Novel ELISA for the measurement of human Semaphorin 4D in dialysis patients

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CONCLUSION

Our novel ELISA provides a reliable and accurate tool for the quantitative determination of soluble, biologically active Semaphorin 4D in human samples and could help to gain insight into the underlying immunoregulatory mechanisms of inflammation in chronic kidney disease.

SUMMARY

Semaphorin 4D or CD100 is a member of a family of trans-membrane and secreted proteins that regulates key cellular functions and is involved in cell-cell communication. Semaphorin 4D is expressed in normal kidneys and has been shown to have a pathogenic role in a model of experimental glomerulonephritis.
The number of studies on circulating levels of soluble Semaphorin 4D (sSEMA4D) in humans is limited due to the lack of a reliable tool to measure the protein in serum or plasma.

Thus, our aim was to develop a specific assay that enables the accurate measurement of sSEMA4D in human samples. The assay utilizes two monoclonal anti-human Semaphorin 4D antibodies, both recognizing conformational epitopes on Semaphorin 4D. The epitopes have been mapped by overlapping constrained peptides and shown to involve amino acids AA30-AA34 and amino acids AA238-AA241, respectively.

Our results demonstrate that sSEMA4D can reliably be measured in various plasma preparations (EDTA, citrate, heparin) with a mean coefficient of variation of <8% between these matrices. Serum measurement of sSEMA4D showed in average a 3 fold higher concentration than plasma, indicating that the measurement of sSEAMA4D in blood samples may be interfered by the in vitro release from platelets. Hence, the assay was optimized for human plasma samples only. The assay covers a wide calibration range between 0 to 2000 pmol/l. Assay characteristics, such as precision, dilution linearity, and spike/recovery as well as sample stability have been analysed and meet the standards of acceptance. The sSEMA4D median plasma concentration was higher in samples from dialysis patients 360 (281-520) pmol/l (n=18) compared with samples from apparently healthy individuals 210 (133-350) pmol/l (n=10).
Our findings indicate that sSEMA4D may be an interesting candidate for further investigations in the field of kidney dysfunction.

Our novel ELISA provides a reliable and accurate tool for the quantitative determination of soluble, biologically active Semaphorin 4D in human samples and could help to gain insight into the underlying immunoregulatory mechanisms of inflammation in chronic kidney disease.

METHODS

The ELISA is based on a sandwich type format with an immobilized monoclonal mouse antibody used to capture human soluble Semaphorin 4D which is subsequently detected with a HRPO labelled monclonal human anti-Semaphorin 4D F(ab)2 antibody.

Assay Protocol	
100µl	Assay buffer
10 µl	Sample/ Standard
3h @ 18-24°C,	Washing
100 µl	Conjugate
1h @ 18-24°C,	Washing
100 µl	Substrate
30 Min @ RT	
50 µl	Stop solution

ASSAY CHARACTERISTICS

Typical standard curve

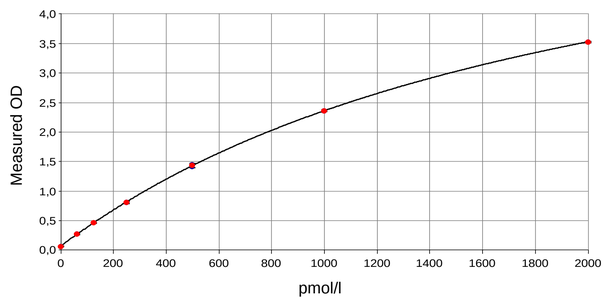

Fig. 1: Assay calibration curve - recombinant human soluble Semaphorin 4D was spiked into human citrate plasma matrix to a range of 62.5 – 2000 pmol/l.

Precision

	Precision[CV in %]	
	Mid calibration range	High calibration range
Intra assay	8%	6%
Inter assay	11%	5%

Tab. 1: Intra- and inter-assay precision:
Intra-assay precision was determined by 1 operator with 2 samples and 5 replicates of each sample within 1 kit lot. The inter-assay precision was determined by 3 operators with 2 samples and at least 2 replicates in different kit lots.

Spike/ Recovery

Matrix	Mean S/R [%]	
	+200 pmol/l	+1,000 pmol/l
EDTA plasma (n=6)	116	92
Heparin plasma (n=2)	94	109
Citrate plasma (n=2)	79	83

Tab. 2: Spike/recovery data for plasma samples:
Samples were spiked with two concentrations of recombinant soluble Semaphorin 4D and recovery was calculated.

Dilution linearity

Matrix	Mean R of dilution steps [%]	
	1+1	1+3
EDTA plasma (n=4)	106	92
Heparin plasma (n=2)	103	94
Citrate plasma (n=2)	111	109

Tab. 3: Dilution linearity data of plasma samples containing endogenous sSEMA4D: Samples were diluted with assay buffer (according to assay protocol) and dilution linearity was calculated.

ANALYTE

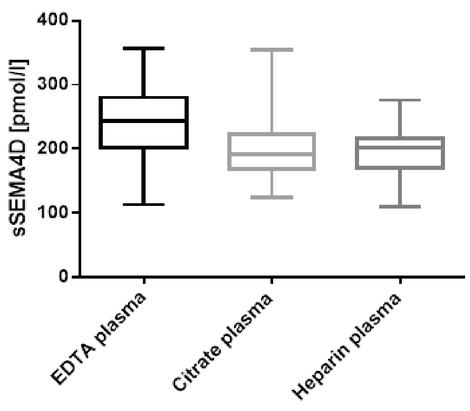

Fig. 2: Comparison of soluble Semaphorin 4D levels from single donors in different matrices (EDTA, heparin and citrate plasma) in an apparently healthy population.

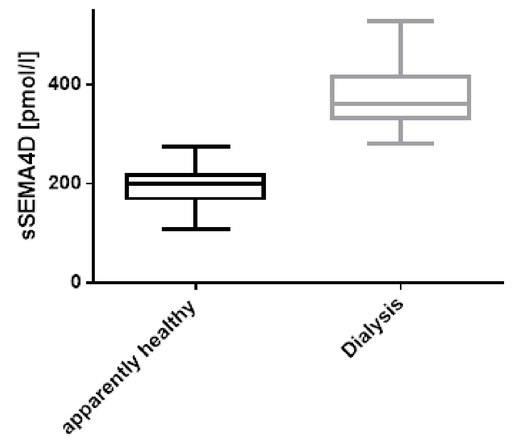

Fig. 3: Comparison of soluble Semaphorin 4D levels in plasma samples from apparently healthy donors with samples from dialysis patients.

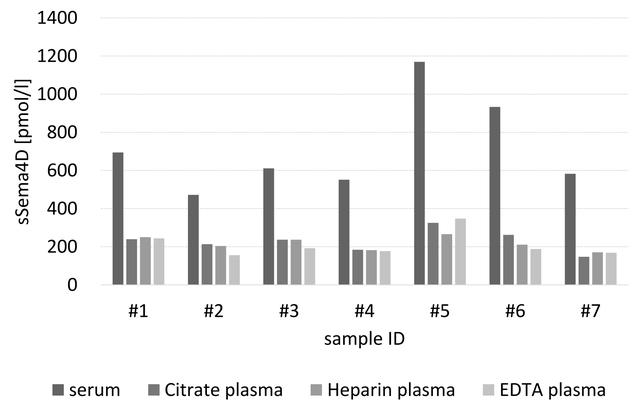

Fig. 4: Comparison of soluble Semaphorin 4D levels from single donors in matrices (serum, EDTA-, heparin and citrate plasma).

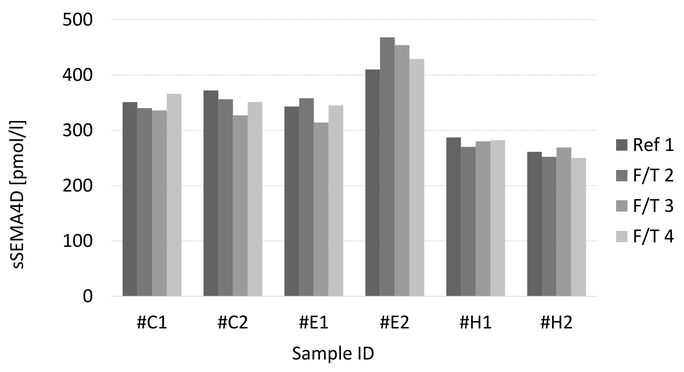

Fig. 5: Freeze/Thaw stability of human soluble Semaphorin 4D in different sample matrices. Recovery was determined for two samples per matrix.

LITERATURE

Li M et al., (2009): Endogenous CD100 promotes glomerular injury and macrophage recruitment in experimental crescentic glomerulonephritis. *Immunology; 128:114–122.*
Maleki KT et al. (2016): Soluble SEMA4D/CD100: A novel immunoregulator in infectious and inflammatory diseases. *Clin Immunol; 163: 52–59.*

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