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Intracerebral haemorrhage due to acquired factor XIII inhibitor—successful response to factor XIII concentrate

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A 63-year-old woman presented with extensive bruising. An inhibitor to factor XIII was detected. Subsequent subcutaneous bruising and soft tissue haemorrhage into the left foot were treated with infusions of pasteurized factor XIII concentrate with good effect. Immunosuppression with cyclophosphamide was attempted but in spite of this she suffered a right cerebral haemorrhage necessitating further intensive therapy with factor XIII concentrate. This overcame the inhibitor, adequate post-infusion factor XIII levels were achieved and she made an excellent recovery. Factor XIII concentrate was well tolerated with no evidence of transmission of hepatitis or HIV infection. The inhibitor appeared to interfere with haemostasis by hindering the fibrin binding site of factor XIII, resulting in interference in clot-solubility tests. Subsequently the inhibitor resolved.

Key words: Acquired inhibitor, factor XIII inhibitor, factor XIII concentrate.

Introduction

Inhibitors to factor XIII are extremely rare and were first described by Lewis *et al.*[1] in 1967. Twelve cases have since been reported.[2–5] Most cases have been associated with isoniazide therapy[3] or autoimmune disease.[2,6–8] Such inhibitors are usually immunoglobulins[1,8] and can inhibit several different aspects of factor XIII activity.[6,7,9] Patients with factor XIII inhibitor can experience severe bleeding[10] and many have died of cerebral haemorrhage.[9,11] In general, therapy has been ineffective[3] although some have resolved spontaneously.[6] We describe a patient, with an acquired inhibitor to factor XIII, in whom life threatening haemorrhage occurred and who was successfully treated with intensive infusions of factor XIII concentrate.

Induction of immune tolerance against factor VIII inhibitors has been achieved with high dose factor VIII concentrate.[12] We report the first case in which an inhibitor to factor XIII was successfully suppressed by intensive therapy with factor XIII concentrate.

Patients, materials and methods

Case report

A 63-year-old female presented with extensive bruising of the trunk, left arm and left leg following minor trauma. There was no previous medical history, no family history of bleeding and she was on no medication. Coagulation screen and platelet function studies were normal. Screening tests for factor XIII deficiency demonstrated that clot from the patient's plasma redissolved in 2% acetic acid in 20 min whereas solution did not occur when a 1 : 1 mixture of normal plasma and patient's plasma was clotted (Table 1). In an antibody-neutralization test,[13] factor XIII antigen (subunit a) in the patient's plasma was found to be less than 5% of normal.

Immunoelectrophoresis[14] did not detect any subunit a in the patient's plasma. Factor XIII deficiency was provisionally diagnosed and in view of the acute onset an inhibitor of factor XIII was suspected. The patient's plasma was found to inhibit the factor XIII transamidase activity[15] of normal plasma. The concen-

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Table 1. Pre-infusion factor XIII studies

	Patient's plasma	Normal range
Factor XIII antigen[13] (% normal)	< 5%	70 – 150
Factor XIII antigen[14] (U/ml)		
Subunit a	≪0.2	84 – 116%
Factor XIII transamidase activity (U/ml)		
^{14}C-putrescine/casein[15]	0.40	**
Dansylcadaverine/casein[20]	0.50	66 – 162
Factor XIII inhibitor (inhibitor U/ml)	4.0	

**No normal range defined for this assay. Patient's results were compared with pooled normal plasma the activity of which was defined as 1.0 U/ml

tration of inhibitor was measured more accurately by a method analogous to that used to quantitate factor VIII inhibitors.[16]

Eight weeks later she developed extensive subcutaneous bruising and soft tissue haemorrhage in the left foot. She was treated with factor XIII concentrate, approximately 50 U/kg, for 1 day, 100 U/kg for 2 days and 50 U/kg for a further 3 days. The bleeding ceased and she was observed over the next 2 weeks. During this period the clot-solubility test continued to indicate factor XIII deficiency and factor XIII inhibitor was detectable, although at a lower concentration than before infusion with factor XIII concentrate. Three weeks after this first course of factor XIII replacement therapy, painful bruising of the buttocks and lower limbs required the resumption of therapy with factor XIII concentrate at a daily dose of 100 U/kg for 5 days. At the same time cyclophosphamide (50 mg/day) was commenced and gradually increased to 100 mg over the next 3 weeks. Twelve days after the second course of factor XIII replacement therapy she was admitted with dense left hemiplegia. The CAT scan confirmed a right intracerebral haemorrhage. Factor XIII inhibitor was still detectable (1.6 U/ml). Factor XIII was given twice daily for 3 days at a total dose of 150 U/kg/day, with a further 100 U/kg on the fourth day and approximately 80 U/kg on the sixth and seventh days (Figure 1). She became unresponsive on the second day but

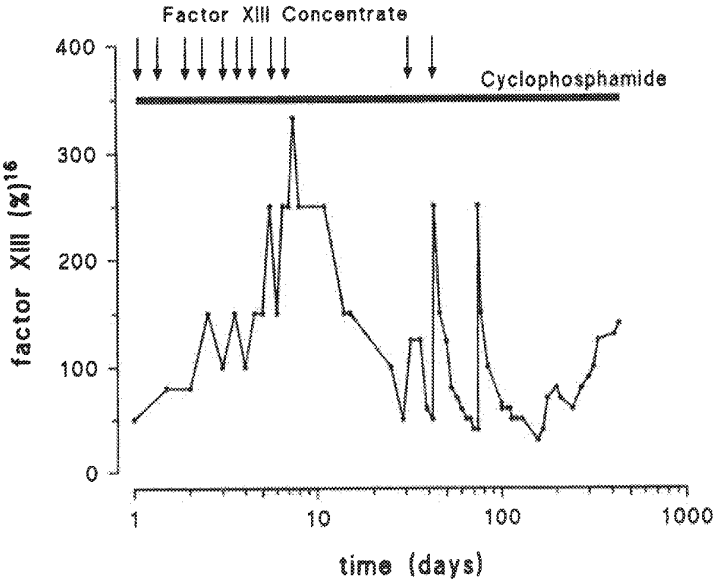

Figure 1. Serial factor XIII levels following treatment of intra-cerebral haemorrhage. The vertical arrows indicate infusions of factor XIII concentrate (for doses see Table 4). The duration of cyclophosphamide (CP) therapy is indicated along the top of graph.

subsequently improved. Three months later, after intensive rehabilitation, she was discharged home with only residual weakness of her left hand. The inhibitor has been undetectable since that time and she remains well two and a half years later.

She received three further doses of factor XIII concentrate at 2–3 week intervals when the factor XIII level fell to 40–50%, while she was in the Rehabilitation Ward. She has required no further doses since then.

Cyclophosphamide was discontinued after 1 year and factor XIII inhibitor remains undetectable. She was vaccinated against hepatitis B [Engerix B (1.0 ml sub-cutaneously)] prior to the second treatment with factor XIII concentrate.

Factor XIII concentrate

This concentrate[17] was supplied by the Bio-Products Laboratory from the Plasma Fractionation Laboratory, Oxford. The process incorporated pasteurization of the concentrate in solution at 60°C for 10 h in order to inactivate blood-borne viruses. The lyophilized concentrate was redissolved to a potency of approximately 50 U/ml (^{14}C-putrescine into casein) and infused. The concentrate from human plasma contains subunit b as well as the active subunit a of factor XIII.

Two batches of factor XIII concentrate were used. Injections were uncomplicated by any undesired side effects. Doses of 50 U/kg initially and 100 U/kg were used, the most intensive treatment being 150 U/kg/day for 3 days.

Analytical methods for factor XIII

The patient's plasma was prepared by adding nine parts venous blood to one part 0.150 M sodium citrate and centrifuging at 3000 × *g* for 10 min. Following separation, solubility tests were performed on the plasma. The remaining plasma was aliquoted and stored at −30°C.

(*i*) *Screening tests for factor XIII deficiency*. This was performed using clot-solubility in 2% acetic acid[18] or 1% monochloracetic acid[19] as an indicator that fibrin cross-linkage had occurred. The appropriate solvent was added to recalcified clots (patient and normal control in parallel) and the time taken for solution noted. (These qualitative tests distinguished factor XIII deficiency at plasma concentrations of only less than 5%.)

(*ii*) *Detection of an inhibitor to factor XIII transamidase activity*. Parallel dilutions of normal plasma were made in the patient's plasma and in Tris buffer as control. After incubation of the dilutions at 4°C for 1–2 h, residual factor XIII was measured using the dansylcadaverine casein assay.[20] This achieved initial detection of the inhibitor.

(*iii*) *Semi-quantitative factor XIII assay (Bohn)*. The antibody neutralization test of Bohn[13] was employed using a commercial kit supplied by Behring Diagnostics (Hoechst Ltd). The assay depends on the ability of a specific anti-factor XIII (subunit a) serum to neutralize the clot-stabilizing activity of the plasma sample. Plasma was added to serial dilutions of the antiserum and, after clotting with thrombin and calcium, the stability of the clot in 1% monochloracetic acid increased with the factor XIII activity of the plasma sample. The antiserum concentration was adjusted so that a dilution of 1 : 10 inhibited a factor XIII activity of 100%. Thus, incomplete solution at a 1 : 50 dilution of antiserum denoted a factor XIII concentration of 20% of normal. Minor modifications of the technique included the use of half volumes of all reagents and expansion of the dilution range to detect extremely low and high values of factor XIII.

(*iv*) *Factor XIII subunit a*. Factor XIII subunit a was measured by Laurell immunoelectrophoresis using a 1% agarose gel preparation in Tris-tricine buffer, containing 3% PEG 4000 and 1/200 monospecific rabbit antiserum (Behringwerke) against subunit a. Gels were run for 16 h at 2 v/cm. The results were expressed by reference to activity in a frozen 'normal plasma' pool from ≥ 30 normal donors, defined as 100%. The normal factor XIII subunit a is in the range of 84–116% (mean ± 2 SD) using electrophoretic separation of a and b.[21] Subunit b was not directly measured.

(*v*) *Incorporation of diamine substrates into casein*. The method of Dvilansky *et al.*[15] was used in which ^{14}C-putrescine was incorporated into casein by factor XIII in the patient's plasma or in a normal plasma control. The method was quantitative down to about 0.3 U/ml (~30%), the activity of a 'normal plasma' pool from ≥ 30 normal donors being defined as 100% [1 U/ml being equivalent to 100%; there is no international standard for factor XIII]. This method was also used to determine the factor XIII potency of the concentrate. The method of Cooke and Holbrook[20] was used to measure the incorporation of dansylcadaverine into casein. The normal range was 66–162%.

(*vi*) *Semi-quantitation of factor XIII inhibitor*. A technique based on the Bethesda assay for factor VIII inhibitors[16] was established to give a semi-quantitation of the factor XIII inhibitor. Test plasma (0.4 ml), or a

Table 2. Initial detection of a circulating inhibitor to factor XIII in the patient's plasma

Dilution of normal plasma	Residual factor XIII activity (% of initial)	
	Diluent Tris buffer	Diluent patient's plasma
× 2	60.0	28.5
× 4	27.0	14.6
× 8	8.6	ND

ND, not done.
Parallel dilutions of normal plasma in the patient's plasma and in Tris buffer were incubated at 4°C for 1–2 h and residual factor XIII transamidase activity assayed by the incorporation of dansylcadaverine into casein.

dilution, was incubated for 2 h with 0.4 ml of normal plasma at 37°C. A normal plasma standard and an imidazole buffer control were incubated in parallel. Residual factor XIII was then measured in the incubated test and control samples.[13] The residual concentration of factor XIII in each test mixture was then related to that in the standard sample. Residual levels of less than 25% and greater than 75% were not used. The concentration of factor XIII inhibitor in the patient's plasma was expressed in inhibitor U/ml where 1 U of inhibitor results in a 50% reduction of factor XIII after 2 h as defined by Kasper *et al.*[16]

Separation of IgG

The IgG fraction was separated from the patient's plasma (IgG level 18.8 g/l) using a Protein G column (Pierce Chemical Co., Chester, UK). Three resulting diluted fractions were checked for purity by immunoelectrophoresis. Minimal levels of IgG remained in the first two fractions (<0.02 g/l and 0.78 g/l), whilst the third fraction contained the vast majority of the IgG (8.3 g/l) in a relatively pure form.

Clinical investigations

The following routine investigations were performed: alanine aminotransferase (ALT), T4, thyroid hormone uptake, TSH, immunoglobulins (nephelometry), hepatitis B surface antigen (HBsAG) by Hepatest (Wellcome), hepatitis B surface antibody (Anti HBs) by sandwich enzyme immunoassay (Enzygnost–Anti HBs, Behring Institute), anti-HIV by indirect ELISA (Wellcome) and anti-HCV by enzyme immunoassay (Ortho).

Results

The following tests were initially performed on a pre-treatment sample.

(i) Clots from the patient's plasma rapidly dissolved in 2% acetic acid or 1% monochloracetic acid, indicating a factor XIII level of less than 5% of normal. Clot lysis was prevented by the addition of an equal volume of normal plasma. We found the acetic acid test both more rapid and convenient for day-to-day assessment of clotting response to concentrate.

(ii) Dilutions of normal plasma in the patient's plasma had much lower factor XIII transamidating activity than corresponding dilutions in Tris buffer, suggesting that the patient had a circulating inhibitor of factor XIII (Table 2).

(iii) Factor XIII antigen (subunit a) was estimated to be <5% of normal by the antibody neutralization technique.[13] In our hands this test showed a precision of 9–13% and gave quantitative information between 1 and 250% of normal plasma factor XIII activity. It took 2 h to perform and was useful for day-to-day monitoring of the patient (Table 3).

(iv) Immunoelectrophoresis confirmed the absence of the subunit a of factor XIII (≪0.2% N). The factor XIII-inhibitor complex resulted in unusual patterns on Laurell immunoelectrophoresis—a fast-moving smudge in advance of very small peaks attributable to the injected concentrate (Table 1).

(v) However, there was low but detectable plasma transamidase activity in pre-infusion samples as measured either by ^{14}C-putrescine (0.4 U/ml) or the dansylcadaverine-casein assay (0.5 U/ml).

(vi) The patient was found to have a weak factor XIII inhibitor which rapidly neutralized about 1 U of factor XIII/ml of the patient's plasma.

Modification of the Bethesda inhibitor assay for factor VIII inhibitors demonstrated an inhibitor of factor XIII in a concentration of 4.0 inhibitor U/ml (Table 1). This assay showed a precision of 19–35%, comparable to published data for the Bethesda factor VIII assay.[16] This method specifically measures inhibition of clot stabilization. The inhibitor was immediate in action and stable, losing no inhibitory activity after 4 days at 20°C.

In the first course of factor XIII replacement ther-

Table 3. Factor XIII levels pre- and post-infusion during the first and second courses of replacement therapy with factor XIII concentrate

Day	Factor XIII concentrate dose (U)	Factor XIII screen (clot-solubility)		Factor XIII antigen[16] % normal		Factor XIII antigen[14] subunit a (U/ml)		Factor XIII transamidase ^{14}C-putrescine–casein[15] (U/ml)		Factor XIII inhibitor (inhibitor U/ml)
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	
1	3125	Pos.*	Pos.	< 5	5	0	0	0.9	3.90	4.00
2	7500	Pos.	Neg.	< 5	70	< 0.20	0.66	0.28	4.60	1.70
3	7500	Pos.	Neg.	< 5	50	< 0.20	1.10	0.80	5.50	1.30
4	3125	Pos.	Neg.	< 5	40	< 0.30	0.30	1.05	5.50	1.40
5	3125	Pos.	Neg.	< 5	20	< 0.20	0.30	1.33	1.04	0.90
27	7500	Pos.	Neg.	< 5	50	< 0.20	0.40	0.66	3.90	2.00
28	7500	Pos.	Neg.	< 5	55	< 0.20	2.40	2.40	> 4.00	1.50
29	7500	Neg.	Neg.	40	> 80	1.40	3.40	> 4.00	> 4.00	< 0.50
30	7500	Neg.	Neg.	40	> 80	1.60	3.30	> 4.00	> 4.00	< 0.50
31	7500	Neg.	Neg.	40	> 80	2.10	3.20	> 4.00	> 4.00	< 0.50

*Factor XIII screen: positive [clot dissolved (< 5% normal)]; negative [clot insoluble (> 5% normal)].

apy, there was a poor response in the clot-solubility test and factor XIII assays to the first 50 U/kg dose of concentrate but a better response to a second dose of 100 U/kg. Satisfactory post-infusion levels[13] were achieved during the rest of the first course of therapy (Table 3); the clot-solubility test showed not only the presence of > 5% factor XIII 1 h post-infusion but also its absence again the following day, in contrast to the usual 7 day half-life of factor XIII concentrate. There was a parallel loss of factor XIII antigen by Laurell immunoelectrophoresis. In contrast, the factor XIII transamidase activity was high in both pre- and post-infusion samples. The inhibitor varied from 0.95–2.4 inhibitor U/ml; no anamnestic response occurred to raise the inhibitor above pre-infusion levels.

After the second course of factor XIII concentrate therapy, the inhibitor became undetectable on the third day and has remained undetectable since (Table 3). The recovery improved to approximately 100%[13] and the half-disappearance time to 2 days. During the third course of factor XIII concentrate therapy, higher plasma levels were maintained initially to provide a good margin of safety until the half-disappearance time was 3 days (Table 4).

Factor XIII concentrate was well tolerated throughout. ALT was tested every 2 weeks during 12 months of follow-up. The highest level noted was 53 (normal range 5–40 IU/ml). Serological markers for hepatitis B (anti-HBs and anti-HBc), hepatitis C (anti-HCv) and HIV infection (anti-HIV) remain negative to date. She did not seroconvert after hepatitis B vaccination, possibly because of age.

Measurement of serum immunoglobulins prior to treatment, revealed an elevated IgG (16.6 g/dl) and normal IgA and IgM. On incubation *in vitro* a mixture of normal plasma and patient's plasma showed a large reduction in IgG (32% and 40%) suggesting that the inhibitor was contained in the immunoglobulin fraction of plasma. The factor XIII inhibitor assay was performed on two occasions on the three fractions collected from the Protein G column. Only the IgG pure fraction showed any factor XIII inhibition, 0.3 IU/ml as compared to 0.6 IU/ml in the pre-treated plasma, thus confirming the factor XIII inhibitor to be an immunoglobulin. The inhibitory action was independent of thrombin and calcium showing that factor XIII was inhibited prior to conversion to factor XIIIa. The patient's autoimmune profile demonstrated strong circulating thyroid antibodies to thyroglobulin and parenchyma. Thyroid function tests were consistent with compensated hypothyroidism [TSH, 11.2 M IU/l (normal range 0.3–4.50)] and remain so.

Discussion

Life-threatening intracerebral haemorrhage occurred in this patient following development of an acquired inhibitor to factor XIII. Factor XIII inhibitor has been described in association with autoimmune disease[6–8]

Table 4. Serial factor XIII levels during treatment of intracerebral haemorrhage with a third course of factor XIII concentrate

Day	Factor XIII concentrate dose (U)	Factor XIII antigen[16] % normal		Factor XIII antigen[14] subunit a (U/ml)		Factor XIII transamidase ^{14}C-putrescine–casein[15] (U/ml)		Factor XIII inhibitor (inhibitor U/ml)
		Pre	Post	Pre	Post	Pre	Post	
1	7500	—	50	—	1.20	—	>4.0	1.40
	3125	50	80	0.60	1.40	3.40	>4.0	0.40
2	7500	80	150	0.80	1.80	2.70	>4.00	—
	3125	100	>150	2.10	2.10	>4.00	>4.00	—
3	7500	>100	>150	0.76	2.10	2.10	3.80	—
	3125	>100	>150	3.00	3.02	3.69	>4.00	—
4	7500	200	>250	2.20	2.40	>4.00	>4.00	—
5	None	150	—	2.50	—	>4.00	—	—
6	6250	150	>250	4.00	6.00	>4.00	>4.00	—
7	6250	250	333	2.00	4.00	>4.00	>4.00	—
8	None	250	—	3.90	—	>4.00	—	0.40

Factor XIII (clot-solubility) is negative throughout.

and it is possible that her compensated immune-based thyroid disease was in some way related. An initial coagulation screen failed to detect the cause of haemorrhage in this patient, emphasizing the need for a specific factor XIII screen in cases of unexplained haemorrhage if such deficiencies and inhibitors are to be detected.

A modification of the Bethesda assay for factor VIII antibodies was sensitive enough to detect the fall in residual factor XIII level and the inhibitor level, which declined as the patient's clinical response to factor XIII replacement therapy improved. The antibody neutralization technique of Bohn, although providing only a semi-quantitative factor XIII activity assay, was rapid, simple and sensitive enough to monitor factor XIII levels and response to therapy.

Before factor XIII replacement therapy, no detectable factor XIII antigen (subunit a) was found in the patient's plasma either by a semi-quantitative neutralization method[13] or by Laurell immunoelectrophoresis, but factor XIII transamidating activity was demonstrated by both dansylcadaverine and ^{14}C-putrescine assays. The patient appeared to have an anti-factor XIII antibody, presumably subunit a, which formed a soluble complex with exogenous factor XIII and interfered with its cross-linking action on fibrin; this resulted in depletion of her factor XIII, as measured in the clot-solubility test, and severe bleeding. The patient's detectable transamidating activity suggests that the inhibitor did not completely block the active site but allowed access to the smaller diamine substrates and receptor casein. Factor XIII may show normal activity when tested on artificial substrates in the presence of inhibitors which block the binding sites of fibrin monomer.[7,9,11,22] The inhibitor was stable and acted very rapidly with factor XIII. Most inhibitors of coagulation factors have been shown to be immunoglobulins.[1,8] This inhibitor was demonstrated to be contained in the IgG fraction of the patient's plasma and is thought to neutralize the action of factor XIII before its activation by thrombin and calcium. Immunoelectrophoretic patterns probably reflected migration of the factor XIII–immunoglobulin complex.

Treatment of patients with factor XIII inhibitors has been unsatisfactory. Immunosuppression, exchange transfusion and plasma exchange[23] have all been attempted with little effect on the inhibitor and no clinical benefit. Many patients died from haemorrhage while an occasional inhibitor resolved spontaneously. We believe this is the first report of such a patient being treated with factor XIII concentrate. She had a very good immediate recovery in excess of 50%, to intensive therapy with factor XIII concentrate. The inhibitor was quickly overcome but the concentrate showed an initial half-disappearance time of less than 24 h which subsequently increased to 2 days as therapy with factor XIII exhausted the inhibitor. During the third course of therapy higher levels of factor XIII were maintained until her clinical state was stable, by which time the response was 100% and the half-disappearance time increased to 3 days. The response of this

patient, whose inhibitor was rapidly overcome by intensive factor XIII concentrate therapy, with control of haemorrhage and excellent functional recovery, is very encouraging. The treatment is simple to deliver to an acutely ill patient and without major side effects. Cyclophosphamide was used in an attempt to immunosuppress but before this would have been effective, massive doses of factor XIII concentrate, which effectively overcame the inhibitor, were needed to control haemorrhage. The role of cyclophosphamide in this patient, therefore, cannot be ascertained, as the inhibitor remained undetectable when cyclophosphamide was withdrawn. Spontaneous resolution may have occurred in this woman.

Had this patient first presented with a cerebral haemorrhage it is unlikely that the investigations, described in this paper, would have been performed and therefore life saving therapy would not have been administered. We suggest that a factor XIII screen be performed in all cases of unexplained haemorrhage. When the clot solubility test is positive, the semi-quantitative factor XIII assay[13] and modified inhibitor assay described in this paper allow a rapid diagnosis. If the patient has serious haemorrhage we recommend early intervention with intensive factor XIII concentrate to overcome the inhibitor. Our patient showed no anamnestic response and prophylactic therapy may be feasible, although an anamnestic response could, theoretically, be provoked by use of factor XIII concentrate. Although the case for or against cyclophosphamide is not proven in our patient we recommend its early use on theoretical grounds because of the potential life-threatening complications of this condition. She was monitored for elevation of transamidase according to ICTH criteria[24] with no elevation of ALT and no serological evidence of transmission of hepatitis B, hepatitis C or HIV infection to date.

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