

An Atypical Case of Idiopathic Nonhistaminergic Angioedema With Anti-C1-INH Antibodies

Bova M^{1,2*}, Suffritti C^{3*}, Joseph K⁴, Caccia S⁵, Gelderman KA⁶, Berra S⁵, Loffredo S^{1,3-7}, Santacroce R⁸, Petraroli A¹, Roem-Haagsma D⁹, Margaglione M⁸, Spadaro G¹, Kaplan AP¹⁰

¹Department of Translational Medical Sciences and Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy

²UOC Internal Medicine 2, AORN Cardarelli, Naples, Italy

³Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy

⁴Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, USA

⁵Department of Biomedical and Clinical Sciences "Luigi Sacco", Università degli Studi di Milano, Milan, Italy

⁶Sanquin Diagnostic Services, Amsterdam, The Netherlands

⁷CNR Institute of Experimental Endocrinology and Oncology "G. Salvatore", Naples, Italy

⁸Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

⁹Sanquin Research, Amsterdam, The Netherlands

¹⁰Department of Medicine, Division of Pulmonary, Critical Care, Allergy and Immunology, Medical University of South Carolina, Charleston, USA

*Equal contributions.

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Recurrent angioedema without wheals can be hereditary or acquired. While the most common form of hereditary angioedema (HAE) is caused by deficiency of C1 esterase inhibitor (C1-INH-HAE), HAE can also occur with normal plasma levels of C1-INH owing to mutations in gene coding for coagulation factor XII, angiopoietin 1, plasminogen, kininogen 1, myoferlin, and heparan sulfate-glucosamine 3-O-sulfotransferase 6. HAE with an unidentified genetic cause is defined as HAE of unknown origin [1].

Acquired angioedema (AAE) includes idiopathic histaminergic AAE (IH-AAE), idiopathic nonhistaminergic AAE (InH-AAE), AAE related to angiotensin-converting enzyme inhibitors (ACEI-AAE), and AAE with C1-inhibitor deficiency (C1-INH-AAE) [2].

C1-INH-AAE is a rare disease characterized by cutaneous swellings, edema of the gastrointestinal mucosa, and life-threatening laryngeal edema [3]. Symptoms first appear after the fourth decade of life in 90% of patients, and a family history of angioedema is absent.

Acquired C1-INH deficiency leads to activation of the complement and contact systems. Complement consumption via the classical complement pathway is associated with reduced C4 in almost all patients and low C1q in >70% of cases. Contact system activation leads to the production of bradykinin and onset of angioedema [4]. Anti-C1-INH antibodies are detected in >70% of cases [5].

It is sometimes difficult to distinguish between C1-INH-AAE and InH-AAE, a condition that comprises nonfamilial, nonhereditary forms in which known causes have been excluded and both C4 and C1-INH are normal [2].

We studied the previously reported case of an 86-year-old woman affected by recurrent angioedema with anti-C1-INH antibodies and normal C1-INH levels [6]. Informed consent for all interventions was obtained from the patient. Her symptoms first appeared at age 66 years, with recurrent episodes of peripheral edema without wheals (1 episode/month), abdominal attacks, and occasional episodes of upper airway edema. She was first treated with antihistamines, corticosteroids, and epinephrine without benefit. None of her relatives had ever experienced angioedema. Since 2011, she has been treating attacks with off-label subcutaneous icatibant (bradykinin B2 receptor antagonist) 30 mg on demand, with improvement after 20-30 minutes and resolution after 8-20 hours. No adverse events were reported.

Since her history was typical of bradykinin-mediated angioedema, we measured levels of C1-INH, C4, and C1q using standardized assays (par.1, Supplementary Material), all of which yielded normal results and remained within normal ranges over 10 years of follow-up. Nevertheless, we suspected C1-INH-AAE. Indeed, at disease onset, consumption of complement components is evident only during angioedema attacks in some patients. We investigated anti-C1-INH antibodies using plasma-derived C1-INH to coat an ELISA plate (Paragraph 2, Supplementary Material) and detected anti-C1-INH IgG (Supplementary Figure 1). In order to evaluate whether C1-INH polysaccharides could be a target for the autoantibodies, we used recombinant C1-INH, whose glycosylation pattern differs from that of plasma-derived C1-INH [7]. We found that the patient's IgG bound both plasma-derived C1-INH and recombinant C1-INH

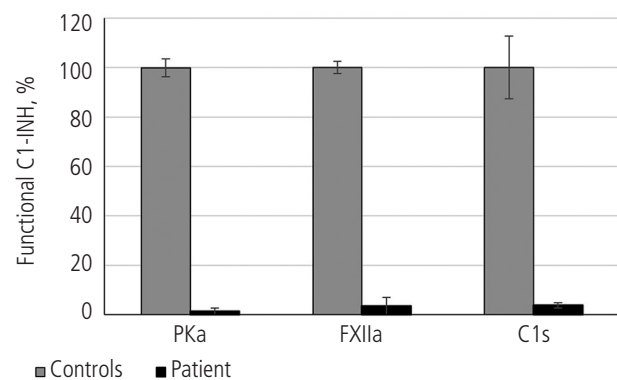


Figure. Functional ELISA based on inhibition of plasma kallikrein (PKa), factor XIIa (FXIIa), and complement C1s (C1s). Results are expressed as the mean (SD) percentage of the controls.

(Supplementary Figure 2). Anti-C1q antibody titers were negative. Total serum IgG levels were within normal limits. We ruled out underlying autoimmune and lymphoproliferative diseases and monoclonal gammopathy of undetermined significance.

In order to rule out a diagnosis of hereditary angioedema, a series of genes implicated in the onset of the disease were evaluated [8], as follows: *SERPING1* (NM_00006.2), *ANGPT1* (NM_001146.5), *PLG* (NM_000301.4), *MYOF* (NM_013451.4), *KNG1* (NM_001102416.3), and *F12* (NM_000505.3). No mutations were found.

Functional C1-INH in the patient's plasma was then evaluated using ELISA based on inhibition of complement C1s, kallikrein, or factor XIIa [9] (Paragraph 5, Supplementary Material). Our results showed that the patient's level of complexed C1-INH was negligible compared to that of healthy controls. Indeed, no inhibitory activity towards the proteases tested was detected (Figure).

We evaluated cleaved high-molecular-weight kininogen (cHK), an indirect measure of bradykinin, (Paragraph 6, Supplementary Material). The patient's plasma was characterized by a high cHK value: 72% vs 33% in 11 healthy individuals (Supplementary Figure 3) [10].

Total IgG from the patient's serum was purified (Paragraph 7, Supplementary Material), and neutralizing activity towards normal C1-INH was assessed using a chromogenic assay. Complexes between the patient's IgG and C1-INH were allowed to form before mixing with kallikrein or C1s. No effect was observed for C1-INH inhibition on kallikrein or C1s (Supplementary Figure 4). While the experimental conditions varied (ie, amount of IgG used or the preparation of complexes), we observed no differences in the kinetic reactions, even after using purified autoantibody isolated from the patient's IgG and passed over a column of purified C1-INH as the ligand (data not shown).

The neutralizing capacity of the patient's anti-C1-INH antibodies was also evaluated using a specifically designed ELISA (Paragraph 10, Supplementary Material). The plate was coated with purified C1-INH. Quality controls and test samples were added. Wells were incubated with either biotinylated C1s, kallikrein, or FXIIa. Streptavidin-HRP was then allowed to bind the biotinylated ligands (Supplementary Figure 5a-c). The index patient showed a neutralizing capacity comparable to quality control 2 for C1s and kallikrein (Supplementary Figure 5a,b), but not for FXIIa (Supplementary Figure 5c). Neutralizing capacity in the index patient was weaker than in the positive control plasma.

We tested the capacity of the autoantibodies to interfere with the formation of a stable covalent complex between C1-INH and its target proteases. In the first protocol we applied, proteases were added exogenously to the patient's plasma, and their complexes were tested with endogenous C1-INH: no complexes were found (Figure), probably owing to the presence of a nonfunctional C1-INH or a neutralizing antibody against C1-INH, reacting with either C1-INH or with the target proteases.

In the second protocol, the patient's plasma had a neutralizing effect on the formation of complexes with exogenous C1s and kallikrein, but not activated factor XII (Supplementary Figure 5a-c). This could be due to an excess

of endogenous activated proteases interacting with exogenous C1-INH and preventing exogenous proteases from forming complexes (in this case endogenous C1-INH has to be nonfunctional) or to release of neutralizing antibodies against C1-INH.

Given that the activity of endogenous C1-INH vs exogenous C1s appeared to be within the normal range in the chromogenic functional assay, the hypothesis of a nonfunctional C1-INH should be ruled out. Mutations in C1-INH were also ruled out.

A proposed hypothesis is that of a neutralizing antibody against C1-INH, whose action would make the complex comprising C1-INH and the protease less stable. In this way, the protease is initially inhibited, although the short half-life of the complex would finally enable the release of an active protease. The reaction conditions of the standard chromogenic assay did not enable us to appreciate this phenomenon, since longer preincubation times would be necessary.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Maria Bova

Department of Medicine

AORN A. Cardarelli

Via Cardarelli, 9

80131 Naples, Italy

E-mail address: bovamaria@virgilio.it