A sensitive and specific assay to characterize plasma kallikrein activity in plasma from hereditary angioedema patients

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Disclosures

- Daniel Lee is a full-time employee of Kalvista Pharmaceuticals
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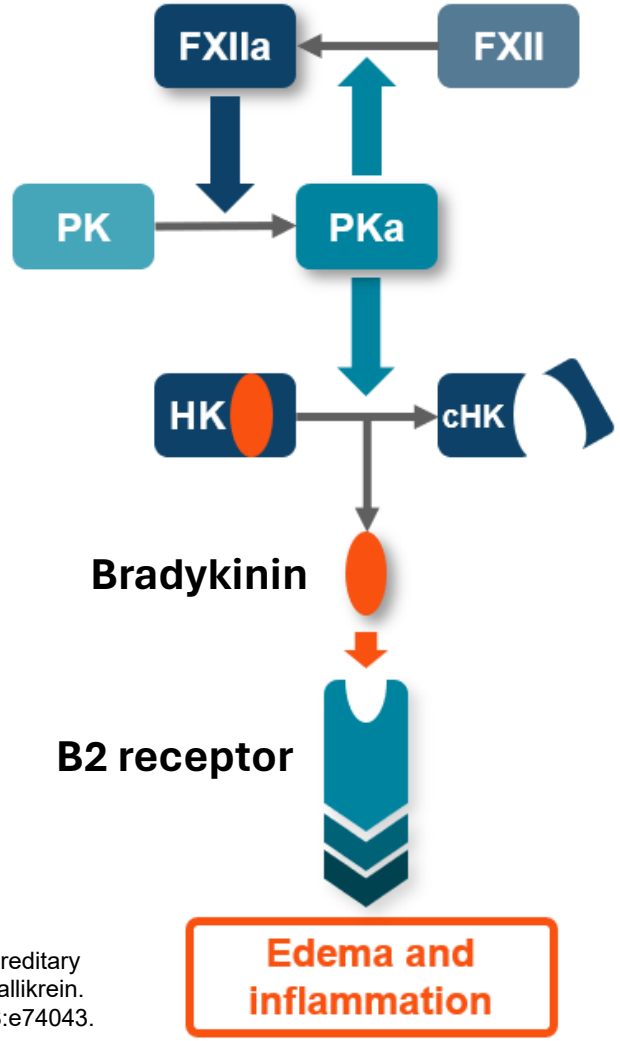
Study Overview

Background

- PKa activity:
  - Is a primary cause for HAE and has been implicated in other KKS-mediated diseases
  - Is increased in plasma of patients with HAE[1-4]
  - Could be a biomarker for other KKS associated diseases
- Exogenous substrates commonly used in PKa assays can be cleaved by multiple plasma proteases, which reduce assay specificity and sensitivity for PKa

Objective

- To establish an assay to detect the specific and sensitive PKa activity as a biomarker for HAE-nC1INH and other KKS-associated diseases



cHK, cleaved high-molecular-weight kininogen; FXII, factor XII; FXIIa, activated factor XII; HAE, hereditary angioedema; HAE-nC1INH, hereditary angioedema with normal C1 inhibitor; HK, high-molecular-weight kininogen; KKS, kallikrein kinin system; PK, prekallikrein; PKa, plasma kallikrein.

1. Charignon D et al. *Mol Immunol.* 2017;85:120-122. 2. Defendi F et al. *PLoS One.* 2013;8:e70140. 3. Konings J et al. *PLoS One.* 2013;8:e74043. 4. Suffritti C et al. *Clin Exp Allergy.* 2014;44:1503-1514.

Measuring sPKa Activity in Plasma

PKa activity was measured in citrated plasma

- Healthy controls (n=57)
- HAE type I/II (n=25) samples obtained during the intercritical period (and the participants were not on HAE therapies) as a pre-dose sample in the open-label pharmacokinetic part 1 of the sebetralstat phase 2 trial[1,2]
- Individuals with presumptive diagnosis with HAE-nC1INH (n=2)

Demographics

Sample	Age range (years)	Sex (%)	
		Female	Male
Healthy (n=57)	20-70	40	60
HAE type I/II (n=25)	19-68	64	36

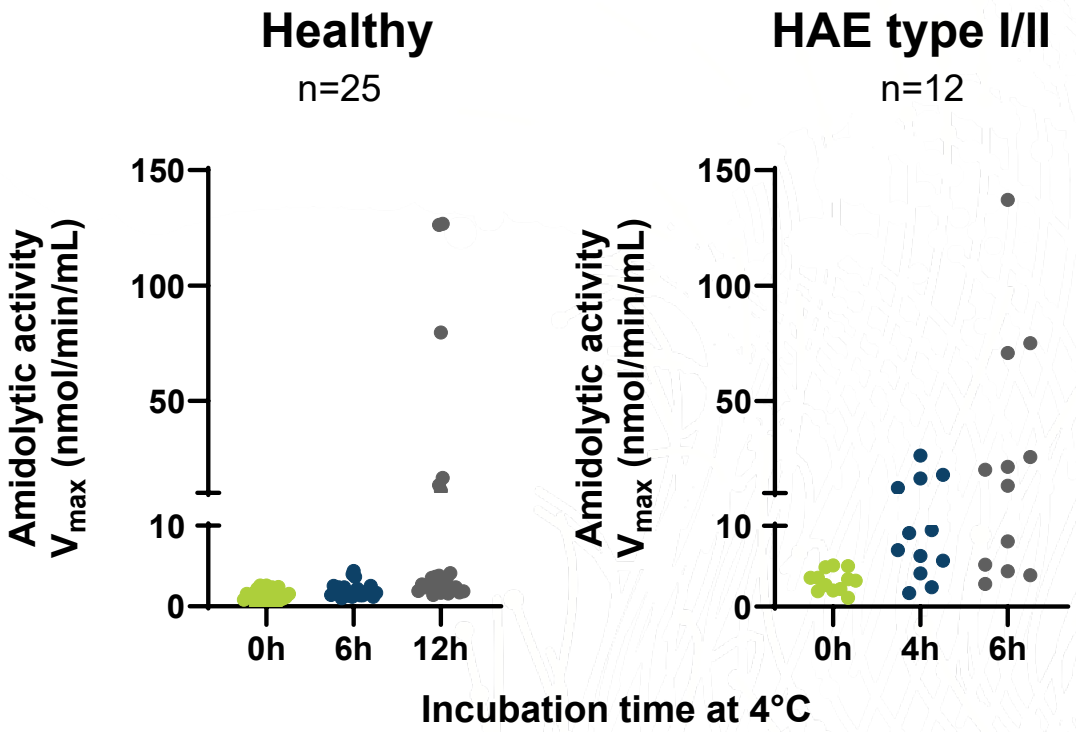
sPKa activity assay

- Amidolytic activity (V_{max}) was measured using H-D-Pro-Phe-Arg-pNA·2HCl in the absence and presence of a specific PKa inhibitor, KV999272
- sPKa was quantified by the subtraction of amidolytic activity not inhibited by KV999272 from the total measured activity

HAE, hereditary angioedema; HAE-nC1INH, hereditary angioedema with normal C1 inhibitor; n, number of participants; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

1. ClinicalTrials.gov. NCT04208412. 2. Aygören-Pürsün E et al. *Lancet.* 2023;401:458-469.

Cold Exposure Increases Amidolytic Activity in Plasma



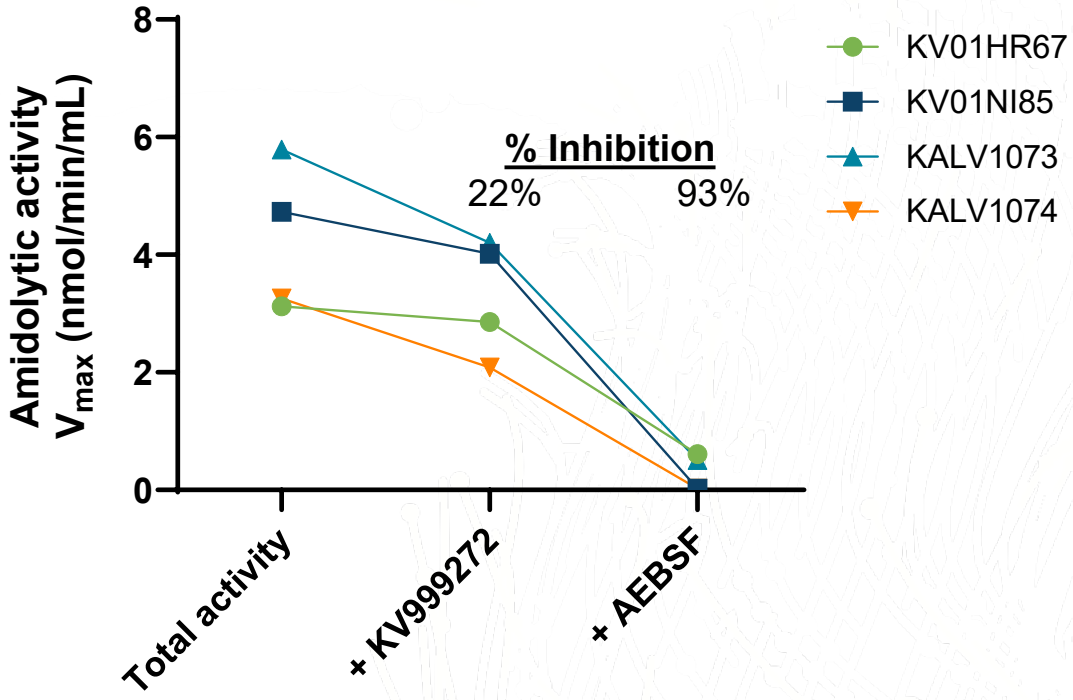
- Exposure of plasma to cold (4°C) has been shown to increase PKa activity[1]
- PKa activity in plasma of healthy controls (healthy plasma) remains low after 6 hours of cold exposure, but some samples show increased activity at 12 hours
- PKa activity in plasma of participants with HAE (HAE plasma) is increased at 4 hours and is further increased at 6 hours of cold exposure

6 h of cold exposure of plasma was chosen as the optimal time point to increase PKa activity in HAE plasma while maintaining low PKa activity in healthy plasma

HAE, hereditary angioedema; n, number of participants; PKa, plasma kallikrein; V_{max}, maximum velocity.

1. Larrauri, Blas et al. *Molecular Immunology vol.* 119 (2020): 27-34.

Using a Specific PKa inhibitor Is Essential to Establishing Assay Specificity

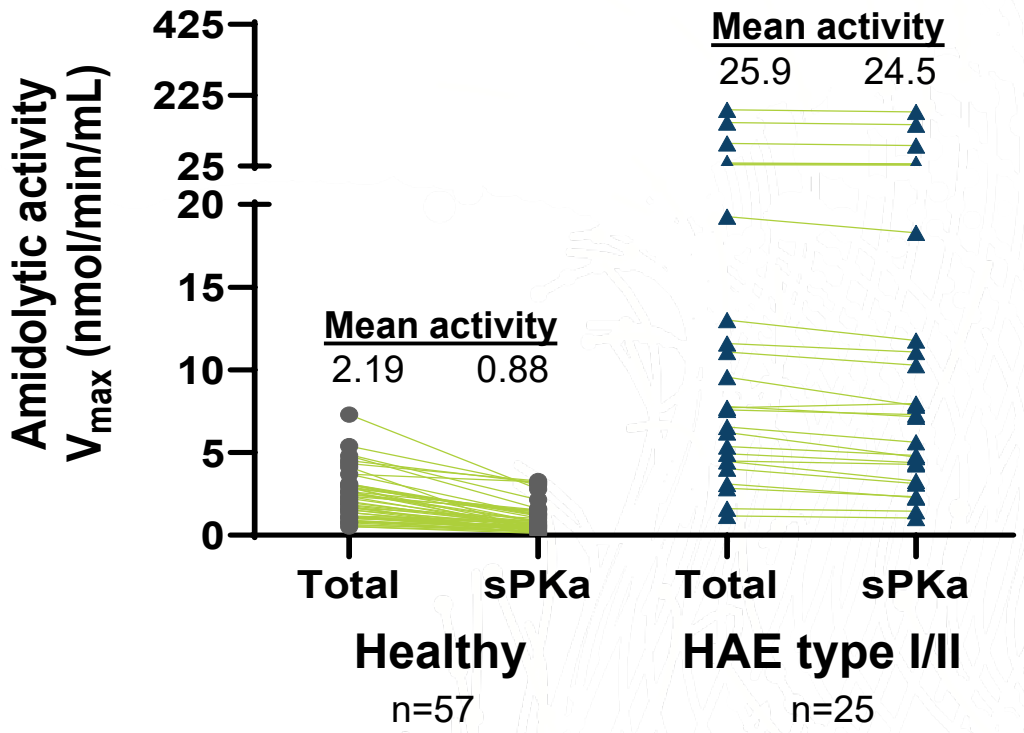


- To quantify the amidolytic activity driven by PKa, the specific inhibitor KV999272 was introduced
- Proteases other than PKa contribute to the cleavage of H-D-Pro-Phe-Arg-pNA·2HCl (S2302) substrate in plasma
  - These proteases include FXIIa,[1] thrombin,[1] trypsin,[1] KLK5,[1] KLK4,[2] KLK2,[2] and tryptase[3]
- A broad-spectrum protease inhibitor AEBSF inhibits the amidolytic activity in healthy samples that was not inhibited by a specific PKa inhibitor (post 6 hours of cold exposure)

AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride; FXIIa, activated factor XII; KLK, kallikrein-related peptidase; PKa, plasma kallikrein; V_{max}, maximum velocity.

1. Data on file, KalVista Pharmaceuticals, Inc. 2. Takayama Tet al. *Biochemistry.* 2001;40:15341-15348. 3. Peng Q et al. *Eur J Biochem.* 2003;270:270-283.

Measuring sPKa Activity Improves Assay Sensitivity

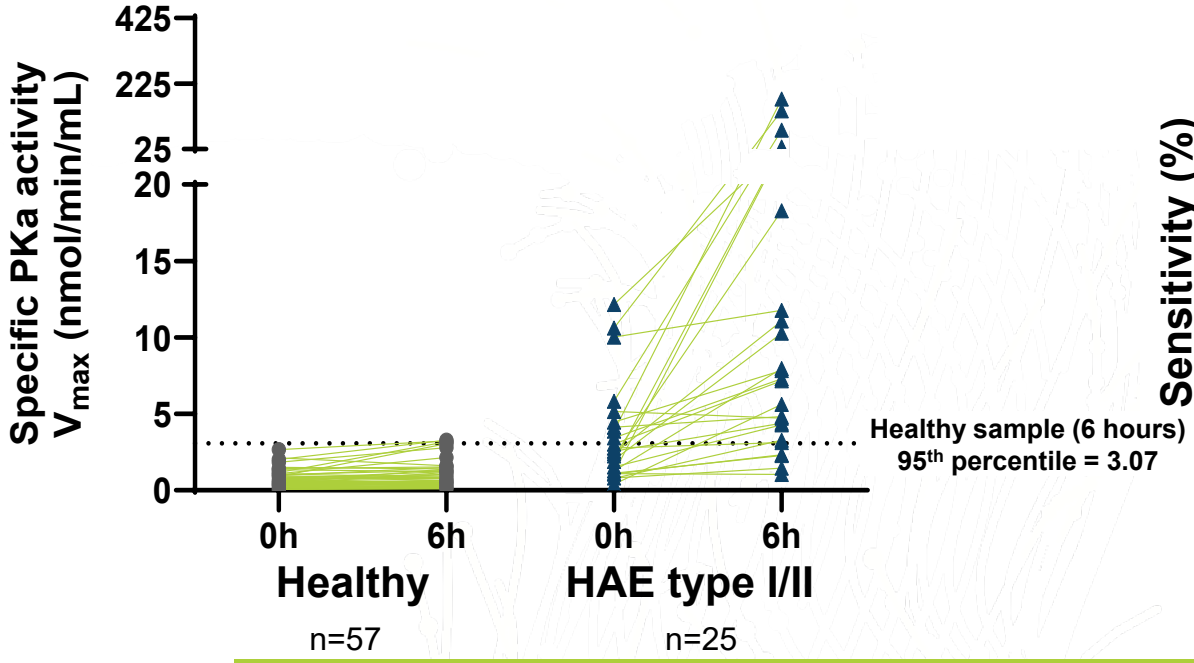


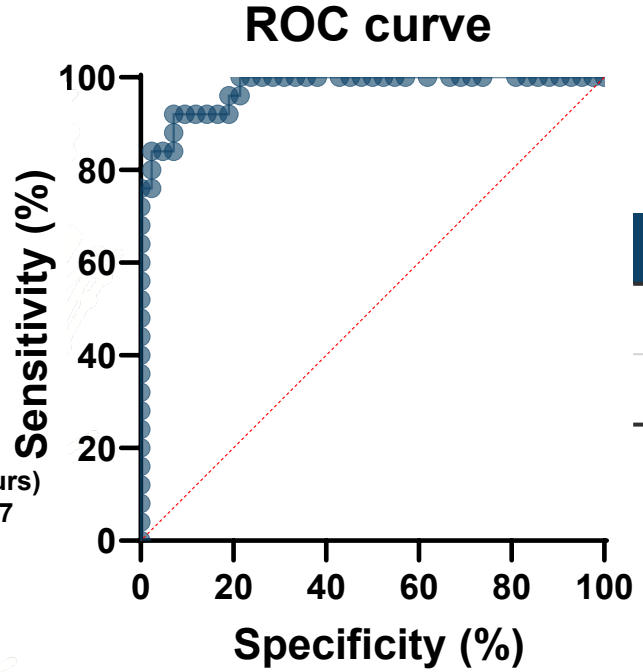
- Total amidolytic activity and sPKa activity (inhibitable by a PKa inhibitor) were measured in plasma after 6 hours of cold exposure
- In healthy plasma, sPKa activity accounted for 40% of the total of amidolytic activity
- In HAE plasma, sPKa activity accounted for >90% of the total amidolytic activity
- Analysis of sPKa activity, rather than total amidolytic activity, increased assay sensitivity to detection of HAE samples from 76% to 84%

Measuring sPKa activity reduces assay non-specific background amidolytic activity in healthy plasma and thereby improved assay selectivity and specificity

HAE, hereditary angioedema; n, number of participants; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

sPKa Activity in Plasma Samples From Healthy Individuals and Those With HAE



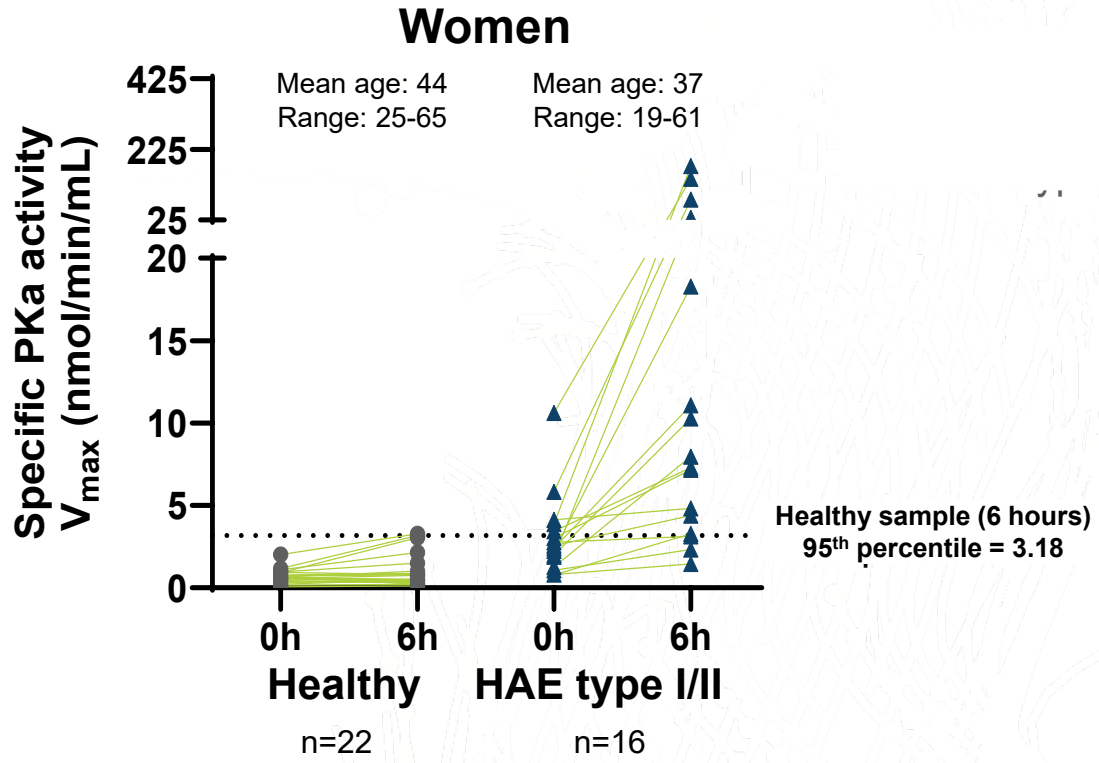


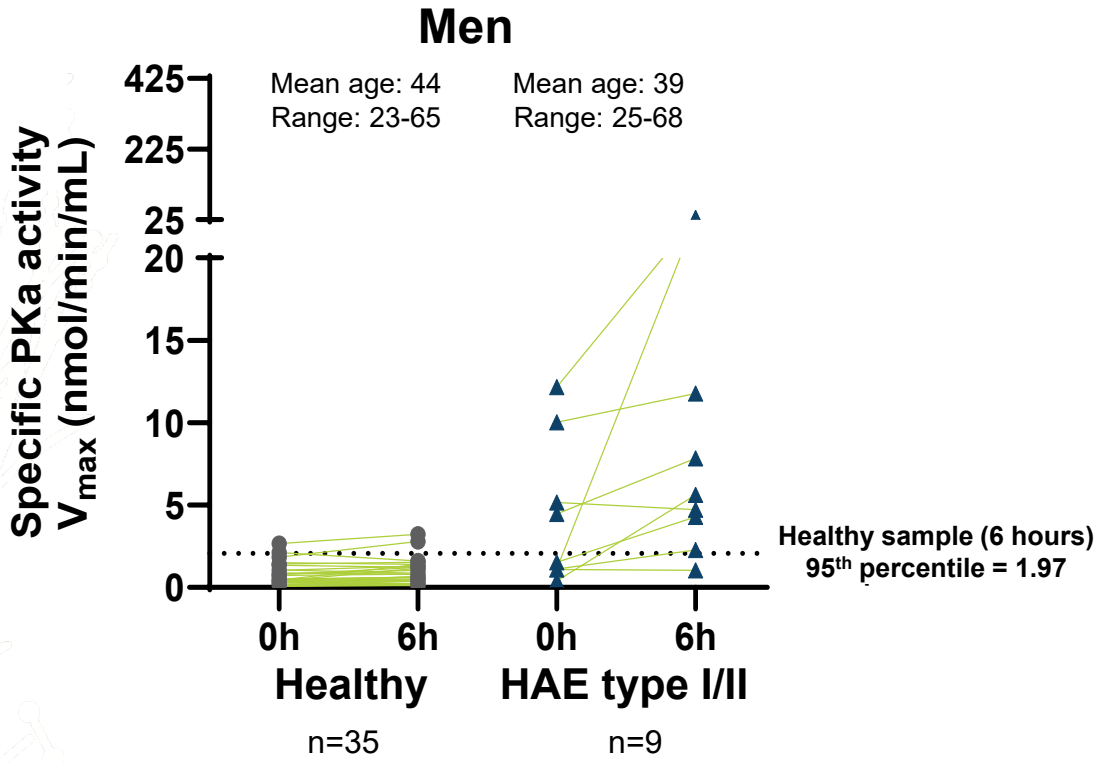
sPKa activity at 6 hours	
AUC	0.98
P value	<0.0001

- **sPKa activity in plasma measured after 6 h of cold exposure can show differentiation between HAE (intercritical period) plasma and healthy plasma, with 84% sensitivity and 95% specificity**
- **ROC curve shows excellent test performance**

AUC, area under the concentration-time curve; HAE, hereditary angioedema; n, number of participants; ROC, receiver operating characteristic; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

sPKa Activity in Plasma Samples From Women and Men

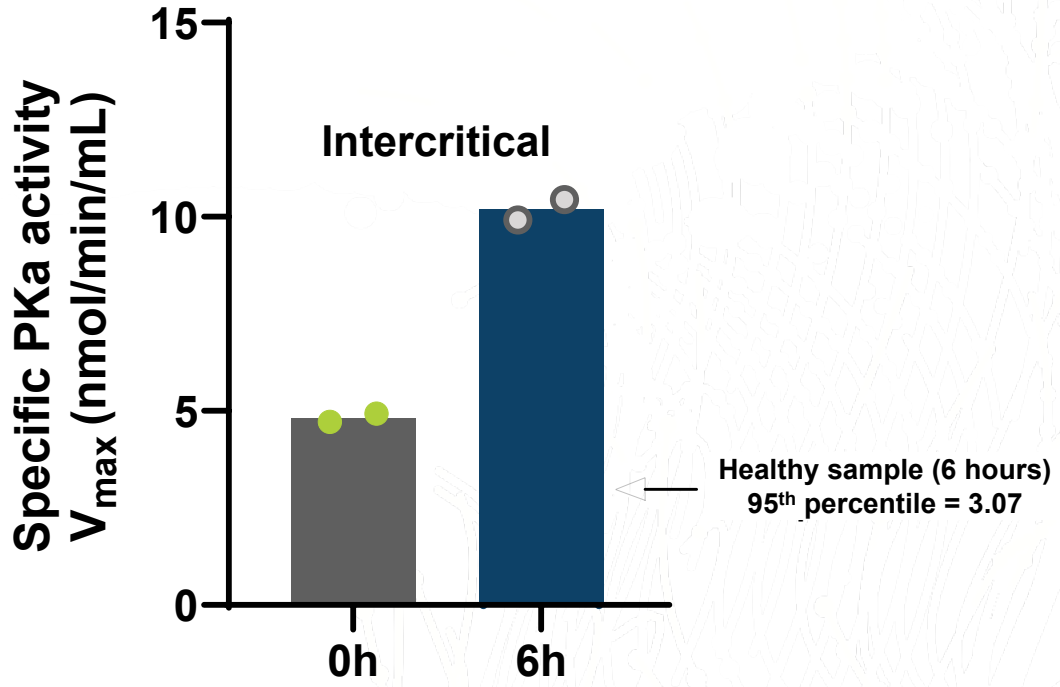




Plasma from healthy women showed higher sPKa activity than plasma from healthy men

HAE, hereditary angioedema; n, number of participants; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

sPKa Activity in HAE-nC1INH: Case Study 1

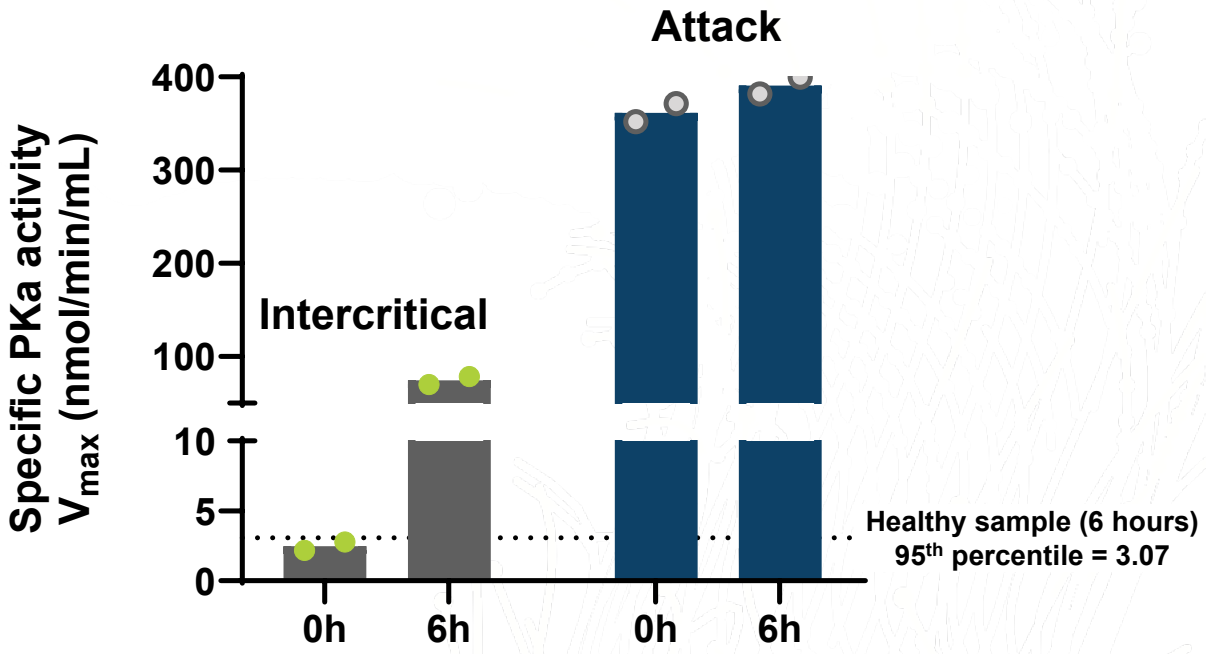


- Background
  - 22-year-old woman
  - Diagnosed with presumptive HAE-nC1INH by physician
  - History of subcutaneous and facial oedema
  - Treated with tranexamic acid (prophylaxis) and Berinert (on demand)
- Plasma sample obtained during intercritical period

The woman in case 1 had increased sPKa activity after cold exposure compared with healthy controls (3.07) or women only (3.18)

HAE-nC1INH, hereditary angioedema with normal C1 inhibitor; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

sPKa Activity in HAE-nC1INH: Case Study 2



- Background
  - 45-year-old woman
  - Diagnosed with presumptive HAE-nC1INH by physician
  - Responsive to on-demand treatment with tranexamic acid and Berinert
  - History of subcutaneous oedema, facial and abdominal attacks
- Intercritical plasma showed increased sPKa activity after cold exposure
- Attack plasma showed increased sPKa activity with and without cold incubation

The woman in case 2 had increased sPKa activity compared with healthy controls

HAE-nC1INH, hereditary angioedema with normal C1 inhibitor; PKa, plasma kallikrein; sPKa, specific plasma kallikrein; V_{max}, maximum velocity.

Conclusions

- Results from current amidolytic activity assays for PKa can be confounded by enzymes other than PKa. Quantifying PKa specifically can be enhanced by using a PKa inhibitor
- The sPKa activity assay can differentiate HAE type I/II plasma collected during the intercritical period and that from healthy controls, with high sensitivity and specificity
- Patients with a presumptive diagnosis of HAE-nC1INH had increased sPKa activity
- Measuring specific PKa activity could be useful as a biomarker for HAE-nC1INH and other KKS-mediated diseases or disorders

HAE, hereditary angioedema; HAE-nC1INH, hereditary angioedema with normal C1 inhibitor; KKS, kallikrein kinin system; PKa, plasma kallikrein; sPKa, specific plasma kallikrein.

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