MASS SPECTROMETRY OF INTACT PROTEIN AND TRYPTIC PEPTIDES IN THE ANALYSIS OF DES LEU ALBUMIN – A NOVEL MARKER FOR CHRONIC PANCREATITIS.

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INTRODUCTION

Chronic pancreatitis is a progressive inflammatory disease leading to pancreatic insufficiency. The diagnosis of chronic pancreatitis is challenging, especially in early disease and the current tests have low sensitivity, may be invasive or have limited availability. At Canterbury Health Laboratories we have previously identified a truncated form of albumin lacking the C-terminal leucine known as desLeu albumin[1]

Albumin ...CFAEEGKKLVAASQAALGL

Carboxypeptidase A ↓ (Removes the C terminal Leucine)

des Leu Albumin ...CFAEEGKKLVAASQAALG

and a truncated form of β-Fibrinogen lacking the C-terminal glutamine known as des Gln β-Fibrinogen[2]

β-Fibrinogen ...YSMRKMSMKIRPFFPQQ

Carboxypeptidase A ↓ (Removes the C terminal glutamine)

des Gln β-Fibrinogen ...YSMRKMSMKIRPFFPQ

During pancreatic inflammation Carboxypeptidase A is released into the circulation along with amylase and lipase. However, unlike amylase, carboxypeptidase A is highly specific for the pancreas, thus the formation of des-Leu albumin and β-Fibrinogen is also likely to be specific for pancreatic inflammation.

With the recent application of Mass Spectrometry in the routine laboratory we now have the technology to develop simple methods for these novel markers and evaluate their clinical utility for the diagnosis and monitoring of pancreatitis[3].

We have investigated two methods of measuring des-Leu albumin in plasma by mass spectrometry;

Method 1 measures the mass spectrum of the intact proteins in plasma
Method 2 measures the mass specta of peptides present in tryptic digests of plasma.

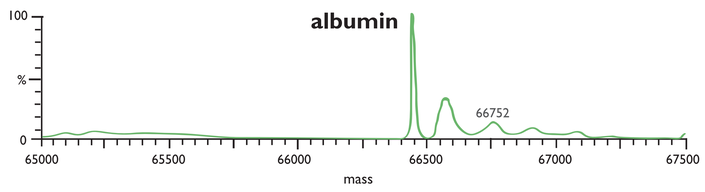
Albumin digest yields LVAASQAALGL and LVAASQAALG
β-Fibrinogen digest yields IRPFFPQQ and IRPFFPQ

We have used these two methods to measure des Leu albumin from patients with and without pancreatitis and demonstrate that des Leu albumin and des Gln beta fibrinogen are potential markers for chronic pancreatitis.

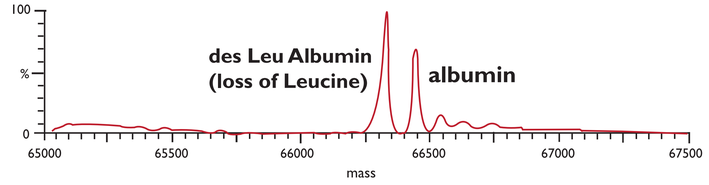
ANALYSIS OF INTACT PROTEIN

Analysis of intact protein by direct aspiration into a VG Platform single quad MS m/z 1200 – 1800 collected for 2 minutes and deconvoluted with Waters MassLynx maximum entropy software

"Normal" Patient des Leu Albumin = <2%

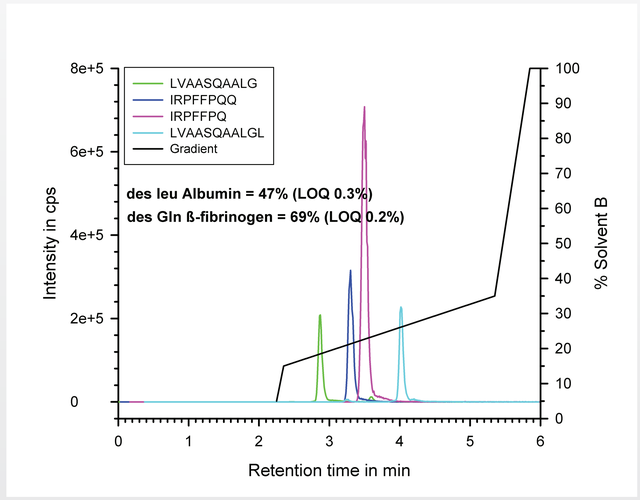


Patient with "Acute Pancreatitis" des Leu Albumin = 58%



ANALYSIS OF PLASMA DIGESTS

Typtic peptides analysed on an Shimadzu LC / ABSciex 4000 tandem MS, Column: Phenomenex Jupiter 4 µm, Proteo 90 Å, 150 x 2.00 mm, Solvent A: 4% Acetonitrile 0.1% Formic Acid, Solvent B 80% Acetonitrile, total flow rate 0.3 mL /min

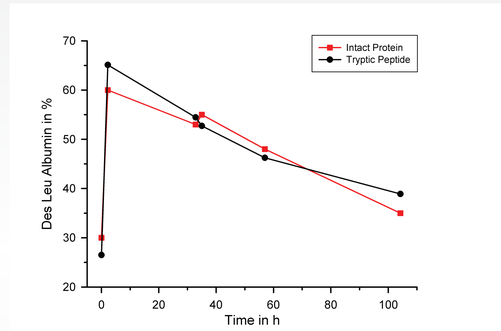


Method for tryptic digestion of plasma

1. Dilute 2.5µL of plasma to 10µl with water.
2. Heat at 95deg for 5min (destroys proteases)
3. Cool, Add 1µL of 2µg Trypsin(2mg/mL), 2µL Ammonium bicarbonate(0.5M)
4. Incubate 37 deg overnight
5. Dilute to 500µL with 5% Acetonitrile, 0.1%Formic acid
6. Inject 6µL into LC for ABSciex 4000

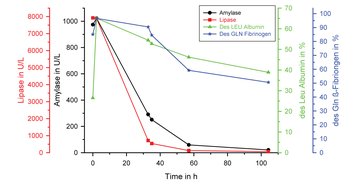
PATIENT RESULTS

Comparison of intact protein v tryptic peptides

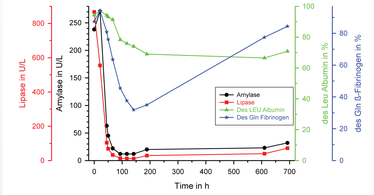


Both methods agree - this supports the science behind this work

Acute Pancreatitis



Chronic Pancreatitis



CONCLUSION

Des Leu Albumin (ref range <10%) and des Gln β-Fibrinogen (ref range <25%) are both raised and sustained in Acute and Chronic Pancreatitis.

REFERENCES

(1) "Three truncated forms of serum albumin associated with pancreatic psuedocyst". Biochem Biophs Acta 2000;1481:337-43. Brennan SO, George PM.
(2) "Modified form of the fibrinogen Bbeta chain (des Gln Bbeta), a potential long-lived marker of pancreatitis". Clin Chem 2007;53:2105-11. Schimdt D, Brennan SO.
(3) "A mass-spectroscopic method for measuring des Leu albumin – a novel marker for chronic panceatitis" Clin Biochem 2012;45: 1664-8. Ireland RO, Brennan SO, Gerrard JA, Walmsley TA, George PM, King RI.

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