT III concentrate was started. Two typical charts are reproduced in Fig. 1. Noteworthy is a rapid rise in plasma AT III activity after

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Table 1. Personal and clinical data of patients with HUS at admission

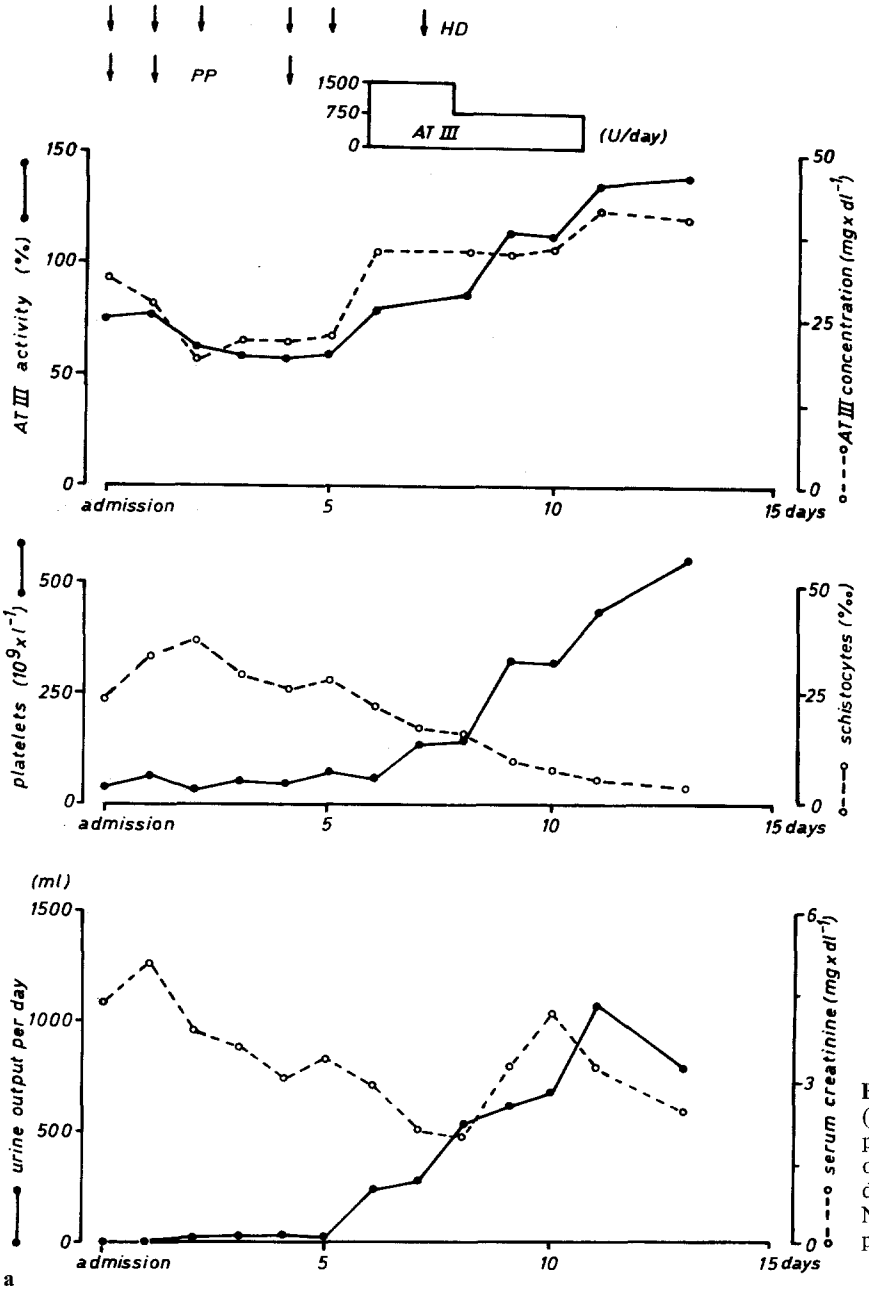
Patient	Sex (f = female; m = male)	Age (years)	Body weight (kg)	Hemoglobin (g/dl)	Schistocytes (‰)	Platelets ($\times 10^9$/l)	Haptoglobin (mg/dl)	LDH (U/l)	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Urine output in ml × kg^{-1} × h^{-1} at admission	SGOT (U/l)	SGPT (U/l)	Quick (%)	Thrombin time (s)	Fibrinogen (mg/dl)
1	F	1 11/12	10.9	9.9	40	60	22.0	2830	197	3.8	1.1	158	170	100	27.3	480
2	F	4	25.0	9.0	24	29	18.0	3000	308	4.9	0.0	32	45	91	35.0	380
3	F	12	47.7	9.0	22	78	28.0	3500	345	10.5	0.0	46	35	100	28.0	400
4	F	3/12	5.9	12.5	18	58	5.7	1200	110	1.2	2.7	10	6	87	25.0	230
5	F	1 8/12	12.8	7.1	38	40	10.5	2830	90	4.2	0.0	65	78	88	24.5	260
6	F	2 6/12	16.0	8.6	30	28	7.6	4550	158	5.5	0.0	91	72	80	30.8	270
7	M	3/12	5.8	8.6	43	80	9.3	4600	168	3.4	0.0	400	516	64	32.0	170
8	M	6/12	6.0	8.9	12	30	18.5	600	54	1.6	3.0	200	144	48	27.0	110
9	F	8 6/12	34.2	9.2	21	53	24.0	2400	87	1.5	2.5	84	92	84	22.3	310

Schistocytes are given per 1000 red cells in a blood smear (upper limit of normal 4‰). Haptoglobin concentration in the plasma was determined by radial immunodiffusion (normal range 28–243 mg/dl, mean 190 mg/dl). Normal range for the thrombin time 17–22 s

Table 2. AT III activities and plasma concentrations in nine patients with HUS and corresponding clinical data on AT III replacement, urine output, hemodialysis and plasmapheresis

Patient	AT III activity (%) on admission	AT III activity (%) at start of replacement	AT III activity (%) on recovery	AT III concentration (mg/dl) on admission	AT III concentration (mg/dl) at start of replacement	AT III concentration (mg/dl) on recovery	Maximum thrombin time during AT III replacement (s)	Start of AT III replacement in days after admission	Urine output in ml × kg^{-1} × h^{-1} at start of AT III replacement	Urine output in ml × kg^{-1} × h^{-1} on 3rd day in AT III replacement	Days of anuria	Number of hemodialyses	Number of plasmaphereses
1	–	47	90	–	22.2	30.2	52.2	14	0.0	1.0	12	17	4
2	74	60	140	31.3	21.7	40.2	27.8	6	0.0	0.4	8	6	3
3	66	66	98	17.9	16.2	33.4	33.9	2	0.0	0.0	22	28	5
4	76	–	90	23.3	–	27.1	–	–	–	–	–	–	–
5	66	66	102	27.1	17.9	29.9	37.0	6	0.0	0.9	8	5	3
6	64	70	112	24.1	25.0	27.1	56.1	3	0.0	0.5	6	5	2
7	40	54	90	10.0	16.2	31.3	43.5	2	0.1	0.6	4	6	2
8	23	34	94	6.0	7.3	40.2	44.0	2	2.0	6.5	–	–	–
9	84	–	124	29.2	–	37.9	–	–	–	–	–	–	–

the commencement of replacement treatment. Hemodialysis alone did not have any clear cut effect on AT III activity (before dialysis 80.3%±25.8%, after dialysis 85.8%±21.9%, $n=20$). The measurements were carried out immediately before and after dialysis. The concentration of immunoreactive AT III was likewise unaffected by dialysis (before dialysis 26.9±6.4 mg/dl, after dialysis 29.9±6.8 mg/dl, $n=20$). Plasmapheresis with fresh frozen plasma had no influence on AT III activity (before plasmapheresis 72.7%±18.3%, after plasmapheresis 74.6%±19.3%, $n=15$). The AT III activity in the fresh frozen plasma itself averaged 105%±14% ($n=15$) and the AT III concentration 19.5±5.3 mg/dl. In contrast to these data, plasmapheresis was followed by a definite reduction in the patients' plasma AT III concentration (before plasmapheresis 23.7±7.4 mg/dl, after plasmapheresis 17.4±5.2 mg/dl, $n=15.2$ $P<0.01$, Wilcoxon rank test for paired groups).



a

Fig. 1a, b. Time course of plasma antithrombin III (AT III activity, plasma AT III concentration, platelets, schistocytes, serum creatinine, and urine output in two patients with hemolytic-uremic syndrome treated with AT III concentrate. **a** Patient No. 2; **b** patient No. 6. HD = hemodialysis, PP = plasmapheresis

During the first 24–36 h of replacement therapy with AT III concentrate an average of 2.1 U/kg in 24 h (range 1.6–2.6 U/kg) was necessary to raise plasma AT III activity by 1%. After correcting the deficit and bringing activity up to 100% the amount of AT III concentrate required to keep plasma activity at a level of between 100% to 120% was approximately 50 U/kg per day. In retrospect it is clear that a dose of AT III amounting to 3 × 500 U/day, as given to patient. No. 3—a girl whose body weight was 47.7 kg—was too low to ensure rapid correction of the deficit in AT III activity. The longest period of anuria (22 days) was noted in this patient. No side effects attributable to AT III therapy were observed, and in particular there was no instance of hepatitis B.

The ultimate outcome with regard to renal function was complicated in only one patient (No. 3) by a persistently reduced creatinine clearance of 72 ml × min^{-1} × 1.73 m^{-2}. In the follow up of at least 6 months the creatinine clearances of all the other patients were found to be in the normal range for their age. Patient No. 1 is still suffering from a mild arterial hypertension controlled by D-propanolol.

Discussion

The most prominent feature of the pathogenesis of HUS is a primary lesion of the endothelial cells of the glomerular capillaries [12] with formation of fibrin deposits in the subendothelial space and also in the lumina of the glomerular capillaries and afferent arterioles [9]. Intravascular fibrin deposition occurs wherever the local equilibrium between the procoagulant and fibrinolytic systems breaks down. Antithrombin III is the principal endogenous proteinase inhibitor of the coagulation system, and as such plays a vital role in the maintenance of this equilibrium. Beside inhibiting the action of thrombin it has other effects, among them being irreversible blocking of activated Factor Xa, IXa, XIa and XIIa. The effects of AT III are enhanced by heparin as a cofactor. A seemingly

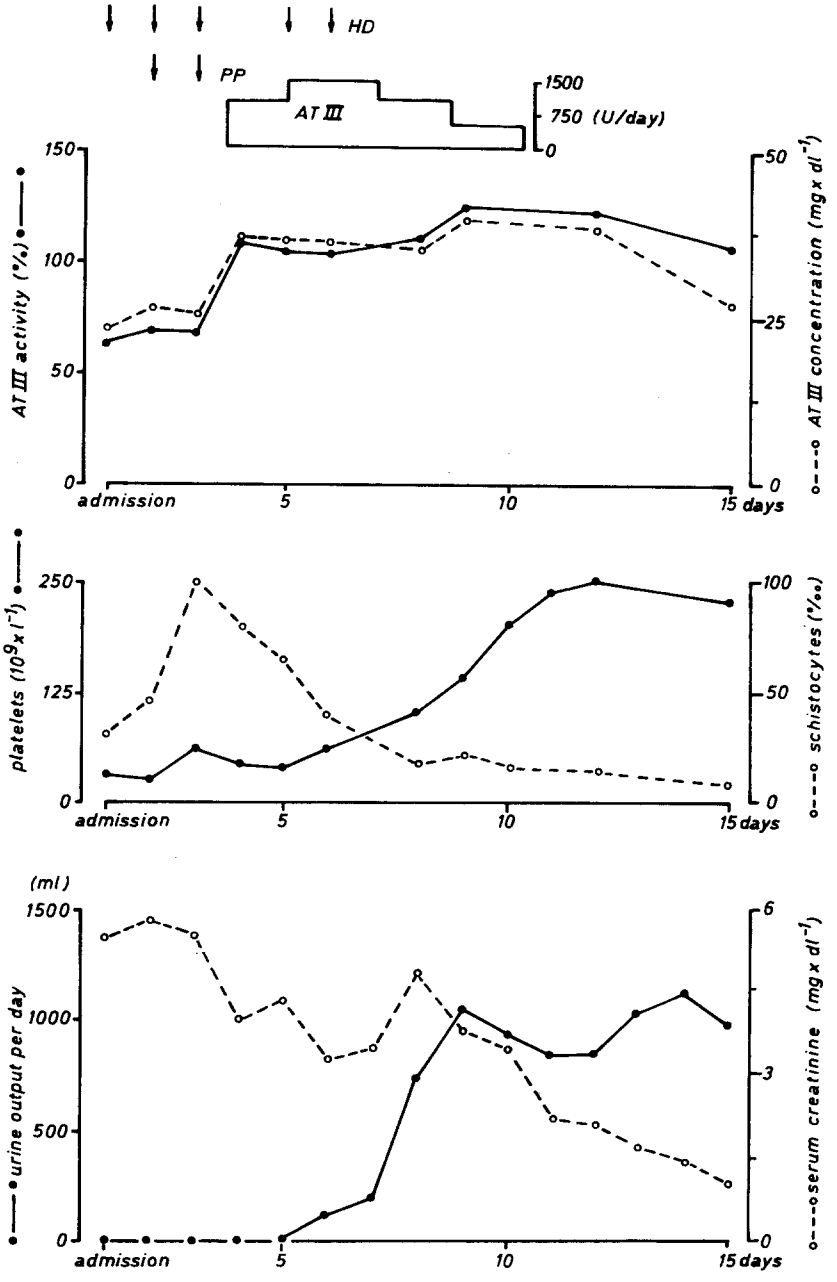


Fig. 1b

slight decrease in plasma AT III activity to levels of 70%–75% of normal is enough to produce a thrombophilic state accompanied by heparin resistance [29].

In seven out of nine children with HUS there was evidence of moderate to marked decrease in AT III activity on admission or during the first 14 days after admission, but only in three of them was there a parallel decrease in the concentration of immunoreactive AT III. This discrepancy can be explained by the presence of immunologically detectable but already inactivated AT III enzyme complex [26, 27]. Plasmapheresis with fresh frozen plasma caused a significant fall in the plasma concentration of this complex without there being any change in AT III activity.

At variance with our data, Monnens et al. [22] found a decrease in AT III activity in only 2 out of 16 children with HUS, though it should be noted that they used a synthetic substrate for assaying AT III activity. The coagulation method [27] that we employed is based on fibrinogen as the natural substrate and may possibly give a better picture of the condition existing in vivo.

AT III is synthesized chiefly in the liver but also in vascular endothelim [7]. Diminished AT III activity is observed in congenital AT III deficiency [25, 29], conditions in which the synthetic capacity of the liver is impaired [2, 10], increased loss of AT III associated with the nephrotic syndrome [30], accelerated turnover or consumption associated with disseminated intravascular coagulation [4], anticoagulant treatment with heparin [20, 31] and during therapeutic plasmapheresis with human albumin solution [23, 28].

The assumption that diminished hepatic synthesis of AT III is the sole mechanism concerned in HUS is obviously implausible, although short-lasting liver involvement with elevation of serum transaminases has been described [19] and was in fact noted in some of our patients. Nor is there any likelihood of significant loss through the kidney, especially as most of our patients with AT III deficiency were anuric.

Our patients underwent repeated hemodialysis and plasmapheresis under heparinization, but these procedures had no directly measurable effect on plasma AT III activity. Turney et al. [32] found a marked rise in AT III activity after hemodialysis. Plasmapheresis itself, provided fresh frozen plasma is used as the exchange medium, does not produce any significant fall in AT III activity. On the other hand, the findings clearly indicate that AT III activity cannot be restored to normal by giving fresh frozen plasma, even in considerable amounts, by means of plasmapheresis. From the available data, however, the possibility that the heparin may have a long-term influence on AT III activity cannot be ruled out and must be considered as a causative factor of the demonstrated AT III deficiency.

Disseminated intravascular coagulation, the most frequent cause of diminution of AT III activity as the result of increased consumption [4], is not usually observed in HUS [16, 17, 19], and this was true in our patients. However, a procoagulant state is often demonstrable in HUS [1] and in this context there is likely to be increased AT III consumption in addition to the effect of heparin. This must be considered in interference with local AT III synthesis by damaged vascular endothelium, a phenomenon which may provoke local coagulation in the kidneys [13] especially in respect to the assumption that renal microvasculature represents a major site for continuous activation of the coagulation system [21].

Diminished plasma AT III activity in HUS can accentuate an already existing procoagulant status and may have unfavorable repercussions on the course of the nephropathy, as a result of local coagulation phenomena confined to the kidneys [13]. By replacing the deficient AT III activity the procoagulant status can be overcome. Although the present data are preliminary, we believe that patients with HUS whose AT III plasma activity is below 70% of normal may benefit from replacement with AT III as an adjunct symptomatic therapy.

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