Advances in Biomarkers for the Differential Diagnosis of Suspected Autoimmune Patients

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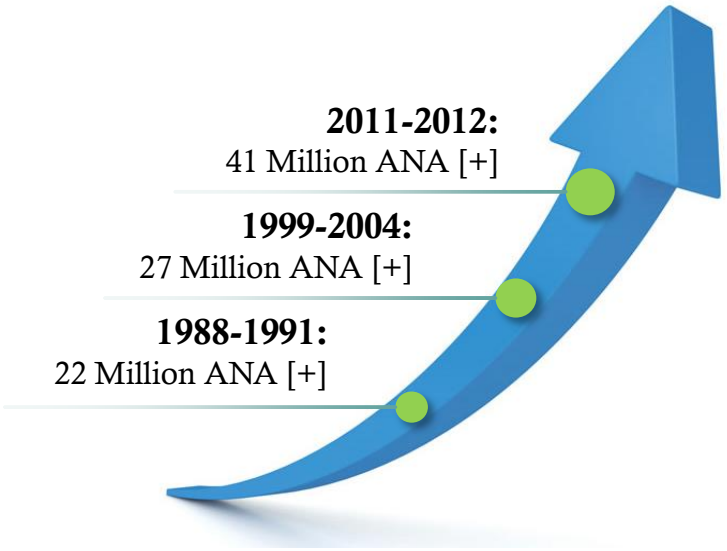
Disclosures

- I am an employee of Exagen Inc, and I receive salary and benefits from Exagen Inc.

Objectives

- Review the limitations in current standard lab testing for autoimmune CTDs, including SLE
- Highlight recent advancements in biomarkers to assess classical complement activation to facilitate earlier SLE diagnosis
- Discuss clinical validity and utility of the biomarkers for diagnosis and monitoring of SLE disease activity

The Frequency of Autoantibodies in the U.S. Population is Rising

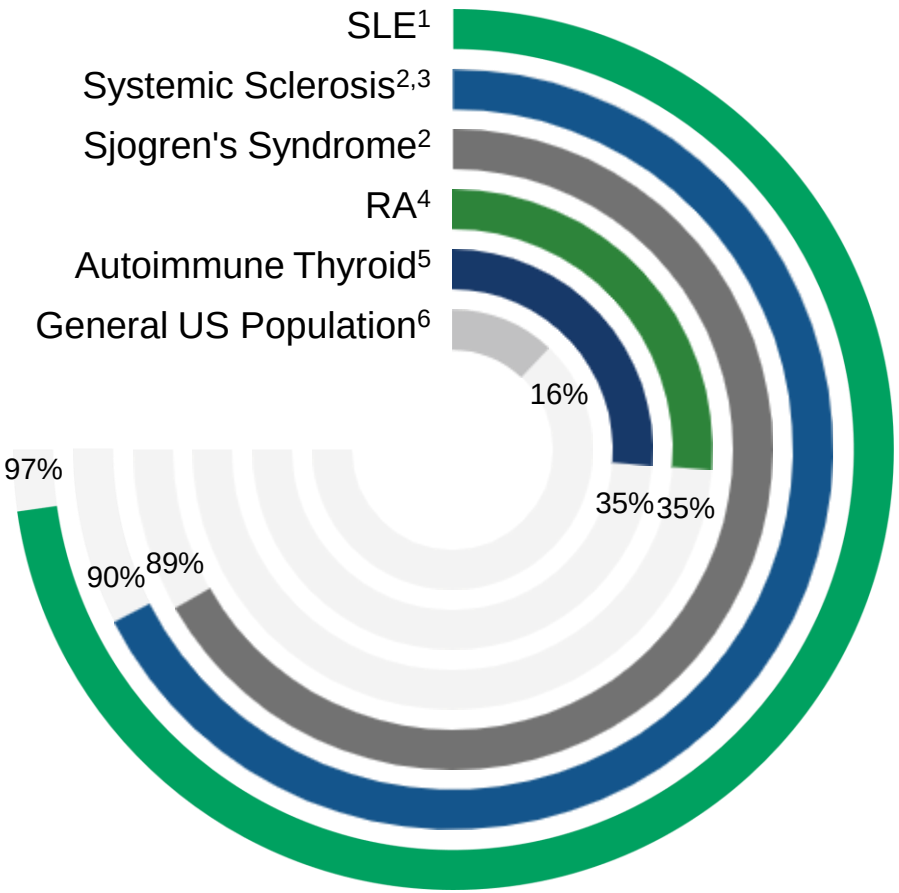


- Recent study findings suggest ANA prevalence reached 41 million in 2011-2012 up significantly from prior decades
- ANA prevalence is up significantly among
  - Both sexes (especially men)
  - Older adults (≥ 50 yrs.)
  - Non-Hispanic whites

Dinse et al. Arthritis and Rheum. 2020. doi:10.1002/art.41214

ANA is Prevalent in Autoimmune CTDs but Lacks the Power to Distinguish One CTD from Another

Prevalence of ANA Positivity in Different CTDs



1) Solomon DH, et al. Arthritis Rheum. 2002,
2) Putterman C, et al. Lupus Science & Medicine. 2014.
3) Virginia D, et al. Arthritis & Rheumatism. 1998.
4) Liao et al., Arthritis Rheumatol 2013
5) Tektonidou M, Ann Rheum Dis 2004.
6) Dinse et al. Arthritis and Rheum. 2020.

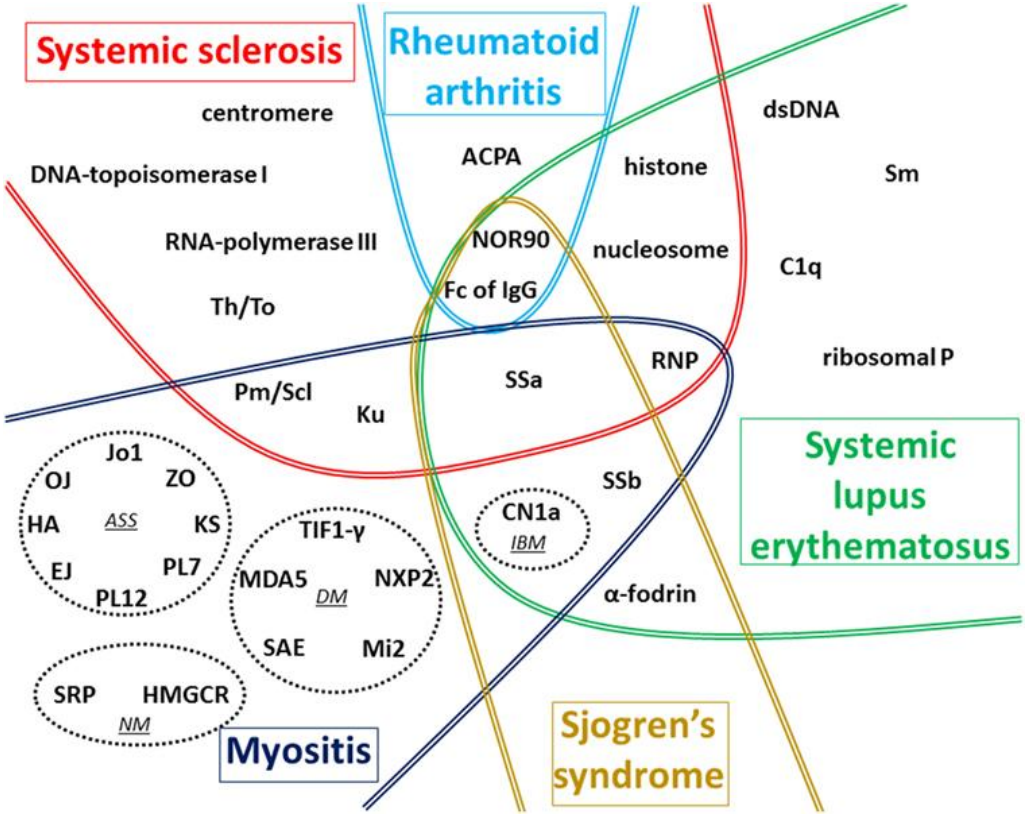
Prevalence of Disease and Overlapping Symptoms

	SLE[1]	Primary Fibromyalgia[2]	Rheumatoid Arthritis[3]
US Prevalence	1,500,000 (1.4 M)	5,000,000 (1.5 M)	2,000,000 (600K)
% of Disease ANA (+)	~90%	~30%	~30%
% of 41M ANA (+)	3.4%	3.7%	1.5%
	Chronic fatigue	Chronic fatigue	Chronic fatigue
	Painful & swollen joints	Painful joints (no swelling)	Painful joints & swelling
	Morning stiffness	Morning stiffness	Morning stiffness
	Myalgias	Myalgias	
	Headaches/Migraine	Headaches/Migraine	Headaches
	Depression/Anxiety	Depression/Anxiety	Depression
	Brain fog	Brain fog	
	Skin rash	Skin - itchy/burning	Extra articular manifestation
	Unexplained fever	Balance problems	Dry eyes and mouth
	Organ damage	Digestive upset (nausea & IBS)	Scarring of lungs
	Hair loss – Alopecia	Non-Restorative sleep	Joint deformity
	Photosensitivity	Tender points	

1) https://www.lupus.org/understanding-lupus. Accessed 2/24/22
2) Helmick et al. Arthritis and rheumatology. 2007. Doi: 10.1002/art.23176
3) Myasedova et al. Arthritis and Rheumatology. 2010. Doi: 10.1002/art/27425

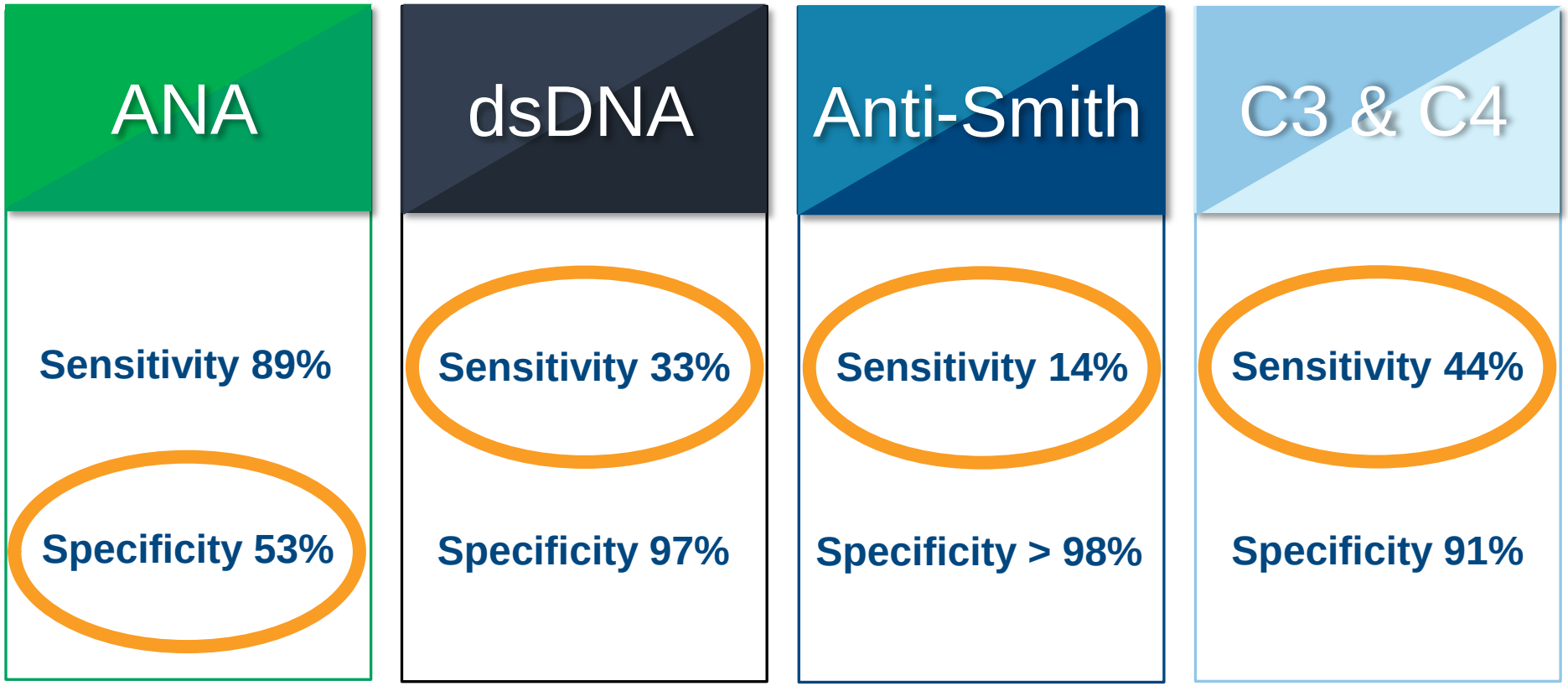
Even Specific Autoantibodies Have Multiple Clinical Associations

- ANA is a characteristic feature of SLE but **very few autoantibodies associate with SLE**
- Testing for **specific autoantibodies** aid in the differential diagnosis of other autoimmune CTDs



Didier et al. Frontiers in Immunology. 2018. doi:10.3389/fimmu.2018.00541

Deficiencies of the Current Testing Paradigm



None of these serological markers provides adequate sensitivity and/or specificity for the diagnosis of CTD or SLE

Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

Delay in Diagnosis Leads to Increased Lupus Activity and Damage

Lula cohort: 585 patients diagnosed with SLE with a mean time to diagnosis of 47 months

Increased duration between onset of symptoms and diagnosis associated with:

Increased disease activity	Increased damage	Increased fatigue
P < 0.0001	P = 0.002	P = 0.003

Kernder et al. Lupus. 2021. doi:10.1177/0961203320983445.

C3 and C4 Testing Has Limited Diagnostic Utility

Interpretation of C3 and C4 levels are subject to the following confounding factors:

Individual genetic variation affecting complement gene copy numbers

Dynamic changes in complement protein synthesis and catabolism

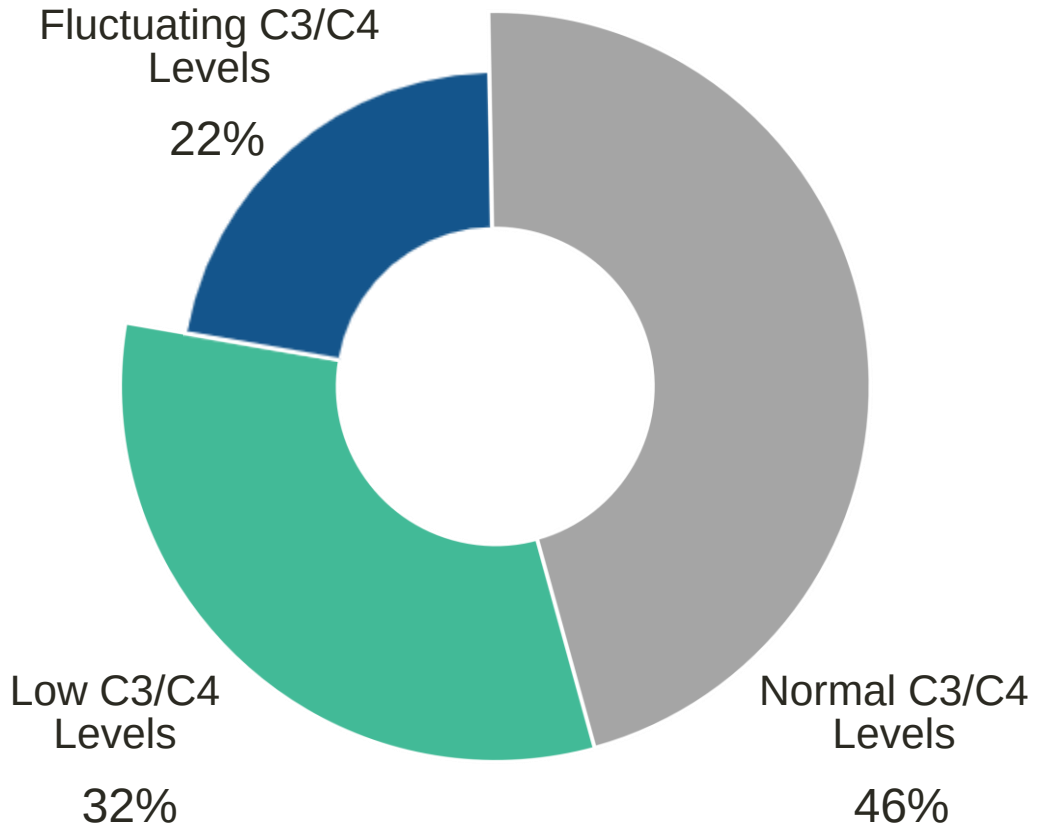
Increases in complement production in response to acute phase reactants

Soluble C3 and C4 Stay Constant for Many Patients

In a combined cohort of 124 SLE patients across 624 visits:

- 46% of patients presented with normal complement levels at all visits
- 32% of patients presented with low C3 or C4 at all visits
- 22% of patients presented with fluctuating complement status

C3/C4 Levels Over Time



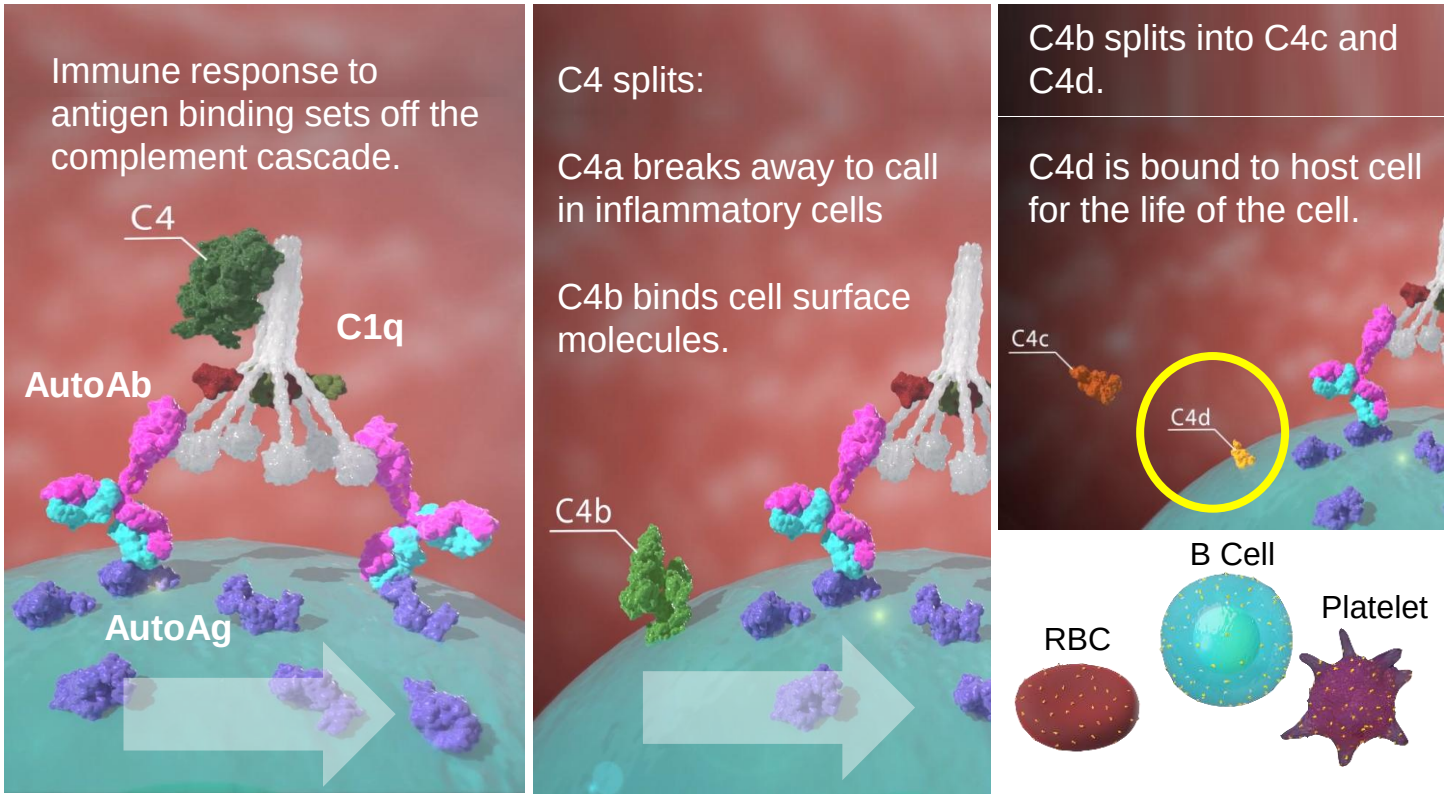
Merrill J, et al. Lupus Science & Medicine 2018. doi:10.1136/ lupus-2018-000263

CB-CAPs

Cell-bound Complement Activation Products

EC4d BC4d PC4d

CB-CAPs: Elements of Classical Complement Activation



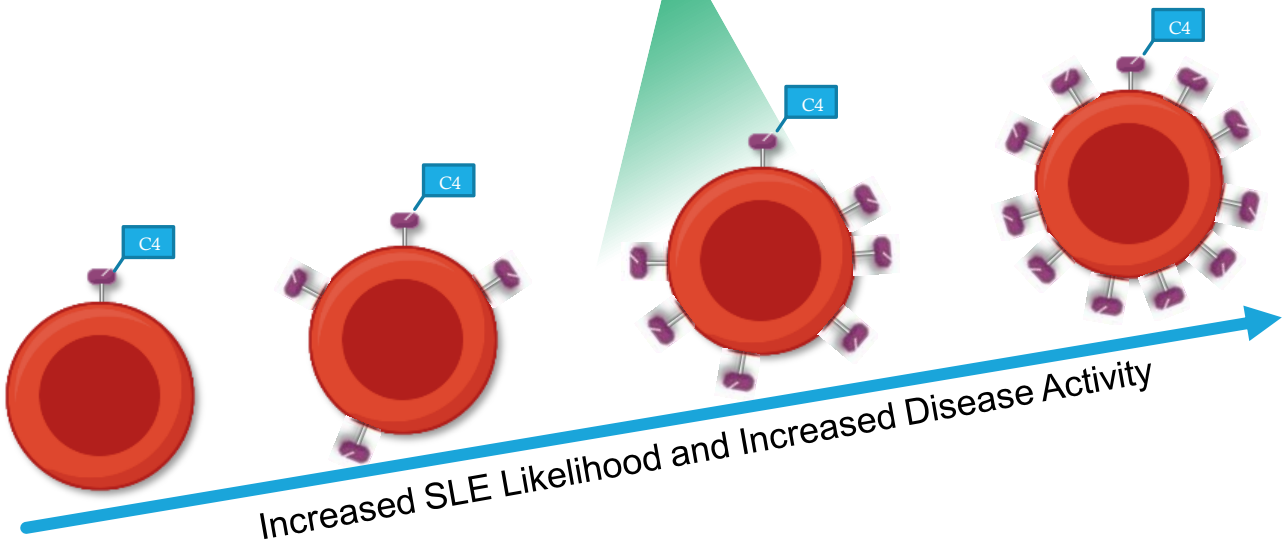
Yellow dots represent C4d bound to the cell.

Sarma et al. Cell Tissue Res. 2011. doi:10.1007/s00441-010-1034-0.

CB-CAPs: Sensitive Indicators of Complement Activation

Autoantibodies and autoantigens lead to inflammation → **Complement proteins recruit cells** → **Inflammation causes organ damage**

Complement pieces bind to cells as markers of activity. EC4d (on RBC), **CB-CAPs**



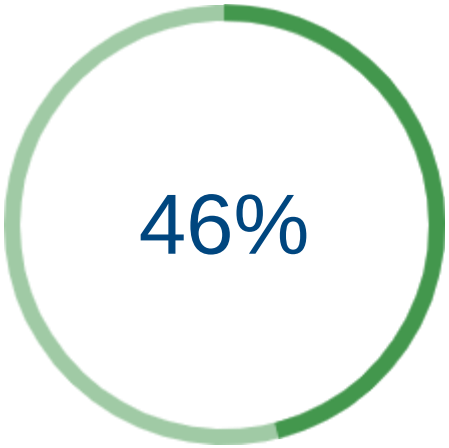
RBCs live 90 days.

EC4d results are the average number of C4d per RBC	**1 C4d/cell**	**4 C4d/cell**	**7 C4d/cell**	**12 C4d/cell**	**100s**

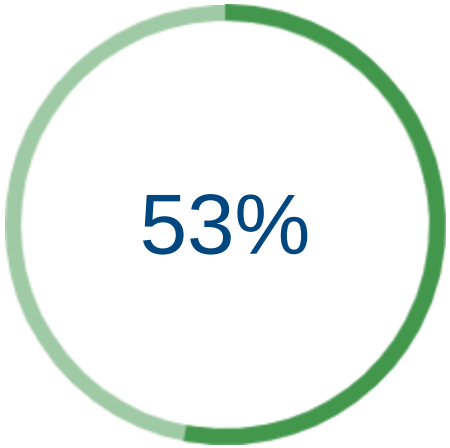
CB-CAPs as a Biomarker for SLE Diagnosis

Elevated CB-CAPs are Primarily Associated with SLE

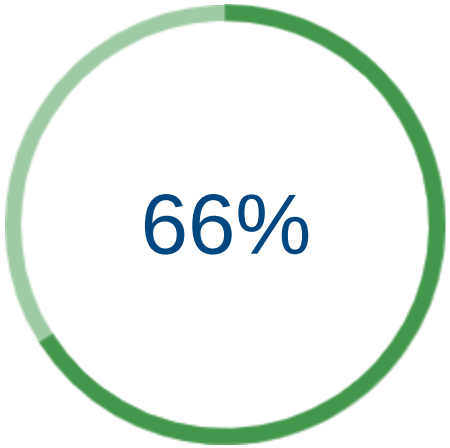
CB-CAPs (EC4d & BC4d) are <u>sensitive for SLE</u> at the low positive threshold levels



46% of SLE patients
test positive for
EC4d > 14 Units



53% of SLE patients
test positive for
BC4d > 60 Units

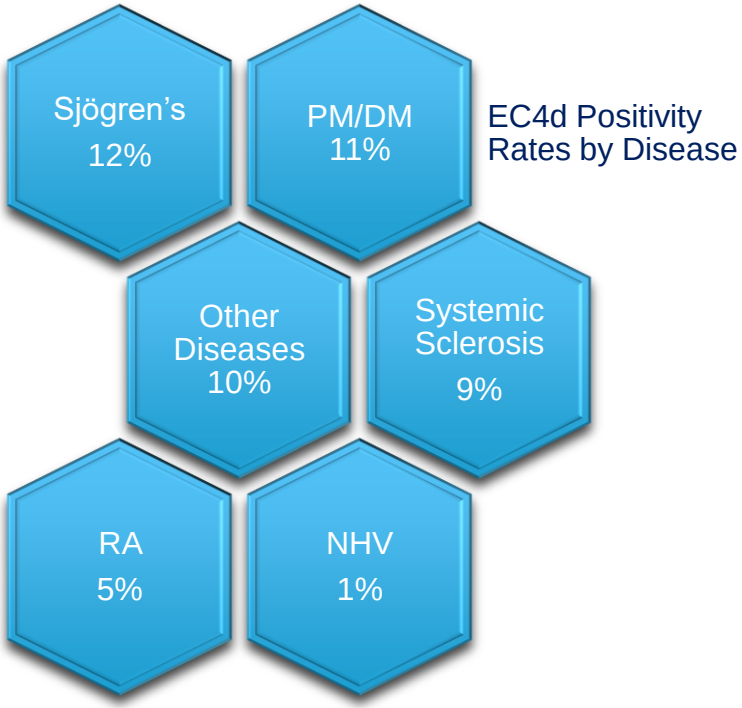


66% of SLE patients
test positive for
EC4d > 14 Units and/or
BC4d > 60 Units

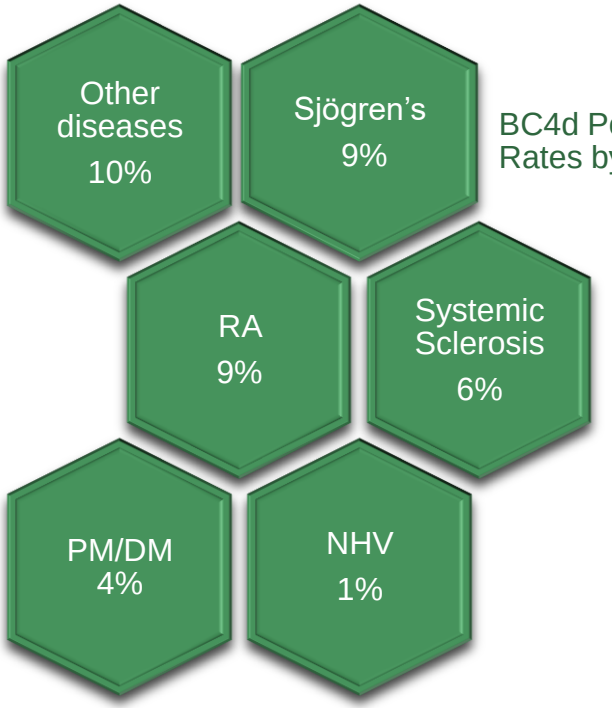
Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

Elevated CB-CAPs are Rarely Found in Other CTDs

CB-CAPs (EC4d & BC4d) are <u>specific for SLE</u> at the low positive threshold levels



EC4d Positivity Rates by Disease



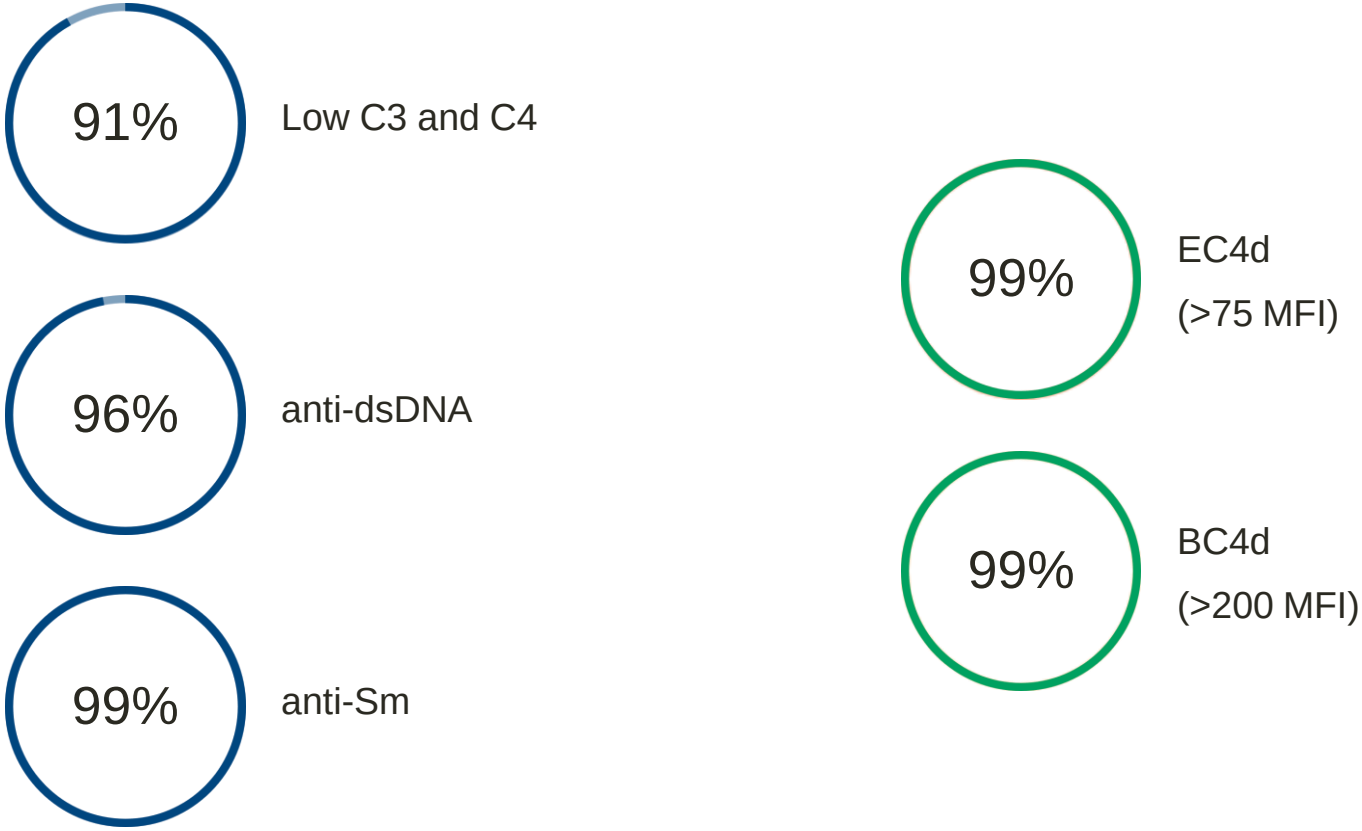
BC4d Positivity Rates by Disease

*Other disease patients include Fibromyalgia, Vasculitis, Granulomatosis with Polyangiitis and Antiphospholipid Syndrome, NHV = Normal Healthy Volunteers

Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

CB-CAPs in the Strong Positive Thresholds are as Specific for SLE as anti-dsDNA and anti-SM

Strong Positive CB-CAPs (EC4d & BC4d) are 99% specific for SLE against other autoimmune CTDs

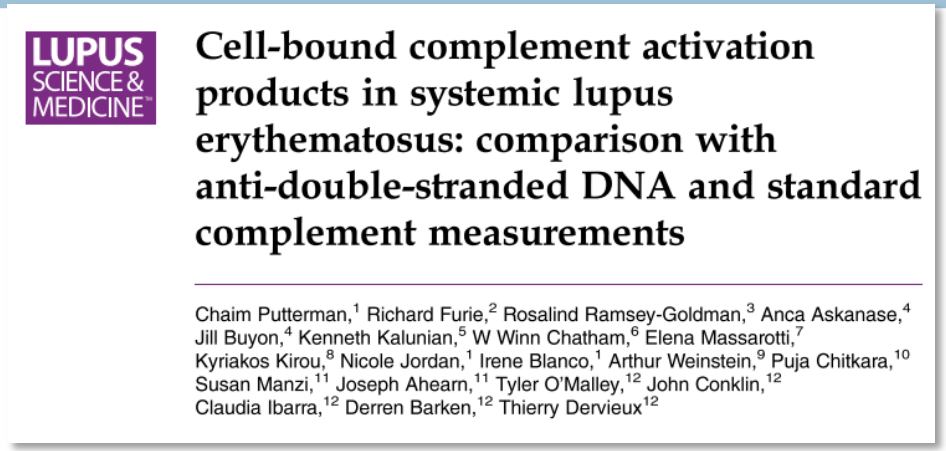


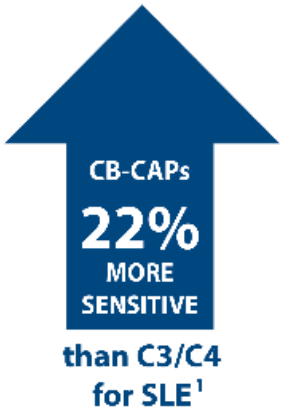
Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

CB-CAPs vs. Serum C3 and C4

In a multi-centered cross-sectional study including 794 total subjects from 15 Lupus centers in the US:

- Elevated EC4d and BC4d yielded a 22% higher sensitivity (66%) for SLE than reduced C3 or C4 (44%)
- CB-CAPs had a higher sensitivity for SLE than standard complement measures alone, which supports the diagnostic utility of CB-CAPs for SLE

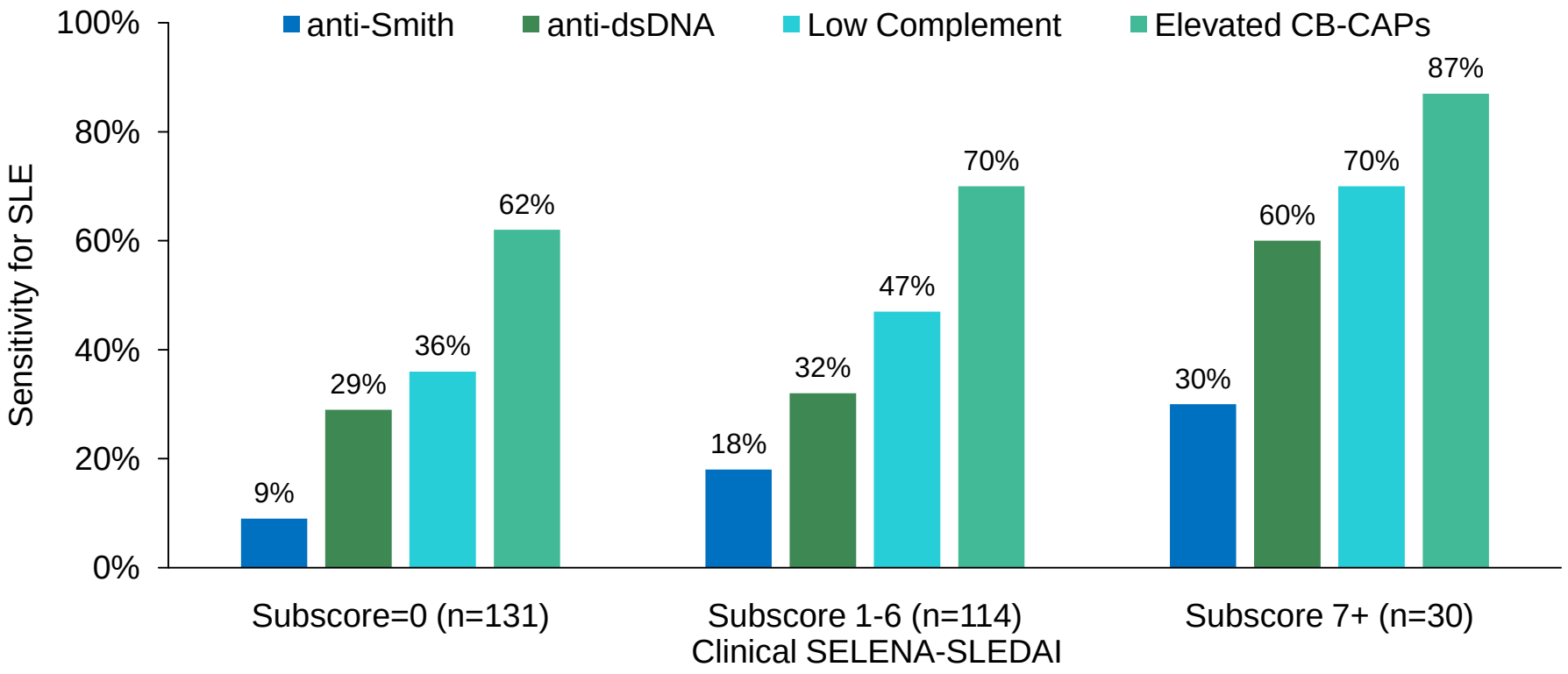




Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

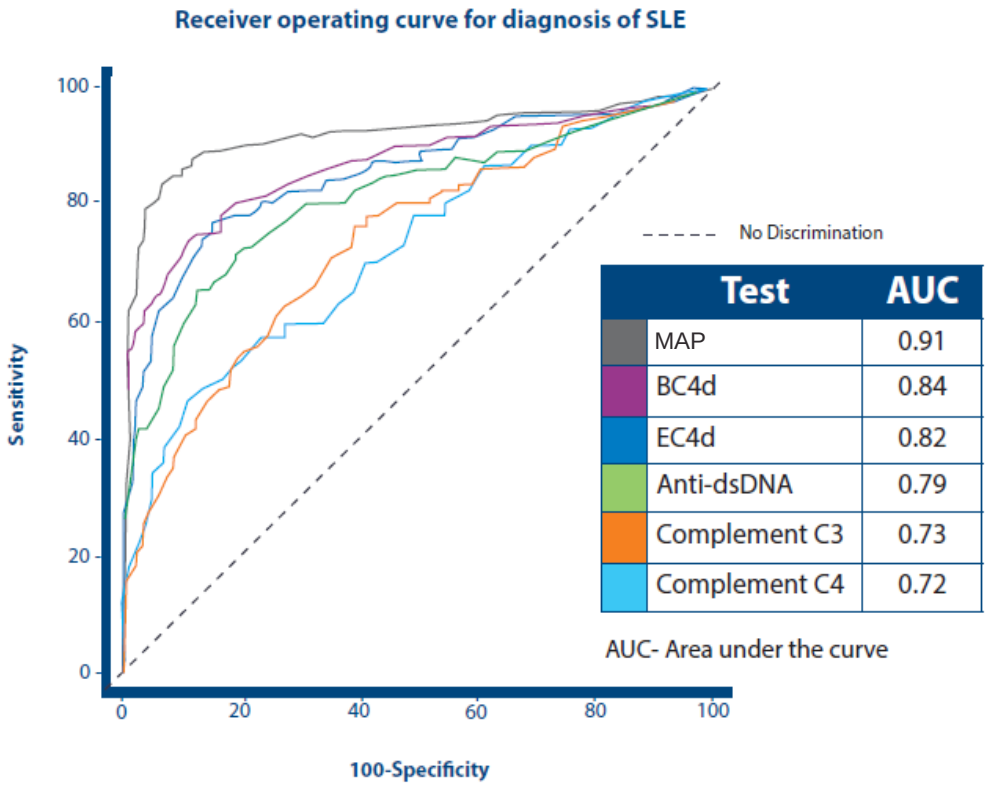
CB-CAPs vs. Traditional SLE Diagnostics Across the Disease Activity Spectrum

In SLE patients, CB-CAPs delivered higher sensitivity compared to anti-Smith, anti-dsDNA, and C3/C4 across the disease activity spectrum



Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

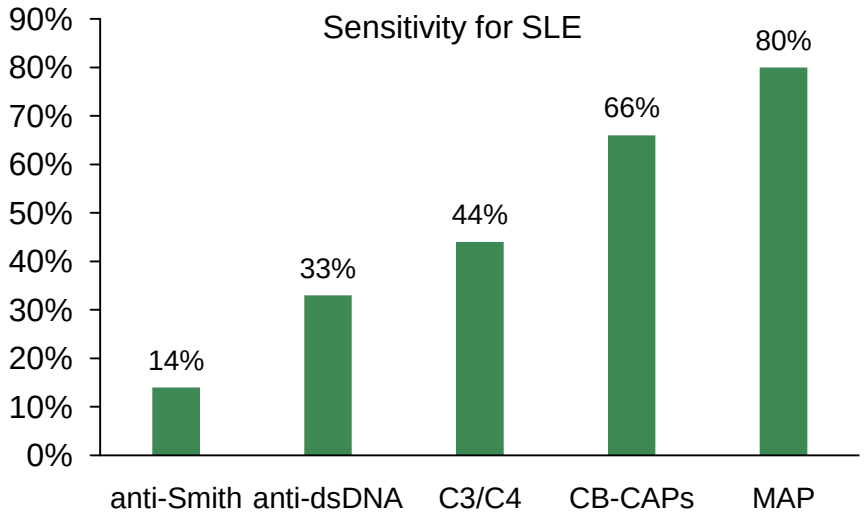
MAP Integrates CB-CAPs with Traditional Markers to Yield High Diagnostic Sensitivity and Specificity



Test	AUC
MAP	0.91
BC4d	0.84
EC4d	0.82
Anti-dsDNA	0.79
Complement C3	0.73
Complement C4	0.72

AUC- Area under the curve

- Multianalyte Assay Panel (MAP) consists of 10 markers including:
  - **ANA**, **anti-dsDNA**, **anti-Sm**, **CB-CAPs** (EC4d and BC4d), and **5 ENA markers** (anti-CCP, anti-SS-B/La, anti-CENP, anti-Scl-70, anti-Jo-1
- MAP and CB-CAPs outperform traditional biomarkers



Putterman C, et al. Lupus Science & Medicine, 2014. doi:10.1136

MAP and CB-CAPs in Fibromyalgia

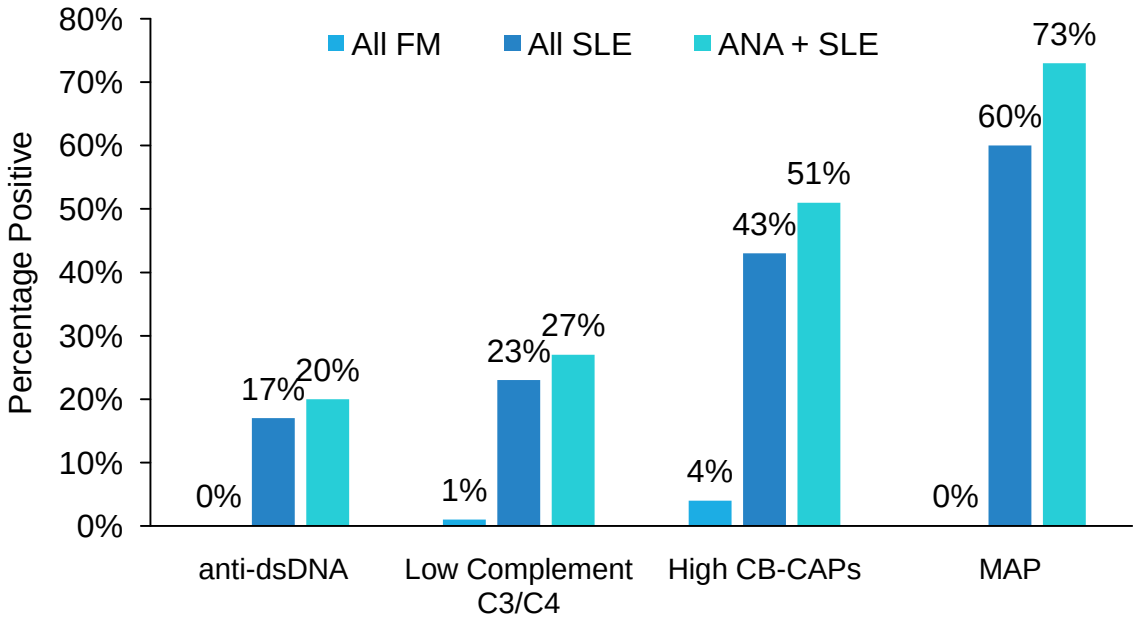
In a prospective study enrolling 75 primary fibromyalgia patients and 75 primary lupus patients from two rheumatology practices:

- CB-CAPs were highly specific for SLE against primary fibromyalgia
  - 96% specific for EC4d
  - 100% specific for BC4d
- MAP was 100% specific for SLE against primary fibromyalgia

LUPUS SCIENCE & MEDICINE

Systemic lupus erythematosus and primary fibromyalgia can be distinguished by testing for cell-bound complement activation products

Daniel J Wallace,[1,2] Stuart L Silverman,[1] John Conklin,[3] Derren Barken,[3] Thierry Dervieux[3]



Wallace D, et al. Lupus Science & Medicine. 2016. doi:10.1136/lupus-2015-000127

MAP and CB-CAPs in Probable SLE

Arthritis & Rheumatology
Vol. 72, No. 1, January 2020, pp 78–88
DOI 10.1002/art.41093

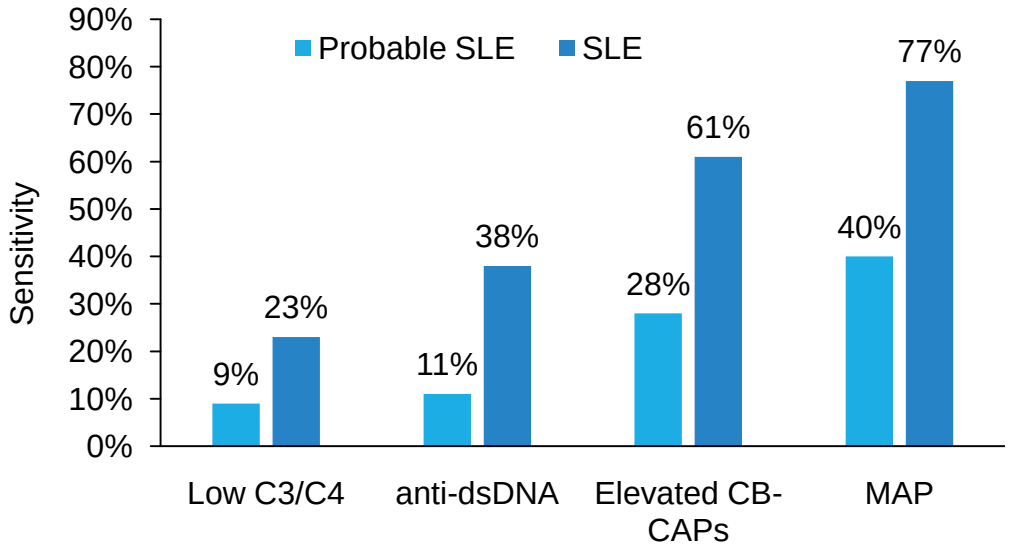
American College of Rheumatology
Empowering Rheumatology Professionals

Complement Activation in Patients With Probable Systemic Lupus Erythematosus and Ability to Predict Progression to American College of Rheumatology–Classified Systemic Lupus Erythematosus

Rosalind Ramsey-Goldman,[1] Roberta Vezza Alexander,[2] Elena M. Massarotti,[3] Daniel J. Wallace,[4] Sonali Narain,[5] Cristina Arriens,[6] Christopher E. Collins,[7] Amit Saxena,[8] Chaim Putterman,[9] Kenneth C. Kalunian,[10] Tyler O'Malley,[2] Thierry Dervieux,[2] and Arthur Weinstein[11]

In a cross sectional cohort of 50 SLE and 92 probable SLE patients (meeting 3 ACR criteria):

- Positive CB-CAPs alone or in MAP were present in a higher percentage of SLE and probable SLE patients compared to anti-dsDNA and low complement levels



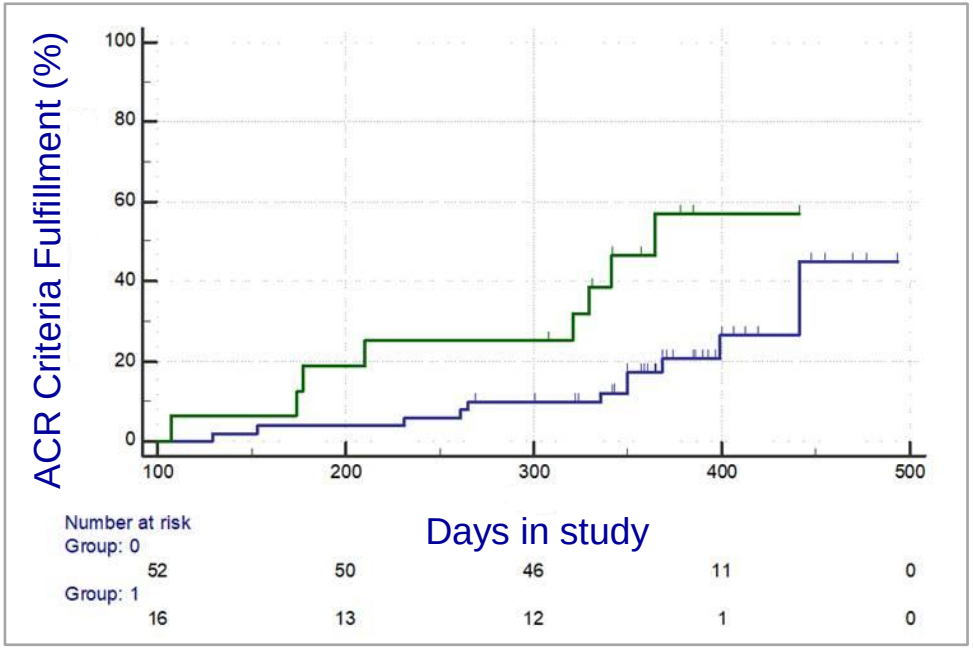
 Ramsey-Goldman R, et al. A&R 2020. doi:10.1002/art.41093

MAP and CB-CAPs in Probable SLE

Patients testing MAP positive (index > 0.8) were significantly more likely to progress to classifiable SLE

Table 3. HRs of biomarkers predicting fulfillment of ACR classification criteria by 18 months in patients with probable SLE*

	HR	95% CI	*P*
Anti-dsDNA†	2.97	0.98–8.99	0.043
Low complement levels‡	1.93	0.44–8.53	0.375
CB-CAPs§	1.66	0.67–4.09	0.275
EC4d >20 MFI	2.61	0.99–6.88	0.053
MAP >0.8	3.11	1.26–7.69	0.0097

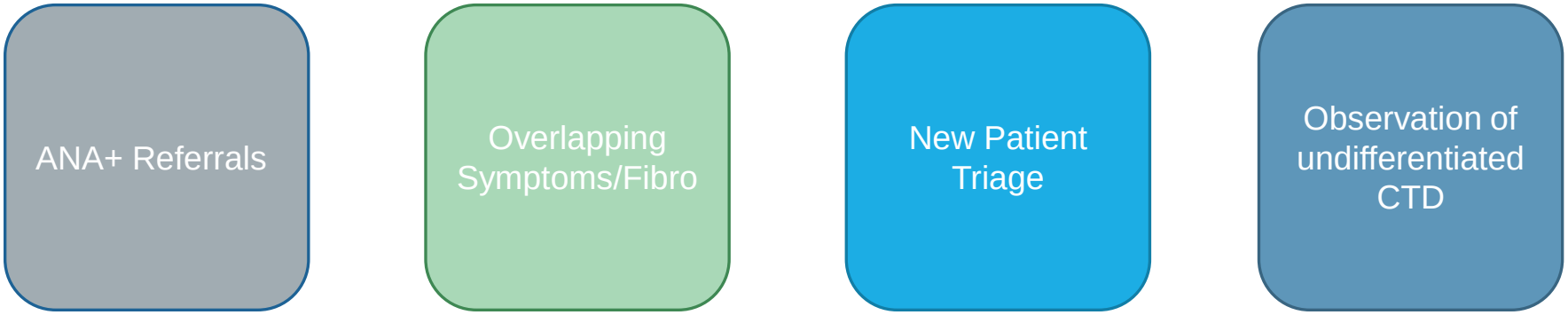


Blue: MAP index ≤ 0.8 | Green: MAP > 0.8

Ramsey-Goldman R, et al. A&R 2020. doi:10.1002/art.41093

Incorporating CB-CAPs with Conventional ENA Autoantibodies Enhances Diagnostic Utility

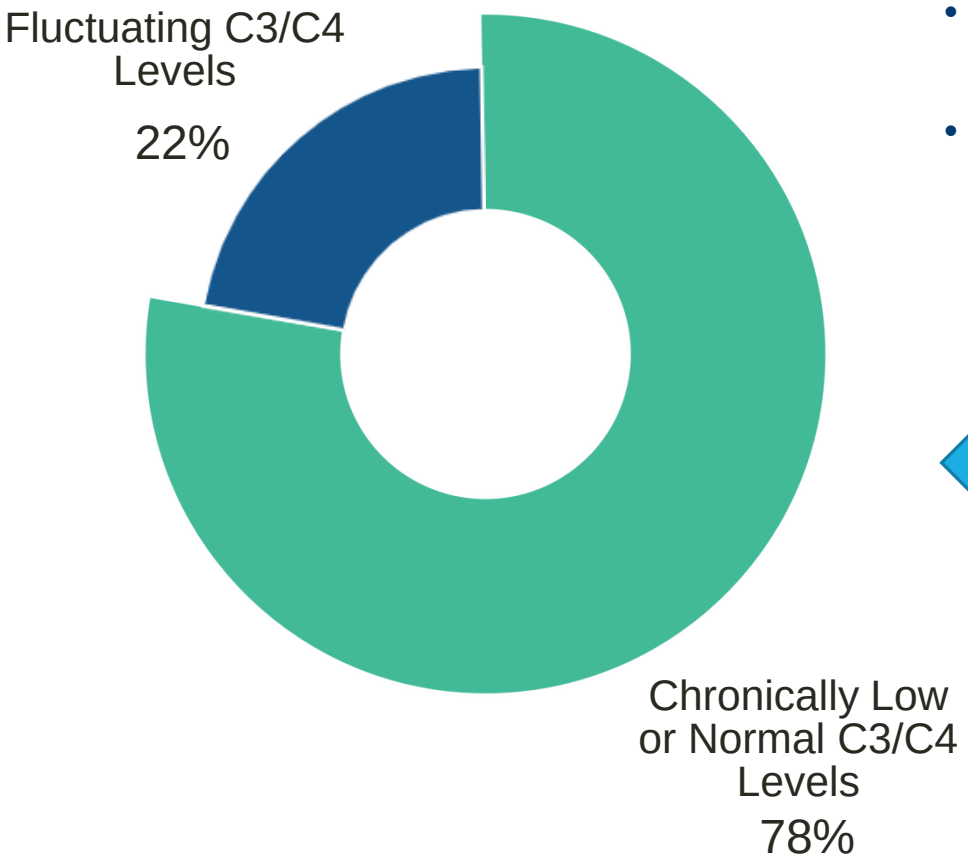
Incorporating CB-CAPs testing in addition to conventional ENA autoantibodies can further enhance diagnostic utility for evaluation of suspected Lupus and other CTDs



CB-CAPs as a Biomarker for SLE Monitoring and Prognosis

EC4d Is Informative Even When C3/C4 is Unchanged

C3/C4 Levels Over Time



- 78% (97/124) patients had chronically low or normal C3/C4.
- EC4d levels fluctuated with disease activity and provided additional information regardless of C3/C4.

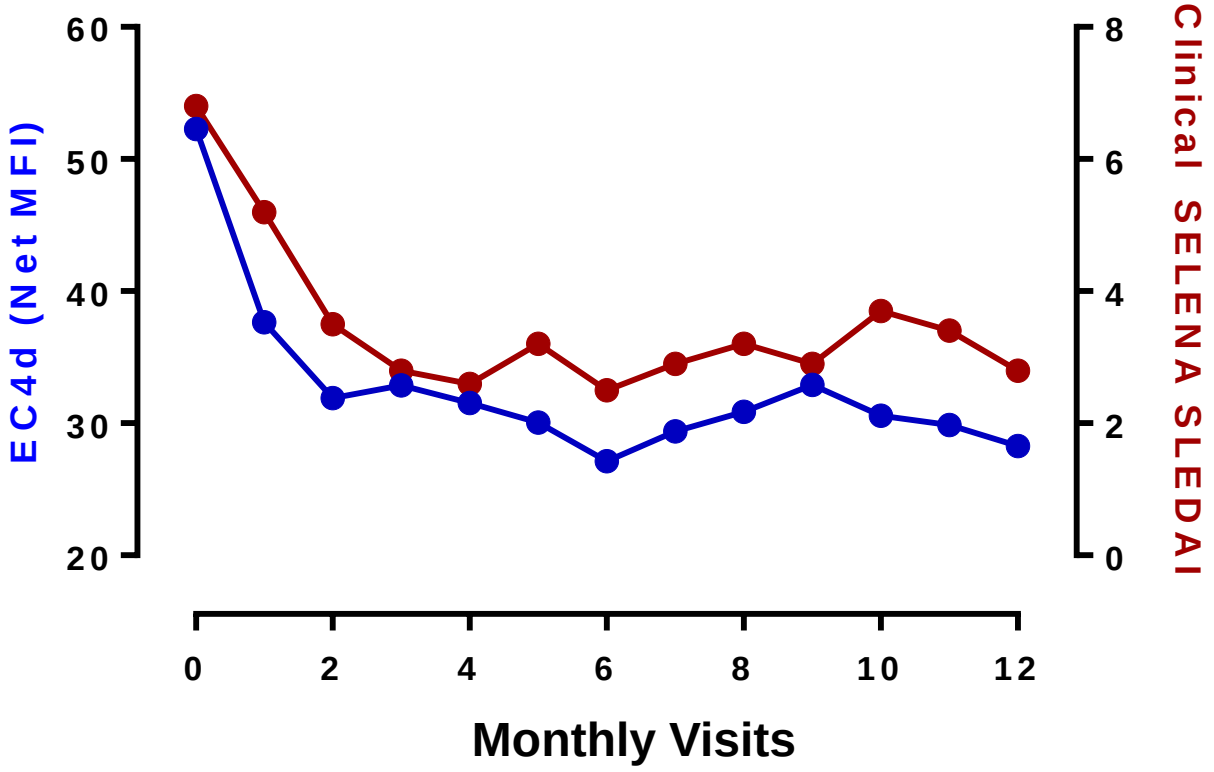
EC4d provided additional information in this group:

Marginal R^2 = 7.9% (p<0.001)

Merrill J, et al. Lupus Sci Med. 2018. doi:10.1136/lupus-2018-000263.

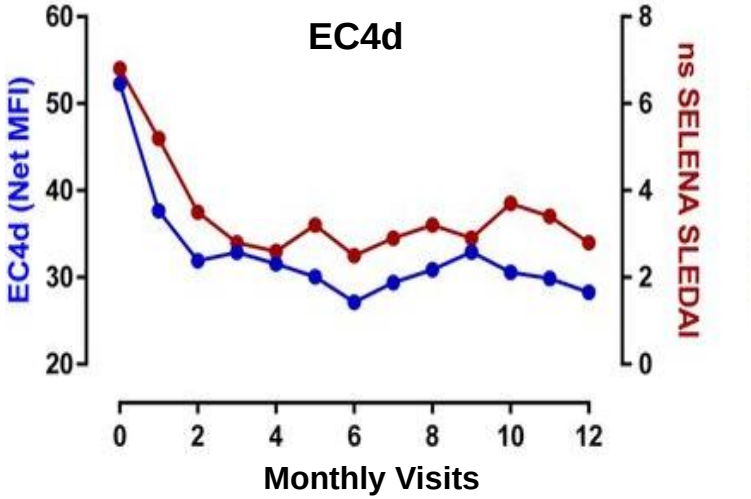
Clinical Improvements and EC4d

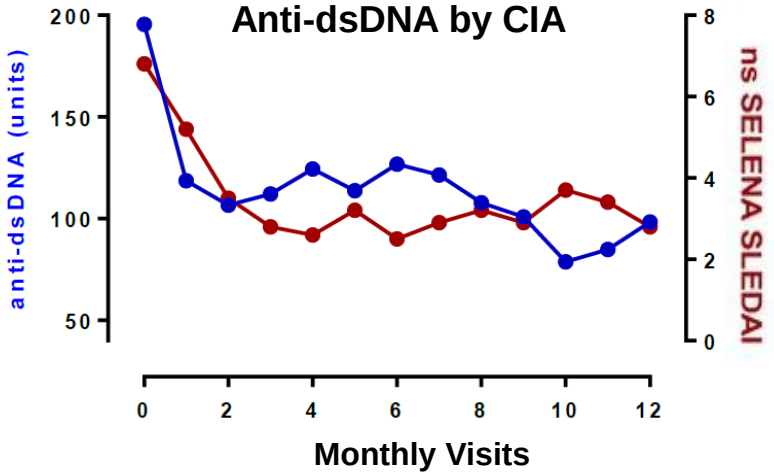
EC4d declines rapidly with clinical improvement ($p < 0.036$)

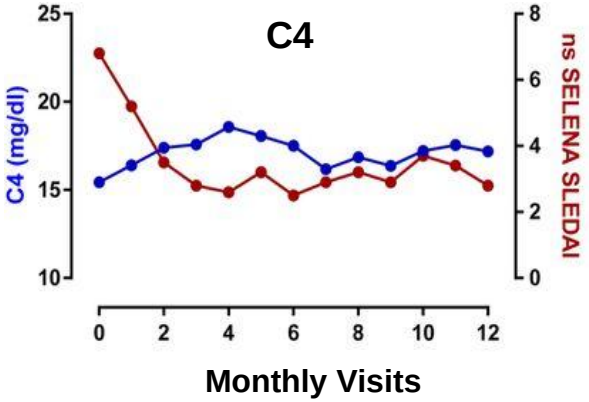


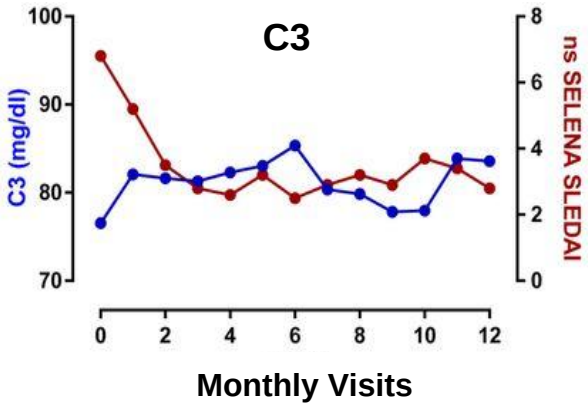
Buyon J, et al. Lupus Science & Medicine 2016. doi:10.1136/lupus-2016-000165

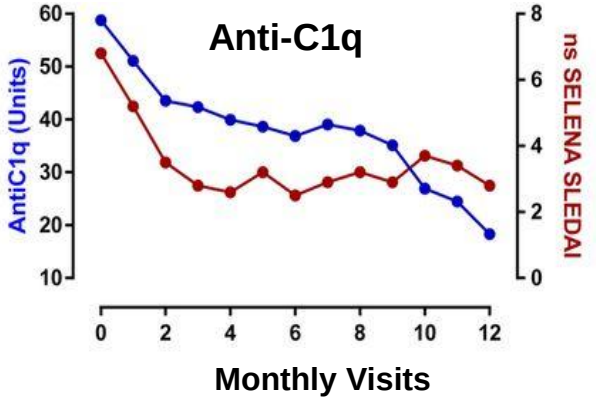
Correlation with Clinical SELENA-SLEDAI Over 12 Monthly Visits







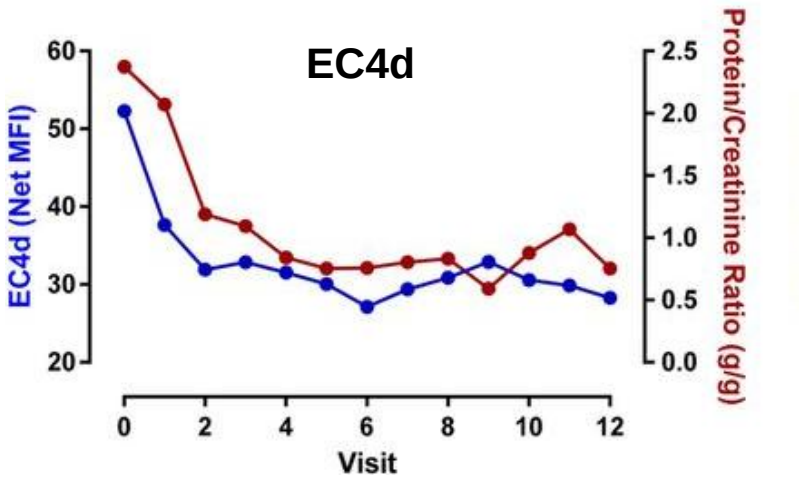


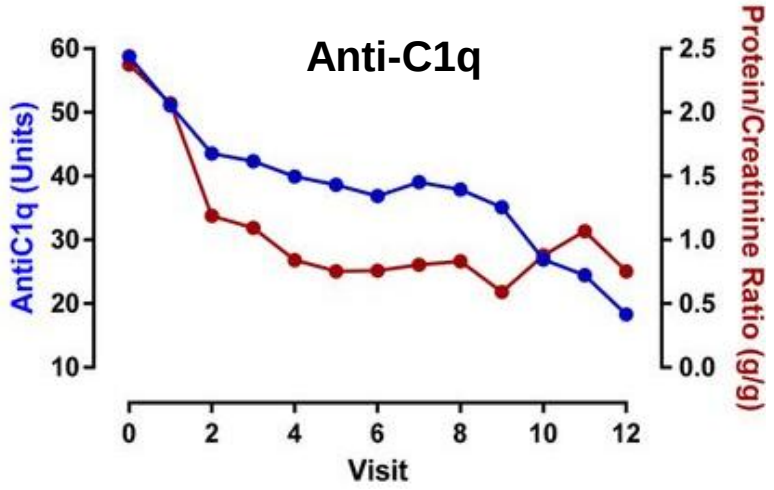


Buyon J, et al. Lupus Science & Medicine 2016. doi: 10.1136/lupus-2016-000165
dsDNA graph source: Data on file Exagen Diagnostics Inc.2017

Correlation to Urine Protein Creatinine Ratio

Similar to anti-C1q, a decrease in uPCR was associated with a decrease in EC4d ($p < 0.001$)



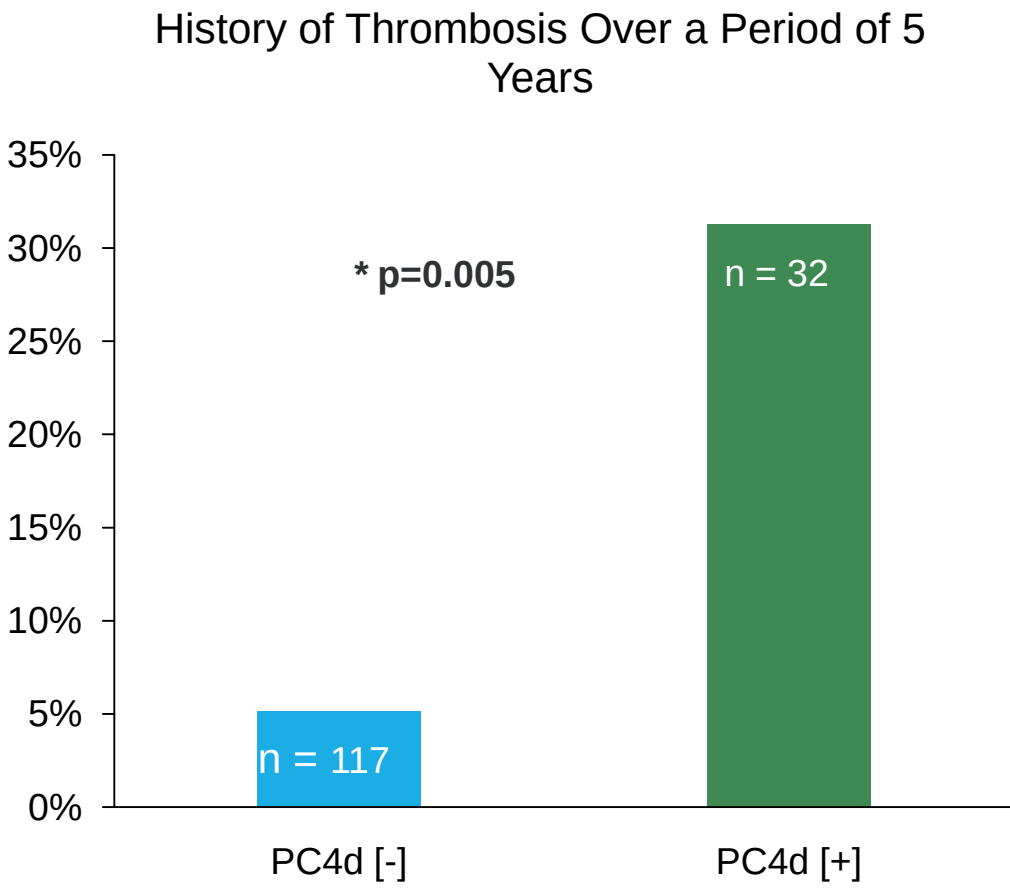


Buyon J, et al. Lupus Science & Medicine 2016. doi:10.1136/lupus-2016-000165

Marker Associated with Thrombosis in SLE: Platelet-bound C4d (PC4d)

In this group of 149 patients with SLE:

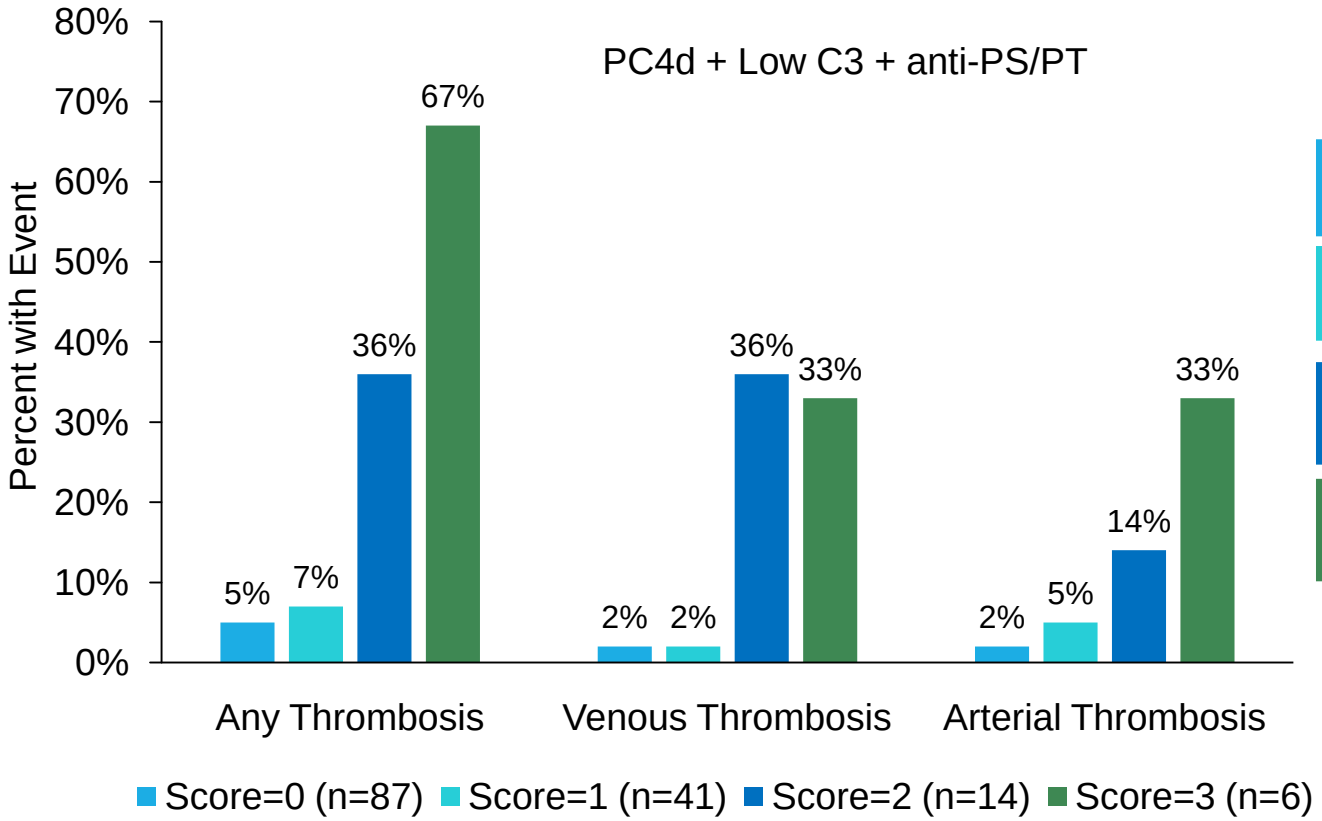
- Abnormal levels of PC4d associated with history of 1 or more thrombotic events over 5 years[1]
- Persistent elevated PC4d associated with thrombosis and ischemic stroke (OR 5.48, 95% CI 1.7–17.6, p=0.004)[2]



1. Petri MA, et al. Lupus Sci & Med. 2019. doi:10.1136/lupus-2019-000318.
2. Kao A, et al.. Transl Stroke Res. 2014. doi:10.1007/s12975-013-0295-9.

PC4d: An Independent Risk Factor for History of Thrombosis

C3, anti-PS/PT IgG, and PC4d independently associate with a history of thrombosis



0 = Negative Low C3 & anti-PS/PT & PC4d>20 net MFI

1 = Positive for either Low C3 or anti-PS/PT or PC4d>20 net MFI

2 = Positive for two Low C3 **or** anti-PS/PT **or** PC4d>20 net MFI

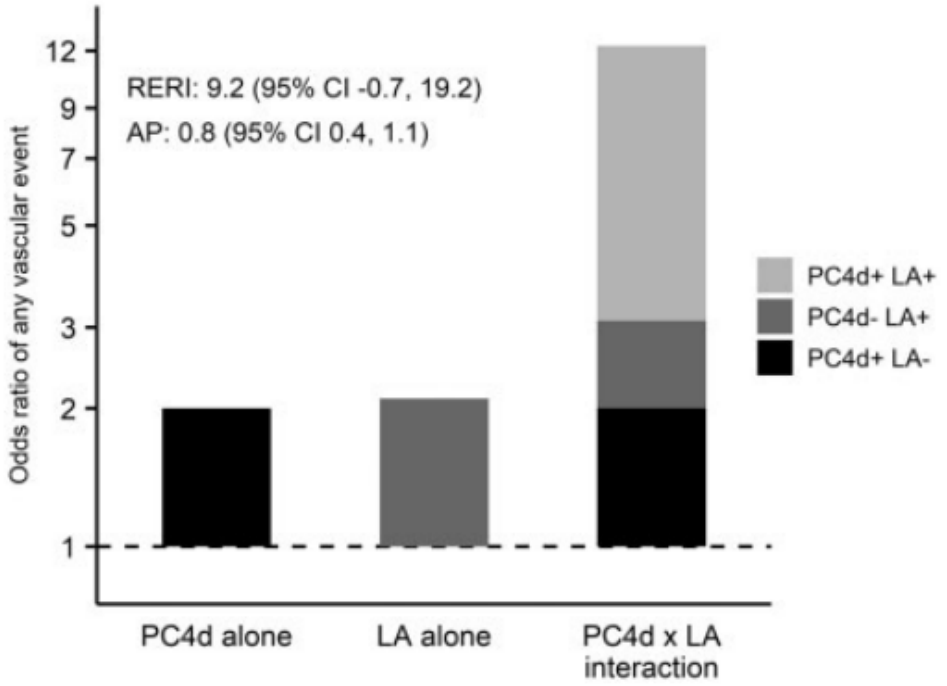
3 = Positive for Low C3 **&** anti-PS/PT **&** PC4d>20 net MFI

Petri MA, et al. Lupus Sci & Med. 2019. doi:10.1136/lupus-2019-000318.

PC4d Independently Associates with Previous Vascular Disease

In this group of 308 SLE patients:

- PC4d and lupus anticoagulant (LA) independently and synergistically associated with vascular events [attributable proportion of 0.8 (95% CI: 0.4, 1.1)]
- PC4d was an independent marker for vascular disease in SLE and can add value to the assessment of the risk of vascular events



Svenungsson E et al. Rheumatology. 2020. doi:10.1093/rheumatology/keaa092

Summary

- SLE is complex with many challenges related to diagnosis, disease monitoring, and prognostic determinates
- CB-CAPs are novel biomarkers with improved performance characteristics over the traditional testing paradigm
- PC4d may add meaningful information in assessing risk for thrombosis
  - The association between PC4d and Thrombosis in SLE appears independent of LAC