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

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.2

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 spectra of peptides present in tryptic digests of plasma.

ANALYSIS OF INTACT PROTEIN

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

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. CooLAdd 1µL of 2ug Trypsin (2ng/mL). 2µL Ammonium bicarbonate(0.5M)
4. Incubate 37 deg overnight
5. Dilute to 500µL with 5% Acetoneitrile. 0.1% Formic acid
6. Inject 6µL into LC for ABSciex 4000

PATIENT RESULTS

Comparison of intact protein v tryptic peptides

ANALYSIS OF PLASMA DIGESTS

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

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