Osteopontin and Lupus Nephritis

By Noah Kava

Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease which hinders the quality of life for millions of people. The most severe SLE cases often develop into lupus nephritis (LN), an intense form of the disease that causes renal inflammation and damage to kidney tissue. Interferons are cytokines that are upregulated in lupus patients, contributing to the proliferation of the disease. The mechanisms that modulate interferon activity are osteopontin, the protein coded for by the Spp1 gene and filtered into urine, and macrophages, immune cells that engulf cellular waste. This study applied advanced image analysis and algorithm development to kidney samples of human LN patients with the goal of quantifying macrophages and osteopontin-positive tubular cells. The results of the study show that osteopontin and macrophage quantities are correlated in lupus patients with the highest Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, as determined by lab clinicians, suggesting that urinary osteopontin concentration could be used as a biomarker to detect risk for the onset of LN.

Review of Literature

Systemic lupus erythematosus (SLE) is an autoimmune disease with over 60 genetic risk markers (Shipman, 2016). Symptoms include prolonged fatigue, butterfly rash, and joint pain (Mayo Clinic, 2017), and there is currently no known cure. Lupus predominantly affects the kidneys – an organ that works to preserve homeostasis by maintaining appropriate mineral balance in the blood and excretes excess mineral waste as urine (Thompson, 1900). The human kidney is comprised of functional units known as nephrons. Nephrons contain tubules (known as proximal and distal tubules) and collecting ducts for urine filtration (Hill, 2019). In lupus, immune complexes deposit in the kidney causing inflammation and tubular injury. This condition called lupus nephritis (LN), causes deterioration of kidney function (Stewart, 1999).

Osteopontin (OPN) is one of the prominent cytokines upregulated in SLE patients (Niewold 2015). OPN is a protein (encoded for by the Spp1 gene) known for stimulating activity in bones (Sodek, 2000) and mediating migration, adhesion, and activates type I interferon (Shinohara, 2006). Type-1 interferons (IFN-α) are cytokines that inhibit viral proliferation (Norfray, 2009) and are upregulated and overexpressed in Lupus patients, contributing to the onset and worsening condition of the autoimmune disease (Ronnblom, 2011; Elkon, 2012). High IFN activity is a heritable trait and a primary factor for Lupus pathogenesis (Niewold, 2007). OPN has a heightened expression in kidney tubules and glomeruli in LN patients (Xie, 2001). Additionally, OPN and IFN-α have a positive correlation with one another (Li, 2004), which represents a potential cycle for disease intensification and inhibition, but this cycle has remained unproven. OPN is expressed by many immune cells, and macrophages are the cells believed to upregulate OPN in diseases such as brain cancer (Chen, 2019)

Macrophages are tissue-sentinel immune cells (Italiani, 2014) that carry out phagocytosis (engulfing) of parasites and microbes (Elhelu,1983). Macrophages are mainly classified in two categories, first the tissue-resident or embryonic-derived and second, monocyte-derived macrophages. The monocyte-derived macrophages originate from circulating monocytes while resident macrophages are produced in the embryonic stage of human development (Perdiguero, 2016). It was observed that the kidney-resident macrophages upregulate osteopontin when phagocytosing the dying cells (Puranik, 2018). The efficiency of phagocytosis is decreased in SLE patients (Herrmann, 2004), and this causes the immune system to recruit monocyte-derived macrophages to infiltrate the diseased kidneys (Maria, 2017).

Hypothesis

Osteopontin expression increases with the severity of lupus nephritis and correlates with macrophage numbers.

Methodology

SLE intensity is measured in terms of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scale. This scale ranges from one to twenty with a higher rating indicating a greater intensity of symptoms. LN intensity is classified into a pathology class ranging from one to five (I to V), with one being the least intense and five being the most intense form of LN.

Kidney biopsies were performed on seven LN patients. Kidney tissue samples were obtained in a double-blind, de-identified format, meaning that the investigators did not access the clinical data prior to experimentation.

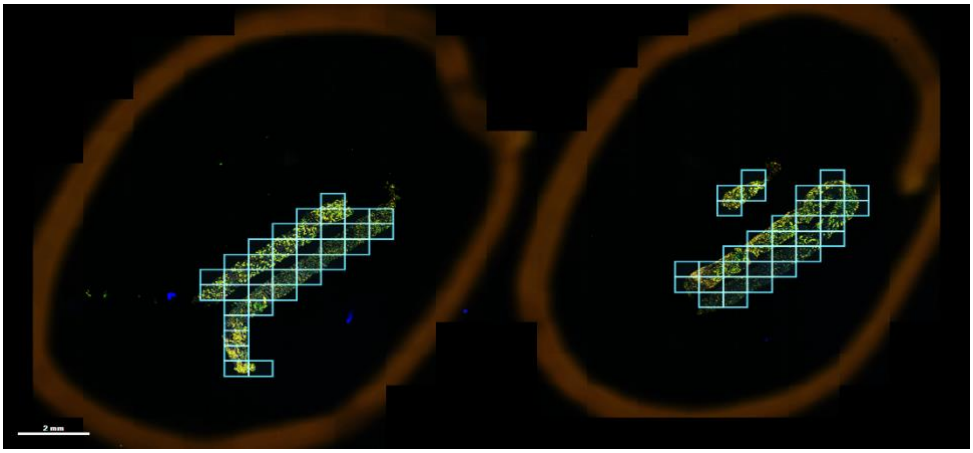


Figure 1. LP Scan from Kidney Biopsy. Tissue samples were collected from lupus nephritis patients. Images were digitally generated and cross-sectioned. This image was provided by the NYU Langone Experimental Pathology core.

The formalin-fixed paraffin-embedded tissue samples were stained with five different fluorophores. Opal 540 indicated SPP1, the gene coding for OPN production, with a yellow hue. Opal 520 indicated monocyte-derived macrophages (via the CD11b+ protein) with a green hue. Opal 570 and Opal 650 indicated the positivity of CD68 and CD163 proteins, both of which are needed to identify a cell like a macrophage. Their hues were cyan and magenta, respectively.

The DAPI fluor detected all nuclei within the kidney sample. After staining, the samples were imaged into LP scans.

LP scans were subsequently divided into smaller magnified square-dimensioned images (n=332). Image quality was confirmed by analyzing each of the five fluor channels and the auto-fluorescence channel using ImageJ software.

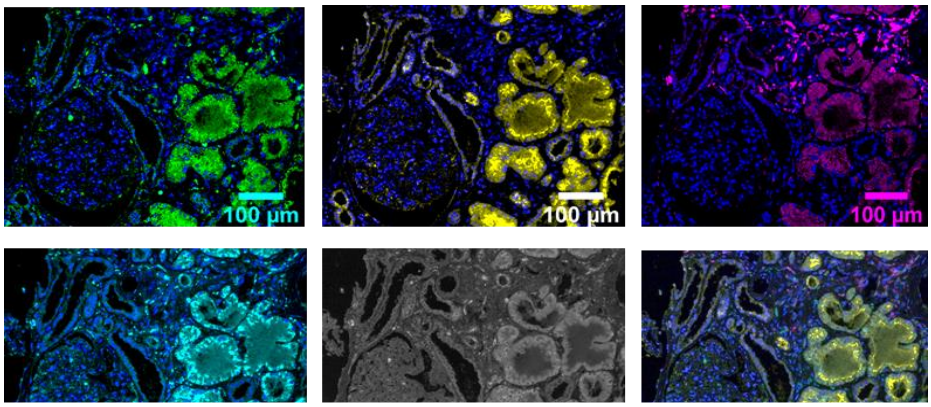


Figure 2. Staining Channels for Lupus Nephritis Kidney Samples. Samples were channeled by experimental phenotype. Nuclei, as indicated by the dark blue points, were identified as CD11b+ (green), Spp1+ (yellow), CD163+ (magenta), CD68+ (cyan), or non-expressive. A gray auto-fluorescence channel was added to maintain the quality of the image. The five channels were stacked onto each other to form a single composite image used for analysis.

Channels were merged into composite images and prepared for analysis on the Inform image analysis software. Within the Inform software, image batches ($n \approx 10$) were segmented on criteria such as tissue type and cell phenotype. The first batch analysis was designed to quantify macrophages, as indicated by the co-expression of Opal 650 and Opal 570.

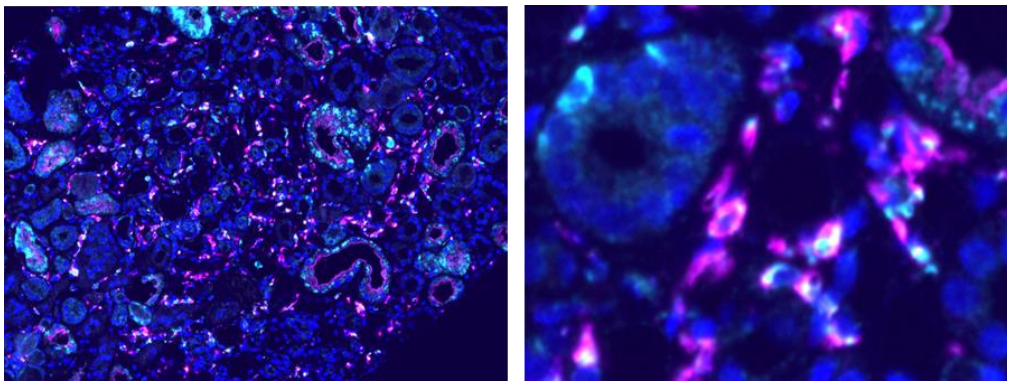


Figure 3. Macrophages in Human Kidneys. Macrophages were identified by nuclei engulfed by CD68 (cyan) with CD163 (magenta) as a membrane.

The second batch analysis was designed to quantify OPN positive tubular cells. Upon performing quantification for the batch, the analysis settings were saved as an algorithm and applied to all of the 332 images in order to get data for every single section of the kidney samples.

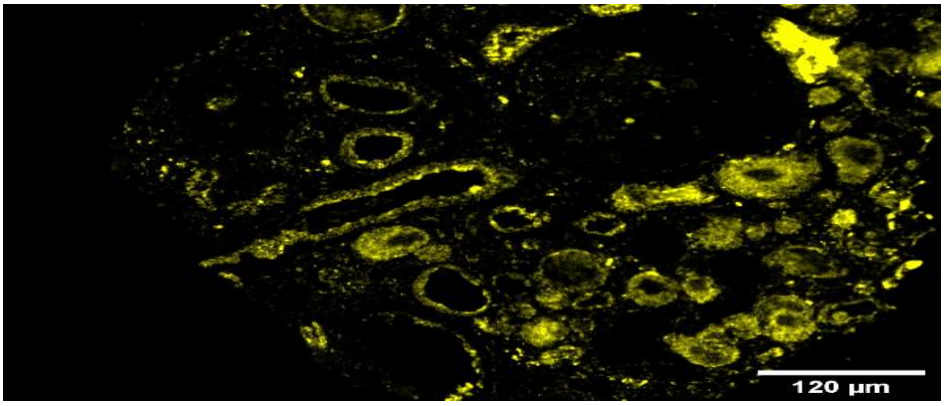


Figure 4. Osteopontin in Tubules. Some tubules in the kidneys express the Spp1 gene (yellow hue) which codes for the production of osteopontin.

Data were compiled onto a spreadsheet and analyzed using a standard two-column p-test, averaging and standard deviation. After the completion of data analysis, the results were compared with the clinical data obtained by Niewold Lab.

Results

There was variety in the quantity of macrophages amongst the seven different patients, suggesting that there is no definitive macrophage concentration in lupus patients. Patient 6010 had the highest median quantity of macrophages.

There was also a great variety in the quantity of OPN+ cells among the patients, where once again, Patient 6010 had the highest median percentage.

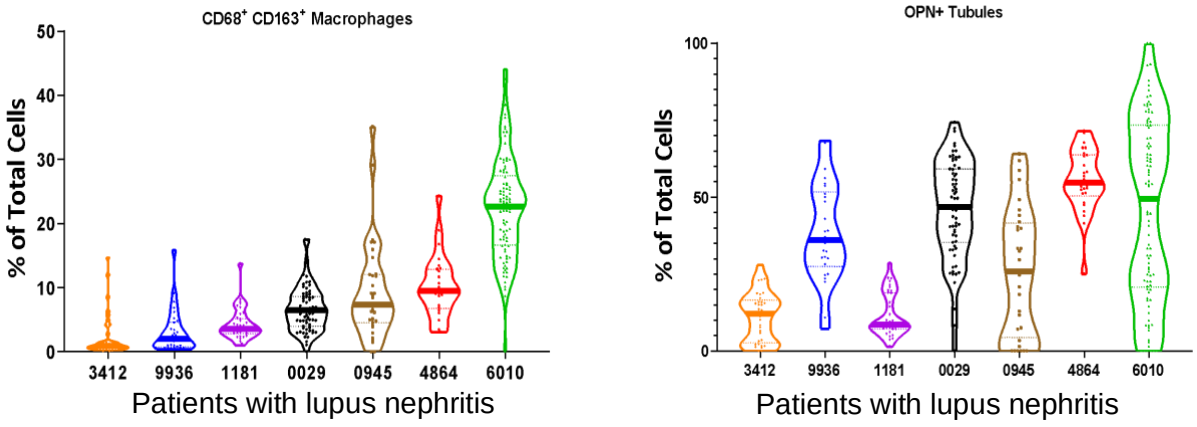


Figure 5. and Figure 6. Quantity of Macrophages and OPN+ Tubular Cells.
The graph shows the percentage of total cells that expressed the macrophage or tubular OPN+ phenotype. Each plot corresponds to a single composite image, and the solid line represents the median quantity.

The majority of OPN was detected apically within the tubules. There was a negligible amount of OPN expressed by macrophages, suggesting that OPN is not significantly produced by macrophages and that there is an alternative source in the kidneys producing OPN.

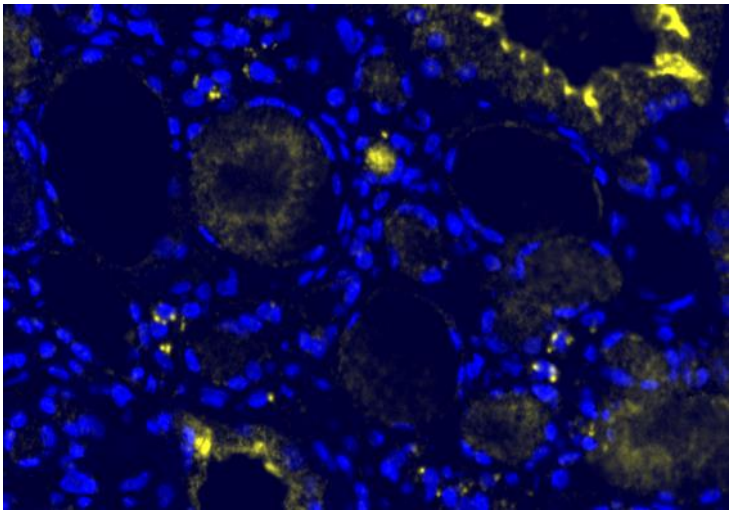
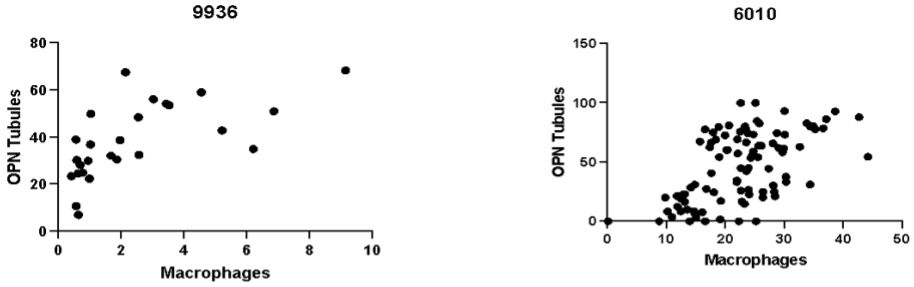


Figure 7. Osteopontin is Predominantly Apical in Kidney Tubules. The majority of OPN was located along the interior of the tubules with some OPN+ tubular cells. There was a negligible quantity of OPN+ endothelial cells.

Two patients, 9936 and 6010, yielded significant p-values ($p < 0.0001$) when macrophage and OPN quantities were compared; however, the r value is not significant for either patient.



	Macrophages vs. OPN Tubules (9936)		Macrophages vs. OPN Tubules (6010)
Spearman r		Spearman r	
r	0.7566	r	0.5541
95% confidence interval	0.5135 to 0.8873	95% confidence interval	0.3908 to 0.6836
P value		P value	
P (two-tailed)	<0.0001	P (two-tailed)	<0.0001
P value summary	****	P value summary	****
Exact or approximate P value?	Approximate	Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes	Significant? (alpha = 0.05)	Yes
Number of XY Pairs	26	Number of XY Pairs	94

Figure 8. Osteopontin and Macrophage Quantities are Correlated in High-Intensity Lupus Nephritis Patients. The scatterplots show the relationship between macrophage and osteopontin concentrations in the two patients with the highest SLEDAI scores. Significant P values ($p < 0.0001$) were calculated for both patients.

After review of the clinical data, it was confirmed that patients 9936 and 6010 had highest SLEDAI (Systemic Lupus Erythematosus, Activity Index) and are class IV lupus nephritis. This was not observed in other patients, suggesting that in class IV lupus nephritis tubular OPN correlates with macrophages. The clinical results showed that patients 6010 and 9936 had a LN pathology class of IV. Furthermore, patient 6010 had a SLEDAI score of 19 while patient 9936 had a SLEDAI score of 14. Overall this suggests that OPN expression correlates with the macrophage numbers in patients with high SLEDAI and class IV nephritis.

Discussion

The correlation between macrophage and OPN quantities in high SLEDAI and class IV lupus nephritis kidneys is a significant finding in lupus research. When considered in conjunction with the observation that most OPN is located apically within kidney tubules, it is possible that urinary OPN could serve as a biomarker for high-intensity LN, as the majority of OPN gets filtered out of the human body. This research indicates OPN as an early warning sign for LN development, as it captures the intensification and peaking of SLE activity as it progresses into LN.

The results confirmed the hypothesis of correlating quantities of macrophages and OPN. The observation of high number of tubular OPN could be indicative of high IFNα activity in this mechanism, which could lead future researchers to investigate the tubules in order to find the source of IFNα production. The next step in this research would be to analyze a control human kidney for macrophage and OPN quantity. The lack of a control sample in addition to the relatively small sample size was a limitation in this study. Additionally, this study could be improved by reducing the time between the biopsy and sample staining in order to ensure that the CD11b could effectively detect infiltrating, monocyte-derived macrophages. This activity is indicative of the auto-immune inflammatory response in lupus patients, and a high presence of monocyte-derived macrophages would add another dimension to the correlation in the high SLEDAI patients.

Conclusion

This study found that the quantities of macrophages and OPN in high SLEDAI patients are correlated with each other despite the deviations in quantities among the seven patients. Since most OPN is located apically within kidney tubules, urinary osteopontin could be used as a biomarker for high SLEDAI lupus patients. Since OPN is not significantly produced by macrophages, there is another source within the human kidneys producing both OPN and IFNα.

Acknowledgements

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