A clinically validated kinin biomarker assay to differentiate bradykinin-mediated from mast cell-mediated angioedema

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Key takeaways

- The validated kinin biomarker assay can be used to assess bradykinin-forming cascade sensitivity in plasma samples from people with hereditary angioedema with C1 inhibitor deficiency (HAE-C1INH) and angioedema with acquired C1INH deficiency (AAE-C1INH) using cold activation.
- The assay allows evaluation of BK-forming pathway sensitivity in plasma samples from people with HAE with normal C1INH (HAE-nC1INH) and from people with HAE or AE of unknown etiology ((H)AE-UNK) following cold activation.
- Importantly, the qualified assay can be used to differentiate bradykinin-mediated from mast cell-mediated angioedema.
- The clinically validated kinin biomarker assay may become a key tool for identifying, studying, and managing BK-mediated diseases including angioedema.

Background

- 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 mast cell-mediated AE and assessing the role of BK in the pathogenesis of other conditions by measuring kinin peptides remains a challenge due to proteolytic instability of the kinin peptides and limitations of most analytical assays.[2,3]

Objective

Establishment and clinical validation of a method to accurately measure BK and kinin related peptides (bradykinin metabolites) could aid in identifying, studying, and managing BK-mediated pathologies, including BK-mediated angioedema.

Methods

- Blood samples were collected from people with hereditary AE (HAE) with C1 inhibitor (C1INH) deficiency (HAE-C1INH), AE with acquired C1INH deficiency (AAE-C1INH), HAE with normal C1INH (HAE-nC1INH, with variants in F12 or PLG genes), HAE-nC1INH of unknown etiology (HAE-UNK), AE of unknown etiology (AE-UNK), mast cell-mediated angioedema (AE-MC) and healthy volunteers (HVs) (**Table 1**). All participants provided their informed consent.
- Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and plasma was prepared (EDTA plasma).

Table 1. Demographics of study population

	HAE-C1INH n=30	AAE-C1INH n=4	HAE-nC1INH n=4	HAE-UNK n=3	AE-UNK n=3	AE-MC n=28	HVs n=30
Age in years, mean (SD)	36.7 (12.8)	53 (13.0)	38.5 (12.8)	40.0 (10.1)	43.3 (18.8)	33.3 (19.8)	34.9 (8.8)
Sex: male/female, n	13 / 17	4 / 0	2 / 2	0 / 3	1 / 2	10/18	17 / 13
Race: white/other, n	27 / 3	4 / 0	4 / 0	0 / 3	2 / 1	28	26 / 4
HAE-1/HAE-2, n	28 / 2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HAE-nC1INH type, n							
HAE-FXII	n.a.	n.a.	1	n.a.	n.a.	n.a.	n.a.
HAE-PLG	n.a.	n.a.	3	n.a.	n.a.	n.a	n.a

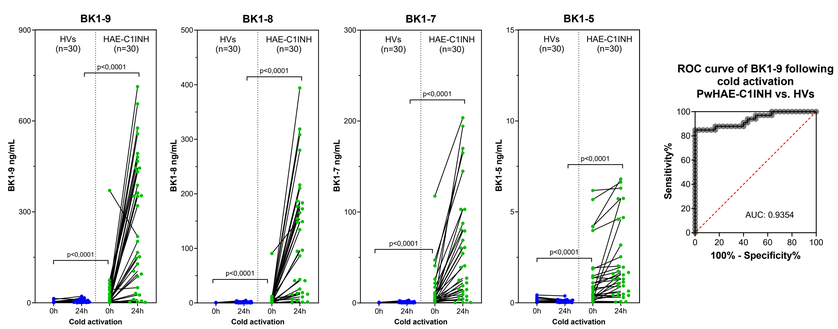
AE: angioedema; AAE-C1INH: AE with acquired C1INH deficiency; AE-UNK: AE of unknown etiology; C1INH: C1 inhibitor; FXII: factor XII; HAE: hereditary angioedema; HAE-1: HAE type 1; HAE-2: HAE type 2; HAE-C1INH: HAE with C1INH deficiency; HAE-FXII: HAE-nC1INH with genetic variant in F12; HAE-nC1INH: HAE with normal C1INH; HAE-PLG: HAE-nC1INH with genetic variant in PLG; HAE-UNK: HAE-nC1INH of unknown etiology; MC-AE: mast cell-mediated angioedema; n: number of individuals; n.a.: not applicable; PLG: plasminogen; SD: standard deviation

- An ultra-high performance liquid chromatography-mass spectrometry method was optimized to measure BK1-9, BK1-8, BK1-7, BK1-5 in EDTA plasma.[4]
- BK and BK metabolite levels were analyzed in EDTA plasma before and after cold activation (exposure to 4°C for 24 hours).

Results

- Kinin levels were analyzed in EDTA plasma before (baseline, 0h) and following cold activation (24h) (**Figure 1**).
- Cold activation led to significant greater increase in BK1-9 and bradykinin metabolites levels compared to baseline in EDTA plasma from people with HAE-C1INH vs. HVs.
- BK1-9 and bradykinin metabolite levels were significantly higher following cold activation in plasma from HAE-C1INH vs. HVs and also at baseline (no cold activation).
- Receiver operating characteristic (ROC) curve analysis (30 people with HAE-C1INH and 30 HVs) showed that BK1-9 levels have significant diagnostic value for HAE-C1INH (**Figure 1**).

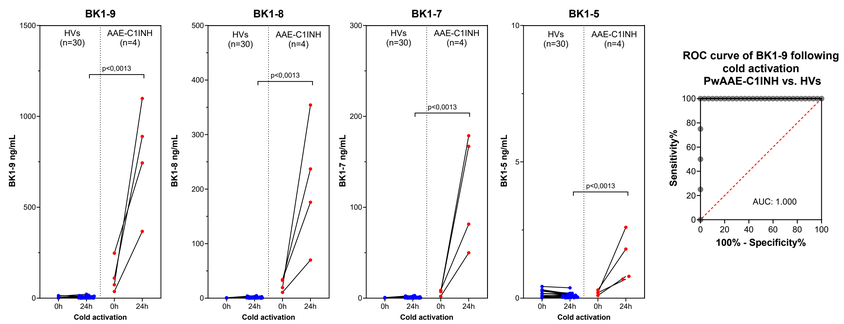
Figure 1. Increased BK1-9 and bradykinin metabolite levels in EDTA plasma from people with HAE-C1INH vs. HVs at baseline and following cold activation



AUC: area under the curve; BK: bradykinin; C1INH: C1 inhibitor; HAE-C1INH: hereditary angioedema with C1INH deficiency; HVs: healthy volunteers; PwHAE-C1INH: people with HAE-C1INH; ROC: receiver operator characteristic

- BK1-9 and bradykinin metabolite levels were significantly increased in plasma from people with AAE-C1INH following cold activation (**Figure 2**).
- The qualified kinin biomarker assay allows clear differentiation between people with AAE-C1INH and HVs.
- ROC analysis (4 people with AAE-C1INH and 30 HVs), showed that BK1-9 levels has significant diagnostic value for AAE-C1INH (**Figure 2**).

Figure 2. Increased BK1-9 and bradykinin metabolite levels in EDTA plasma from people with AAE-C1INH vs. HVs at baseline and following cold activation

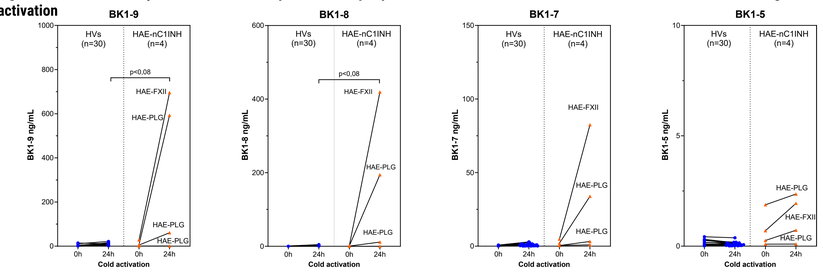


AUC: area under the curve; BK: bradykinin; C1INH: C1 inhibitor; AAE-C1INH: angioedema with acquired C1INH deficiency; HVs: healthy volunteers; PwAAE-C1INH: people with AAE-C1INH; ROC: receiver operator characteristic

Results

- Cold activation caused elevated BK1-9 and bradykinin metabolite levels in samples from people with HAE-FXII and HAE-PLG, indicative of increased BK-forming cascade sensitivity to triggers (**Figure 3**).
- Two individuals with HAE-PLG without history of AE attacks did not respond to cold activation.

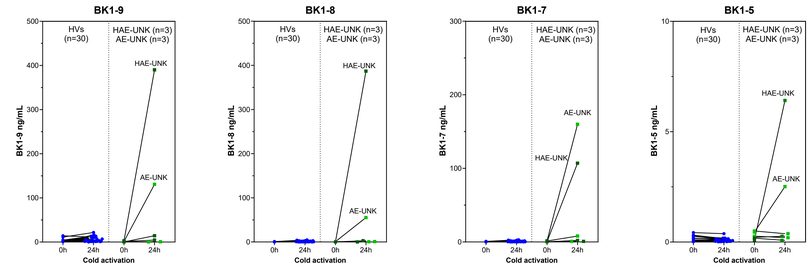
Figure 3. Increased bradykinin levels in EDTA plasma from people with HAE-FXII and HAE-PLG vs. HVs at baseline and following cold activation



BK: bradykinin; C1INH: C1 inhibitor; FXII: factor XII; HAE: hereditary angioedema; HAE-FXII: HAE with genetic variant in FXII; HAE-nC1INH: HAE with normal C1INH; HAE-PLG: HAE with genetic variant in PLG; HVs: healthy volunteers; PLG: plasminogen

- Cold activation caused increased BK1-9 and bradykinin metabolite levels in samples from people with HAE-UNK and AE-UNK, indicative of BK-forming cascade sensitivity to triggers (**Figure 4**).
- AE attacks in these individuals are believed to be bradykinin-mediated.

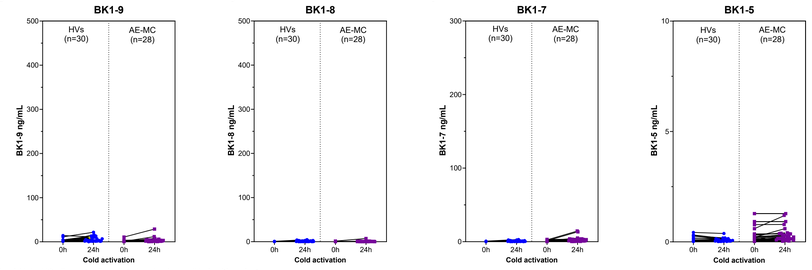
Figure 4. The cold activation kinin biomarker assay could be used to assess a bradykinin signature in people with (H)AE-UNK



AE-UNK: angioedema of unknown etiology; BK: bradykinin; C1INH: C1 inhibitor; HAE: hereditary angioedema; HAE-UNK: hereditary angioedema with normal C1INH of unknown etiology; HVs: healthy volunteers

- There was no increase in BK1-9 and bradykinin metabolite levels in plasma from people with AE-MC following cold activation (**Figure 5**).

Figure 5. No increase in BK1-9 and bradykinin metabolite levels in EDTA plasma from people with AE-MC and HVs following cold activation



BK: bradykinin; MC-AE: mast cell-mediated angioedema; HVs: healthy volunteers

References

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E.P.: employee of Pharvaris, holds stocks/stock options in Pharvaris; **H.R.H.:** received travel grants from Takeda and CSL Behring; **J.J.Y.:** employee of Virant Diagnostics, no conflicts of interest; **L.W.:** employee of Virant Diagnostics / Institute for Asthma and Allergy / Angioedema Center of Reference and Excellence, no conflicts of interest; **M.d.L.:** has received speaker fees from Takeda; **O.D.:** CEO of Attoquant Diagnostics GmbH; **D.v.O.:** employee of Attoquant Diagnostics GmbH; **D.S.:** Principal at Sexton Bio Consulting, LLC; **G.Z.:** employee of Globalization Partners GmbH, consultant to Pharvaris, holds RSU; **D.M.C.:** has received speaker or consultancy fees from Astria, BioCryst, CSL Behring, Intellia, Ionis, KalVista, Otsuka, Pharvaris, and Takeda and research funding from CSL Behring, Ionis, KalVista and Pharvaris; **H.H.L.:** employee of Virant Diagnostics / Institute for Asthma and Allergy / Angioedema Center of Reference and Excellence, received speaker or consultancy fees from Biocryst, CSL Behring, Ionis, Kalvista, Pharming, and Takeda and research grants from Pharvaris, Ionis, CSL Behring, Biocryst, Kalvista, Intelia, Argo, and AdaRx; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma; **H.F.:** received research grants from CSL Behring, Pharming, Takeda and served as an advisor for these companies and BioCryst, Intellia, KalVista, ONO Pharmaceutical, Pharvaris; has participated in clinical trials/registries for BioCryst, CSL Behring, KalVista, Pharming, Pharvaris, Takeda.