

A specific, sensitive assay measuring patient sample plasma kallikrein activity

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Disclosures

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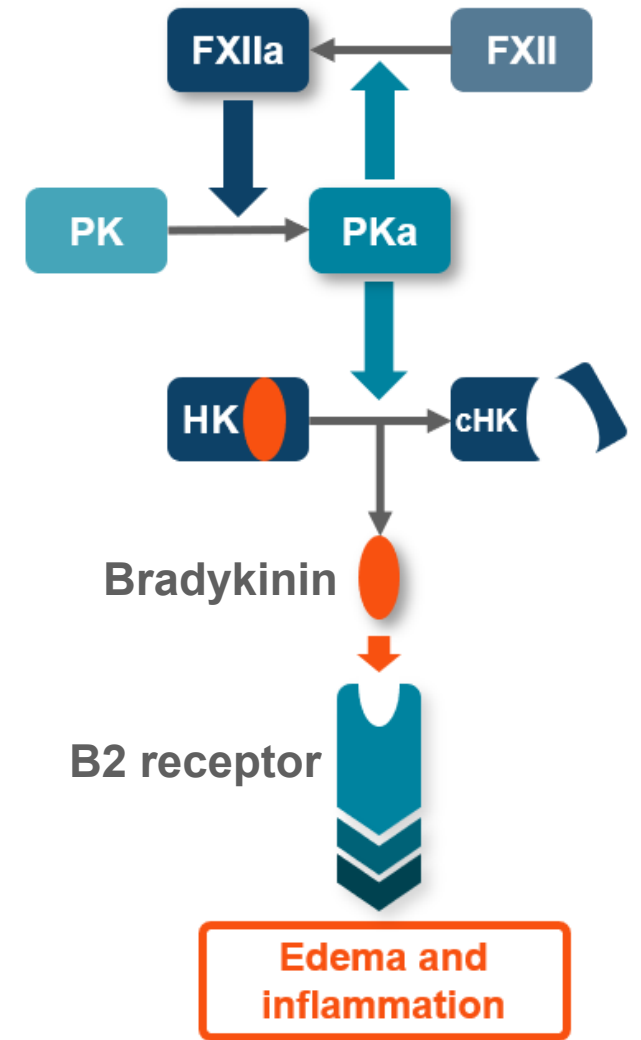
Study Overview

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 HAE¹⁻⁴
 - 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)

sPKa activity assay

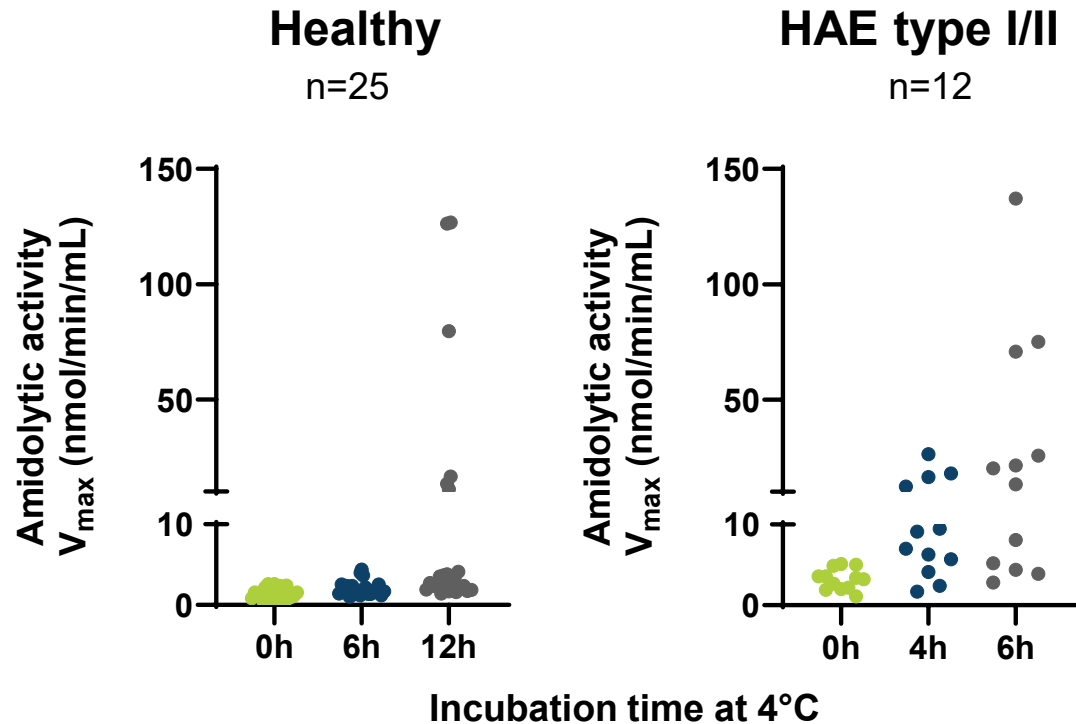
- Amidolytic activity (V_{max}) was measured using H-D-Pro-Phe-Arg-pNA·2HCl in the absence and presence of a specific PKa inhibitor, KV999272
- sPKa was quantified by the subtraction of amidolytic activity not inhibited by KV999272 from the total measured activity

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

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.

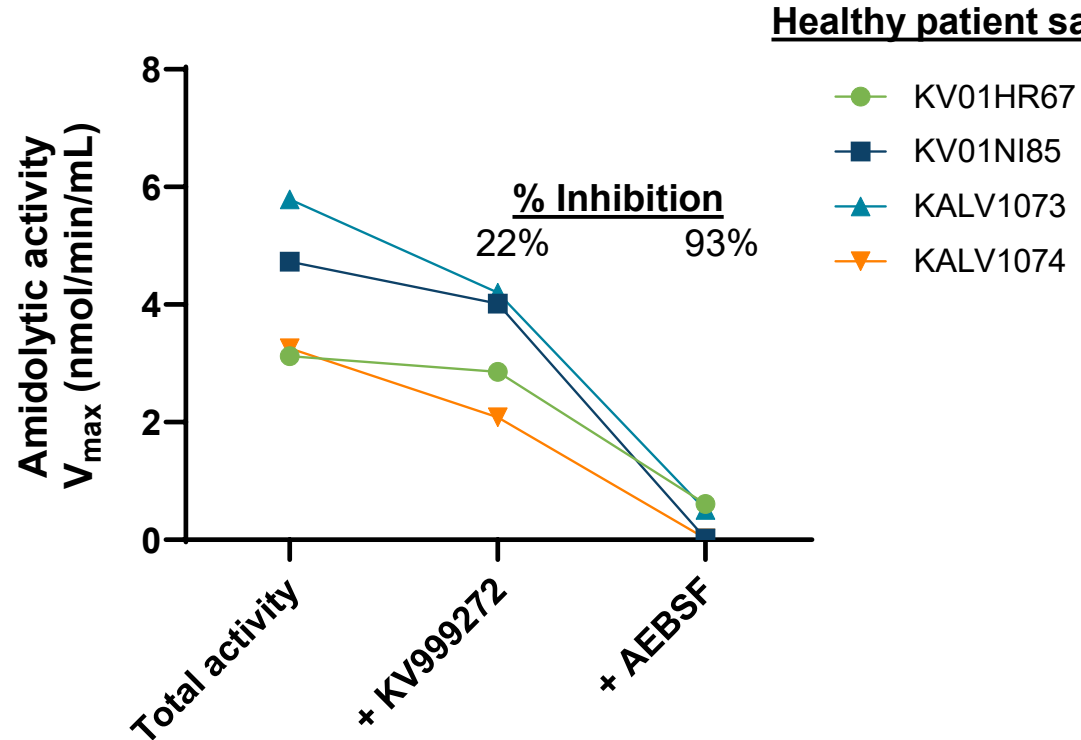
Cold Exposure Increases Amidolytic Activity in Plasma



- Exposure of plasma to cold (4°C) has been shown to increase PKa activity¹
- 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

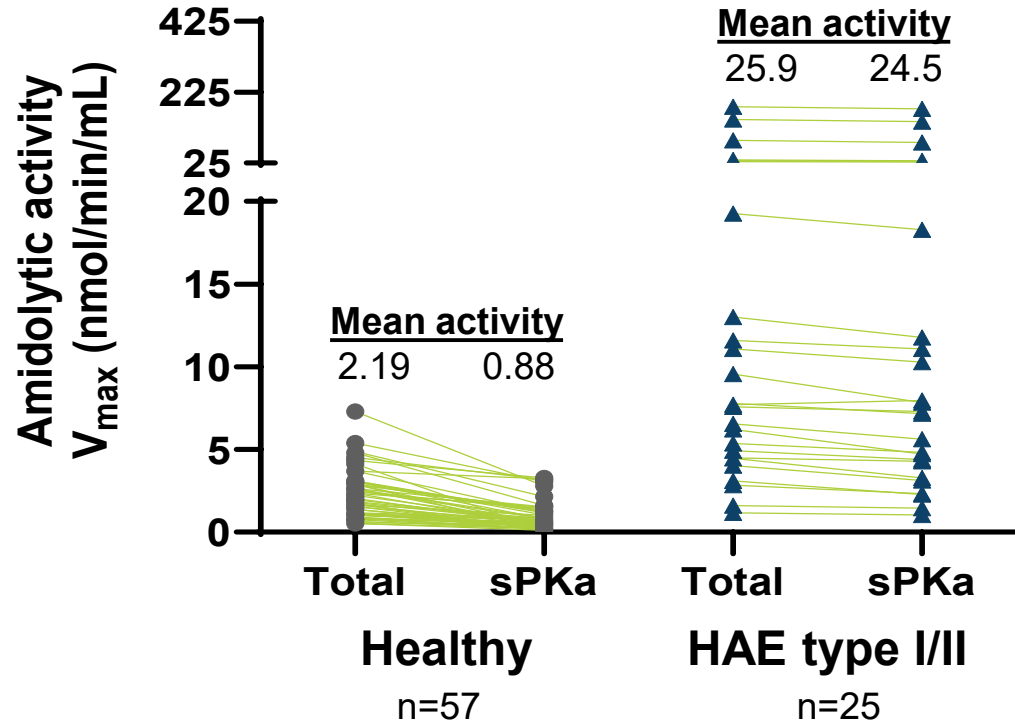
6 h of cold exposure of plasma was chosen as the optimal time point to increase PKa activity in HAE plasma while maintaining low PKa activity in healthy plasma

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 tryptase³
- 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)

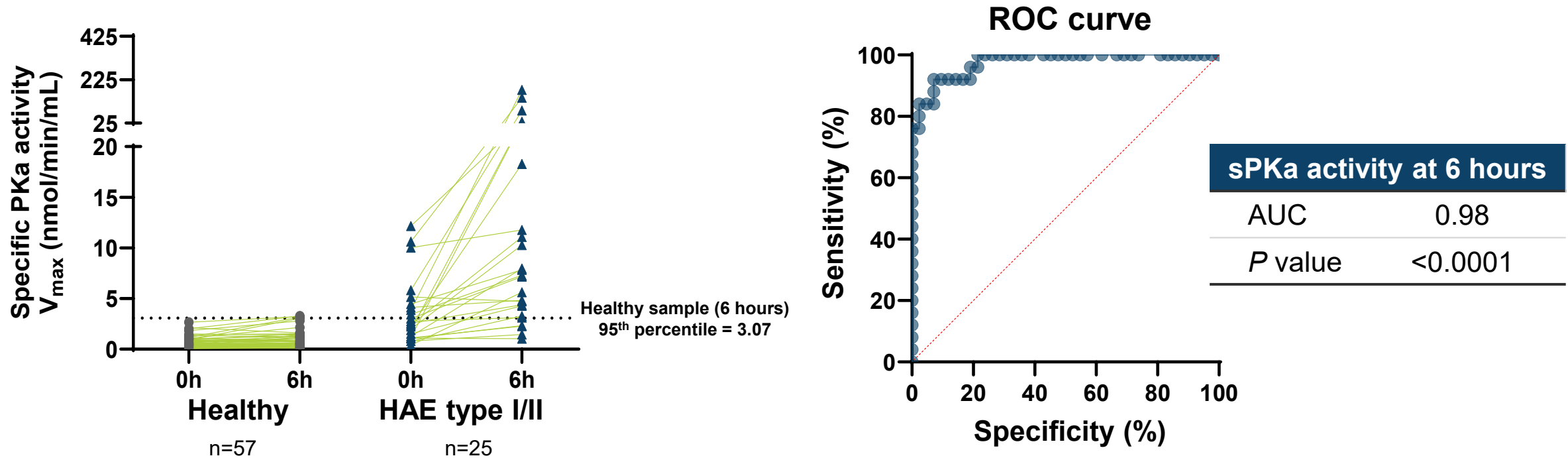
Measuring sPKa Activity Improves Assay Sensitivity



- 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%

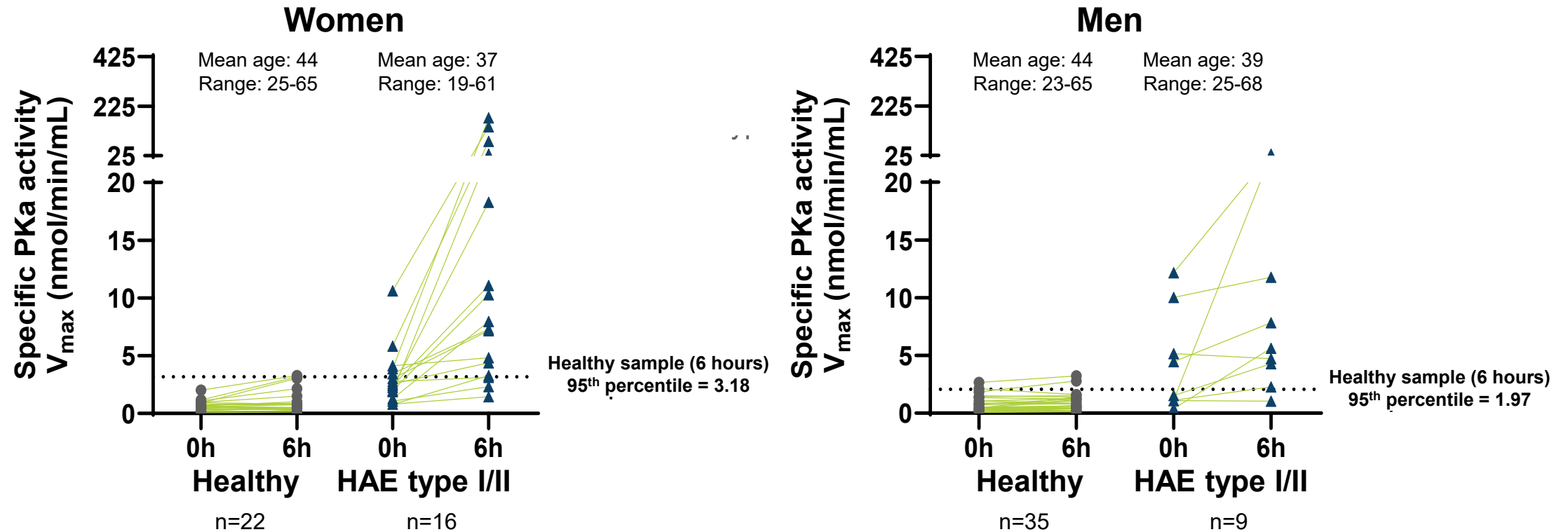
Measuring sPKa activity reduces assay non-specific background amidolytic activity in healthy plasma and thereby improved assay selectivity and specificity

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



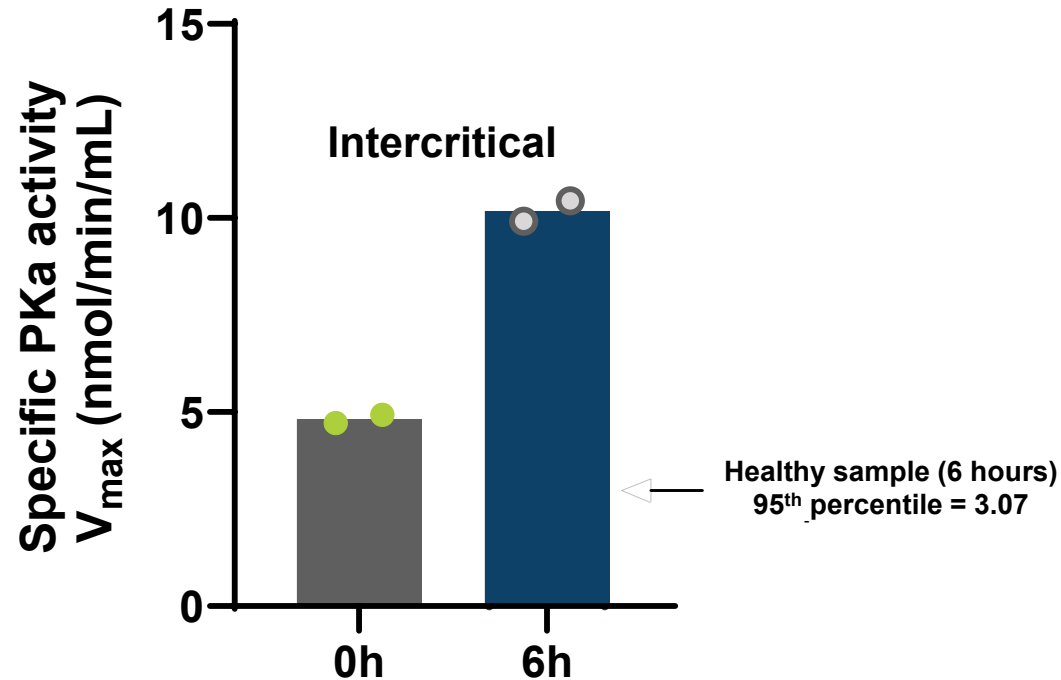
- sPKa activity in plasma measured after 6 h of cold exposure can show differentiation between HAE (intercritical period) plasma and healthy plasma, with 84% sensitivity and 95% specificity
- ROC curve shows excellent test performance

sPKa Activity in Plasma Samples From Women and Men



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

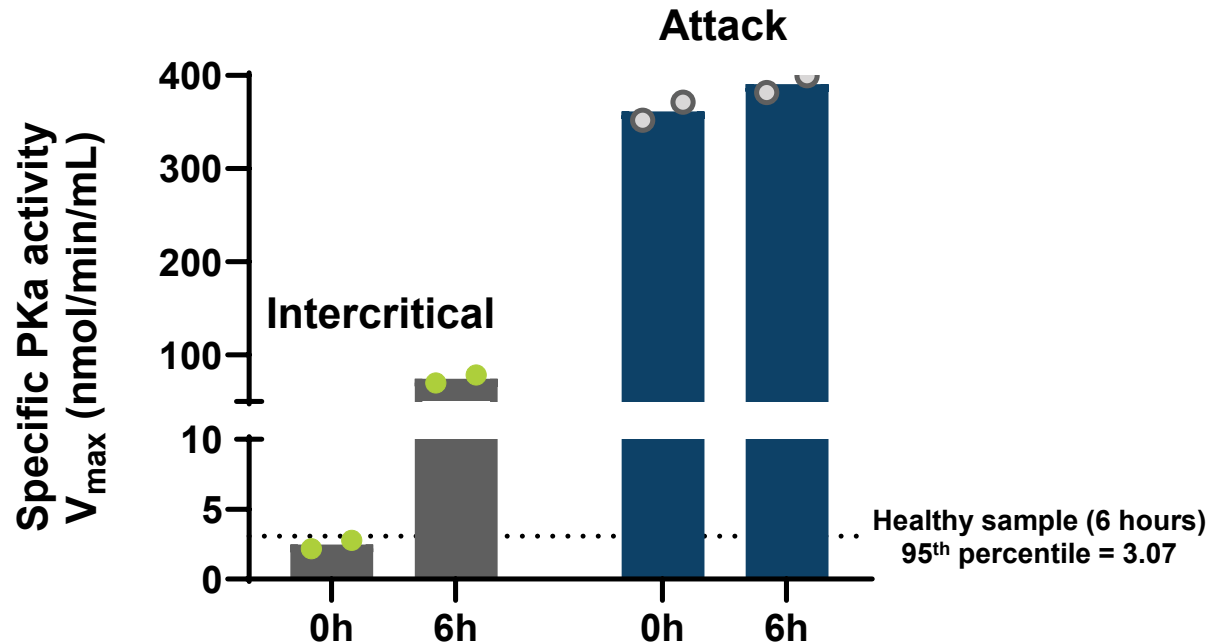
sPKa Activity in HAE-nC1INH: Case Study 1



- 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)

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 sample and during-attack sample

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

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

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