Multiplex Planar Microarray Assay for Simultaneous Detection of Autoantibodies in ANCA-associated Vasculitis and Anti-GBM or -C1q-associated Glomerulonephritis

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Introduction:

Simultaneous measurement of a number of correlated analytes is useful for reasons of reduced specimen volume and reagent costs, and enhanced clinical utility wherein a combination of markers facilitates disease- or organ-oriented profiling, prognostic studies of disease activity and severity and therapeutic monitoring.[1] SQI Diagnostics' IgX PLEX™ planar microarrays allow multiplexing of both antigen and antibody class in an automation compatible 96 well format. Assays which facilitate the detection of multiple biomarkers from multiple patients in a fully automatable platform better reflect the complexity of autoimmune disease processes.[2] Use of specifically labelled reporter molecules permit simultaneous measurement of autoantibody immunoglobulin classes.[3]

Objective:

Clinical performance of multiplex testing was evaluated in a novel planar microarray assay (Figure 1) by detecting the frequency of anti-PR3 (proteinase-3), anti-MPO (myeloperoxidase), anti-GBM (glomerular basement membrane antigen alpha3-collagen IV) and anti-C1q IgG antibodies in diagnostic, clinically defined (Chapel Hill and American College of Rheumatology) subsets of vasculitis and nephritis.

Materials and Methods:

Vasculitis sample sets included: 40 ANCA-associated vasculitis (AAV) comprised of Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome; 25 systemic lupus erythematosus (SLE) plus vasculitis (SLEV); and 20 other vasculitides (OV) comprised of cutaneous vasculitis, Henoch Schonlein purpura and mixed cryoglobulinemia.

Renal sets included: 23 rapidly progressive (cresentic) glomerulonephritis (RPGN type III); 19 Goodpasture's syndrome (GS, RPGN type I); 71 lupus nephritis (LN) and 20 nephropathy patient samples. 29 healthy controls (HC) were also included. Autoantibodies in patient samples were detected with a research use only version of the IgX PLEX™ Vasculitis and Nephritis assay on a manual adaptation of the fully automated SQiDworks™ Diagnostic Platform.

Clinical sensitivity and specificity were determined with specificity measured against relevant disease controls and healthy controls. Overall correct classification was determined as a measure of differential diagnostic efficiency.

Results:

The clinical performance results support the application of IgX PLEX™ planar microarray technology to differential diagnosis involving ANCA testing in AAV and RPGN type III, GBM antibodies in RPGN type I and C1q antibodies in LN. Sensitivity, specificity, positive and negative predictive values, and overall correct classification for all relevant diagnostic comparisons are listed in Table 1. As a serological test to support clinical symptomatology and histopathology results, the IgX PLEX™ Vasculitis and Nephritis Assay was most efficient for diagnosing AAV, RPGN type III and Goodpasture's syndrome.

Discussion:

The majority of patient sera contained autoantibodies typically associated with their disease state (Table 1. sensitivity values). Multiplex testing for 4 vasculitis- and glomerulonephritis-associated autoantibodies in the IgX PLEX™ microarray detected autoantibody multireactivity that may be overlooked in single antigen assays. The importance of the multiplex assay is best exemplified by the detection of IgG anti-C1q or -GBM antibodies in 55% of RPGN type III but not in AAV. Thus, these kidney antibodies help differentiate the two diseases in the spectrum of ANCA positivity. Additionally, several patients classified as having one disease but exhibiting antibodies typically associated with another disease (e.g. ANCA positive SLEV, LN or GS) may have an overlap syndrome (e.g. ANCA disease[4]) and require additional diagnostic testing. Our results confirm the prevalence of ANCA antibodies in SLE with an increased frequency of ANCA PR3 or MPO antibodies seen in SLE + vasculitis (24%) vs. SLE + nephritis (8.5%).[5]

Substantiating an earlier study[6], our results demonstrate the coexistence of GBM antibodies and C1q antibodies in a limited number of patients (8.5% of LN), as well as coexistence of GBM antibodies and MPO or PR3 antibodies in 4.7% of AAV and RPGN type III patients. Whether this multireactivity contributes to the etiopathogenesis or severity of the associated disease requires further histopathological and prognostic studies.

Obtaining multiple autoantibody results from one patient sample will enhance clinical decision making based on histopathology results and disease activity scores. Additional biomarkers for inflammation, as well as detection of therapeutic monoclonal antibodies can further expand the clinical utility of the microarray.

Conclusion:

SQI's IgX PLEX™ Vasculitis and Nephritis assay provides a sensitive and specific means of detecting MPO and PR3 antibodies in AAV and RPGN type III, and GBM antibodies in Goodpasture's disease. C1q antibody testing was specific to lupus nephritis. The clinical implementation of this microarray assay will aid physicians in characterizing and monitoring vasculitis and nephritis patients. Further studies are ongoing to determine the clinical utility of IgA antibodies to these antigens.

References:

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Figure 1. IgX PLEX™ Multiplex Planar Microarray Assay for Vasculitis and Nephritis

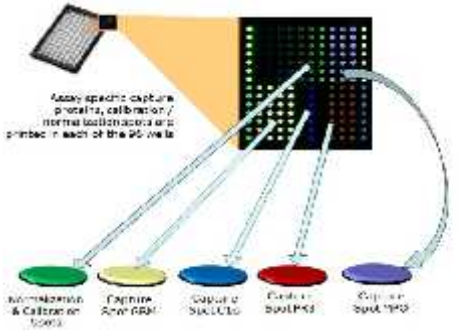

Table 1. Clinical Sensitivity, Specificity and Overall Correct Classification of Vasculitis and Nephritis Phenotypes using the IgX PLEX™ Vasculitis and Nephritis Assay

Goodpasture's vs. All other nephritis		Diagnosis Present	Diagnosis Absent	
Anti-GBM	Test Positive	17	6	23
	Test Negative	2	125	127
		19	131	150
Sensitivity		0.895		
Specificity		0.954		
Odds ratio (OR)		177.083		
Likelihood Ratio+ (LR+)		19.535		
Likelihood Ratio- (LR-)		0.069		
Positive Predictive Power (PPP)		0.739		
Negative Predictive Power (NPP)		0.984		
Overall Correct Classification (OCC)		0.947		

AAV vs. SLE+vasculitis and other vasculitis		Diagnosis Present	Diagnosis Absent	
Anti-MPO or PR3	Test Positive	32	7	39
	Test Negative	8	38	46
		40	45	85
Sensitivity		0.800		
Specificity		0.844		
Odds ratio (OR)		21.714		
Likelihood Ratio+ (LR+)		5.143		
Likelihood Ratio- (LR-)		0.237		
Positive Predictive Power (PPP)		0.821		
Negative Predictive Power (NPP)		0.826		
Overall Correct Classification (OCC)		0.824		

Lupus nephritis vs. All other nephritis		Diagnosis Present	Diagnosis Absent	
Anti-C1q	Test Positive	57	11	68
	Test Negative	14	48	62
		71	59	130
Sensitivity		0.803		
Specificity		0.814		
Odds ratio (OR)		17.065		
Likelihood Ratio+ (LR+)		4.306		
Likelihood Ratio- (LR-)		4.126		
Positive Predictive Power (PPP)		0.838		
Negative Predictive Power (NPP)		0.774		
Overall Correct Classification (OCC)		0.808		

RPGN type II vs. All other nephritis		Diagnosis Present	Diagnosis Absent	
Anti-MPO or PR3	Test Positive	21	13	34
	Test Negative	3	97	100
		24	110	134
Sensitivity		0.875		
Specificity		0.882		
Odds ratio (OR)		52.231		
Likelihood Ratio+ (LR+)		7.404		
Likelihood Ratio- (LR-)		7.007		
Positive Predictive Power (PPP)		0.618		
Negative Predictive Power (NPP)		0.970		
Overall Correct Classification (OCC)		0.881		