Brief Report

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Cut-off value of C1-inhibitor function for the diagnosis of hereditary angioedema due to C1-inhibitor deficiency

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SUMMARY Hereditary angioedema caused by C1-inhibitor (C1-INH) deficiency (HAE-C1-INH) is a rare autosomal dominant disease. Primary care physicians sometimes face difficulties in diagnosing HAE-C1-INH owing to fluctuations in C1-INH function levels influenced by blood sampling conditions. International major guidelines do not stipulate a cut-off value of C1-INH function for the diagnosis. We aimed to explore the distribution of C1-INH function levels in patients with HAE-C1-INH and elucidate the influence of blood sampling conditions using healthy volunteers' samples to confirm the cut-off value of C1-INH function. In 48 patients with HAE-C1-INH who visited the Juntendo University Hospital in Japan between 2013 and 2019, C1-INH function levels were evaluated for 160 samples during symptom-free periods and 147 samples during an acute attack. Fluctuations of C1-INH function level were also evaluated for 8 healthy volunteers, wherein the samples were divided into 3 groups according to different sampling conditions. C1-INH function levels in all patients with HAE-C1-INH were found to be < 50%. The average C1-INH function level in healthy volunteers measured soon after blood collection in an appropriate sampling condition was 77% (61-92%) with some having lower C1-INH function levels than the reference value. C1-INH function levels fluctuated unstably in inappropriate sampling conditions. In conclusion, we can confirm that a < 50% C1-INH function level can be used as the diagnostic cut-off value for HAE-C1-INH. Moreover, it is necessary to repeat measurements of C1-INH function level in appropriate blood sampling conditions to accurately diagnose HAE-C1-INH.

Keywords cut-off value, C1-inhibitor, guideline, hereditary angioedema, Japan

1. Introduction

Hereditary angioedema caused by C1-inhibitor (C1-INH) deficiency (HAE-C1-INH) is an autosomal dominant disease that produces excess bradykinin, inducing unpredictable and recurrent acute subcutaneous or submucosal angioedema (*1-4*). Although HAE-C1-INH is a rare disease, estimated to affect around 1 in 50,000 individuals with no reported bias among different ethnic groups, only approximately 400-500 patients, much less than the estimated prevalence, have been diagnosed in Japan. The responsible gene *SERPING1* has been detected, although the severity and frequency of the disease vary even in the same family members (*5*). HAE-C1-INH can be life-threatening when severe edema develops in the upper respiratory tracts, and patients might undergo unnecessary abdominal surgical procedures for severe abdominal pain resulting from gastrointestinal edema without appropriate treatment for HAE-C1-INH (*6-8*). Additionally, it has been reported that it takes an average of 13.8 years from the onset of the initial symptoms to be diagnosed with HAE-C1-INH due to the low awareness of the disease in Japan (*8*). To improve these conditions, an early diagnosis of HAE-C1-INH with clear criteria is important.

Concerning the diagnosis of HAE-C1-INH, the guideline of the World Allergy Organization/the European Academy of Allergy and Clinical Immunology (WAO/EAACI) recommends that all patients suspected to have HAE-C1-INH should be assessed for blood levels of C1-INH function, C1-INH protein, and C4, and the tests should be repeated to confirm the diagnosis of HAE-C1-INH, if any of the levels are abnormally low (*9*). The C1-INH protein level test is not covered by health insurance in Japan, and the measurement is not necessarily performed, because the C1-INH function

level test is sufficient for the diagnosis of HAE-C1-INH. Moreover, the guideline of the Japanese Association for Complement Research revised in 2014, recommends that low levels of C1-INH function and C4 are effective indicators, but the number of blood analyses of these markers needed for the diagnosis of HAE-C1-INH is not suggested (*10*). Both guidelines did not refer to the cut-off value of C1-INH function for diagnosis of HAE-C1-INH. Therefore, primary care physicians sometimes face difficulties in the diagnosis of HAE-C1-INH owing to confusingly low C1-INH function levels (*11,12*).

In the present study, we aimed to investigate the distribution of C1-INH function levels in patients with HAE-C1-INH to confirm the cut-off value of C1-INH function for an early diagnosis of HAE-C1-INH. Furthermore, because we sometimes encounter some patients with low C1-INH function levels, we also aimed to explore the influence of blood sampling conditions before measuring the C1-INH function level and fluctuations in the C1-INH function levels using healthy volunteers' samples.

2. Materials and Methods

2.1. Patients and blood samples

The study enrolled 48 patients (16 males, 32 females) with a confirmed diagnosis of HAE-C1-INH at the Juntendo University Hospital in Tokyo, Japan. The mean age of the patients was 41.1 years (range, 20-71 years) during their inclusion in this study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Juntendo University (No. 25-325). Written informed consent was obtained from the patients. We collected serum samples from the patients, as well as data on C1-INH function levels from 160 samples during symptom-free periods and 147 samples during an acute attack (total: 307 samples) between 2013 and 2019. Serum samples were appropriately measured soon after blood collection.

We planned to mimic the actual situations of blood sampling in the primary clinics. Serum samples were also obtained from 8 healthy volunteers (volunteers A-D: male, volunteers E-H: female) with no clinical symptoms of angioedema or other basic diseases. The samples were divided into the following 3 groups to measure C1-INH function levels: *i*) centrifuge the samples immediately after blood collection before the measurement, which should be ideally performed in clinical situations, *ii*) allow the samples to stand at room temperature for 6-24 h after the blood collection until centrifugation and the measurement, and *iii*) centrifuge the samples immediately after the blood collection and allow it to stand at room temperature for 6-24 h before the measurement. The *ii*) and *iii*) conditions were set as experimental controls.

2.2. Laboratory data

All serum samples were evaluated for C1-INH function levels by chromogenic assay in a commercial company (Special Reference Laboratories: SRL, Tokyo, Japan). The reagent for the C1-INH function level measurement was produced by a commercial company (Berichrom C1-inhibitor, Siemens, Munich, Germany). Thus, the testing procedures and methods were unified with their automated analysis methods. The reference value of C1-INH function was 70-130% set by the manufacturer; SRL (*13*).

3. Results and Discussion

All enrolled patients with HAE-C1-INH presented < 50% of C1-INH function levels (Table 1). Particularly, C1-INH function levels were ≤ 25% in 114 samples (71.3%) during symptom-free periods and 136 samples (92.5%) during an acute attack, respectively. Moreover, the enrolled patients had not received plasma-derived human C1-INH concentrate within 3 days before each blood collection, whose half-life period is reported to be 64 h (*14*).

The average C1-INH function level in 8 healthy volunteers immediately after the blood collection in an appropriate condition was 77.0% (61-92%).

For the samples left to stand at room temperature for 6-24 h after the blood collection and until the centrifugation and measurement, the C1-INH function levels were continuously decreased in 3 volunteers (B, D, G), continuously increased in 2 volunteers (C, F), and inconsistently changed in the others (Figure 1). The maximum rate of fluctuation in C1-INH function level was 22.8% in volunteer G.

For the samples immediately centrifuged after the blood collection and left to stand at room temperature for 6-24 h until the measurement, the C1-INH function levels continuously decreased in 2 volunteers (D, G), continuously increased in a volunteer (C), and inconsistently changed in the others (Figure 2). The maximum rate of fluctuation in the C1-INH function level was 31.7% in volunteer B.

Theoretically, C1-INH production declines by 50% in patients with HAE-C1-INH, because this is an autosomal dominant disease with the *SERPING1* gene mutation. However, C1-INH is continuously consumed by the kallikrein-kinin system, complement system, fibrinolysis system, contact system, and coagulation system. Indeed, the C1-INH function levels in all 307 of the HAE-C1-INH samples did not reach 50% (Table 1), although the C1-INH function levels fluctuated in many patients. We can therefore conclude that HAE-C1-INH is mostly ruled out, if the C1-INH function level is ≥ 50%, and that the cut-off value of C1-INH function for diagnosing HAE-C1-INH is considered likely to be 50%.

Table 1. The distribution of C1-INH function levels in patients with HAE-C1-INH

Pt no.	Number of patients presenting C1-INH function level									
	During symptom-free periods					During an acute attack				
	C1-INH function level (%)					C1-INH function level (%)				
	≤ 25	26-29	30-39	40-49	≥ 50	≤ 25	26-29	30-39	40-49	≥ 50
1	6	1	1			4				
2	6	2								
3	3		1			7				
4	3	2				6	1			
5	3					22			1	
6	5	3				2				
7	7					21	2			
8	5	1				5				
9	2					35				
10	1	1	1							
11	1									
12	1	3	2							
13			1							
14	7									
15	1									
16	1									
17				1						
18		1								
19			1							
20	2	1					1			
21	3									
22	5	1				5			1	
23	5					2				
24		1		4				1	1	
25										
26	5	1				1				
27	4					1		1		
28	3	1				10	1		1	
29			1							
30	4	1								
31	7									
32	1	4	1							
33	4									
34	2					9				
35	1	3	1							
36	1									
37	1									
38	2									
39	1		1			5				
40	1									
41	1									
42	3									
43	4									
44	1					1				
45		1								
46	1									
47		1								
48		1								
	114	30	11	5	0	136	5	2	4	0
Total	160					147				
	307									

All enrolled patients with HAE-C1-INH presented < 50% of C1-INH function levels. C1-INH function levels were ≤ 25% in 114 samples (71.3%) during symptom-free periods and 136 samples (92.5%) during an acute attack, respectively. Moreover, the enrolled patients had not received plasma-derived human C1-INH concentrate within 3 days before each blood collection, whose half-life period is reported to be 64 h. C1-INH, C1-inhibitor; HAE, Hereditary angioedema; Pt no., Patient number.

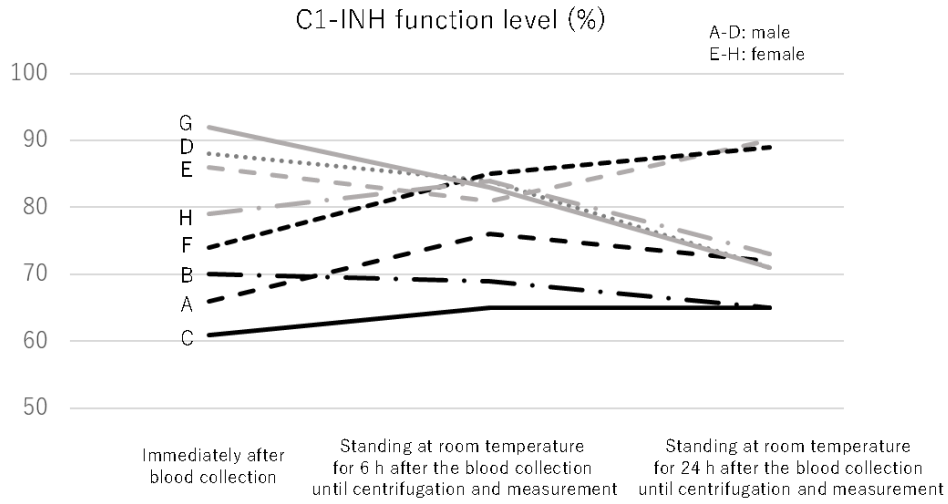

Figure 1. C1-inhibitor (C1-INH) function levels of the samples from 8 healthy volunteers standing at room temperature for 6-24 h after blood collection until centrifugation and measurement. The figure shows the fluctuations of C1-INH function levels in 8 healthy volunteers. The samples were left to stand at room temperature for 6-24 h after blood collection until centrifugation and measurement. C1-INH function levels were continuously decreased in volunteers B, D, and G, continuously increased in volunteers C and F, and inconsistently changed in the others. The maximum rate of fluctuation in C1-INH function level was 22.8% in volunteer G.

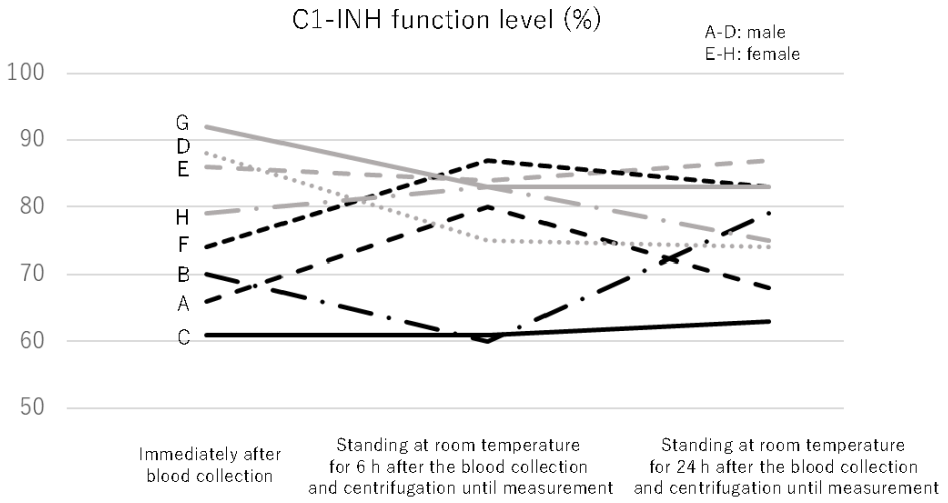

Figure 2. C1-inhibitor (C1-INH) function levels of samples from 8 healthy volunteers standing at room temperature for 6-24 h after blood collection and centrifugation until measurement. The figure shows fluctuations of C1-INH function levels in 8 healthy volunteers. The samples were centrifuged immediately after blood collection and left to stand at room temperature for 6-24 h before measurement. C1-INH function levels were continuously decreased in volunteers D and G, continuously increased in volunteer C, and inconsistently changed in the others. The maximum rate of fluctuation in C1-INH function level was 31.7% in volunteer B.

The reference value of C1-INH function is set as a wide range of 70-130%, because C1-INH function levels can fluctuate due to some biological factors. C1-INH is reported to decline during late phase of pregnancy and it also decreases due to liver disease, malignant disease, extracorporeal circulation therapy, disseminated intravascular coagulation, multiple organ failure, arteriosclerosis obliterans, and infection (*15*). Moreover, in patients with low levels of C1-INH function, the influence of blood sampling conditions before measurement should be taken into consideration. We have previously experienced some cases of non-HAE-C1-INH patients with angioedema, showing low C1-INH function levels at the first measurement, but not at the second measurement as presented below.

Case 1, 15-year old, female: The patient experienced recurrent swelling around the mouth at the age of 15 years. After blood analysis, her family doctor suspected HAE-C1-INH because of the C1-INH function level being < 25%. Although she had a history of angioedema in her paternal family, her blood analysis result in our hospital showed normal C1-INH function levels (96%).

Because she originally had urticaria and pollinosis with a high serum level of IgE (269 IU/mL), she was diagnosed with histamine-mediated angioedema (*16*). We found that the result of blood analysis for C1-INH function level performed in the previous hospital had been obtained using stocked serum 7 days after blood collection, which might have led to the incorrect C1-INH function level.

In this study, the fluctuations of C1-INH function level were observed at about 20-30% according to the inappropriate conditions as shown in Figures 1 and 2. Furthermore, the results of measuring the samples would be naturally impaired by poor testing conditions as we experienced in case 1.

Moreover, we experienced another case of a non-HAE-C1-INH patient with angioedema as presented below.

Case 2, 32-year old, female: After beginning to take estrogen pills at the age of 30 years, the patient experienced facial swelling, for which she visited her home doctor. The result of her blood analysis indicated HAE-C1-INH owing to the presence of low C1-INH function levels (63%). She had no family history of angioedema, and the result of her blood analysis in our hospital showed normal levels (C1-INH function level of 93.8%). We were unable to identify the primary problem associated with the blood collection and measurement procedure in the previous hospital; therefore, we considered that the estrogen pills may have caused drug-induced angioedema (*17*).

Thus, the measurement results of C1-INH function levels might originally show wide variations that cannot be explained simply by blood sampling conditions before the measurement. First, in this study, the obvious difference or tendency was not observed between different blood sampling conditions before measurement as shown in Figure 1 and Figure 2. Second, considering the accuracy and precision of the reagent for C1-INH function levels measurement used in the present study, because Siemens reveals that its accuracy is < 15%, and its coefficient of variation is < 10%, we can say that the reagent used for C1-INH function levels is reliable (*18*). Third, because the measurement of C1-INH function levels was usually outsourced in most of the Japanese hospitals, the testing procedures and methods were unified according to their automated analysis methods. Thus, if blood sampling conditions have an influence on the results, the C1-INH function levels observed in this study should show consistent changes and tendencies over time. Therefore, we need to recognize that the measurement of C1-INH function levels might be unexpectedly unstable.

Additionally, in this study, 2 healthy volunteers (A, C) presented lower C1-INH function levels than the reference value. In particular, volunteer C who showed the lowest C1-INH function level immediately after blood collection, consistently showed 61-65% in all conditions as shown in Figure 1 and Figure 2. Because we were assured that volunteer C did not have any disease including angioedema, this result means that some healthy people could present lower C1-INH function levels than the reference value.

In conclusion, we can confirm that < 50% of C1-INH function level can be used as the diagnostic cut-off value for HAE-C1-INH. Moreover, it is necessary to repeat measurements of C1-INH function level in an appropriate blood sampling condition to accurately diagnose HAE-C1-INH as recommended by the guideline of WAO/EAACI. We hope that more samples will be included in the future to provide more sufficient data as evidence to support this conclusion.

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Conflict of Interest: D.H has received honoraria as a speaker from Takeda Pharmaceutical Company. I.O has received honoraria as a speaker/advisor from BioCryst, CSL Behring and Takeda Pharmaceutical Company. S.M, H.R, Y.T, and Y.S have no financial conflicts of interest to declare.

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