SHORT REPORT

A multicentre evaluation of the diagnostic efficiency of serological investigations for C1 inhibitor deficiency

M M Gompels, R J Lock, J E Morgan, J Osborne, A Brown, P F Virgo

Aim: To determine the diagnostic efficiency of assays routinely used in the investigation of hereditary angio-oedema.

Methods: Over a four year period, 1144 samples were received for analysis from 907 patients suspected of C1 inhibitor deficiency. Analyses were performed for C4 and C1 inhibitor (functional and immunochemical). Notes were reviewed retrospectively on patients with low serological indicators to determine diagnosis.

Results: These are the first data to indicate the sensitivity, specificity, and predictive values of the assays most frequently used to screen for C1 inhibitor deficiency. A combination of low C4 and low C1 inhibitor function has 98% specificity for C1 inhibitor deficiency in this population and a 96% negative predictive value, and is thus a very effective screen. All patients with untreated C1 inhibitor deficiency had a low C4 value.

Conclusions: All patients considered for a diagnosis of C1 inhibitor deficiency should have serum examined to measure both C4 and functional C1 inhibitor. If either is normal at presentation this essentially excludes a diagnosis of C1 inhibitor deficiency. These tests can be performed sequentially. If C4 is normal it is not necessary to proceed to C1 inhibitor analysis. If C1 inhibitor function and C4 are both low then a repeat sample should be obtained to confirm the findings.

C1 inhibitor is a serine protease inhibitor that is involved in the regulation of several enzymes including C1r, C1s, plasmin, kallikrein, factor XIa, factor XIIa, and factor XIIf.[1] Deficiency results in recurrent oedema affecting primarily the extremities, face, larynx, and gastrointestinal mucosa. Insufficient normal C1 inhibitor leads to uncontrolled activation of the classical complement pathway, with subsequent reduction of serum C4 and C2 concentrations.[1]

C1 inhibitor deficiency may be either hereditary (hereditary angio-oedema; HAE) or acquired.[1 2] HAE has two major variants. In type 1, the classic form, found in 85% of patients, concentrations of C1 inhibitor are low at presentation. The remaining 15% have type 2 HAE, where a dysfunctional C1 inhibitor is produced in normal or increased amounts.[3] The disorder is rare, with HAE affecting around 1 in 10 000–50 000 of the population,[4] and the acquired form affecting a 10th of that number. The diagnosis is important because there is a high associated morbidity and mortality. There are potent and effective treatments available, particularly the androgenic drugs. These may result in serum concentrations of C1 inhibitor and C4 reaching normal values,[5] but the side effects are potentially serious and include hepatocellular adenoma.[6]

To our knowledge, there are no data in the literature to indicate the sensitivity, specificity, and predictive values of the assays most frequently used to screen for C1 inhibitor deficiency (C1inhD); namely, serum C4, C1 inhibitor protein,

Table 1 Reference ranges for serological assays

	C4 (g/l)	C1 inhibitor (g/l)	Functional C1 inhibitor (% mean normal)
SMH	0.15–0.50	0.15–0.35	70–130
BRI	0.17–0.65	0.15–0.35	70–130

BRI, Bristol Royal Infirmary; SMH, Southmead Hospital.

and C1 inhibitor function. We reviewed the data from two laboratories over four years on samples referred from three centres for C1 inhibitor values and reviewed the notes in all cases where, based on the laboratory findings, C1inhD was considered a possibility.

METHODS

All samples referred over a four year period (1996–9) for the investigation of possible C1 inhibitor deficiency from Southmead Hospital, Bristol (SMH), the Bristol Royal Infirmary (BRI), and Musgrove Park Hospital, Taunton were included in the study. Serological assays were undertaken at either of two sites (SMH, BRI). At SMH, C1 inhibitor and C4 were quantified nephelometrically (BNII nephelometer; Dade Behring, Behring Diagnostics UK Ltd, Milton Keynes, UK) according to the manufacturer's instructions. Functional C1 inhibitor was measured using the Berichrome chromogenic assay (Dade Behring), according to the manufacturer's instructions. At BRI, C1 inhibitor and C4 were measured turbidimetrically (Kone Pro; Labmedics, Stockport, UK), according to the manufacturer's instructions. Functional C1 inhibitor was measured using the Immunochrom C1-Inh chromogenic assay (Technoclone Ltd, Dorking, UK), according to the manufacturer's instructions. Samples from Taunton were referred to either one of the other hospitals for analysis. Reference ranges were similar for each of the assays across the two sites (table 1). Data considered positive for the purposes of screening and statistical analysis were low C4, low C1 inhibitor, low functional C1 inhibitor, and combined low C4 and functional C1 inhibitor.

Notes for all patients with low C4 or low C1 inhibitor were subject to retrospective review by one author (MMG). Patients were categorised on the basis of their history (recurrent acute oedema of the skin, airways, gastrointestinum, or extremities, low C1 inhibitor function in serum when off treatment, with subsequent serum C4 analysis also being low[1]) as to whether they were likely to have true C1inhD.

The first sample analysed for each patient during the study period, from whichever site, was used to calculate the sensitivity, specificity, positive predictive value, and negative

Table 2   Number of samples low for analyte for each group examined

	C4	C1 inhibitor (immunochemical)	Functional C1 inhibitor	C4 and functional C1 inhibitor
All samples	144 (n=1022)	133 (n=1141)	309 (n=1138)	100 (n=1018)
First samples	63 (n=806)	67 (n=906)	161 (n=904)	26 (n=805)
All C1inhD samples in study period	80 (n=114)	75 (n=115)	112 (n=114)	79 (n=113)
First C1inhD samples in study period	15 (n=23)	15 (n=23)	22 (n=23)	15 (n=23)
C1inhD samples off treatment	20 (n=20)	17 (n=20)	20 (n=20)	20 (n=20)

C1inhD, C1 inhibitor deficiency; n, number of samples analysed in each group.

Table 3   Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of assays for a diagnosis of C1 inhibitor deficiency

	C4	C1 inhibitor (immunochemical)	Functional C1 inhibitor	C4 and functional C1 inhibitor
Using all samples				
Sensitivity	70%	65%	97%	70%
Specificity	93%	94%	81%	98%
PPV	56%	56%	36%	79%
NPV	96%	96%	99%	96%
Using first samples in study period				
Sensitivity	65%	65%	96%	65%
Specificity	94%	94%	84%	99%
PPV	23%	22%	14%	58%
NPV	99%	99%	100%	99%
Using untreated C1inhD v first sample from non-C1inhD				
Sensitivity	100%	85%	100%	100%
Specificity	94%	94%	84%	99%
PPV	29%	25%	13%	65%
NPV	100%	99%	100%	100%

The PPV associated with the immunochemical C1 inhibitor measurement incorporates type 2 hereditary angioedema. The C1 inhibitor values will be normal or raised despite functional deficiency.
C1inhD, C1 inhibitor deficiency.

Table 4   Analysis of patients where repeat sampling was used (first two referred samples used in all cases)

	Patients with C1inhD		Patients without C1inhD	
	C4	C1inhF	C4	C1inhF
Normal on at least one occasion	7	1	18	34 (17/34 normal on both)
Original and repeat serology both abnormal	16	22	5	5

C1inhD, C1 inhibitor deficiency; C1inhF, C1 inhibitor function.

predictive value of the assays for C1inhD. It has been noted previously that the serological parameters may normalise and remain stable on treatment.[5][7][8] Several patients with true deficiency had been diagnosed before our study start date. Thus, we also reviewed the serology at a time when the patients were not undergoing specific treatment during the study period. Data were not available on all patients owing to post-treatment referrals from other centres.

RESULTS
During the study period, 1144 samples were received for analysis (787 BRI, 357 SMH). These were from 907 patients, including 23 patients with C1inhD (22 HAE, one acquired) from whom 115 of 1144 samples were received. Table 2 summarises the results. In all cases of C1inhD, untreated patients had at least one sample with very low C4 (< 0.1 g/litre) and very low functional C1 inhibitor (< 30%). Table 3 gives the parameters of clinical usefulness. Note that calculations in the third section of the table use data from 20 patients with C1inhD who were untreated and compare these with the first samples received from all patients without C1inhD. This mirrors the data one would see at presentation and allows an estimate of the diagnostic efficiency at that time.

Table 5   False positive rates of functional C1 inhibitor by referring centre

	Analysed	Low C1inhF	True low (C1inhD)	False positive (%)
Centre 1	272	63	6	90
Centre 2	296	74	27	64
Centre 3	570	172	79	54

C1inhD, C1 inhibitor deficiency; C1inhF, C1 inhibitor function.

Repeated testing of patients without C1inhD was uncommon in our study (table 4). However, of 23 patients without C1 inhibitor deficiency in which both C4 and C1 inhibitor were repeated, only one was low on two consecutive occasions for both C4 (0.13 and 0.15 g/litre) and functional C1 inhibitor (50% and 55%). In contrast, 15 of 23 patients with C1inhD were low for both ($p < 0.001$, Fisher's exact test). Although the numbers are small, 15 of 16 patients with low C4 and functional C1inh on two occasions were shown to have true deficiency (positive predictive value, 94%).

Table 5 shows the false positivity rate seen in each centre.

DISCUSSION

We present the first data to examine the usefulness of assays for C1inhD in routine practice. The data include only a small group of patients with C1inhD; nevertheless, the figures provide some important information.

Although the assays appear to be straightforward for the C1 inhibitor, with several alternative kits available, falsely low results are not infrequent (table 5). Quality control of the assays is difficult and no UK External Quality Assurance scheme is available for either the functional or immunochemical measurement of C1 inhibitor.

With such a rare disease, a low false positive rate in the normal population will reduce the positive predictive value. In the data presented, not only is there a small disease population, but all of the patients were offered treatment during our study and 30% of samples showed a normalised C4. Six patients with C1inhD (28 samples) had normal C4 throughout our study. However, even in the unselected population a combination of low C4 and low C1 inhibitor function has a 98% specificity for C1inhD and a 96% negative predictive value and is thus a very effective screen. These figures are better than using functional C1 inhibitor alone. Probably this is because of the elimination of those low results that are caused by the degradation of this sensitive enzyme during transit to the laboratory. This is supported by the fact that the false positive rate for functional C1 inhibitor is higher from centre 1 than the other two sites (table 5), where transit times from sample acquisition to the testing laboratory are unavoidably longer.

The accuracy of diagnosis is significantly improved if, wherever the C1 inhibitor function is low, a repeat sample is analysed to confirm the result. In only one case without true C1 inhibitor deficiency were both the C4 and C1 inhibitor function low on repeat testing. However, in this case the C4 and C1 inhibitor results were borderline low on both occasions and were much higher than is seen in active C1inhD. Typically, in HAE functional values of C1 inhibitor are less than 50%.[9] This patient had idiopathic urticaria.

It has been suggested that the C1q values should be determined to distinguish HAE from acquired C1inhD.[2] However, this assay is offered in only a few UK laboratories and the distinction can usually be made on the basis of the patient's medical history.

Functional C1 inhibitor assays are preferred to immunochemistry so as not to miss type 2 HAE. Table 2 shows that three of 20 patients with HAE had normal immunochemical values, in accordance with published data.[1]

We recommend that all patients considered for a diagnosis of C1 inhibitor deficiency should have serum examined to measure both C4 and functional C1 inhibitor. As the data from centre 1 show, it is important that the receiving laboratory assays C4 to check for sample integrity. If either is normal at presentation this essentially excludes a diagnosis of C1 inhibitor deficiency. Indeed, it has been advocated that serum C4 alone can be used as a screen for C1inhD.[10] Our data (table 3) support this view and it would not be unreasonable to exclude the diagnosis on the basis of normal serum C4 alone. If C1 inhibitor function and C4 are both low then a repeat sample should be obtained to confirm the findings. In conclusion, we have shown that, using routine assays, the diagnosis of C1 inhibitor deficiency can be made with 98% specificity and, equally importantly, excluded with 96% accuracy (96% negative predictive value). This can best be achieved by testing both C4 and functional C1 inhibitor. To diagnose or exclude HAE correctly both values must be obtained.

....

Authors' affiliations
M M Gompels, R J Lock, P F Virgo, Immunology and Immunogenetics, Southmead Hospital, Bristol BS10 5NB, UK
J E Morgan, J Osborne , Department of Chemical Pathology, Bristol Royal Infirmary, Bristol, UK
A Brown , Immunology and Coagulation, Musgrove Park Hospital, Taunton, Devon, UK

Correspondence to: Dr MM Gompels, Immunology and Immunogenetics, Southmead Hospital, Bristol BS10 5NB, UK; GOMPELS_M@SOUTHMEAD.SWEST.NHS.UK

REFERENCES

1 **Davis AE**. C1-inhibitor and hereditary angioneurotic edema. *Annu Rev Immunol* 1988;**66**:595–628.
2 **Markovic SN**, Inwards DJ, Frigas EA, *et al.* Acquired C1 esterase inhibitor deficiency. *Ann Intern Med* 2000;**132**:144–50.
3 **Circolo A**, Strunk RC. Hereditary angioedema—understanding the basis of C1 inhibitor deficiency. *The Immunologist* 1997;**5**:166–70.
4 **Cicardi MC**, Agostoni A. Hereditary angioedema. *N Engl J Med* 1996;**334**:1666–7.
5 **Gadek JE**, Hosea SW, Gelfand JA, *et al.* Response of variant hereditary angioedema phenotypes to danazol therapy. *J Clin Invest* 1979;**64**:280–5.
6 **Bork K**, Pitton M, Harten P, *et al.* Hepatocelluar adenomas in patients taking danazol for hereditary angio-oedema. *Lancet* 1999;**353**:1066–7.
7 **Pitts JS**, Donaldson VH, Forristal J, *et al.* Remissions induced in hereditary angioneurotic edema with an attenuated androgen (danazol): correlation between concentrations of C1-inhibitor and the forth and second components of complement. *J Lab Clin Med* 1978;**92**:501–7.
8 **Waytes AT**, Rosen FS, Frank MM. Treatment of hereditary angioedema with a vapor-heated C1 inhibitor concentrate. *N Engl J Med* 1996;**334**:1630–4.
9 **Quastel M**, Harrison R, Cicardi M, *et al.* Behavior in vivo of normal and dysfunctional C1 inhibitor in normal subjects and patients with hereditary angioneurotic edema. *J Clin Invest* 1983;**71**:1041–6.
10 **Sim TC**, Grant JA. Hereditary angioedema: its diagnostic and management perspectives. *Am J Med* 1990;**88**:656–64.