ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

New diagnostic approach to hereditary angioedema using filter paper

Nova proposta para diagnóstico do angioedema hereditário com papel filtro

Maine Luellah Demaret Bardou[1], Rosemeire Navickas Constantino-Silva[1], Maria Luiza Oliva-Alonso[2], Ana Júlia Ribeiro Teixeira[3], Pedro Giavina-Bianchi[3], Eli Mansour[4], Solange Oliveira Rodrigues Valle[2], Anete Sevciovic Grumach[1]

ABSTRACT

Introduction: Hereditary angioedema (HAE) is a rare disease characterized by edema in the subcutaneous tissue, gastrointestinal tract, and upper airways. C1 inhibitor deficiency is caused by a *SERPING1* mutation (HAE-C1-INH) that results in bradykinin accumulation and increased endothelial permeability. HAE may also present with normal C1-INH. Functional assessment of C1-INH allows the detection of both forms of HAE-C1-INH. This study aimed to evaluate patient screening using dried blood spots (DBS) on filter paper compared with the current technique using chromogenic assays. **Methods:** A multicenter prospective study of patients with HAE divided into: G1 – HAE-C1-INH (n = 53; types 1 = 48 and 2 = 5); G2 – HAE with FXII mutation (HAE-FXII) (n = 30); G3 – HAE with unknown mutation (HAE-UNK) (n = 10); and G4 – Control (n = 10). C4 and C1-INH levels were measured (radial immunodiffusion); functional C1-INH levels were assessed using chromogenic assay and DBS. Statistical analysis used GraphPad Prism. The protocol was approved by the Ethics Committee (CAAE:41812720010010082). **Results:** The median C4 and C1-INHq levels were, respectively, 9.14 and 8.9 mg/dL for G1; 33.2 and 34.2 mg/dL for G2; 35.3 and 29.4 mg/dL for G3; and 32.2 and 35.3 mg/dL for G4. The median fC1-INH levels obtained by chromogenic assay and by DBS were, respectively, 35% and 0% for G1; 120% and 81% for G2; 120% and 91% for G3; and

RESUMO

Introdução: O angioedema hereditário (AEH) é raro, caracterizado por edema em subcutâneo, trato gastrointestinal e vias aéreas superiores. A deficiência do inibidor de C1 decorre da mutação em *SERPING1* (AEH-C1-INH) que resulta em acúmulo de bradicinina e maior permeabilidade endotelial. O AEH também pode apresentar-se com C1-INH normal. A avaliação funcional do C1-INH permite detectar as duas formas de AEH-C1-INH. O objetivo do estudo foi avaliar a triagem de pacientes usando amostras de gota de sangue seca (DBS - Dried blood spot) em papel filtro comparada à técnica atual por ensaio cromogênico. **Métodos:** Estudo multicêntrico, prospectivo, em pacientes com AEH subdivididos em: G1 - AEH-C1-INH (n = 53; tipos 1 = 48 e 2 = 5); G2-AEH com mutação de FXII (AEH-FXII) (n = 30) e G3–AEH sem mutação conhecida (AEH-UNK) (n = 10) e G4 - Controle (n = 10). Realizadas dosagens de C4 e C1-INH (imunodifusão radial); C1-INH funcional (ensaio cromogênico e DBS). A avaliação estatística utilizou o programa GraphPad Prism. O protocolo foi aprovado pelo Comitê de Ética (CAAE:41812720010010082). **Resultados:** Valores de C4 e C1-INHq resultaram em mediana de 9,14 e 8,9 mg/dL para G1; G2 de 33,2 e 34,2 mg/dL; G3 de 35,3 e 29,4 mg/dL e G4 32,2 mg/dL e 35,3 mg/dL respectivamente. Valores de fC1-INH pelo ensaio cromogênico e por DBS resultaram em mediana de 35% e 0% em G1; G2 de 120% e 81%; G3 de 120% e 91%; e em G4

1. Centro Universitário FMABC, Imunologia Clínica - Santo André, SP, Brazil.
2. Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Serviço de Imunologia, Departamento de Clínica Médica - Rio de Janeiro, RJ, Brazil.
3. Universidade de São Paulo, Divisão de Imunologia Clínica e Alergia - São Paulo, SP, Brazil.
4. Universidade Estadual de Campinas, Imunologia Clínica e Alergia, Departamento de Clínica Médica - Campinas, SP, Brazil.

This study won the Antônio de Oliveira Lima Prize at the 2023 ASBAI Brazilian Congress of Allergy and Immunology, Maceió, Alagoas, Brazil.

Submitted May 30 2024, accepted Aug 04 2024.

Arq Asma Alerg Imunol. 2024;8(3):235-43.

http://dx.doi.org/10.5935/2526-5393.20240036-en

32.2% and 35.3% for G4. G1 had 42/53 samples (79.2%) and 53/53 (100%) with reduced fC1-INH, respectively. In G2, 30/30 (100%) and 28/30 (93.7%) had fC1-INH above 50%, respectively. In G3 and G4, fC1-INH was normal with both techniques. **Conclusion:** The fC1-INH measured in DBS samples allows a more precise identification of patients with HAE-C1-INH than using the chromogenic assay. DBS samples are simple to collect and easy to transport, facilitating the diagnosis of HAE-C1-INH.

Keywords: Hereditary angioedema, C1 inhibitor, factor XII, C1-INH, complement C4, biomarker, diagnosis.

de 32,2% e 35,3%, respectivamente. G1 com 42/53 amostras (79,2%) e 53/53 (100%) com fC1-INH reduzida respectivamente. Em G2 30/30 (100%) e 28/30 (93,7%) com fC1-INH acima de 50% respectivamente. Para G3 e para G4 a fC1-INH estava normal em ambas as técnicas. **Conclusão:** O fC1-INH pela técnica em DBS permite identificar os pacientes com AEH-C1-INH com maior precisão que o ensaio cromogênico. Trata-se de coleta simples, de fácil transporte, facilitando o diagnóstico de AEH-C1-INH.

Descritores: Angioedema hereditário, inibidor de C1, fator XII, C1-INH, Complemento C4, biomarcador, diagnóstico.

Introduction

Hereditary angioedema (HAE) is a term used to describe isolated and/or recurrent episodes of angioedema in subcutaneous and submucosal tissues. It often involves the face, extremities, gastrointestinal tract, genitalia, and upper airways, but is not associated with urticarial-type skin lesions. It often affects multiple family members due to its autosomal dominant inheritance, with 75% of patients having a family history and 25% a *de novo* mutation.[1] There are different types of HAE; the most common subtype is HAE with C1 inhibitor (C1-INH) deficiency (HAE-C1-INH), which is caused by more than 800 mutations affecting the *SERPING1* gene.[2,3]

Most mutations in *SERPING1* lead to a quantitative deficiency in the synthesis and secretion of C1-INH (HAE-C1-INH type 1), with diagnosis based on low plasma levels of C1-INH (C1-INHq). In approximately 15% of HAE-C1-INH cases, the mutation results in an abnormal protein, detected by functional C1-INH assay (fC1-INH), which is characterized as HAE-C1-INH type 2.[4] In these patients, quantitative C1-INH levels are normal or high, while C4 levels and functional C1-INH activity are low.[1,4,5]

C1-INH is a protease inhibitor that inhibits the complement, fibrinolytic, coagulation, and kinin-kallikrein pathways; its deficiency results in uncontrolled release of bradykinin. Bradykinin binds to the B2 receptor on endothelial cells, leading to vasodilation and a release of nitric oxide.[6,7] However, some patients have normal C1-INH in both value and function (HAE-nC1-INH), which has to date been associated with 8 different mutations. The identified gene mutations affect coagulation factor XII gene (HAE-FXII)[8], plasminogen[9], angiopoietin-1[10], kininogen 1[11], myoferlin[12], heparan sulfate-glucosamine 3-O-sulfotransferase 6[13], carboxypeptidase N subunit 1[14] and disabled homolog 2-interacting protein[15,16]. Despite the variety of diagnostic tools, the difficulty lies in accessing the tests and appropriately collecting and handling the samples, given that complement system proteins are thermolabile, i.e., they must be collected quickly and aliquoted at -80 °C for subsequent laboratory assessment.[1,5,17,18] Moreover, few clinical laboratories in Brazil can determine quantitative and functional C1-INH levels.[19,20] It is costly to ship samples to distant locations, since they must be packaged in dry ice and delivered quickly. Providing a way to send samples without compromising the functional evaluation of C1-INH would be essential to improving access to HAE-C1-INH diagnosis. In light of the above, the present study aimed to compare functional C1-INH (fC1-INH) levels using filter paper samples (dried blood spot - DBS) to chromogenic assay for HAE-C1-INH diagnosis.

Methods

This multicenter, prospective study included patients > 1 year of age with a confirmed diagnosis of HAE based on clinical presentation, positive family history, and confirmatory biochemical tests for patients with C1-INH deficiency and/or genetic tests for HAE with normal C1 inhibitor. After initial screening, 93 patients were divided into 4 groups: (G1) HAE-C1-INH

(type 1 [n = 48] and type 2 [n = 5]); (G2) HAE-FXII (n = 30); (G3) HAE with normal C1 inhibitor with an unknown mutation (HAE-UNK) (n = 10), because exome sequencing revealed no mutations currently described in the literature; and finally (G4), controls (n = 10). Patients unable to provide informed consent or who were diagnosed with acquired angioedema and/or diseases that could result in complement system dysfunction, such as nephropathies and hepatopathies, were excluded.

The samples were separated by centrifuging freshly collected blood at 3000 rpm for 10 minutes at 4 °C. They were immediately aliquoted and stored at -80 °C to determine plasma levels of C1-INH (C1-INHq), C4, and functional C1-INH (fC1-INH) by the chromogenic method. C4 and C1-INHq were measured by radial immunodiffusion, while fC1-INH was assessed by chromogenic assay and DBS. A commercial Technochrom C1-INH kit was used to determine the functional activity of C1-INH by the chromogenic method. In this technique, synthetic C1 esterase (C1s) substrate is incorporated in the plasma sample to measure the inhibitory activity of C1-INH protein. Through the chromogenic method, fC1-INH is measured by the reaction between C1s and its artificial substrate, Z-Lys-SBzl•HCl, to produce Cbz-Lys and thiomethyl-benzene. Thiomethyl-benzene is measured by chromogenic assay, after derivatization with 5,5'-dithiobis-(2-nitrobenzoic acid). Less intense color indicates greater C1 inhibitor activity due to lower production of thiomethyl-benzene, which is a product of the reaction of C1s and the substrate. Hence, more intense color suggests reduced C1 inhibitor function.

Blood was also collected in EDTA Vacutainer tubes. After collection, the tubes were inverted to resuspend the blood cells, and 60 µL aliquots were placed on the filter paper, which was dried for ≥ 3 hours at room temperature, stored at -20 °C for up to 180 days, and sent to PerkinElmer Genetics for fC1-INH testing via DBS.

Standardized material provided by PerkinElmer Genetics was used for the DBS sampling procedure, in which liquid chromatography/mass spectrometry was used to measure fC1-INH. In this assay, C1-INH is first extracted from the DBS cards and bound to the excess C1s. After that, a reaction is performed with the C1s that was not bound with its substrate in the previous steps. This produces Cbz-Lys and thiomethyl-benzene. The Cbz-Lys product is analyzed directly by liquid chromatography/mass spectrometry. Thus, this method measures the inhibitory activity of C1s through the C1-INH present in the samples.[21]

We considered normal C4 values to be 16.7-38.5 mg/dL for women and 16.2-44.5 mg/dL for men. Normal C1-INHq values were 19.5-34.5 mg/dL. Normal fC1-INH values were > 69% according to the Technochrom kit and > 62.8% for DBS, according to PerkinElmer Genetics. However, in the literature, values > 50% are used for diagnosis.[3,17,22]

The study was approved by the Centro Universitário FMABC Research Ethics Committee (decision 41812720010010082). Patients and volunteers provided written informed consent prior to inclusion.

Results

All groups were predominantly female. In G1, the mean age was 40 (SD, 17.7) years for HAE-C1-INH type 1, and 27 (SD, 13.4) years for HAE-C1-INH type 2. In G2, the mean age was 38 (SD, 18.6) years, while in G3 it was 40 (SD, 14.4) years. The majority of patients in all groups were symptomatic at diagnosis, with the mean age at symptom onset ranging from 10 to 26 years (Table 1).

The median serum C4 and C1-INHq levels were 9.14 and 8.9 mg/dL, respectively, in G1; 33.2 and 34.2 mg/dL, respectively, in G2; and 35.3 and 29.4 mg/dL, respectively, in G3 (Table 2). Two patients with HAE-C1-INH type 1 had above normal C4 values (a 4-year-old child and a 27-year-old adult). All patients with HAE-C1-INH type 2 had low C4 values. All values in G2, G3, and G4 were normal (Figure 1).

C1-INHq values were low in 100% of the HAE-C1-INH type 1 patients and high in the 5 patients with HAE-C1-INH type 2, as well as in the G2, G3, and G4 groups (Figure 2).

For fC1-INH by chromogenic assay, the median values were 35% for G1 and 120% for G2 and G3. For fC1-INH by DBS, the median values were 0% for G1 81% for G2, and 91% for G3 (Table 2).

For chromogenic fC1-INH, the G1 values were < 50% in 42 of the 53 patients (79.2%), with 10 HAE-C1-INH type 1 patients and 4 HAE-C1-INH type 2 patients having values > 50%. fC1-INH was > 50% in G2, G3, and G4, (Table 2 and Figure 3). For DBS fC1-INH, the G1 values were < 50% in 100% of the patients. In G2, 2 pregnant women had low values and 28 patients had normal values. In G3 and G4, all values were > 50% (Table 2 and Figure 4).

Table 1
Distribution and characteristics of patients with HAE according to group

	G1		G2	G3	G4
	HAE-C1-INH type 1	HAE-C1-INH type 2	HAE-FXII	HAE-UNK	Control
Distribution	48	5	30	10	10
Women n (%)	35 (72.9%)	4 (80 %)	20 (66.6%)	10 (100%)	4 (40%)
Mean age (years) (SD)	40 (±17.6)	28 (±13.4)	38 (±18.6)	40 (±14.1)	23 (±25.8)
Mean age at symptom onset (years) (SD)	15 (±11.2)	10 (±4.5)	20 (±10.2)	26 (±10.2)	–
Symptomatic	44 (91.6%)	5 (100%)	23 (76.6%)	10 (100%)	–
Asymptomatic	4 (8.4%)	0	7 (23.4%)	0	10 (100%)
Total	53		30	10	10

HAE = hereditary angioedema, FXII = coagulation factor XII mutation, UNK = unknown mutation, C1-INH = C1 esterase inhibitor.

Table 2
Distribution of biochemical values according to group among patients with HAE

	G1		G2	G3	G4
	HAE-C1-INH type 1	HAE-C1-INH type 2	HAE-FXII	HAE-UNK	Control
Median C4 (mg/dL)	9.14		33.2	35.3	32.20
Median C1-INHq (mg/dL)	8.9		34.2	32.25	35.30
Chromogenic fC1-INH n (%)					
≤ 50%	38 (79.1%)	4 (71.5%)	–	–	
≥ 50 %	10 (20.9%)	1 (28.5%)	30 (100%)	–	10 (100%)
Median chromogenic fC1-INH (%)	35		120	120	119.80
DBS fC1-INH n (%)					
≤ 50%	48 (100%)	5 (100%)	2[a] (6.6%)	–	–
≥ 50 %	–	–	28 (93.4%)	10 (100%)	10 (100%)
Median DBS fC1-INH (%)	0		81.8	109.63	101.20
Total patients	48	5	30	10	10

[a] Pregnant.
HAE = hereditary angioedema, FXII = coagulation factor XII mutation, UNK = unknown mutation, C1-INHq = amount of C1 esterase inhibitor, fC1-INH = function of C1 esterase inhibitor.

Discussion

When not diagnosed or properly treated, HAE is potentially fatal, with a mortality rate of 25% to 40% due to glottis edema and asphyxia. (23, 24) An HAE mortality study found a mortality rate of around 17.5%, with only 13% being diagnosed before death.[25] This reflects a myriad of factors, such as the ability to recognize the disease, a lack of specialists, and difficulty accessing treatment and diagnostic tests.[20,26] For example, in Brazil's Unified Health System, only two laboratories are capable of performing all the diagnostic tests[27], i.e., C4, C1-INHq, and fC1-INH measurement. When evaluating these components, the blood samples must be processed as quickly as possible in serum and/or EDTA plasma. If the analysis is not performed immediately, the samples must be frozen at -80 °C until they are analyzed or must be shipped in dry ice to specialized laboratories. Another point is that repeated freezing and thawing can lead to in vitro activation of the complement.[18]

Currently, to diagnose patients with suspected HAE, the investigation can begin by requesting C4 screening, which several laboratories provide. However, this strategy has low specificity, since C4 can still be normal in 27% of those with HAE-C1-INH.[28,29] Another relevant test is C1-INHq, which will only be reduced in patients with HAE-C1-INH type 1. Thus, measuring fC1-INH is important for diagnostic purposes due to its potential to diagnose both HAE-C1-INH type 1 and 2.

Given this difficult scenario, in which samples must be shipped to distant locations, it became evident that the collection method and climatic conditions can affect HAE diagnosis. Eleven patients from G1 (HAE-C1-INH) in our study had normal fC1-INH values in the chromogenic assay. With DBS (filter paper), all 53 patients in G1 had C1-INHf values < 50% (HAE-C1-INH), despite sending the samples to another country long after collection. The samples were kept at -20 °C (common freezer) and processed within 180 days (6 months), which did not compromise the results.

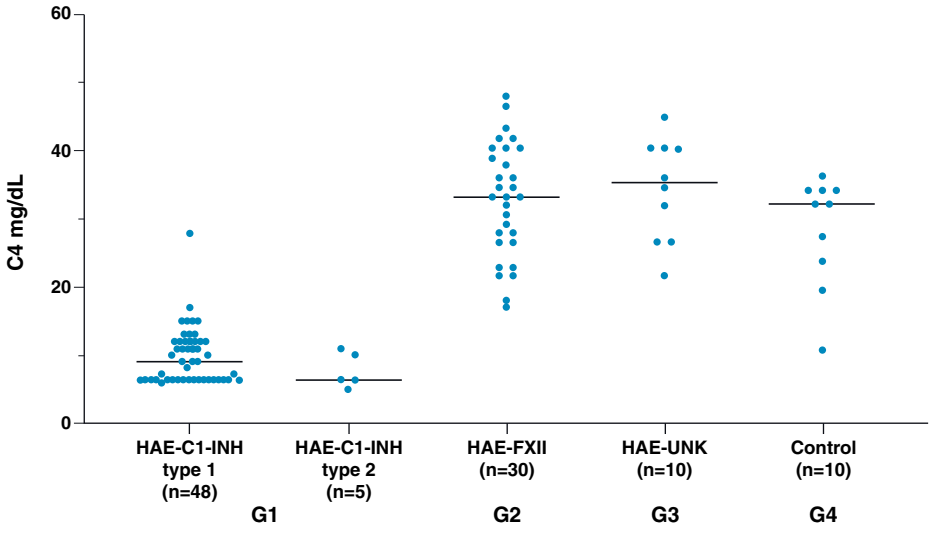

Figure 1

C4 values according to group among patients with HAE

HAE-1 = hereditary angioedema type 1, HAE-2 = hereditary angioedema type 2, HAE-FXII = hereditary angioedema with coagulation factor XII mutation, UNK = hereditary angioedema with unknown mutation.

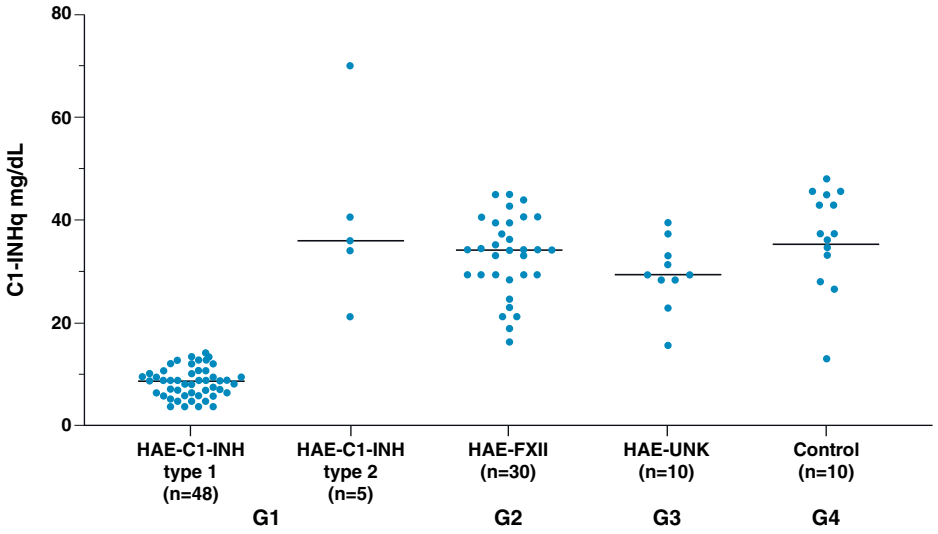

Figure 2

C1-INH values according to group among patients with hypertension

HAE-C1-INH type 1 = hereditary angioedema type I, HAE-C1-INH type 2 = hereditary angioedema type 2, HAE-FXII = hereditary angioedema with coagulation factor XII mutation, UNK = hereditary angioedema with unknown mutation, fC1-INH = amount of C1 esterase inhibitor.

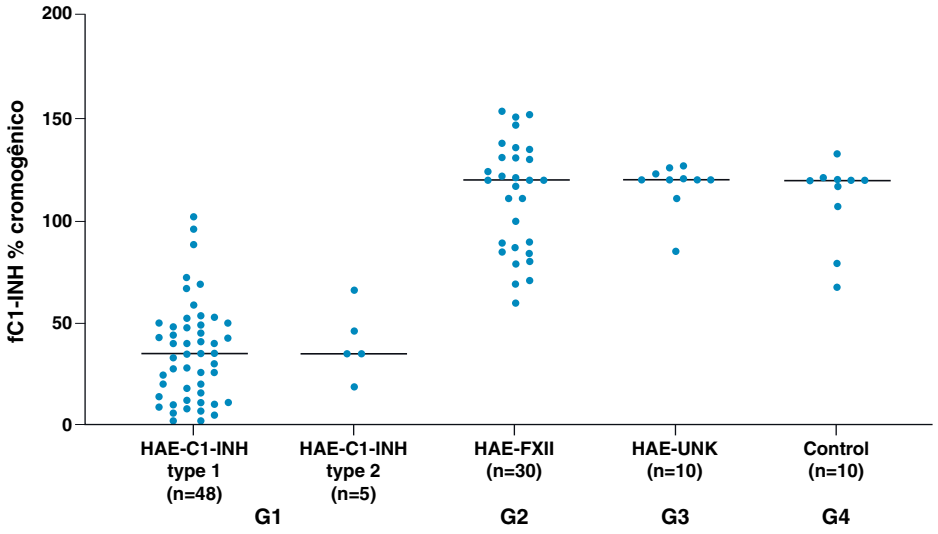

Figure 3

C1-INH function values in patients with HAE according to chromogenic assay

HAE-C1-INH type 1 = hereditary angioedema type I, HAE-C1-INH type 2 = hereditary angioedema type 2, HAE-FXII = hereditary angioedema with coagulation factor XII mutation, UNK = hereditary angioedema with unknown mutation, fC1-INH = C1 esterase inhibitor function.

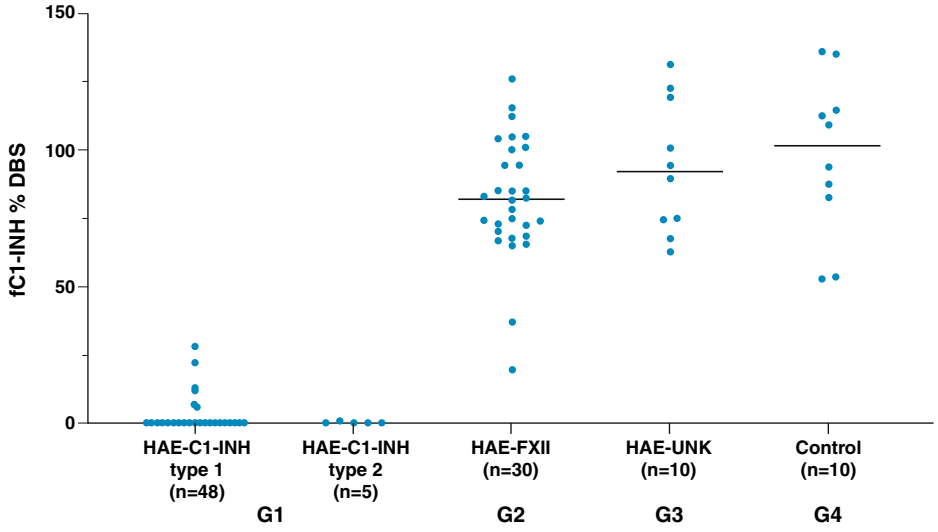

Figure 4

C1-INH function values in patients with HAE according to DBS assay

HAE-C1-INH type 1 = hereditary angioedema type I, HAE-C1-INH type 2 = hereditary angioedema type 2, HAE-FXII = hereditary angioedema with coagulation factor XII mutation, UNK = hereditary angioedema with unknown mutation, fC1-INH = C1 esterase inhibitor function.

From a methodological point of view, the chromogenic assay is based on 2 reaction steps, while the liquid chromatography/mass spectrometry assay measures the direct product of the C1s reaction, i.e., the assay variation may be smaller.[21]

Two patients with HAE-C1-INH type 1 had above-normal C4 values, i.e., 4% of patients with HAE-C1-INH type 1 could remain undiagnosed if only this screening method were used. It is worth noting that after DBS sample standardization, fC1-INH in whole blood remained stable for 7 days at 4 °C, for up to 3 days at 45 °C, and for 134 days when stored at room temperature[21], which could facilitate the testing process by reducing shipping costs.

It is also worth mentioning that the DBS technique was applied to patients with normal C1 inhibitor levels, since its validation study only involved patients with C1-INH deficit. Interestingly, in the DBS results for the group with HAE-nC1-INH and FXII mutation, pregnant women had reduced (< 50%) fC1-INH.

Conclusions

The study demonstrated that, in comparison with the chromogenic test, assessing fC1-INH with the DBS technique is a reliable method of HAE-C1-INH diagnosis. It has superior reproducibility and greater diagnostic accuracy due to its simple, easy-to-transport, and thermostable samples.

Acknowledgements

The authors would like to thank Takeda for the financial and infrastructure support, the participating centers, and the FMABC team.

References

1. Cicardi M, Aberer W, Banerji A, Bas M, Bernstein JA, Bork K, et al. Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. Allergy. 2014;69(5):602-16.
2. Busse PJ, Christiansen SC. Hereditary Angioedema. N Engl J Med. 2020;382(12):1136-48.
3. Maurer M, Magerl M, Betschel S, Aberer W, Ansotegui IJ, Aygören-Pürsün E, et al. The international WAO/EAACI guideline for the management of hereditary angioedema-The 2021 revision and update. Allergy. 2022 Jul;77(7):1961-90.
4. Longhurst HJ, Bork K. Hereditary angioedema: an update on causes, manifestations and treatment. Br J Hosp Med (Lond). 2019;80(7):391-8.
5. Wagenaar-Bos IG, Drouet C, Aygören-Pursun E, Bork K, Bucher C, Bygum A, et al. Functional C1-inhibitor diagnostics in hereditary angioedema: assay evaluation and recommendations. J Immunol Methods. 2008;338(1-2):14-20.
6. López Lera A. Pathophysiology and underlying mechanisms in hereditary angioedema. Balkan Med J. 2021;38(2):82-8.
7. Sainz IM, Pixley RA, Colman RW. Fifty years of research on the plasma kallikrein-kinin system: from protein structure and function to cell biology and in-vivo pathophysiology. Thromb Haemost. 2007;98(1):77-83.
8. Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem Biophys Res Commun. 2006;343(4):1286-9.
9. Bork K, Wulff K, Steinmüller-Magin L, Braenne I, Staubach-Renz P, Witzke G, et al. Hereditary angioedema with a mutation in the plasminogen gene. Allergy. 2018;73(2):442-50.
10. Bafunno V, Firinu D, D'Apolito M, Cordisco G, Loffredo S, Leccese A, et al. Mutation of the angiopoietin-1 gene (ANGPT1) associates with a new type of hereditary angioedema. J Allergy Clin Immunol. 2018;141(3):1009-17.
11. Bork K, Wulff K, Rossmann H, Steinmüller-Magin L, Braenne I, Witzke G, et al. Hereditary angioedema cosegregating with a novel kininogen 1 gene mutation changing the N-terminal cleavage site of bradykinin. Allergy. 2019;74(12):2479-81.
12. Ariano A, D'Apolito M, Bova M, Bellanti F, Loffredo S, D'Andrea G, et al. A myoferlin gain-of-function variant associates with a new type of hereditary angioedema. Allergy. 2020;75(11):2989-92.
13. Bork K, Wulff K, Möhl BS, Steinmüller-Magin L, Witzke G, Hardt J, et al. Novel hereditary angioedema linked with a heparan sulfate 3-O-sulfotransferase 6 gene mutation. J Allergy Clin Immunol. 2021;148(4):1041-8.
14. Vincent D, Parsopoulou F, Martin L, Gaboriaud C, Demongeot J, Loules G, et al. Hereditary angioedema with normal C1 inhibitor associated with carboxypeptidase N deficiency. J Allergy Clin Immunol Glob. 2024;3(2):100223.
15. D'Apolito M, Santacroce R, Vazquez DO, Cordisco G, Fantini CA, D'Andrea G, et al. DAB2IP associates with hereditary angioedema: Insights into the role of VEGF signaling in HAE pathophysiology. J Allergy Clin Immunol. 2024 Sep;154(3):698-706.
16. Germenis AE, Rijavec M, Veronez CL. Leveraging Genetics for Hereditary Angioedema: A Road Map to Precision Medicine. Clin Rev Allergy Immunol. 2021;60(3):416-28.
17. Germenis AE, Cicardi M. Driving towards Precision Medicine for angioedema without wheals. J Autoimmun. 2019;104:102312.
18. Grumach AS, Kirschfink M. Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach. Mol Immunol. 2014;61(2):110-7.
19. Veronez CL, Mendes AR, Leite CS, Gomes CP, Grumach AS, Pesquero JB, et al. The Panorama of Primary Angioedema in the Brazilian Population. J Allergy Clin Immunol Pract. 2021;9(6):2293-304.e5.
20. Serpa FS, Cruz AAS, Condino Neto A, Silva ECF, Franco JM, Mello JML, et al. O atendimento médico de pacientes com doenças imunoalérgicas no Brasil: reflexões e propostas para a melhoria - Carta de Belo Horizonte. Arq Asma Alerg Imunol. 2017;1(4):327-34.
21. Lai Y, Zhang G, Zhou Z, Inhaber N, Bernstein JA, Chockalingam PS, et al. A novel functional C1 inhibitor activity assay in dried blood spot for diagnosis of Hereditary angioedema. Clin Chim Acta. 2020;504:155-62.
22. Betschel S, Badiou J, Binkley K, Borici-Mazi R, Hébert J, Kanani A, et al. The International/Canadian Hereditary Angioedema Guideline. Allergy Asthma Clin Immunol. 2019;15:72.
23. Bork K, Siedlecki K, Bosch S, Schopf RE, Kreuz W. Asphyxiation by laryngeal edema in patients with hereditary angioedema. Mayo Clin Proc. 2000;75(4):349-54.
24. Giavina-Bianchi P, Arruda LK, Aun MV, Campos RA, Chong-Neto HJ, Constantino-Silva RN, et al. Brazilian Guidelines for Hereditary Angioedema Management - 2017 Update Part 1: Definition, Classification and Diagnosis. Clinics (Sao Paulo). 2018;73:e310.
25. Minafra FG, Gonçalves TR, Alves TM, Pinto JA. The Mortality from Hereditary Angioedema Worldwide: a Review of the Real-World Data Literature. Clin Rev Allergy Immunol. 2022;62(1):232-9.
26. Serpa FS, Urrutia-Pereira M, Costa E, DiGesu RW, Guidacci MFRC, Cruz AS, et al. A especialidade de Alergia e Imunologia Clínica nos diferentes níveis de atenção à saúde no Brasil. Arq Asma Alerg Imunol. 2018;2(3):335-43.
27. Grupo de Estudos Brasileiro em Angioedema Hereditário, GEBRAH [Internet]. Available from: https://gebraeh.com.br/diagnostico-laboratorial/. Accessed may/2024.
28. Tarzi MD, Hickey A, Förster T, Mohammadi M, Longhurst HJ. An evaluation of tests used for the diagnosis and monitoring of C1 inhibitor deficiency: normal serum C4 does not exclude hereditary angio-oedema. Clin Exp Immunol. 2007;149(3):513-6.
29. Charest-Morin X, Betschel S, Borici-Mazi R, Kanani A, Lacuesta G, Rivard G-É, et al. The diagnosis of hereditary angioedema with C1 inhibitor deficiency: a survey of Canadian physicians and laboratories. Allergy, Asthma & Clinical Immunology. 2018;14(1):83.

Financing: Maine L. D. Bardou received a LASID researcher initiative grant in 2022-2023 (Takeda Scholarships). Anete S. Grumach is a CNPq productivity fellow (309824/2021-4) and holds a Shire/Takeda researcher initiative grant.

Conflict of interests: Maine L. D. Bardou and Ana Júlia Ribeiro Teixeira declare no conflicts of interest. Anete S. Grumach is a speaker and/or consultant for Takeda, CSL Behring, Pharvaris, Kalvista, Exeltis, Pint-Pharma, Biomarin, Binding site, and

Multicare. She holds a CNPq productivity grant and a grant from Shire/Taked to support investigator-initiated research.
Maria Luisa O. Alonso, Pedro Giavina-Bianchi, Solange O. R. Valle and Eli Mansour have received financial support and/or honoraria from Takeda and CSL Behring.

Corresponding author:
Maine Luellah Demaret Bardou
E-mail: maine_bardou@hotmail.com