Towards a better test for managing arthritis

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What is the problem?

Rheumatoid Arthritis (RA) is a debilitating chronic inflammatory disease of the joints which results in their eventual degeneration (see Figure 1). A 2010 report estimated RA cost the UK economy £3.8 to £4.8 billion per year with this predicted to rise as the population ages and, thus, the prevalence of RA increases.

A range of treatments exist for RA from classical anti-inflammatories, such as ibuprofen, to modern targeted therapies, such as anti-TNF agents. However, knowing how best to treat patients is difficult due to disease variability. A broad spectrum of responsiveness to therapies is observed in different patients and their disease progression varies.

Tests are available for the diagnosis of disease but they provide limited or no information to aid in managing a patient's treatment. There is a clear need for biological markers which could provide information that will predict a patient's responsiveness to therapy and their disease progression.

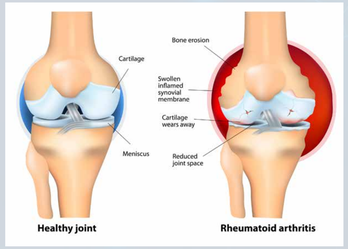

Figure 1: A representation of a healthy and arthritic joint. Image source: https://commons.wikimedia.org/wiki/File:Rheumatoid-Arthritis.png created by National Library of Medicine US.

What are we interested in?

Tenascin-C (TNC) is a pro-inflammatory protein which has been implicated as a driving factor in RA. It has been shown to be deposited in the inflamed joint where it interacts with cells to activate and support pro-inflammatory processes. For example, TNC has been shown to stimulate immune cells to produce Tumour Necrosis Factor α (TNFα), a pro-inflammatory signalling molecule well known for playing a role in RA. Furthermore, production of TNC itself has been shown to be driven by other pro-inflammatory factors, including TNFα. Thus, a positive feedback loop is created whereby TNC drives the production of pro-inflammatory factors which in turn drive the production of more TNC (see Figure 2). In this role, it is thought that TNC is preventing the inflammation from subsiding, thereby supporting its progression to a long term diseased state.

We wanted to investigate if TNC could be a good marker of disease that could be measured in RA patients.

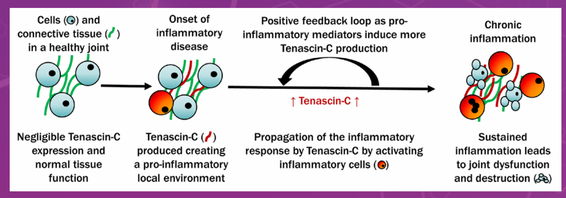

Figure 2: Diagrammatic representation of the role of TNC in the disease process of arthritis.

What did we do?

We predicted that as TNC accumulates at the site of inflammation in the joint fluid it will also make its way into the blood at elevated levels in RA patients. As such, we developed a way of measuring TNC in the blood using an Enzyme Linked Immunosorbent Assay (ELISA) technique (see Figure 3).

Using known concentrations of purified TNC we set up a standard curve so we could convert the test's absorbance output into ng/ml of TNC. The test performed well and was able to detect tiny amounts of TNC, down to about 1ng/ml (1ng = 1 billionth of a mg) (see Figure 4).

We used our ELISA test to analyse blood samples from 81 healthy controls and 106 RA patients.

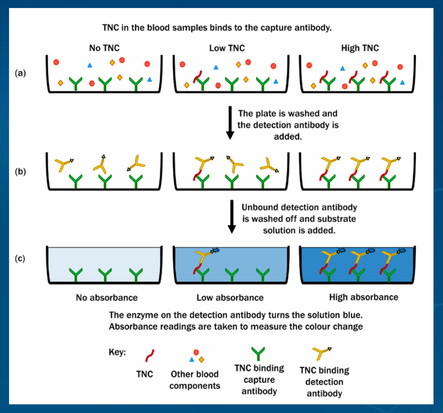

Figure 3: Schematic representation of the Tenascin-C (TNC) Enzyme Linked Immunosorbent Assay (ELISA).

A blood sample is added to a well of a 96 well plate and the TNC is captured by an antibody which specifically binds to it (a). Other blood components, which do not bind, are washed away and a second TNC detecting antibody, which has an enzyme attached to it, is added which also binds specifically to the TNC (b). Unbound detecting antibody is washed away and TNC is detected by addition of a solution which is turned blue by the enzyme on the detecting antibody (c). The degree of colour change is proportional to the amount of bound TNC and is measured by reading the solution's absorbance.

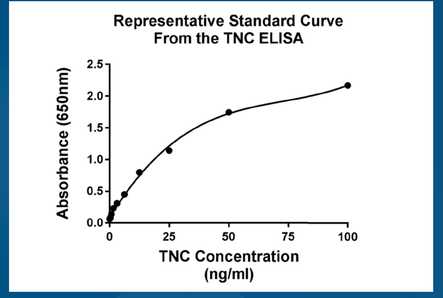

Figure 4: A standard curve made using serial dilutions of purified TNC in the TNC ELISA.

What did we find?

We found that the healthy controls had an average blood TNC concentration of 530ng/ml. This was elevated to a significant degree in RA patient samples, which had an average concentration of 902ng/ml (see Figure 5).

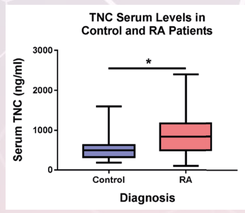

Figure 5: TNC levels in healthy control (n=81) and RA patient's (n=106) serum (part of the blood) as measured by custom ELISA. TNC was significantly elevated in the RA patients compared to controls (* P<0.001).

What does this mean?

We have developed a test to measure the levels of TNC and used it to confirm that TNC is elevated in RA. We are continuing to validate the test by analysing more samples from a wider range of sources and conditions other than RA. We hope that measuring the levels of TNC with our test may help predict the likely disease progression of patients and how they will respond to a particular treatment.

Who am I?

I am a PhD student at the Roslin Institute, University of Edinburgh, working in the laboratory of Professor Colin Farquharson. I started my PhD in 2015 after completing an undergraduate degree in Molecular and Cellular Biology at the University of Bath. I am currently enjoying the world of biomedical research and upon completing my project I am looking to go into industrial research. I would like to acknowledge and thank my academic supervisors, Professor Colin Farquharson, Professor Kim Midwood and Dr Gerry McLachlan, as well as Dr Jeff Brady and Dr Mel Lewis who work with me on this project at Axis Shield Diagnostics Ltd.

James started his PhD project entitled *"Development of assays to assess the clinical utility of tenascin-C"* in 2015 and while principally based at the University of Edinburgh, supervised by Professor Colin Farquharson, he is also working closely with Axis Shield Diagnostics Ltd.