



Curtin University

Anti-C1q Antibodies Concentrations by Elisa in Systemic Lupus Erythematosus

Audrey Margery-Muir

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Systemic Lupus Erythematosus (SLE) - Overview

- Female bias disease in reproductive years
 - Ratio ≈ 10
 - BUT 1.2 in neonates
- Multiple organ involvement & tissue damage
- Deficiencies complement components
 - Complements C1q, C2, C3, C4

Mahajan, A., *et al.*, *Clearance Deficiency and Cell Death Pathways: A Model for the Pathogenesis of Sle*. 2016

Margery-Muir, A.A., Bundell, C., Nelson, D., Groth, D.M. and Wetherall, J.D., *Gender Balance in Patients with Systemic Lupus Erythematosus*. *Autoimmunity reviews*, 2017.



Systemic Lupus Erythematosus (SLE) - Overview

- Complement C1q activates classical pathway
 - Directly binds immunoglobulins
 - Recognition patterns on microorganisms & apoptotic material
- High affinity binding C1q to Fc receptors
 - → formation neo-antigen on collagen-like tails of C1q molecules
 - → Production auto-antibodies (anti-C1q antibodies - aC1q ab)

Pickering, M.C. and Botto, M., *Are Anti-C1q Antibodies Different from Other Sle Autoantibodies?* 2010; Beurskens, F.J., *et al.*, *C1q, Antibodies and Anti-C1q Autoantibodies.* 2015; Fang, Q.Y., *et al.*, *Anti-C1q Antibodies and Igg Subclass Distribution in Sera from Chinese Patients with Lupus Nephritis.* 2009. Margery-Muir *et al.*, *Anti-C1q antibodies concentrations by ELISA in systemic lupus erythematosus.* 2017.



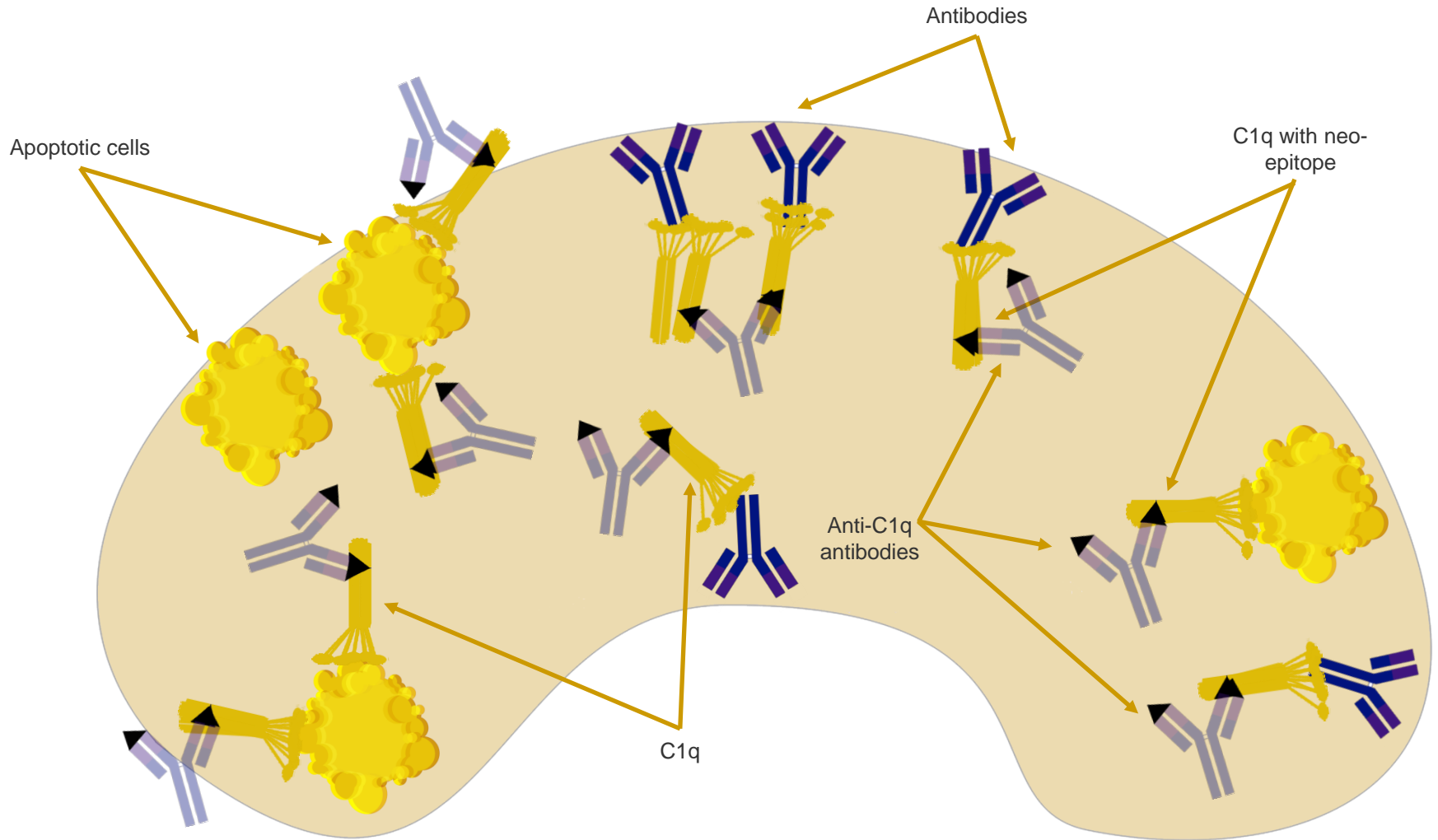
Systemic Lupus Erythematosus (SLE) - Overview

- Evidence impaired clearance apoptotic material → aberrant inflammation
 - ↑ dsDNA antibodies
 - ↓ Complement levels (i.e. C3, C4)
- Levels of aC1q ab ↑ in SLE - Reports strong association with active renal disease
 - 1/3 SLE have anti-C1q ab, associated with disease severity, nephritis
- Concentrations indicative renal flares
- Concentrations aC1q ab correlate with disease activity, complement levels, dsDNA ab & presence immune complexes

Pickering, M.C. and Botto, M., *Are Anti-C1q Antibodies Different from Other SLE Autoantibodies?* 2010; Beurskens, F.J., et al., *C1q, Antibodies and Anti-C1q Autoantibodies.* 2015; Fang, Q.Y., et al., *Anti-C1q Antibodies and Igg Subclass Distribution in Sera from Chinese Patients with Lupus Nephritis.* 2009.



How are aC1q ab generated?



Cartoon representation of a kidney

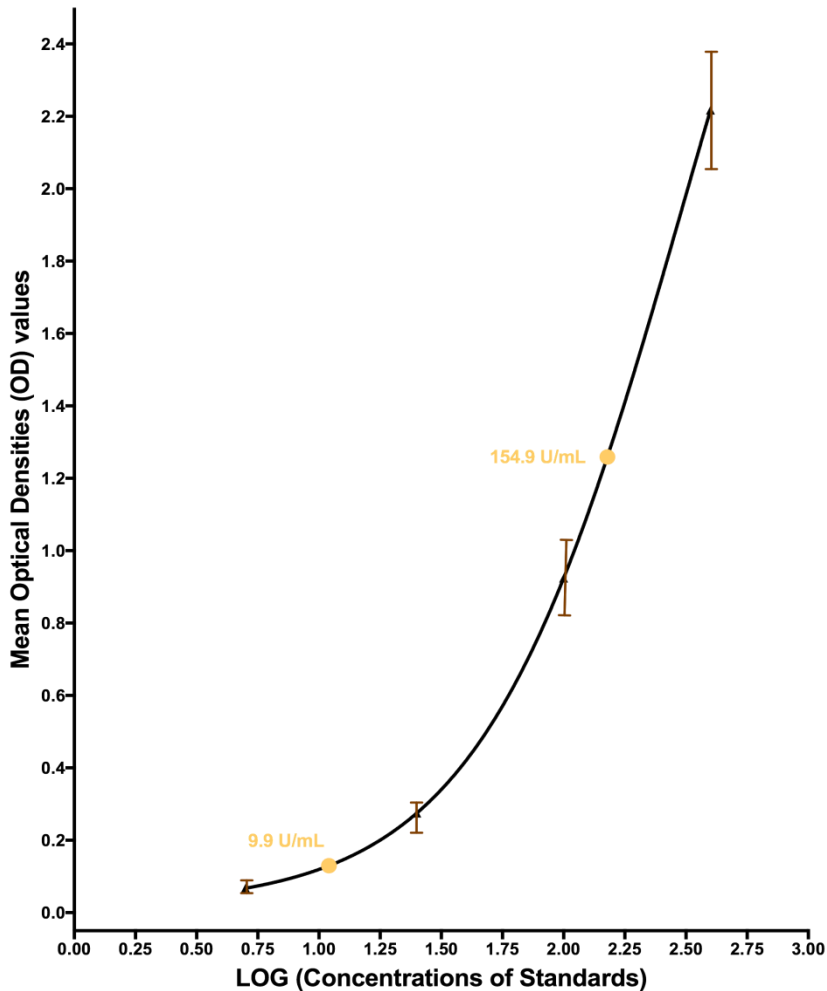
Rationale: Anti-C1q antibodies as a diagnostic tool?

- 56 Western Australian SLE & 33 age/sex matched controls
- Measured aC1q ab by commercial Elisa kit (Bulhmann – Germany)
 - No internationally agreed standard methods at present
 - A number of commercial kits, each with their own secondary standards

Katsumata, Y. , et al., Anti-C1q Antibodies Are Associated with Systemic Lupus Erythematosus Global Activity but Not Specifically with Nephritis: A Controlled Study of 126 Consecutive Patients. 2011; Zivković, V. , et al., Anti-DsDNA, Anti-Nucleosome and Anti-C1q Antibodies as Disease Activity Markers in Patients with Systemic Lupus Erythematosus; Chen, Z. , et al., Anti-C1q Antibody Is a Valuable Biological Marker for Prediction of Renal Pathological Characteristics in Lupus Nephritis. 2012; Marto, N. , et al., Anti-C1q Antibodies in Nephritis: Correlation between Titres and Renal Disease Activity and Positive Predictive Value in Systemic Lupus Erythematosus. 2005; Bock, M. , et al., Anti-C1q Antibodies as a Follow-up Marker in SLE Patients. 2015; Magro-Checa, C. , et al., Complement Levels and Anti-C1q Autoantibodies in Patients with Neuropsychiatric Systemic Lupus Erythematosus. 2016.



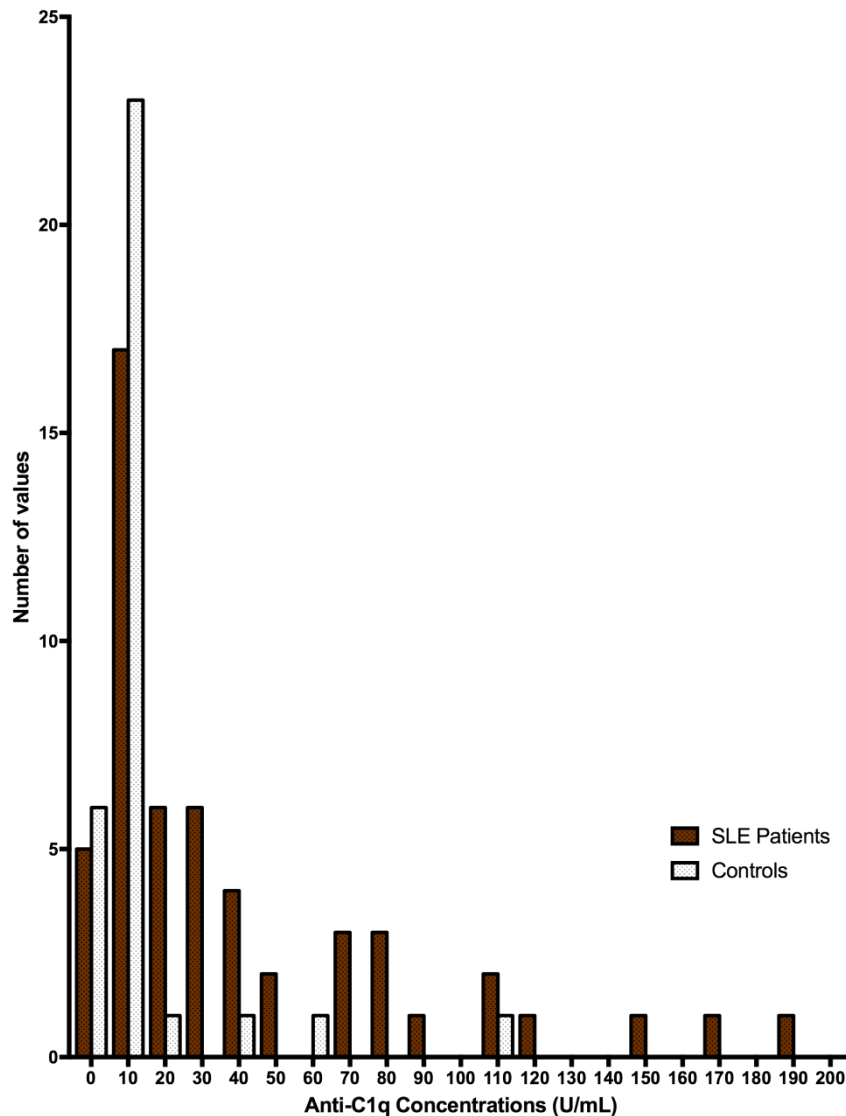
Assay calibration & performance



- Cut off value provided by manufacturer 15 U/mL
 - Internal calibrators provided in the kit
 - Positive and negative controls
 - Expected ranges 4.0 – 7.9 U/mL & 118 – 233 U/mL
- Positive control performed within range
- Negative control average 9.9 U/mL

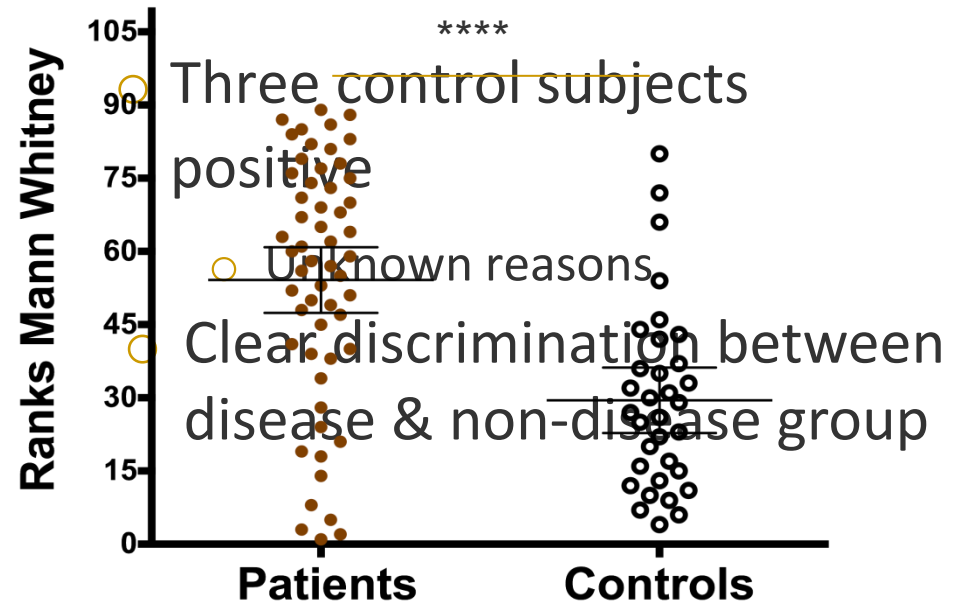
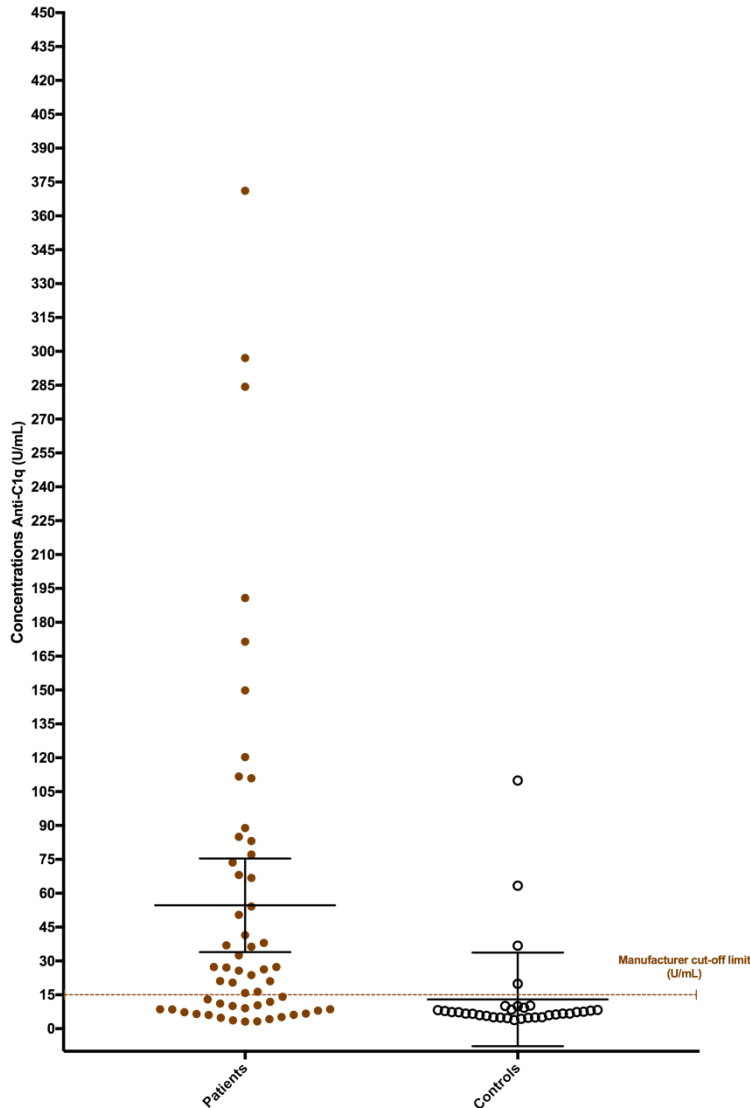
A four-parameter logistic standard curve over the range of 5, 25, 100 and 400 U/ml (mean \pm standard deviation of optical density values).

Distribution of α C1q ab serum concentrations



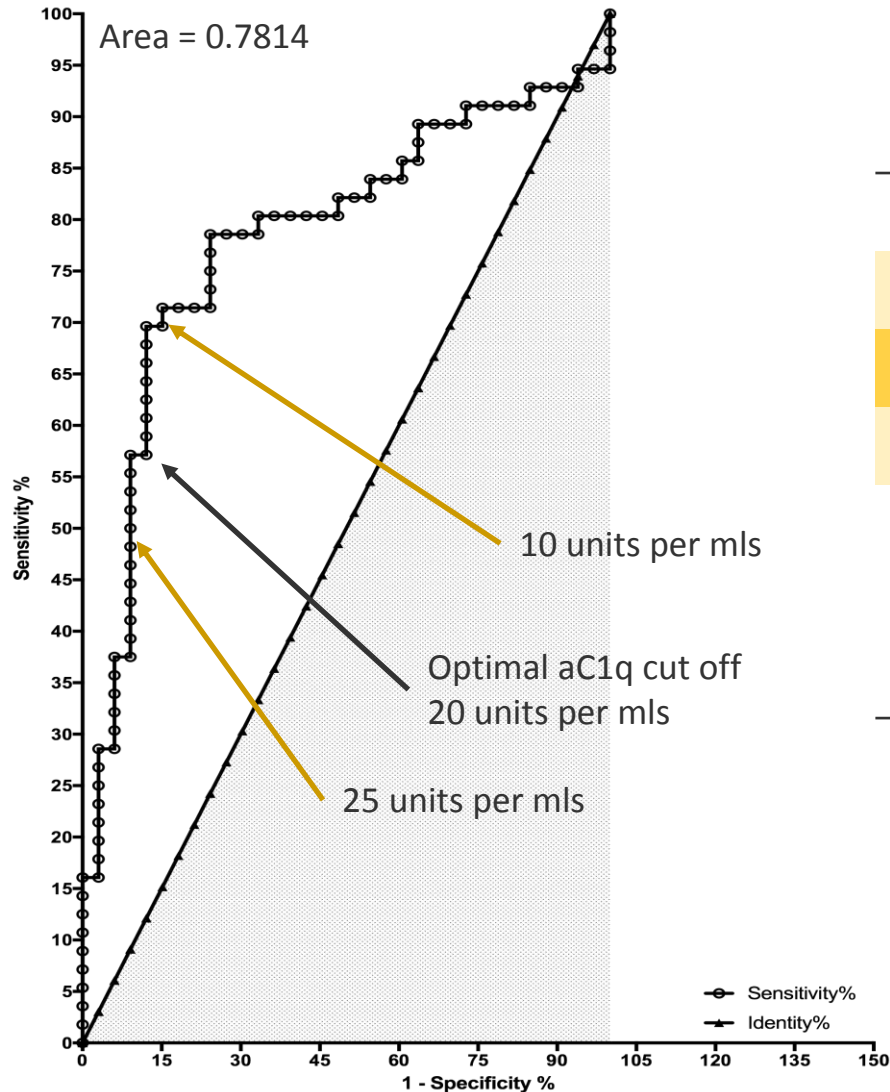
- Non-Gaussian distribution of α C1q ab concentrations
- Frequency distribution of the α C1q ab for SLE patients and controls respectively (mean concentrations - α C1q ab SLE patients: 54.70 U/mL; controls: 12.84 U/mL).

Discrimination of disease groups by [anti-C1q ab]



- Ranking of anti-C1q ab concentrations and means of ranks \pm 95% CI (Mann Whitney rank test analysis $p \leq 0.0001$).

Results Assay Specificity & Sensitivity



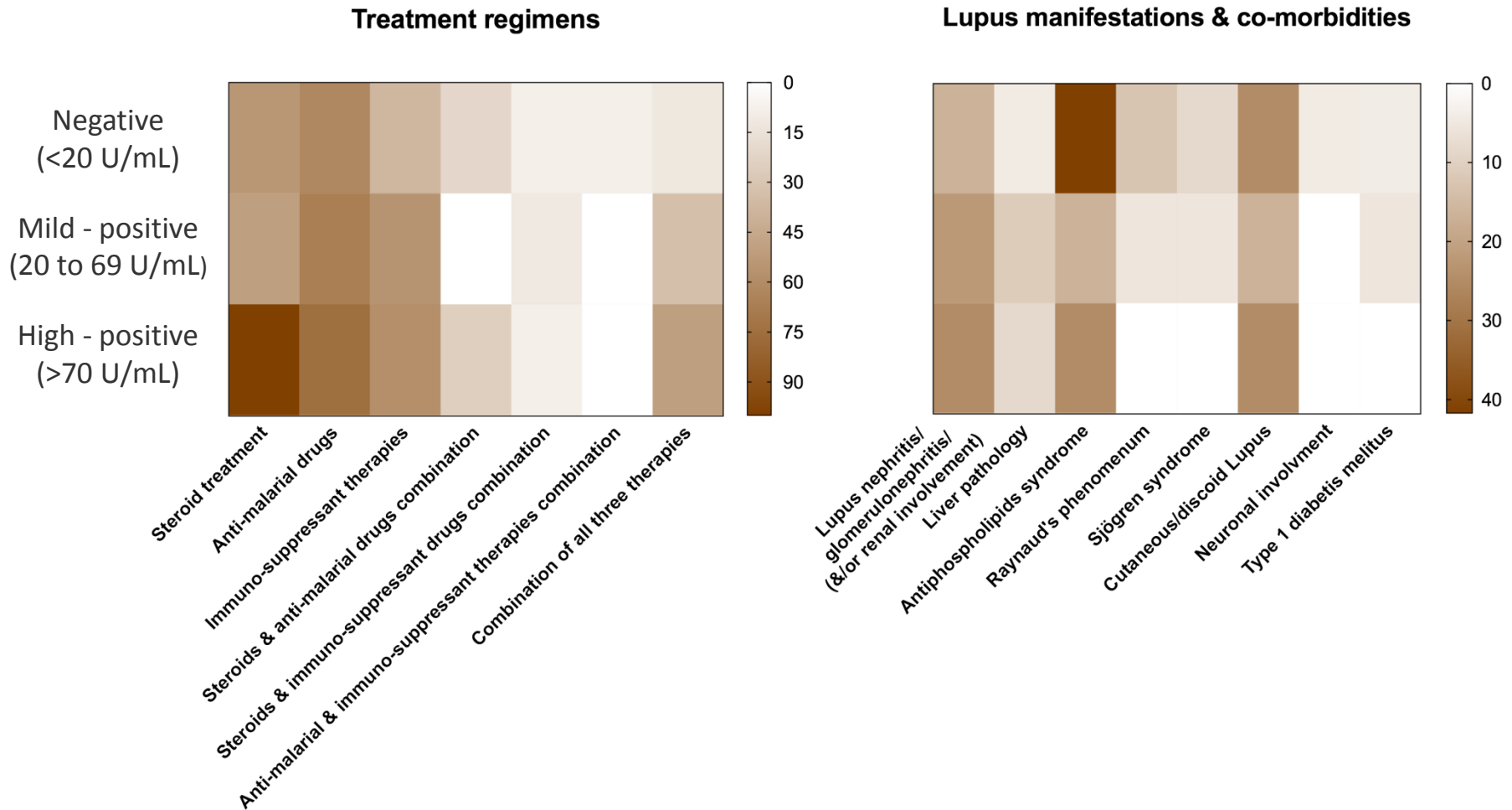
α C1q ab	Sensitivity	Specificity	*PVP+	*PVN-
Units/ml	% SLE in true positive (TP)	% non SLE in true negative (TN)	[TP/(TP+FP)]%	[TN/(TN+FN)]%
5	89.3	24.2	66.7	57.1
10	69.6	84.8	88.6	62.2
20	57.1	90.9	91.4	55.6
25	50.0	90.9	90.3	51.7
30	41.1	90.9	88.5	47.6
40	33.9	93.9	90.5	45.6
110	16.1	100.0	100.0	41.3

- * PVP+ve = likelihood a positive result indicates presence of disease
- ** PVP -ve = likelihood a negative result indicates absence of disease

Correlations with other laboratory parameters

	Age	(C4)	(C1q)	(C3)	(CRP)	(Ds-DNA ab)	(DNASE-I)	(Anti-C1q ab)
Age		-0.073	0.070	0.063	0.014	-0.275 (P=0.042)	0.034	-0.146
(C4)	-0.073		0.085	0.653 ≤0.0001	0.203	-0.087	-0.045	-0.274 (P=0.041)
(C1q)	0.070	0.085		0.248	0.325 (P=0.024)	0.241	0.263 (P=0.050)	0.088
(C3)	0.006	0.116	-0.020		0.239	-0.118	0.017	-0.131
(CRP)						0.406 (P=0.005)	-0.063	0.136
(Ds-DNA ab)							0.059	0.502 (p≤0.0001)
(DNASE I)	0.140	-0.141	0.230	0.225				0.038
(Anti-C1q ab)	-0.566 (P=0.001)	-0.246	-0.024	0.057			-0.368 (P=0.035)	

Heat Maps: Relating aC1q ab with disease severity



Conclusions

- Based on this study
 - Patients with more severe disease requiring multiple treatment therapies to reduce organ damage have higher concentrations of aC1q ab
 - Quantitation and standardisation of aC1q ab have useful roles in classification of SLE subtypes, diagnosis confirmation and monitoring of disease progression and therapy
- However:
 - Recent reports have identified monoclonal aC1q ab reacting with epitopes on the A and B collagen-like tails of human C1q (ie. not present on C-chain).
 - Future studies will require identification of aC1q ab based on antigenic specificities.

Vanhecke, D. Roumenina, L., T. Wan, H. Osthoff, M. Schaller, M. Trendelenburg, M. Identification of a major linear C1q epitope allows detection of systemic lupus erythematosus anti -C1q antibodies by a specific peptide-based enzyme-linked immunosorbent assay. 2012.



Acknowledgements

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- Staff & students at Curtin University for their support



Contingency table for test outcomes

	Disease present	Disease absent
Positive Test result	Group (a) True Positive TP	Group (b) False Positive FP
Negative Test result	Group (c) False Negative FN	Group (d) True Negative TN

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN}) = \text{Group(a)} / [\text{Group(a)} + \text{Group(c)}]$$

$$\text{Specificity} = \text{TN}/(\text{FP} + \text{TN}) = \text{Group(d)} / [\text{Group(b)} + \text{Group(d)}]$$

	Steroids (1)	Anti-malarial (2)	Immuno- suppressants (3)	1 + 2	1 + 3	2 + 3	Combination of 1, 2 & 3	Other therapeutics
Renal symptoms (N =11)	8 (72.7%)	7 (63.6%)	8 (72.7%)	1 (9.1%)	3 (27.3%)	0 (0%)	4 (36.4%)	8 (72.7%)
Hepatic involvement (N= 4)	2 (50%)	4 (100%)	2 (50%)	1 (25%)	0 (0%)	1 (25%)	1 (25%)	3 (75%)
Anti-phospholipid (N= 17)	13 (76.5%)	9 (52.9%)	7 (41.2%)	3 (17.6%)	3 (17.6%)	0 (0%)	4 (23.5%)	16 (94.1%)
Raynaud’s phenomenon (N= 4)	2 (50%)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	4 (100%)
Sjögren’s disease (N= 4)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)
Cutaneous/discoid symptoms (N= 12)	5 (41.7%)	6 (50%)	7 (58.3%)	1 (8.3%)	0 (0%)	0 (0%)	3 (25%)	9 (75%)
Neuronal symptoms (N= 1)	1 (100%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
T1DM (N= 3)	3 (100%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	1 (33.3%)	0 (0%)	1 (133.3%)	3 (100%)
Other symptoms (N= 13)	9 (69.2%)	11 (84.6%)	7 (53.8%)	2 (15.4%)	2 (15.4%)	1 (7.7%)	4 (30.8%)	13 (100%)

Bulhmann Elisa Kit

- A solid-phase ELISA kit Quantify IgG α C1q ab specific for the neo-antigen generated on solid phase C1q.
- Stored patient sera were diluted in high salt buffer (0.5M NaCl)
- Incubated in microtiter wells coated with human C1q.
- HRP- labeled anti-human IgG added then TMB
- A washing step between each incubation.
- Addition 0.25M sulphuric acid
- Absorbance at 450 nm
- Cut-off suggested 15 U/mL

Authors	Assay used	Cut-off value
[5] previously described by [21]	In house ELISA	55 arbitrary units (AU)
[10]	ELISA commercial kit by Buhlmann	15 U/mL (manufacturer) & 40U/mL in comparison assay
[22]	ELISA commercial kit by Orgentec Diagnostika	10 U/mL
[13]	ELISA commercial kit by Euroimmun	10 U/mL
[23]	ELISA commercial kit by Orgentec Diagnostika	20 U/mL
[15]	ELISA commercial kit by Orgentec Diagnostika	12 U/L
[24]	Autostat II by Hycor Biomedical INC	50 μ L/mL
[14]	Diagenics UK	19 U/mL
	ELISA commercial kit by Orgentec Diagnostika	10 U/mL
[25] previously described by [26]	In house ELISA	16 AU/mL
[6]	In house ELISA &	55 AU/mL
	Quanta Lite™ ELISA commercial kit by Inova Diagnostics	20 U/mL
[16]	ELISA commercial kit by Inova Diagnostics	10 U/mL
[17]	ELISA commercial kit by Buhlmann	15 U/mL
[12]	ELISA commercial kit by Inova Diagnostics	10 U/mL
[18]	Quanta Lite™ ELISA commercial kit by Inova Diagnostics	20 U/mL



