CHIEF SCIENTIST OFFICE

CODE: ASM/14/11

RESEARCH PROJECT BRIEFING

STABILITY-RA

How Stable are Biomarkers of Rheumatoid Arthritis?

The STABILITY RA Study

AIMS

The aim of the Stability-RA study was to investigate the stability of biomarkers (molecules in the body that are measurable and change in the presence of disease) under different sample collection and processing conditions that reflect the real world clinical practice. We investigated the following conditions to assess their impact on biomarker expression; 1) time of sample collection, looking over the course of 1 day, 1 week and 1 month, 2) overnight fasting (not eating any food since the night before blood samples are taken) and 3) sample processing delays. The biomarkers we were looking for can be found in whole blood, blood serum (the liquid remaining following removal of white and red blood from whole blood) and urine.

Potential biomarkers have been identified for rheumatoid arthritis, however, we don't know if factors such as those listed above would impact their expression and therefore their usefulness as biomarkers. Thus, the samples collected here were assessed to see if these factors had a significant impact on the expression of the previously identified biomarkers.

For this report we specifically focussed on 12 biomarkers that are used to calculate the Vectra disease activity (VectraDA) score. The VectraDA score is a blood test that assesses disease activity by measuring 12 protein markers and applying a pre-specified algorithm (a mathematical equation that that's into consideration all of the factors that influence the score) generating a score between 0-100. A score of 1-29 indicates a low disease activity score, 30-44 indicates a moderate disease activity score and 45-100 indicates a high disease activity score.

KEY FINDINGS

- The time of day blood is taken from a patient and overnight fasting can have an effect on the outcome of the VectraDA test for rheumatoid arthritis.

- Delays in the laboratory processing of blood samples has a marked effect on the outcome of the VectraDA test for rheumatoid arthritis due to non-disease specific changes in biomarker expression .

WHAT DID THE STUDY INVOLVE?

A total of 10 patients with active RA were recruited from across Glasgow for the STABILITY RA study, all of the patients who entered into the trial met the inclusion criteria laid out in the study protocol.

Patients that were recruited into the study and asked to attend 5 study visits; day 1, day 2, day 3, day 4 and day 28. On day 1 the patient was asked to give blood samples before any food (overnight fasted samples) at 8am, and then again at 9am, 11am, 1pm and 3pm. On day 2 the patient gave blood at 9am, on day 3 the patient again gave fasted bloods at 8am and on day 4 the patient gave bloods at 9am. The final visit was on day 28 where again the patient gave blood samples at 9am. Acute phase reactants (proteins in the blood that increase with any form of inflammation), physical examination and disease activity score (DAS28) were assessed at the day 1 and day 28 visit, gaining a better understanding of the participants disease state.

Bloods were processed according to a standard protocol, leaving the blood for 30 minutes before the removal of any cells and collecting the serum. Some samples were subject to delays in the processing protocol, reflecting delays that could be experienced in the real world clinical setting. Blood that was subject to processing delays were left to sit on the bench for an additional 30, 90 and 210 minutes before processing.

As mentioned above, for the purpose of this report we will focus on VectraDA analysis. To this end a VectraDA score for each sample was generated by Crescendo Bioscience in the USA. Here we have investigated the stability of the VectraDA score specifically when blood is sampled over 5 time points in day 1 or after processing delays.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

One of the outcomes of Stability RA was to assess if certain collection conditions, that could be encountered within the real world clinical setting, have an impact on the stability of rheumatology tests, such as the VectraDA test for rheumatoid arthritis

Here, one of the factors investigated was the impact of the time of day that blood sample was collected. In figure 1, the mean VectraDA scores of all 10 patients was investigated at 5 time points throughout day 1. The first blood sample was taken at 8am before the patients had eaten any food, known as an overnight fasted sample, the rest of the samples were non-fasted and were obtained at 9am, 11am, 1pm and 3pm. The results show that at 8am and 9am most of the patients have a high disease activity score, meaning that their VectraDA score is greater than 50. At 11am there is a statistically significant drop in the score resulting in the a moderate disease activity score (a VectraDA score between 30-44). By 1pm and 3pm the average score returns to greater than 50, with the patients once again having a disease activity score classified as high. It is possible that the drop in the score observed at 11am, taking the patients from a high disease activity score to a moderate score, is due to the processing of food.

The patients had not eaten anything since the night before their appointment and gave their first blood samples before eating. Therefore, by the time the 3rd sample was taken at 11am, the ingested food may have impacted the level of expression of the VectraDA biomarkers. Further to this, time of day can also alter the level of certain proteins in the blood. The body has its own clock, known as the circadian rhythm, that can help us release the right hormones to make us sleepy at night and more awake during the day. This clock has also been shown to have lots other functions, such as modifying the release of inflammatory cytokines, such as the ones that are measured in the VectraDA analysis. Therefore, circadian rhythm may play a role in altering the expression of these markers and hence modify the VectraDA score depending on when the blood samples are taken.

Another factor investigated with regards to the VectraDA score was sample processing delays. Standard practice is to allow the blood tubes to sit for 30 minutes before processing, which here we have called time 0. Tubes were also left to sit for an additional 30, 90 or 210 minutes before processing, assessing what impact this may have on the VectraDA score. In figure 2, we can see that the scores at the normal processing time (time 0) are statistically different from the scores calculated when the blood that was left to sit for either an additional 30 or 90 minutes. The VectraDA disease activity scores change from high at time point 0, to moderate at the 30 and 90 minute time points. It is possible that the proteins measured initially at time 0 are being broken down in the tube. It is also interesting that the high disease activity returns at the 210 minute time point. This is possibly due to cells beginning to dye within the tube and causing other cells to release the markers we measure in response to cell death and not because of any factors related to disease.

Time of day that the blood sample is taken has an effect on the VectraDA score.

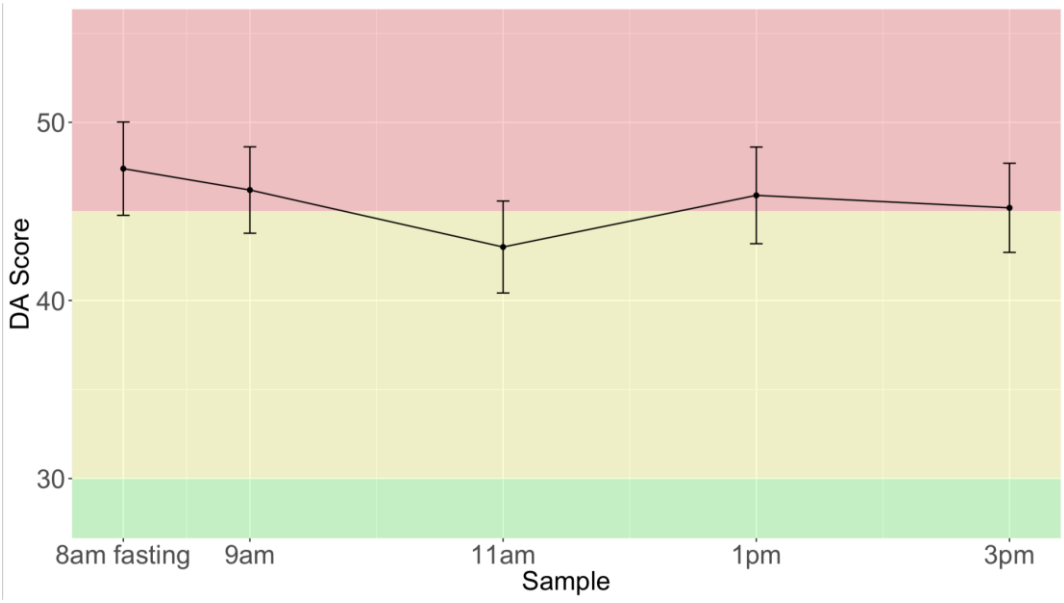

Figure 1: Mean disease activity scores when blood is taken form 10 RA patients at multiple time points throughout the day 1 (8am (fasted), 9am, 11am,1pm and 3pm) and subject to Vectra DA analysis. Bars show the standard error between the 10 patients.

Delaying the processing of the blood has a significant effect on the VectraDA score.

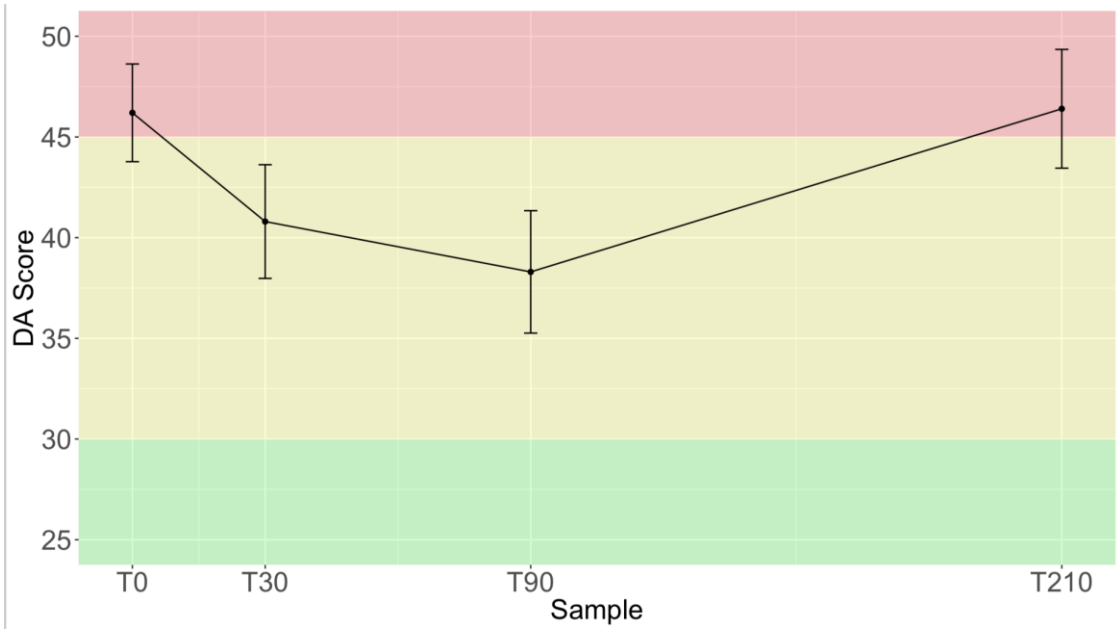

Figure 2: Mean disease activity score when blood is taken form 10 RA patients at one time point and subject to delays in processing T0 (standard processing), T30 (30 minute delay), T90 (90 minute delay) T210 (210 minute delay) and subject to Vectra DA analysis. Bars show the standard error between the 10 patients.

Further to the analysis that we have carried out with regards to disease activity through the VectraDA test, we will also be conducting analysis on several other data sets produced throughout study. These will included, looking at RNA transcripts in the serum (messages made from DNA), looking at proteins in the serum (the final product made from the transcript messages) and looking at metabolites in the serum (products from chemical process in the body). Once each has been analysed we will work to integrate these data sets to enhance our understanding of the stability of potential biomarkers.

Despite the small sample number we have been able to find statistically significant changes in disease activity scores, depending on the blood drawl time and the processing methods.

WHAT IMPACT COULD THE FINDINGS HAVE?

The findings of this study, outlined within this report, may have potential impact on:

- Clinical Practice: These findings suggest that when we take blood from patients to carry out certain test relating to rheumatoid disease activity, factors such as circadian rhythm and fasting may have a non-disease related impact on their interpretation.
- Laboratory Processing: These findings could help to generate supporting data regarding the strict adherence to blood processing protocols. As by leaving the bloods for longer than stated in the standard protocol, biomarkers may be degraded (broken down) or increased in a non-disease specific manner. This again can have implication on the interpretation of disease activity scores based on biomarkers.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We will share the findings of the study by publishing in peer-reviewed journals and by presenting the data at national and international conferences. We will also present this data to political and public engagement groups, e.g the arthritis Cross Party Group in Holyrood, at Rheumatosphere public engagement events and at NRAS meetings.

We will make our data generally available by submission to public databases and also use the data to implement changes to our clinical work locally. Overall these findings will support the strong foundations of precision medicine that is becoming a major theme for the medical communities and life science sector in Scotland.

CONCLUSION

When designing disease activity tests, such as the VectraDA test, that involve biomarker measurement in blood, investigation into the potential affects of factors such as, circadian rhythm, fasting and processing delays that reflect the real world clinical practice must considered, assessing the robust nature of the test.

Our expert statistical reviewers and bio-informatitions consider that this exemplar project was more than sufficient (in terms of the number of participants) to elucidate the core changes and stability of molecular patterns in people with rheumatoid arthritis.

RESEARCH TEAM & CONTACT

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Additional Information

Analysis for the transcriptomic metabolimic and proteomic data is still on-going.