How to predict lupus nephritis (non-invasively)

Presenter: Eleni Frangou, Limassol, Cyprus
Chairs: Eleni Frangou, Vladimir Tesar
Written by: Jasna Trbojevic-Stankovic

Lupus nephritis (LN) affects up to 40 percent of adults with systemic lupus erythematosus (SLE) and despite advances in care and management it is still associated with increased morbidity and mortality. The pathogenesis of LN is complex and elusive. The interaction between genetic, epigenetic, and environmental factors ultimately leads to autoantibody formation and an immune complex deposition to multiple end organs, including the kidneys. The kidneys themselves take part in the pathogenesis of the disease, through the activation of kidney resident cells, such as podocytes and mesangial cells, and through the activation of mechanisms of tissue injury and response. Different models demonstrate the potential to avert autoantibody deposition and resulting immune responses within kidneys, suggesting that pre-emptive therapy could represent a valid therapeutic concept in LN.

![Figure 1. Clinical trial design for investigating LN](Image)

Novel approaches to LN – Basic studies, high-throughput studies, and machine learning

Several agents have been tested as novel therapies against LN, but most clinical trials have failed to meet their primary endpoints. The failure of clinical trials can be in part explained by their design, failing to take into account the heterogeneous nature of the disease and the molecular mechanisms occurring within the kidneys and other tissues. To improve outcomes, including kidney failure and death, patient recruitment into clinical trials should integrate kidney biopsy data, preferably non-invasive molecular profiling data and clinical phenotypic data. This would provide tools to predict the risk of progression or relapse of the disease, the risk of the onset of LN, or the response to treatment, through big science and high throughput studies.

Gene expression studies (transcriptomics), which have been performed in LN on mouse models and patients with LN, demonstrated a shared immune cell transcriptome in these species. Blood transcriptomics with microarrays demonstrated that molecular signatures could stratify patients into distinct groups, with a neutrophilic signature characterizing the patients with active LN. Also, different signatures in response to treatment were identified in different LN classes. In the lupus cohort, RNA sequencing in the blood was performed and results showed that the ‘susceptibility signature’ persisted in inactive LN patients despite clinical remission in agreement with clinical observations.

Tissue transcriptomics at the single cell level of the kidney and the skin demonstrated that renal tubular cell cells and keratinocytes share common gene expression profiles. Therefore, type I interferon...
response signature in both cell types could distinguish healthy individuals from LN patients. High interferon response signatures and fibrotic signatures were markers of failure of response to treatment.

Machine learning models represent another novel approach that might help with the prediction of relapse and outcome in LN. Several models were developed based on histological and clinical features to predict response to treatment, disease flares, histology patterns based on clinical features, and renal outcome. Although extensive, these invasive methods could not predict the onset of the disease and failed to identify kidney-specific mechanisms in LN, but some were successful in predicting renal flares.

Figure 2. High-throughput studies in LN: Non-hypothesis-driven and unbiased approaches

**Mouse transcriptome in LN**
Performing serial biopsies for multiple tissue samples is not feasible in humans with LN. To identify kidney-specific signatures in LN, the group led by Elena Frangou used the NZB/W-F1 mice model, the classical model for researching human SLE. The mice used were in the pre-puberty and nephritic stage, and the human cohort consisted of 106 healthy individuals and 261 lupus patients both with active and inactive disease and SLE patients without renal involvement. To identify the mechanisms that characterize the progression from the pre-clinical to the clinical stage, research focused on 500 genes which are uniquely differentially expressed only in the kidneys. These genes define the kidney-specific signature characterizing the transition toward clinical LN. The research group questioned whether the mouse kidney-specific signature of lupus also existed in the blood of patients with LN, i.e., whether it could serve as a non-invasive marker of kidney disease in human SLE. After performing whole blood RNA sequencing in the human cohort, the researchers examined whether the kidney-specific signature of lupus mice at the clinical stage of the disease could be found in the blood transcriptome of patients with LN versus healthy individuals. A similar analysis was performed to examine whether the mouse kidney-specific signature of lupus mice proved that the clinical versus the pre-clinical stage of the disease intersects with a blood transcriptome of patients with LN versus lupus patients without kidney involvement. Ninety-seven genes were common between the two groups defining a shared gene signature characterizing the transition from the pre-clinical to the clinical stage of the disease.

The next step was to identify novel drugs or small molecule compounds that could reverse this cross-species signature. The group used the web engine L1000CDS2 to upload the relevant genes and identify matching signatures of multiple human cell lines that are treated with more than 20,000 drugs or small molecule compounds. With this method, the top 50 drugs were identified that are likely to reverse the
shared cross-species signature, among them an adenosine A3 receptor agonist, NMDA receptor modulator, and HDAC inhibitor.

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<th>HEMADO</th>
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<td>(Adenosine A3 receptor agonist)</td>
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Figure 3. Novel drugs predicted to reverse the shared cross-species signature

Early identification and prompt therapy in LN are linked to improved outcomes. However, demographic, clinical, and serological data cannot predict the onset of kidney disease in SLE. The group used the human orthologues of the mouse kidney-specific signature to answer whether mouse genes and human blood genes could predict SLE patients who will develop LN. The same approach was used to examine whether renal involvement could be predicted in SLE patients. Feature selection was performed and the top 20 genes were elected out of the 500 differentially expressed genes. Using this model, 20 mouse genes, together with the human age, gender, and the presence of Anti ds-DNA could non-invasively discriminate between patients who will and those who will not develop LN, with an accuracy of 82%. As expected, a younger age, male gender, and the presence of Anti ds-DNA were associated with a higher probability of LN. The kidney-specific set of gene predictors may be used for monitoring SLE in humans and enrolment in prevention and early treatment trials.

Key points

1. Early identification and prompt therapy in LN are associated with improved outcomes. However, demographic, clinical, and serological data cannot predict the onset of renal involvement in SLE.
2. Common cross-species genes could be prioritized as potential kidney-specific targets for predicting LN or tested as an alternative non-invasive ‘liquid biopsy’ marker of kidney disease in SLE patients.
3. The mouse kidney-specific set of gene predictors (20 genes) could be used towards enhanced vigilance in the monitoring of LN development in SLE patients.
Further reading


