A Novel Kinin Biomarker Assay for Characterisation of Bradykinin-Mediated Disorders

Abstract ID 116577

Evangelia Pardali[1], Oliver Domenig[2], Dan Sexton[3], Grit Zahn[4], Anne Lesage[5]

[1]Pharvaris B.V., Leiden, The Netherlands; [2]Attoquant Diagnostics, Vienna, Austria; [3]Sexton Bio Consulting, LLC, Melrose, MA, US; [4]Globalization Partners, Munich, Germany; [5]GrayMatters Consulting, Schilde, Belgium

Introduction

- Bradykinin (BK) is involved in various physiological and pathological processes, including angioedema (AE).[1] AE is a predominant manifestation in multiple medical conditions and is generally mediated by BK and/or histamine.[2]
- Differentiating BK-mediated from histamine-mediated AE and assessing the role of bradykinin in the pathogenesis of other conditions by measuring kinin peptides remains a challenge due to their proteolytic instability and limitations of current analytical assays.[3]
- In addition, unspecific activation of the plasma kallikrein-kinin system (KKS), resulting in cleavage of high-molecular-weight kininogen (HMWK) and production of kinins, could have a significant impact on the results.[3]
- Establishment of a method to accurately measure BK and related peptides could aid in identifying, studying, and managing BK-mediated disorders.

Materials and Methods

- To inhibit *ex vivo* activation of KKS proteases and proteolytic degradation of BK, a protease inhibitor (PI) cocktail was manufactured in a liquid form (Liquid PI).
- Blood samples were collected from healthy volunteers (HV) by Fidelis Research AD (Sofia, Bulgaria) in accordance with the Declaration of Helsinki and approved by The National Bioethics Committee of Medicines and Medical Devices (protocol no. FRT-19101). All participants provided their written informed consent before enrolment.
- Plasma was collected using S-Monovettes (Sarstedt) with a single venipuncture with a 21G x 3/4'' Safety-Multifly® needle using the aspiration technique, into tubes containing either liquid PI or ethylenediaminetetraacetic acid (EDTA) as a control.
- An ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC)-MS/MS protocol was optimized to separately measure BK1-9, BK1-8, BK1-7, BK1-5, and kallidin (KD) (Attoquant Diagnostics GmbH, Vienna, Austria).
- Qualification of the UPLC-MS/MS was performed using plasma from HVs collected under different conditions.

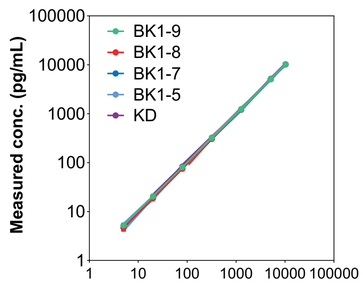
Results

- Calibration curves were prepared by spiking different kinin peptides in surrogate blank matrix.
- For all tested kinins, the back-calculated concentrations of the calibrator standards were within ±15% of the nominal value and met qualification criteria (**Figure 1**).

Figure 1. Calibration curves of BK1-9, BK1-8, BK1-7, BK1-5, and KD

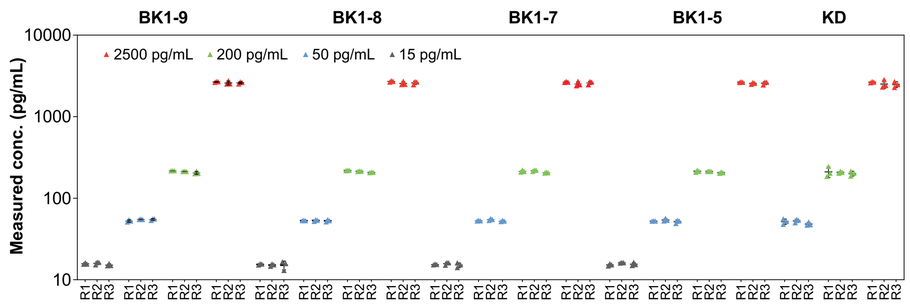
	Linearity range of quantification	
Kinin peptide	**LLoQ**	**ULoQ**
BK1-9	5 pg/mL	10240 pg/mL
BK1-8	5 pg/mL	10240 pg/mL
BK1-7	5 pg/mL	10240 pg/mL
BK1-5	5 pg/mL	10240 pg/mL
KD	20 pg/mL	10240 pg/mL

LLoQ: lower limit of quantification; ULoQ: upper limit of quantification



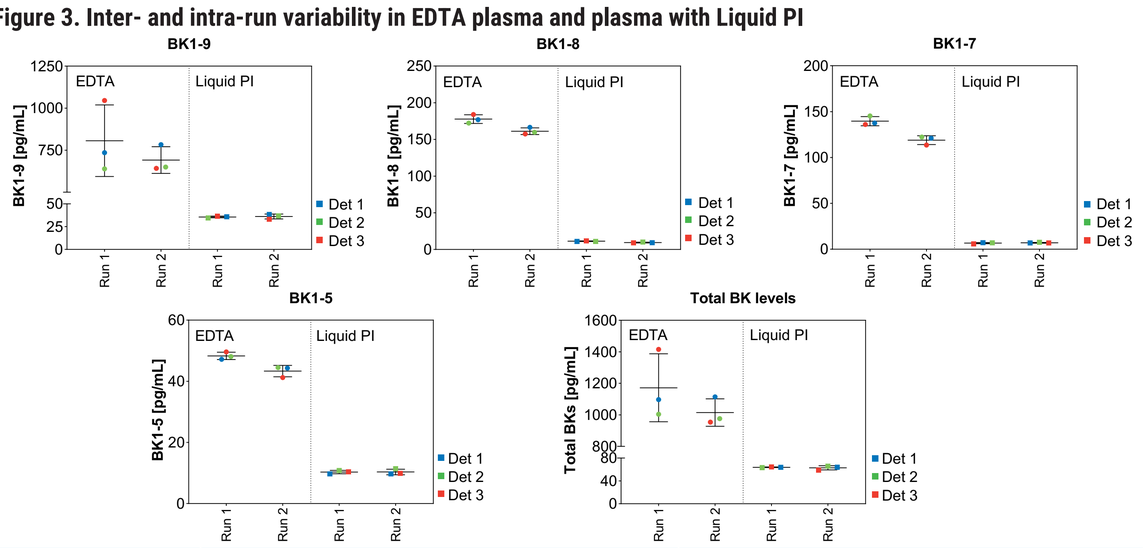
- Kinin UPLC-MS/MS assay in blank matrix met qualification criteria for within- and between-run accuracy and precision.
- Accuracy ±15 % deviation from nominal value. Precision Coefficient of variation (CV) <15% for (**Figure 2**).

Figure 2. Analysis of quality control (QC) samples for BK1-9, BK1-8, BK1-7, BK1-5 and KD in blank matrix



- KD levels were below the limit of detection in all types of human plasma analysed (not shown).
- Liquid PI efficiently inhibited KKS activation and stabilized levels of all BK peptides compared with EDTA alone, resulting in low inter-run and intra-run variability (CV <15%) for BK1-9, BK1-8, BK1-7, BK1-5 and total BK levels (**Figure 3**).

Figure 3. Inter- and intra-run variability in EDTA plasma and plasma with Liquid PI

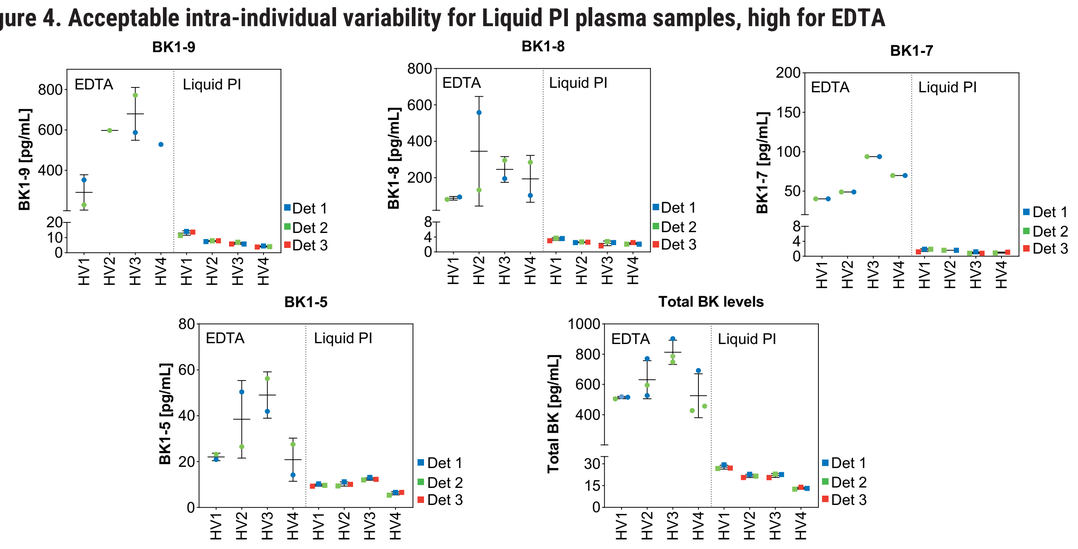


	EDTA plasma		Liquid PI plasma	
Kinin peptide	**Mean ± SD (pg/mL)**	**CV%**	**Mean ± SD (pg/mL)**	**CV%**
BK1-9	748.9 ± 81.3	10.9	35.9 ± 0.4	1.0
BK1-8	169.4 ± 11.7	6.9	10.4 ± 1.3	12.8
BK1-7	129.3 ± 14.6	11.3	6.8 ± 0.3	3.9
BK1-5	45.8 ± 3.5	7.6	10.3 ± 0.0	0.1
Total BK	1093.5 ± 111.1	10.2	63.4 ± 0.7	1.1

Results

- EDTA plasma presented higher levels of all BK peptides and high intra-individual variability in plasma from HVs.
- Low intra-individual variability, CV <15 % for all BK peptides in Liquid PI plasma samples from all HVs (**Figure 4**).
- Liquid PI efficiently inhibited *ex vivo* KKS activation and kinin degradation.

Figure 4. Acceptable intra-individual variability for Liquid PI plasma samples, high for EDTA

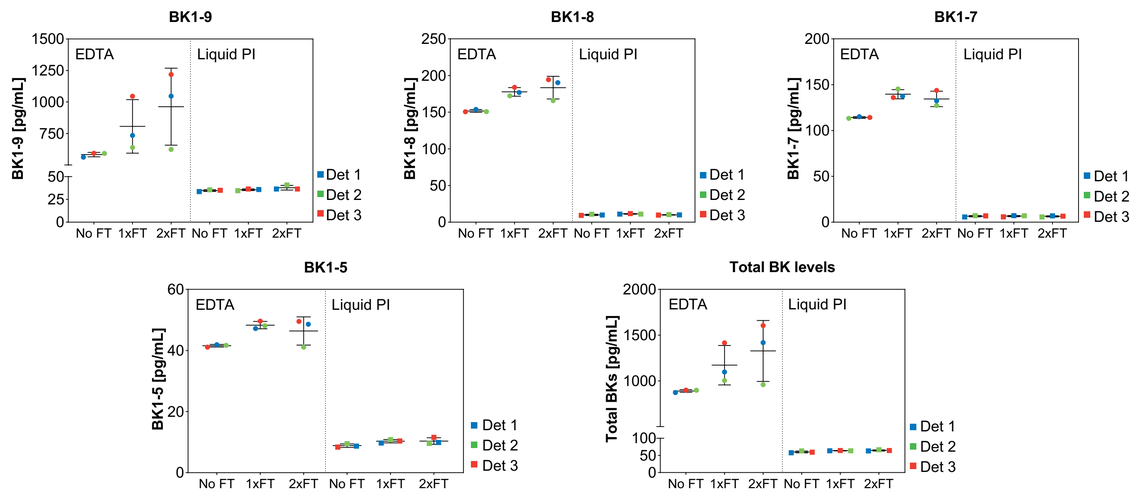


EDTA	BK1-9		BK1-8		BK1-7		BK1-5		Total BKs	
	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%
HV1	291.1±87.7	30.1	87.0±10.0	11.5	43.9±5.2	11.8	22.1±1.6	7.3	444.0±101.2	22.8
HV2	2323.6±2440.4	105.0	345.3±301.2	87.2	105.7±80.4	76.1	38.4±17.0	44.1	2813.0±2839.1	100.9
HV3	679.8±130.5	19.2	245.1±70.7	28.8	85.9±11.0	12.8	49.1±10.2	20.7	1059.9±222.4	21.0
HV4	1230.5±992.7	80.7	193.3±129.1	66.8	58.5±15.8	27.0	20.9±9.4	45.1	1503.2±1147.1	76.3
Liquid PI	**BK1-9**		**BK1-8**		**BK1-7**		**BK1-5**		**Total BKs**	
	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%	Mean±SD (pg/mL)	CV%
HV1	13.1±1.4	11.0	3.4*±0.4	na	1.5*±0.4	na	9.8±0.5	5.2	27.7±1.4	4.9
HV2	7.7±0.3	3.8	2.6*±0.1	na	1.6*±0.0	na	10.2±0.9	9.1	21.7±1.1	4.9
HV3	6.2±0.7	11.2	2.3*±0.6	na	1.1*±0.2	na	12.4±0.6	4.7	22.0±1.4	6.2
HV4	4.1±0.4	8.6	2.2*±0.2	na	1.0*±0.2	na	6.2±0.7	11.0	13.2±0.7	5.5

* Concentrations are below LLoQ; na: not applicable

- Liquid PI efficiently inhibited *ex vivo* KKS activation and stabilized BK peptides following 2 cycles of freeze and thaw (FT) as compared with EDTA (**Figure 5**).
- FT induced KKS activation in EDTA plasma resulting in high levels and r variability in BK levels (**Figure 5**).

Figure 5. Effects of Freeze and thaw on BK peptide levels and stability



	EDTA plasma					Liquid PI plasma				
	Mean (pg/mL)			Deviation %		Mean (pg/mL)			Deviation %	
Kinin peptide	**No FT**	**1xFT**	**2xFT**	**1xFT**	**2xFT**	**No FT**	**1xFT**	**2xFT**	**1xFT**	**2xFT**
BK1-9	582.4	806.4	962.9	38.5	65.3	34.7	35.6	37.9	2.5	8.9
BK1-8	151.7	177.7	183.4	17.1	20.9	10.0	11.3	10.0	13.5	0.5
BK1-7	114.2	139.6	134.5	22.2	17.7	6.5	6.7	6.4	1.6	-3.0
BK1-5	41.6	48.3	46.4	16.1	11.6	8.9	10.3	10.4	15.7	16.8
Total BK	889.9	1172.0	1327.2	31.7	49.1	60.2	63.9	64.6	6.2	7.4

No FT, no freeze/thaw; 1xFT, 1 freeze/thaw cycle; 2xFT, 2 freeze/thaw cycle

Conclusions

- **The Liquid PI cocktail was shown to be effective in inhibiting non-specific activation of KKS and generation of BK1-9 as compared to EDTA without PI.**
- **The established qualified kinin UPLC-MS/MS biomarker assay can be used to reliably measure KKS biomarkers in human plasma.**
- **The assay could become a key tool for identifying, studying, and managing BK-mediated diseases, including BK-mediated AE.**

References

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

COI: E.P.: employee of Pharvaris, holds stock in Pharvaris; **O.D.:** CEO of Attoquant Diagnostics GmbH; **D.S.:** principle at Sexton Bio Consulting, LLC, consultant to Pharvaris; **G.Z.:** employee of Globalization Partners GmbH, consultant to Pharvaris, holds RSU; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma.
Funding statement: Funding towards this study was provided by Pharvaris.