Clinical validation of a novel kinin biomarker assay for characterisation of bradykinin-mediated pathologies in US subjects with hereditary angioedema

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Introduction

- Angioedema (AE) is a predominant manifestation in multiple medical conditions and is generally mediated by bradykinin (BK) and/or histamine.[1]
- Differentiating BK-mediated from histamine-mediated AE and assessing the role of BK in the pathogenesis of other conditions by measuring kinin peptides remains a challenge due to their proteolytic instability and limitations of current analytical assays.[2,3]
- Establishment and clinical validation of a method to accurately measure BK and related peptides (BK-metabolites) could aid in identifying, studying, and managing BK-mediated pathologies, including BK-mediated angioedema.

Methods

- A protease inhibitor (PI) cocktail was manufactured to inhibit *ex vivo* contact activation of kallikrein kinin system (KKS) system and increased cleavage of HMWK and to increase stability of kinin peptides.[4]
- Blood samples were collected from 5 subjects with hereditary angioedema (HAE) due to C1 inhibitor deficiency (HAE-C1INH) and from 6 healthy volunteers (HVs) who had provided their informed consent.
- Two of the HAE-C1INH individuals received a single 30 mg dose of icatibant subcutaneously one day before blood collection to treat an AE attack.

Table 1: Demographics of study population

	HVs n=6	HAE-C1INH (Type 1) n=5
Age in years, mean (SD)	34.0 (12.4)	43.2 (18.8)
Sex: Male/female, n (%)	1 (16.6) / 5 (83.4)	2 (40.0) / 3 (60.0)
Race: White/other, n	3 / 3	4 / 1

C1INH: C1 inhibitor; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers

- Plasma was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) with and without liquid PI.
- An ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS/MS) protocol was optimized to measure BK1-9, BK1-8, BK1-7, BK1-5, and kallidin.[4]
- A capillary-based immunoassay was also developed to quantify the intact HMWK (iHMWK) and cleaved HMWK (cHMWK).[5]

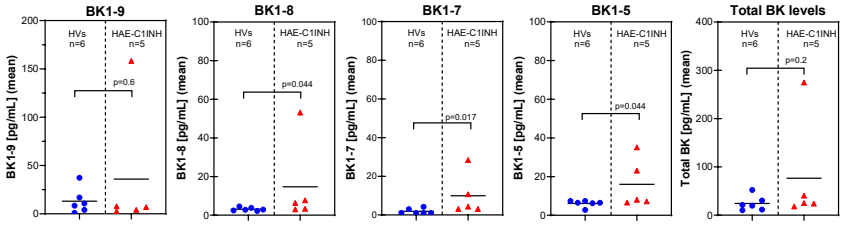
Reference

1. Maurer M, et al. *Clin Rev Allergy Immunol.* 2021;61:40-9. **2.** Kaplan AP, et al. *Adv Immunol.* 2014;121:41-89. **3.** Kaplan AP, et al. *Front Med (Lausanne).* 2017;4:206. **4.** Pardali E, et al. Ann Allergy Immunol. 2024; 6:S30: R088. **5.** Pardali E, et al. Ann Allergy Immunol. 2024; 6:S30: R090.

Results

- Kinin levels were analysed in EDTA plasma with PI using the qualified kinin assay.
- Absolute bradykinin levels were low in plasma samples from people with HAE-C1INH at remission **(Figure 1)**.
- Individual kinin and total kinin levels were slightly increased in people with HAE-C1INH vs HVs.

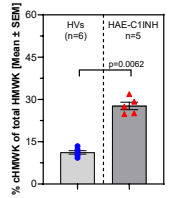
Figure 1: Low absolute kinin levels in plasma from people with HAE-C1INH at remission



BK: Bradykinin; C1INH: C1 inhibitor; HAE: Hereditary angioedema; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers

- Levels of iHMWK and cHMWK were analysed in EDTA plasma with PI by the qualified immunoblotting assay.
- cHMWK levels were expressed as percentage of total HMWK levels (intact and cleaved) **(Figure 2)**.
- cHMWK levels were significantly increased in plasma from people with HAE-C1INH at remission vs HVs .

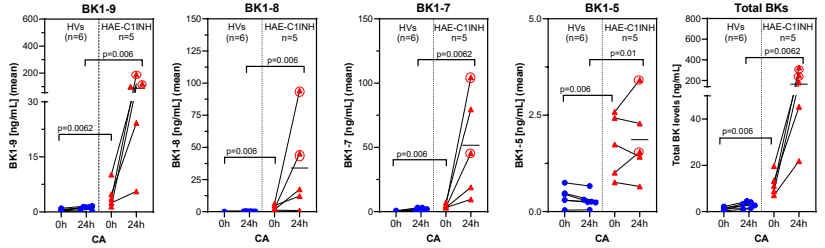
Figure 2: Increased cHMWK levels in plasma from people with HAE-C1INH at remission



C1INH: C1 inhibitor; cHMWK: cleaved high molecular weight kininogen; HAE: Hereditary angioedema; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers; iHMWK: intact high molecular weight kininogen

- Kinin levels were analysed in EDTA plasma before (baseline) and following exposure to cold (4 °C) temperature **(Figure 3)**.
- Cold activation (CA) led to further elevated BK levels in people with HAE-C1INH vs HVs.
- Icatibant treatment had no effects on cold-induced KKS activation and BK generation.
- Significantly higher BK1-9 and total BK levels in EDTA plasma from people with HAE-C1INH also at baseline (no CA).

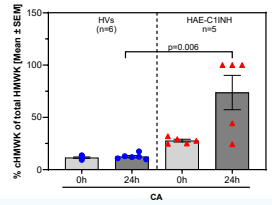
Figure 3: Increased kinin levels in EDTA plasma from people with HAE-C1INH at baseline and following CA



BK: Bradykinin; CA: cold activation; C1INH: C1 inhibitor; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers; Ica: Icatibant treated

- iHMWK and cHMWK levels were analysed before (baseline) and after CA in EDTA plasma **(Figure 4)**.
- CA-induced cleavage of iHMWK resulted in significantly increased cHMWK levels in people with HAE-C1INH vs HVs.
- % cHMWK of total HMWK in EDTA plasma was significantly higher from people with HAE-C1INH also at baseline (no CA).
- Results from iHWMK and cHMWK analysis were in line with the results from kinin analysis.

Figure 4: Increased cHMWK levels in EDTA plasma from people with HAE-C1INH at baseline and following CA



C1INH: C1 inhibitor; CA: cold activation; cHMWK: cleaved high molecular weight kininogen; HAE: Hereditary angioedema; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers; iHMWK: intact high molecular weight kininogen

Conclusions

- **The developed and qualified UHPLC-MS/MS kinin assay and the capillary-based immunoassay assessing iHMWK and cHMWK can be used to identify subjects with BK-mediated AE.**
- **Increased kinins and cHMWK following exposure to cold temperature reveals the KKS pathway hypersensitivity in people with HAE-C1INH.**
- **Using the qualified biomarker assays, evaluation of baseline BK levels may differentiate people with HAE and BK-AE, and cold activation may allow the assessment of the hyperactivatable state of KKS in people with BK-mediated AE.**
- **This clinically qualified BK assay may become a key tool for identifying, studying, and managing BK-mediated conditions, including BK-mediated AE.**

COI: E.P.: employee of Pharvaris, holds stock in Pharvaris; **O.D.:** CEO of Attoquant Diagnostics GmbH; **D.v.O.:** employee of Attoquant Diagnostics GmbH; **J.Y.:** employee of Virant Diagnostics, Inc./MedBio; L.W.: employee of Virant Diagnostics, Inc./MedBio; H.H.L.: employee of Virant Diagnostics, Inc./MedBio; **D.S.:** Principal at Sexton Bio Consulting, LLC; **G.Z.:** employee of Globalization Partners GmbH, consultant to Pharvaris, holds RSU in Pharvaris; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma.

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