Student: Regan Clarke
Title: A new monoclonal assay for BNP signal peptide
Supervisor: Associate Professor Chris Pemberton
Sponsor: Upstream Medical Technologies Limited

Introduction:
Acute coronary syndrome (ACS) comprises approximately 10 % of ED admissions. ACS encompasses several cardiac conditions, one of which, unstable angina (UA), is not easily diagnosed, and can lead to further cardiac complications. UA currently requires stress testing, imaging, and family history checks to confirm a diagnosis. There is a clinical need for a rapid biomarker based diagnostic tool for UA to better assess risk in ED patients in a time appropriate manner.

BNP is a hormone released in cardiac stress and is indicative of heart failure. Processing of BNP precursors involves cleavage of a signal sequence. It was thought that this sequence was degraded, but a fragment of this sequence has been found to circulate in the bloodstream, in greater amounts following an episode of UA. BNPsp, when combined with a suite of cardiac biomarkers, forms a ratio able to diagnose UA with a diagnostic power (AUROC) of 0.79 (closer to 1 is more useful as a diagnostic tool), and may also predict the risk of further cardiac complications. A radioassay for determining BNPsp concentrations is available, but this is time-consuming, requires radioactive isotopes, and uses specialised equipment. A rapid monoclonal antibody based assay has not yet been developed.

An ELISA (enzyme-linked immunosorbent assay) was employed to determine plasma BNPsp concentrations. Both competitive and sandwich ELISAs were developed. A competitive ELISA requires tagged BNPsp (to create a signal) and untagged BNPsp from human plasma (in unknown concentrations) competing for binding to an antibody until equilibrium is reached. The signal produced is inversely proportional to the concentration of the non-tagged peptide in the sample, so the concentration of BNPsp in a patient sample can be determined by comparison with a standard curve. A sandwich ELISA uses 2 antibodies which bind different regions of BNPsp and is used for increased specificity and sensitivity. ELISA can be performed on a 96-well plate for high-throughput testing.

Aim:
The aim of this project was to develop an antibody based assay in the form of a sandwich or competitive ELISA, for the determination of the BNP signal peptide concentration in human plasma. This assay could be used clinically in the diagnosis of UA, or as a tool for laboratory research purposes.

Impact:
Development of an ELISA for BNPsp could be used as a clinical risk assessment tool for patients in the emergency department presenting with chest pain. The assay could also be used for research purposes in studying cardiovascular disease.

Method:
Cell supernatant from antibody producing cells raised against the protein of interest were collected and purified. The resulting antibodies were used in development of the ELISA. 96 well plates were coated with antibody and various conditions were systematically changed (buffer types, antibody concentrations, tagged peptide concentrations, incubation times,

incubation temperatures) to determine the conditions required to form a standard curve with an appropriate absorbance range.

Once a standard curve was developed, a set of patient samples from the INSPIRE study were tested in the assay after being extracted and concentrated. The concentrations determined by comparison against a standard curve were compared to that of the previous assay which was carried out on the same samples. Regression statistics were calculated to determine the effectiveness of the assay at determining concentrations of BNPsp in comparison to a previously characterised assay.

Results:
Antibodies were successfully purified and used to coat 96-well plates. Suitable antibody and tagged peptide concentrations, as well as incubation times were determined in the competitive format ELISA which produced a standard curve. It was found that a 24 hour incubation was necessary for the assay to reach equilibrium. This could then be used to measure patient samples. 19 samples from the INSPIRE study were assayed and the BNPsp concentrations were measured. The BNPsp concentrations were compared to the concentrations determined by the previously characterised radioassay and were found to be 20 x higher in the competitive ELISA. Pearson regression analysis did not find a correlation between the two assays ($r = -0.180$). Further testing is required to determine why this might be the case. Complementary work on a sandwich ELISA produced a similar standard curve with only a 3 hour sample incubation time.

Conclusion:
A standard curve for a competitive ELISA was produced. This was able to be used on patient samples, but results did not correlate with previous analysis. The low sensitivity and 24 hour incubation required for signal generation makes this assay currently insufficient for emergency department diagnosis. Further development of the competitive format ELISA may be able to improve sensitivity and sample turnaround such that the test is suitable as a diagnostic or research tool. The sandwich format ELISA shows promise in determining BNPsp concentrations in human plasma in a more time-sensitive manner.