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Alternative phosphorylation states of protein tyrosine kinases SHP-1 as a biomarker for renal activity in lupus nephritis patients

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Background: The management of lupus nephritis (LN) is significantly hampered by our reliance on clinical biomarkers that poorly reflect inflammation occurring in the kidney. Protein tyrosine kinases (PTKs) are enzymes responsible for the phosphorylation of tyrosine residues in critical cell signaling molecules, that are switched on prior to cell activation. Recent data (Mkaddem et al., 2017) has suggested that unique PTK signatures in peripheral leukocytes are associated with active lupus nephritis. However, it is unknown if these signatures change with disease activity and could be used as clinical biomarkers. We hypothesised that active and refractory patients with LN have a different PTK profile, with lower expression of pSHP1-Y536 and greater expression of pSHP1-S591, in comparison to remission patients and healthy controls. In addition, longitudinal measurement of these profiles may better inform management decisions resulting in improved outcomes.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation of whole blood venesected from patients with LN or healthy age matched controls. Western blotting of PBMC lysates for SHP1-phospho-S591 and-Y536 was performed. Densitometry of bands was carried out and a ratio of S591:Y536 expression calculated (both normalized to actin).

Results: We tested 14 healthy controls (50% female; median age 34.5 years; interquartile range [IQR] 31-38 years) and 10 LN patients (70% female; median age 36 years; IQR 31-40 years), two with clinically active disease and eight considered to be in remission. LN patients had a median eGFR 90ml/min/1.73m2 (IQR 86-90 ml/min/1.73m2) and a proliferative class (III or IV) in 80% of the renal biopsies. The median relative protein expression of SHP1-pS591:pY536 in PBMC was similar between remission patients and healthy controls (p=0.13). However, clinically active/refractory patients had significantly higher ratios than remission patients (Figure 1). Interestingly the overall ratio in patients was strongly correlated to proteinuria (r=0.74, 95% CI=0.21-0.93, p=0.014), but not levels of C3, C4 or dsDNA.

Conclusion: We show for the first time that the ratio of PBMC phospho-SHP1 expression (pS591: pY536) normalizes during disease remission, and could identify patients with active disease or at risk for worse renal outcomes. Longitudinal follow up and correlation with biopsy features may help define if this is a better marker of ongoing disease rather than proteinuria which may reflect inflammation or scarring. We are currently testing a clinically applicable flow cytometry based method to assess these markers.