Falsely normal C4 in a case of acquired C1 esterase inhibitor deficiency

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A 59-year-old lady presented with recurrent angioedema without urticaria. The clinical history and examination were consistent with an acquired C1 esterase deficiency secondary to lymphoproliferative disease. Despite a low C1 esterase level, the C4 level assayed by nephelometry on our automated analyser was normal. Analysis using different nephelometric analysers revealed consistently low C4, despite consistent normal readings in our analyser. Further investigation revealed an IgM- κ paraprotein that seemed to interfere with both this and haematology coagulation assays. Splenic marginal zone lymphoma was confirmed on bone marrow biopsy. Monoclonal paraproteins may interfere with nephelometric, turbidimetric and immunological assays in a non-antibody-specific manner and should be considered when there are unusual or unexpected results, particularly in a patient with lymphoproliferative disease.

A 59-year-old woman was referred with a 13-month history of recurrent angioedema. These attacks came on over a few hours, resolved over 2–3 days and occurred approximately every 2 weeks. Attacks predominantly involved the peripheries and were not associated with urticaria. There were no identifiable triggers. She denied any abdominal pain or vomiting. On systemic enquiry she described fatigue, weight loss, drenching night sweats, bruising, tinnitus and paraesthesia of her hands, worsening over this 13-month period. She had no significant medical history and was not taking any drugs associated with angioedema. Initial investigations at the district general hospital showed a microcytic anaemia and a markedly increased erythrocyte sedimentation rate (136 mm/h). Previous C3 and C4 levels performed during an episode of angioedema in our laboratory on an Array 360 analyser (Beckman Coulter, High Wycombe, UK) were normal (table 1). A C1 esterase level performed at a different laboratory on a Mira analyser (Roche Diagnostics, Welwyn Garden City, UK) using a turbidimetric method was low, and a C4 level at the same laboratory performed on a BN2 analyser (Dade Behring, Marburg, Germany) was markedly low, and the patient was referred for assessment.

In the clinic she was pale, with a tachycardia at 130 beats/min with normal heart sounds. On respiratory examination sparse crepitations were audible at both bases. Abdominal examination revealed splenomegaly extending towards the umbilicus. There was no hepatomegaly or ascites. There was no evidence of cervical, inguinal or axillary lymphadenopathy. Further investigations confirmed a microcytic anaemia with a haemoglobin concentration of 7.2 g/dl. Erythrocyte sedimentation rate remained increased at 130 mm/h with a C reactive protein level of 52 mg/l. Liver and renal function were normal but her lactate dehydrogenase was increased at 354 U/l (normal range 0–215 U/l). Her prothrombin time was 20 s, which corrected to 13 s on 50:50 mix with normal plasma. The activated partial thromboplastin time was markedly prolonged at 66 s, which only corrected to 59 s on 50:50 mix. Anti-cardiolipin (aCL) IgG and IgM tests were performed using

commercial antibody kits (Orgentec, Mainz, Germany). aCL IgM antibody was positive, but levels were untitratable despite serial dilutions (>1:1600). The aCL IgG antibodies were increased at 26.8 (normal <10). The dilute Russel viper venom time ratio was increased at 2.0 (normal 0.9–1.2). Autoantibody screen was antinuclear antibody negative with an increased double-stranded (ds)-DNA antibody of 87 IU/ml (normal <20 IU/ml), anti-smooth muscle antibody titre of 1:320 and weakly positive antibodies to soluble liver antigens. IgG level was normal with a mildly increased IgA level (4.28 g/l), and serum electrophoresis identified a γ band. Initial immunofixation showed non-specific staining, but following treatment of the sample with 2-mercaptoethanol an IgM κ paraprotein at a concentration of 6.6 g/l was identified. Cryoglobulin activity was negative. Rheumatoid factor activity could not be assayed owing to non-specific agglutination on the latex agglutination test. Immunophenotyping by flow cytometry of peripheral blood revealed a clonal B cell population suggestive of a non-Hodgkin's lymphoma and a trephine bone marrow biopsy confirmed splenic marginal zone lymphoma. A C1 esterase test performed by radial immunodiffusion using a commercial kit (Binding Site) was low. Repeat testing of the C4 on the Array 360 remained within the normal range, but when performed using a BN2 analyser in our laboratory, the C4 was low. Functional C1 esterase activity was also low at 15% (normal range 70–120%). IgM depletion performed by anti-IgM antibody precipitation as previously described¹ showed a dose-dependent correction in C4 levels. Analysis of IgM-depleted serum confirmed the presence of IgG anti-smooth muscle antibody, weak positive soluble liver antigens and positive ds-DNA of 42 IU/ml. The artefactually increased C4 results on the Beckman Array were believed to be due to paraprotein interference. The patient started receiving treatment with tranexamic acid, prednisolone and chlorambucil. Her C1 esterase levels increased (table 1), and she has not had any further attacks of angioedema.

DISCUSSION

Serum C4 levels are considered to be a highly sensitive and specific screen for untreated C1 esterase inhibitor deficiency. In the absence of low C4, it is usually considered not necessary to proceed to analysis of C1 esterase levels or function.^{2–3} Acquired C1 esterase inhibitor deficiency usually manifests in two forms: one associated with lymphoproliferative disease, which is typically low grade (low-grade lymphoma, chronic lymphocytic leukaemia or monoclonal gammopathy of uncertain significance), and less commonly autoimmune diseases, with autoantibodies directed against the C1 esterase inhibitor molecule.⁴ Monoclonal paraproteins have been shown to interfere with several automated assays of different methodologies, including nephelometry, turbidimetry and immunological assays via the formation of precipitates, non-specific antibody binding, hyperviscosity, cryoglobulinaemia and gel formation.^{5–7} The phenomenon does not depend on the antibody specificity of the paraprotein but on peculiarities of their physiochemical properties.⁸ Although both

Abbreviation: aCL, anti-cardiolipin

Table 1 Results of C4 and C1 esterase levels

	15.05.04 24 weeks prior to assessment	09.08.04 12 weeks prior to assessment	09.11.04 Results on assessment	01.02.05 8 weeks of treatment
C4*	0.22	0.32	0.47	0.49
C4†		<0.04	0.08	0.07
C1 esterase		<0.06‡	0.06§	0.1§

*C4 levels (normal range 0.18–0.60 g/l) were performed on an Array 360 Nephelometer (Beckman Coulter).

†C4 levels (normal range 0.18–0.60 g/l) were performed on a BN2 analyser (Dade Behring).

‡C1 esterase levels (normal range 0.18–0.54 g/l) were performed on a Mira turbidometer (Roche).

§C1 esterase levels (normal range 0.18–0.54 g/l) were performed by radial immunodiffusion (Binding Site, Birmingham, UK).

Take-home messages

- Monoclonal paraproteins may interfere with immunological assays.
- This interference may be non-specific and not related to the antibody specificity of the paraprotein.
- Monoclonal antibodies should be suspected if there are unusual or unexpected results for the clinical picture, particularly in patients with lymphoproliferative disease.

the Beckman Array and the BN2 analyser use a nephelometric method, the BN2 analyser seems to be unaffected by the paraprotein, possibly owing to differences in buffers or the anti-C4 antibodies used in these assays. Both analysers used a rabbit anti-human C4 antibody, making heterophilic activity an unlikely cause for the interference. A positive ds-DNA with negative ANA is unusual. Multiple autoantibodies have been described previously in splenic marginal zone lymphoma, independent of the paraprotein.⁹ The paraprotein does seem to interfere with the aCL assay. In a study of patients with monoclonal gammopathies, all sera of patients with IgA paraproteins were positive for IgA aCL activity.¹⁰ Although less common in IgM and IgG paraproteins, interferences on aCL ELISAs have also been reported.^{10–13} In these patients, the factor detected by ELISA should not be interpreted as a specific aCL antibody as it may represent non-specific phospholipid binding by the paraprotein. In patients with unusual results or those discordant with the clinical picture, especially in patients with known or suspected lymphoproliferative disease, interference from a paraprotein should be considered.

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REFERENCES

- 1 Yu A, Pira U. False increase in serum C-reactive protein caused by monoclonal IgM-lambda: a case report. *Clin Chem Lab Med* 2001;**39**:983–7.
- 2 Gompels MM, Lock RJ, Abinun M, et al. C1 inhibitor deficiency: consensus document. *Clin Exp Immunol* 2005;**139**:379–94.
- 3 Gompels MM, Lock RJ, Morgan JE, et al. A multicentre evaluation of the diagnostic efficiency of serological investigations for C1 inhibitor deficiency. *J Clin Pathol* 2002;**55**:145–7.
- 4 Markovic SN, Inwards DJ, Frigas EA, et al. Acquired C1 esterase inhibitor deficiency. *Ann Intern Med* 2000;**132**:144–50.
- 5 Berth M, Delanghe J. Protein precipitation as a possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2 case reports and a review of the literature. *Acta Clin Belg* 2004;**59**:263–73.
- 6 Sheppard CA, Allen RC, Austin GE, et al. Paraprotein interference in automated chemistry analyzers. *Clin Chem* 2005;**51**:1077–8.
- 7 Yamada K, Yagihashi A, Ishii S, et al. Interference with nephelometric assay of C-reactive protein and antistreptolysin-O by monoclonal IgM-κ from a myeloma patient. *Clin Chem* 1997;**43**:2435–7.
- 8 Lammers M. Interference with nephelometric assay of C-reactive protein by monoclonal immunoglobulin. *Clin Chem* 1998;**44**:1584–5.
- 9 Ziakas PD, Giannouli S, Tasidou A, et al. Multiple autoimmune phenomena in splenic marginal zone lymphoma. *Leuk Lymphoma* 2006;**47**:772–5.
- 10 Bizzaro N, Pasini P, Finco B. False-positive reactions for IgA anti-phospholipid and anti-beta(2)-glycoprotein I antibodies in patients with IgA monoclonal gammopathy. *Clin Chem* 1999;**45**:2007–10.
- 11 Hardiman KL, Horn S, Manoharan A, et al. Rheumatic autoantibodies in the sera of patients with paraproteins. *Clin Exp Rheumatol* 1994;**12**:363–8.
- 12 Hughes TP, Jones P, Rozenberg MC. Hypergammaglobulinemia and the anticardiolipin antibody test. *Arthritis Rheum* 1989;**32**:813.
- 13 Cowchock S, Fort J, Munoz S, et al. False positive ELISA tests for anticardiolipin antibodies in sera from patients with repeated abortion, rheumatologic disorders and primary biliary cirrhosis: correlation with elevated polyclonal IgM and implications for patients with repeated abortion. *Clin Exp Immunol* 1988;**73**:289–94.