Complementary medicine:

The hunt for lupus biomarkers

Systemic lupus erythematosus (SLE) is a chronic autoimmune and potentially fatal disease that can affect every organ in the body. SLE typically manifests in waves with flare-ups followed by periods of remission. However, existing biomarkers that evaluate disease activity are ineffective. By focusing on a vital part of the immune system known as the complement system, Dr Alfred Kim, Assistant Professor of Medicine and Pathology & Immunology and Co-Director of the Lupus Clinic at Washington University School of Medicine, and collaborators aim to develop and test biomarkers that could be crucial to improving patient care.

Systemic lupus erythematosus (SLE) is an autoimmune disorder sometimes referred to simply as lupus. SLE can affect numerous organs, resulting in a confusing constellation of signs and symptoms including rash, joint pain, chest pain, hair loss, sun sensitivity, cognitive dysfunction, and renal failure. Consequently, patients are often misdiagnosed initially, leading to a delay of the correct diagnosis of up to five year after onset of symptoms on average.

Lupus affects approximately 1 in 1,000 people in the UK, and approximately five million people worldwide. The disease tends to disproportionately affect women and is more likely to be diagnosed in people of colour, with most cases spotted in early to mid-adulthood. At present, there is no cure for lupus and most patients are required to take immunosuppressive medications to counteract inflammation and manage symptoms. Symptoms of SLE can be distressing and can take a toll on a patient's quality of life.

The immune system protects the body's tissue by attacking foreign pathogens. In the case of autoimmune disorders such as SLE, the immune system attacks the patient's own tissue, for reasons that remain largely unknown. Inflammation is a key weapon in the immune system's arsenal, but when inflammation occurs chronically as a restyle of a long-term attack on healthy tissue, as in SLE, this can cause substantial damage.

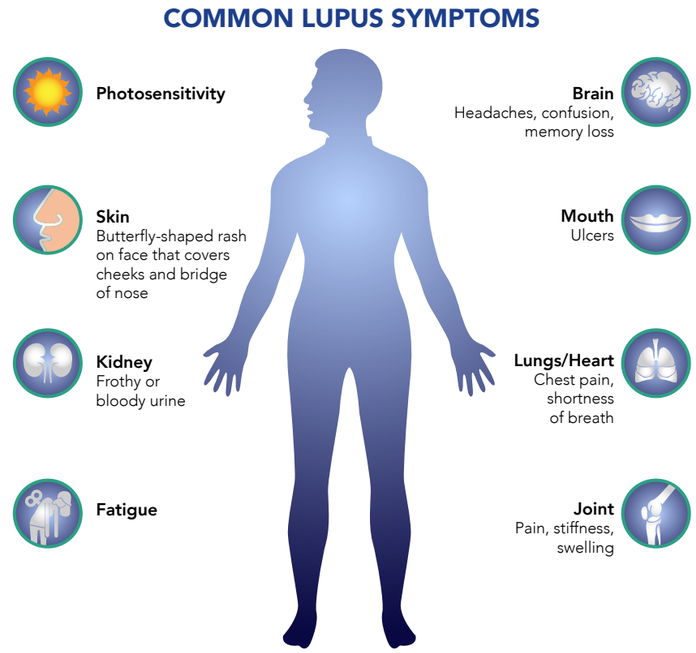
In severe cases, inflammation as a result of SLE can interfere with the function of major organs including the kidneys, heart and brain, causing a serious threat to life. A key characteristic of the disease, however, is its heterogeneity and patients present with tremendous clinical diversity. This heterogeneity has made it difficult to develop treatments that are suitable for all patients. Heterogeneity has also made it more difficult for researchers to characterise the mechanisms that underpin disease, causing SLE to remain poorly understood.

Nancy Mathis, CASTLE study coordinator, is testing blood iC3b levels using a novel lateral flow assay platform engineered by Kyphia, Inc. This test can accurately determine iC3b and C3 levels without cross-reactivity to other C3b fragments within 20 minutes.

FLARE-UPS

Patients with SLE tend to experience symptoms in bouts, with its effects often remising for many months. Flare-ups can vary in length, but their onset can be very unpredictable with triggers for flares remaining largely unknown. Detecting these stages of active disease are key to effectively managing symptoms, thus improving a patient's quality of life and survival. Accurately pinpointing disease activity with tests utilising biomarkers – biological flags, such as a change in protein level or gene that specifically associates with disease pathophysiology – is key to ensuring the best outcomes for patients. Existing biomarkers available for SLE, however, lack sufficient specificity or sensitivity, making it difficult for clinicians to correctly associate the patient's clinical presentation with SLE versus an alternative etiology. The lack of sensitivity also impedes the interpretation of clinical trials, which are vital to finding much-needed treatments for patients. Biomarkers are essential to these studies as they act as a measurable factor that changes in the presence of an intervention, offering researchers a way to determine if a therapy is efficacious.

One researcher dedicated to improving biomarkers for SLE is Dr Alfred Kim, Assistant Professor of Medicine and Pathology & Immunology at the Washington University School of Medicine in St. Louis, Missouri. Dr Kim, who is also founder and co-director of the Lupus Clinic at Washington University, is working with collaborators to embark on the CASTLE trial – Complement Activation SignaTures in Systemic Lupus Erythematosus. The team aims to identify better biomarkers and nail down the role of key mechanisms that are central to the disease. They have fixed their sights on the complement system, a vital part of the immune system that could hold the key to effective biomarkers, and therefore better care for patients living with SLE.



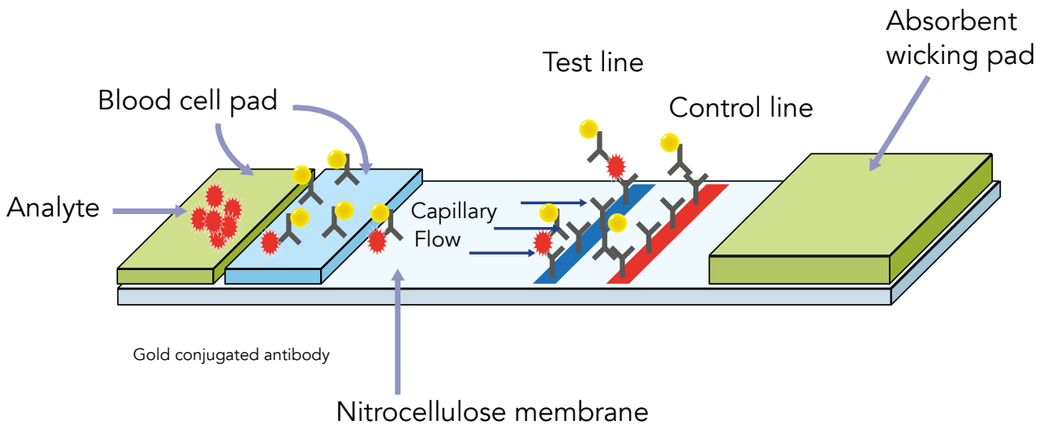
Lupus is a clinically heterogeneous syndrome with over 50 signs and symptoms involving multiple organs that are commonly observed in patients.

The CASTLE pilot study has shown the potential of the complement split product, iC3b, as a new biomarker for disease activity.

COMPLEMENT SYSTEM

The complement system is an integral part of the innate immune system and its activation is a core hallmark of SLE. In SLE, the complement system is activated by autoantibodies bound to the patients' cells, leading to inflammation that can cause tissue damage. Serum measures of complement components, known as C3 and C4, are currently used to detect disease activity as levels of both of these factors fall as the complement system is activated. However, these tests can be inaccurate as the genetic make-up of some patients can lead to lowered levels of C4. Furthermore, the liver generates higher levels of C3 and C4 during systemic inflammation as occurs in SLE, potentially masking low concentrations of these proteins.

Kim has teamed up with colleagues from Washington University, Saint Louis University and Stanford to use an innovative testing platform to understand more about the complexities of complement activation in SLE. Their aim is to fully delineate the numerous fragments that result from complement activation and that are found in patient immune cells and in blood. By characterising complement fragments that promote inflammation, the team hopes to create better measures of disease activity, which could lead to better outcomes for patients and better-informed decision-making for clinical teams. Furthermore, by identifying the key complement fragments that denote disease activity, the team will



The lateral flow assay (LFA) design uses matched pair antibodies to either iC3b or C3 to form an immunochromatography assay. The cassette contains an anti-iC3b or anti-C3 specific monoclonal antibody conjugated to colloidal gold, and the antigen-specific capture antibody bound to the membrane strip to form the solid-phase enzyme immunoassay. iC3b and C3 values determined with the LFA reader correlate highly with ELISA results.

build the most comprehensive picture yet of the mechanisms that are driving disease, potentially opening avenues to investigate new therapies.

NEW DIRECTIONS

The CASTLE pilot study has shown the potential of the complement fragment, or split product, iC3b as a biomarker for disease activity. In a pilot study of over 150 patients with SLE, the iC3b/C3 ratio correlated with clinical changes and appeared to be a more accurate predictor of SLE disease activity than existing biomarker tests. Moreover, this ratio differed between patients with flare-ups and those with inactive SLE.

Photo credit: Wellcome Collection

Herpes esthiomenos (gnawing dermatosis) was described by Hippocrates, which was likely the first observation of a lupus rash. Following more complete descriptions in the 1700-1800's, Sir William Osler was the first to use "systemic lupus erythematosus" around 1900. Despite its long history, biomarkers that accurately track disease activity have been an elusive goal.

Over the course of CASTLE, Kim and colleagues will work towards establishing the long-term relationships between iC3b, C3, the iC3b/C3 ratio, disease activity and clinical observations in SLE.

Importantly, the pilot study utilised a novel investigational device that relies on a lateral flow assay, a simple paper-based indicator – similar to those used in home pregnancy testing – that returned a result within 20 minutes, much faster than current biomarker tests. If brought into clinical diagnostic practice, these time frames could allow doctors to shed light on the disease state at the point of care.

By characterising complement fragments, the team hopes to create better measures of disease activity, leading to better outcomes for patients with SLE.

A subsequent study in CASTLE will also evaluate whether iC3b/C3 ratios can predict treatment response. Most SLE therapies take several weeks to months to evaluate clinical response, but with an improved biomarker, the time to observing treatment efficacy may be shorted substantially.

Whilst the CASTLE study's promising pilot data holds hope for patients who are living with SLE, another ongoing study could also change our understanding of the disease. Using a technique known as mass cytometry, the research team hope to characterise complement fragments on circulating immune cells, relating these to changes in disease activity. Mass cytometry – a cutting-edge technology that characterises cellular networks at unprecedented depth – allows researchers to identify individual complement fragments on each cell and build a holistic picture of complement effects on the immune system in SLE. In doing so, they may also flag new mechanisms that could become targets for the development of future pharmacological interventions.

FUTURE HOPE

Final results from the CASTLE study will not be available for a number of years as researchers continue to characterise the variations in numerous complement split product levels in patients with SLE. At the end of the study, researchers hope to have the most detailed understanding of the role of the complement system in SLE. Ultimately, these findings will be a real step forward in understanding the disease, providing more hope for those living with this complex condition.

Behind the Research

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Research Objectives

Dr Kim and his collaborators aim to identify better biomarkers and nail down the role of key mechanisms that are central to SLE. The team is focusing on the complement system, a vital part of the immune system that could hold the key to more effective biomarkers, and therefore better care, for patients living with SLE.

Detail

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Dr Kim is an Assistant Professor of Medicine and Assistant Professor of Pathology & Immunology at Washington University School of Medicine. He is also the Founder and Co-Director of the Lupus Clinic at WashU. Dr Kim's research combines clinical with translational approaches to improve our understanding of human SLE.

Funding

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Collaborators

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References

Schramm EC, Staten NR, Zhang Z, Bruce SS, Kellner C, Atkinson JP, Kyttaris VC, Tsokos GC, Petri M, Sander Connolly E, Olson PK. 'A quantitative lateral flow assay to detect complement activation in blood'. *Anal Biochem*. 2015; 477:78-85. PMC4404182. https://doi.org/10.1016/j.ab.2015.01.024.

Kim AHJ, Strand V, Sen DP, Fu Q, Mathis NL, Schmidt MJ, Bruchas RR, Staten NR, Olson PK, Stiening CM, Atkinson JP. 'Blood concentrations of complement split product iC3b and serum C3 associate with SLE disease activity'. (*In review*).

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Personal Response

Do you think that by characterising the complement signature, the CASTLE trial could potentially identify new targets for drug development?

"I do for two reasons. First, complement split products have the potential to serve as a more effective biomarker of SLE disease activity. This has important implications for SLE clinical trials, as current outcome measures still lack the precision we desire. Measuring complement split products may improve our ability to assess treatment efficacy. Second, we suspect that subsets of patients with SLE utilise different pathways to activate complement. By identifying the pathway(s) utilised, it can be blocked by novel therapies under development. Thus, we may be able to predict which patients will respond to a particular complement inhibitor, serving as an example of personalised medicine."