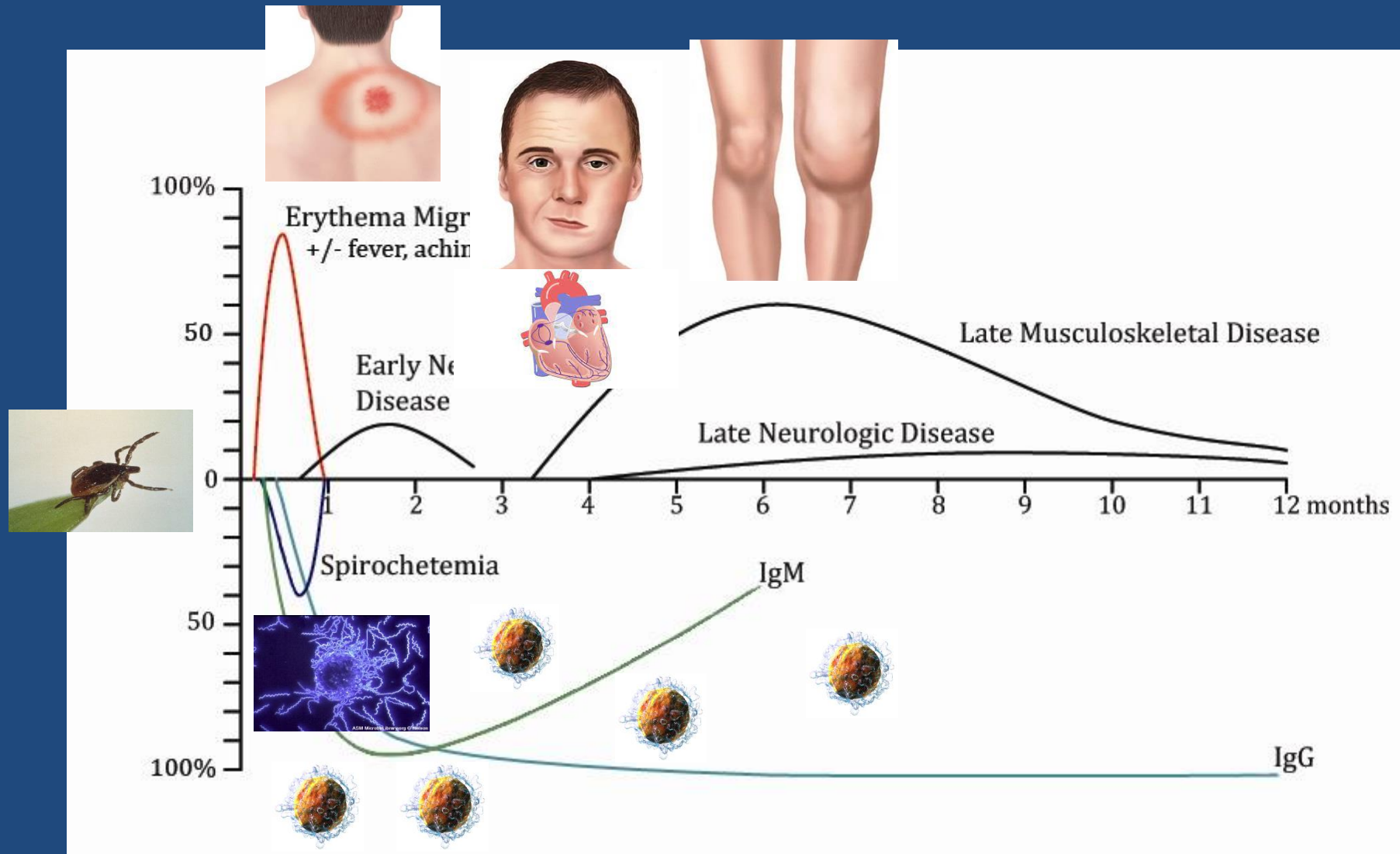
Biomarkers for Lyme Disease and Post-treatment Lyme

Future opportunities and research priorities in diagnostics

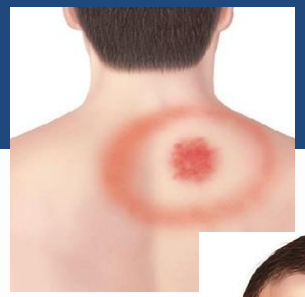
John Aucott, MD
Barbara Townsend Cromwell Professor in Lyme Disease and Tickborne Illness
Director, Lyme Disease Research Center
Associate Professor of Medicine
Division of Rheumatology
Johns Hopkins University School of Medicine

JOHNS HOPKINS
MEDICINE

The Diagnosis of Untreated Lyme Disease Depends on the Stage of the Disease: Early, Early Disseminated, and Late Lyme Disease



Lyme Disease and Persistent Symptoms



Persistent Symptoms without Textbook Objective Findings

Post-treatment Lyme Disease

Arvikar SL, Crowley JT, Sulka KB, Steere AC. Autoimmune Arthritides, Rheumatoid Arthritis, Psoriatic Arthritis, or Peripheral spondyloarthritis Following Lyme Disease. *Arthritis Rheumatol*. 2017;69(1):194-202. doi:10.1002/art.39866

Clinicians Goal for Lyme Testing

- The perfect test! High sensitivity and specificity
- Test that works at all stages of infection
  - Early seronegative windows
    - IgM
    - IgG
  - Identifies bacteria in bloodstream
- Test to diagnose post-treatment Lyme disease
  - Marker of past exposure (like a PPD skin test for tuberculosis)
  - Test of cure (like VDRL or RPR for syphilis)

Opportunities and Research Priorities in Diagnostics

Currently Available Tests
Antibody tests

ELISA, Western Blot

Limitations in sensitivity and specificity

DIRECT TESTS FOR PATHOGEN

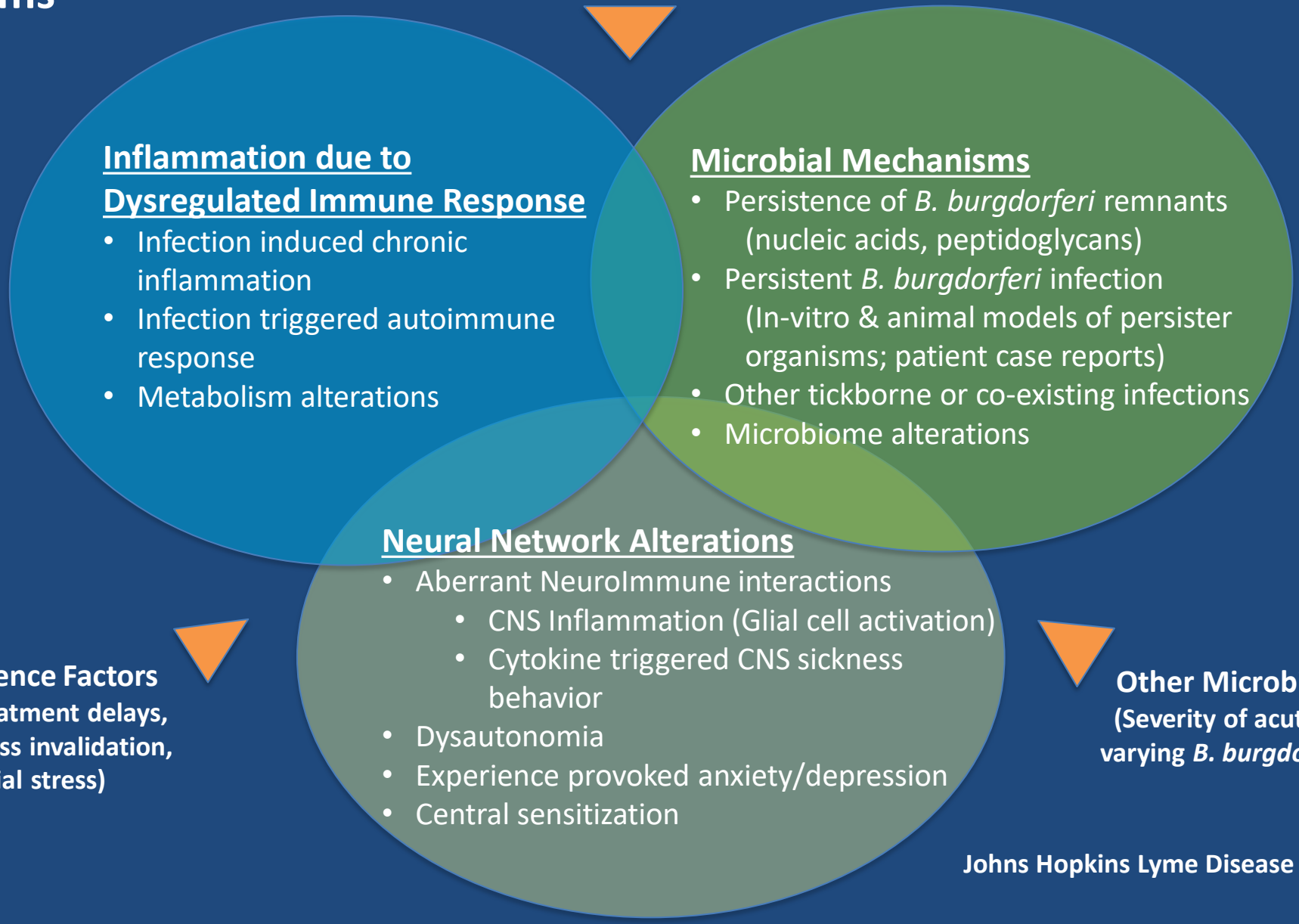
- Culture
- Direct tests for molecular components of the microbe
  - PCR tests
  - Metagenomics
  - Antigen detection
    - Urine proteomics
    - Peptidoglycan

INDIRECT TESTS FOR HOST RESPONSE TO PATHOGEN

- Blood Proteomics
  - Cytokines/chemokines
  - Autoantibodies
- Metabolomics
- Transcriptomics
- Epigenetics

Multifactorial Potential Mechanisms of Lyme Disease Associated Chronic Illness

Host, Genetic, and Environmental Factors
(Genetic variation in key immune system mediators, epigenetic changes, sex, age, race/ethnicity, environmental exposures, co-morbidities)



Illness Experience Factors
(Diagnosis & treatment delays, uncertainty, illness invalidation, psychosocial stress)

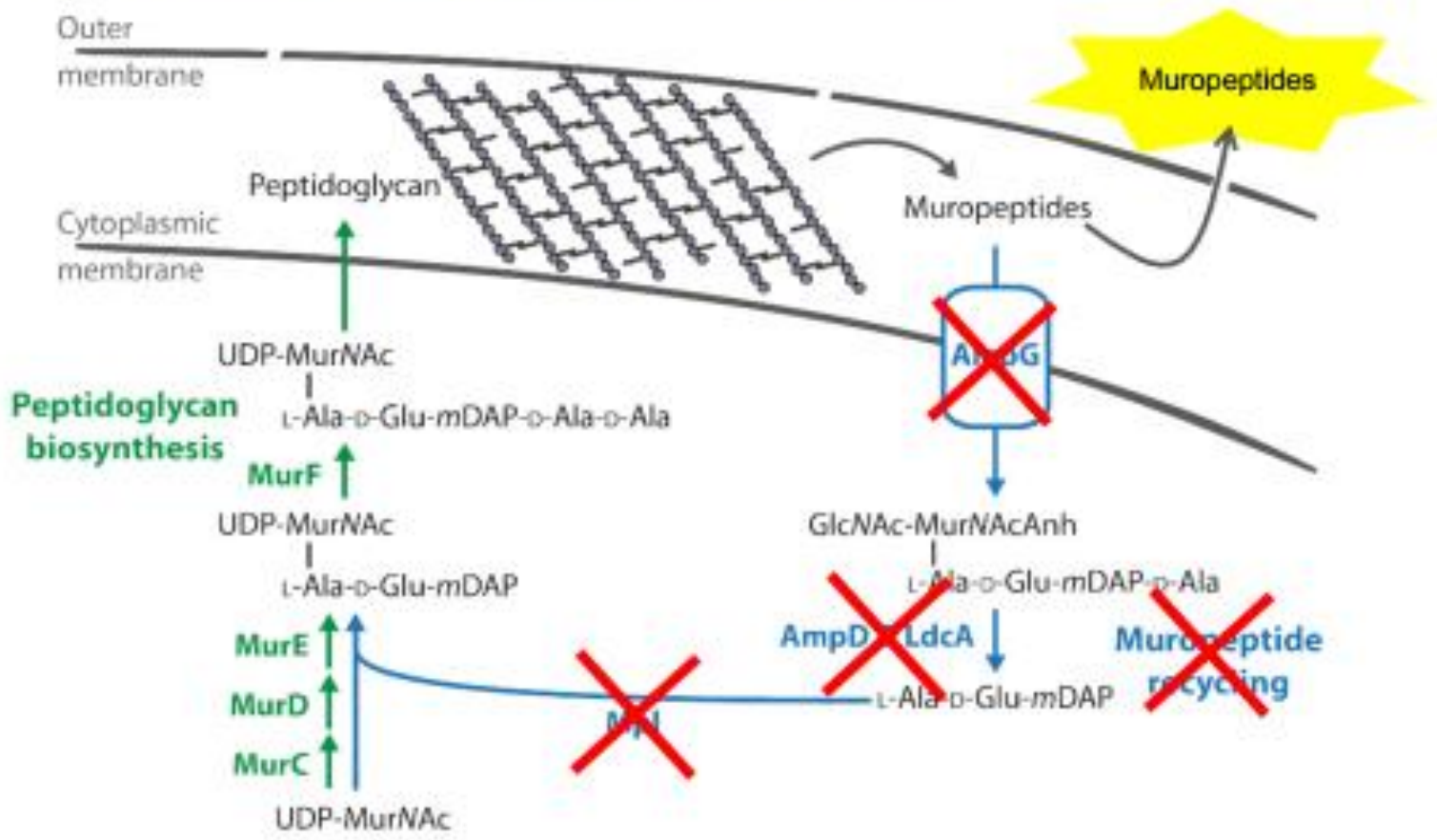
Other Microbial Factors
(Severity of acute infection, varying *B. burgdorferi* strains)

Johns Hopkins Lyme Disease Research Center

Borrelia burgdorferi peptidoglycan is a persistent antigen in patients with Lyme arthritis

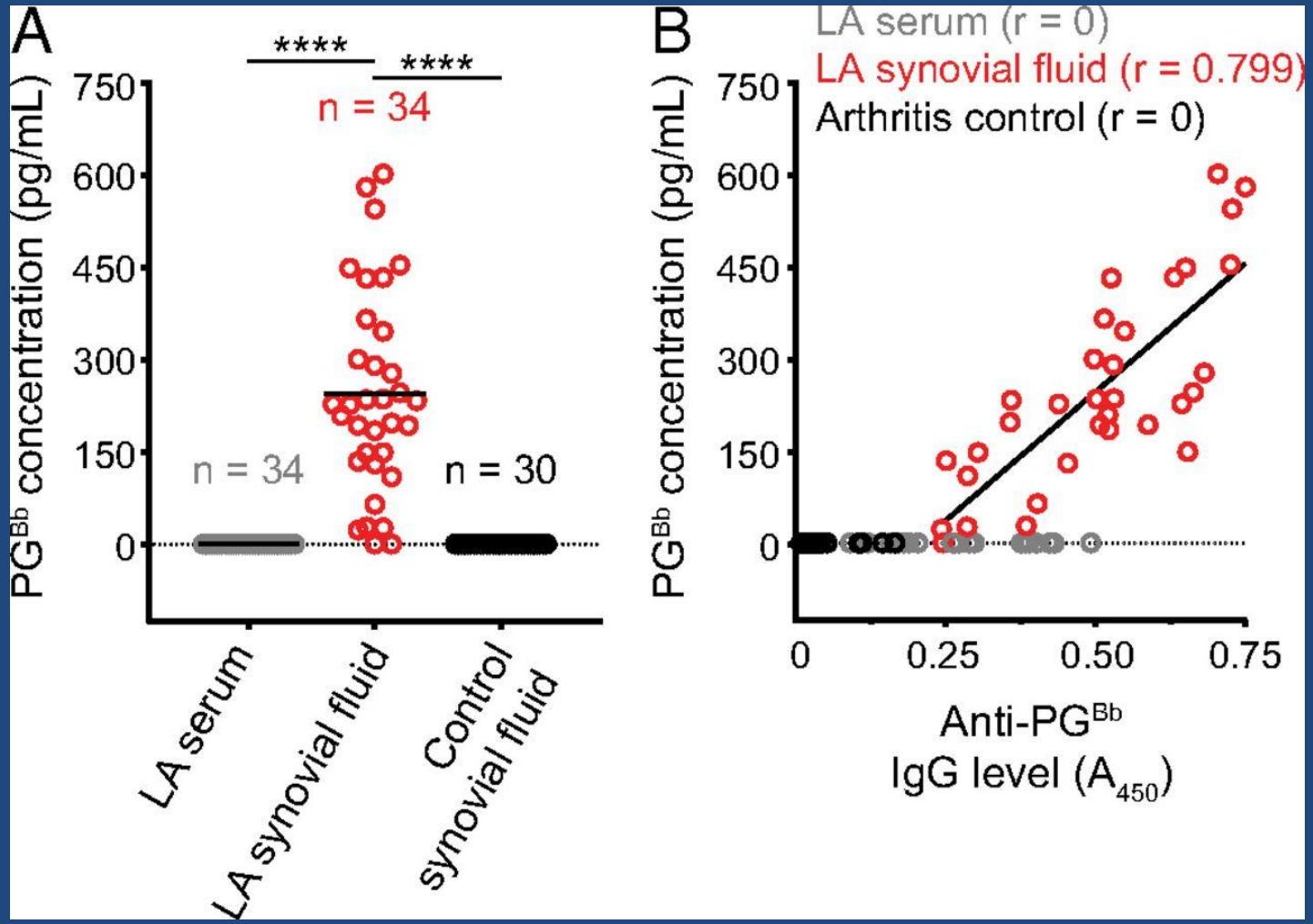
Brandon L. Jutras[a,b,c,1], Robert B. Lochhead[d,2], Zachary A. Kloos[a,e], Jacob Biboy[f,g], Klemen Strle[d], Carmen J. Booth[h], Sander K. Govers[a,b], Joe Gray[i], Peter Schumann[j], Waldemar Vollmer[f,g], Linda K. Bockenstedt[k], Allen C. Steere[d], and Christine Jacobs-Wagner[a,b,c,l,3]

B. burgdorferi sheds muropetides during growth

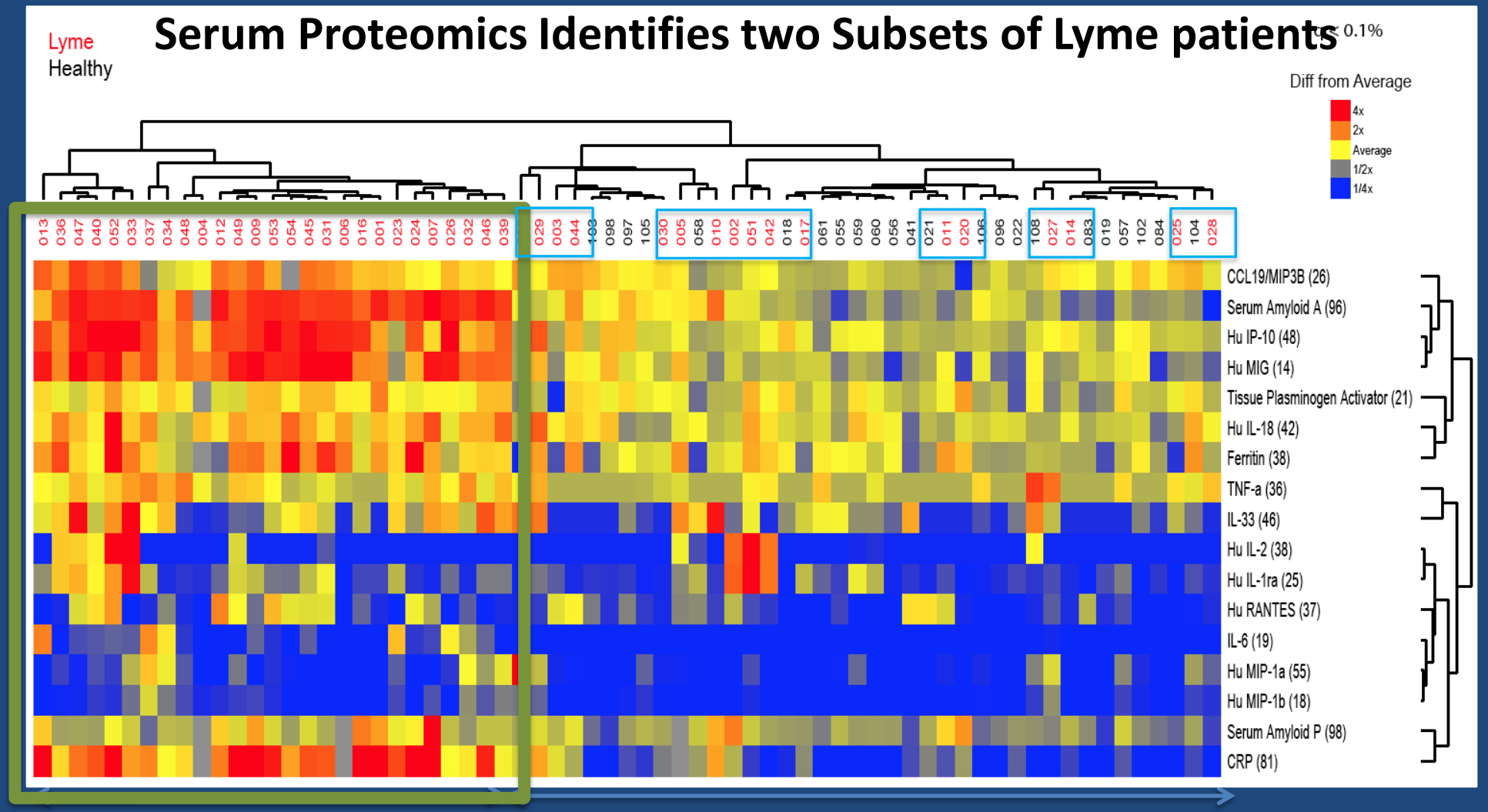


Detection of *Bb* PG in Synovial Fluid Samples of Patients with LA

Jacobs-Wagner. 507 PNAS July 2, 2019. vol. 116 no. 27 P 13499



Serum Proteomics Identifies two Subsets of Lyme patients



Acute Lyme: mediator-I

- Lymphopenia
- High liver enzyme levels
- High seroconversion rate
- More Symptoms

Acute Lyme: mediator-II

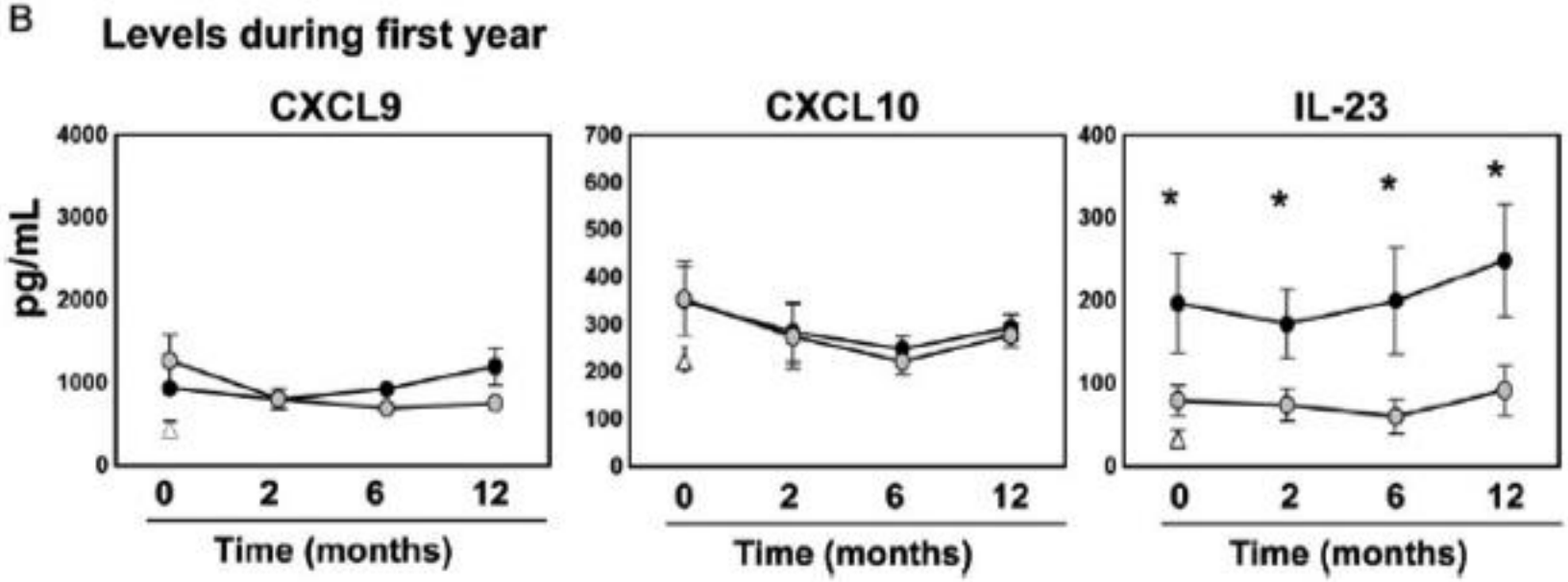
- Normal wbc levels
- Normal liver enzyme
- Lower seroconversion rate
- Mild Symptoms

SLICE STUDIES

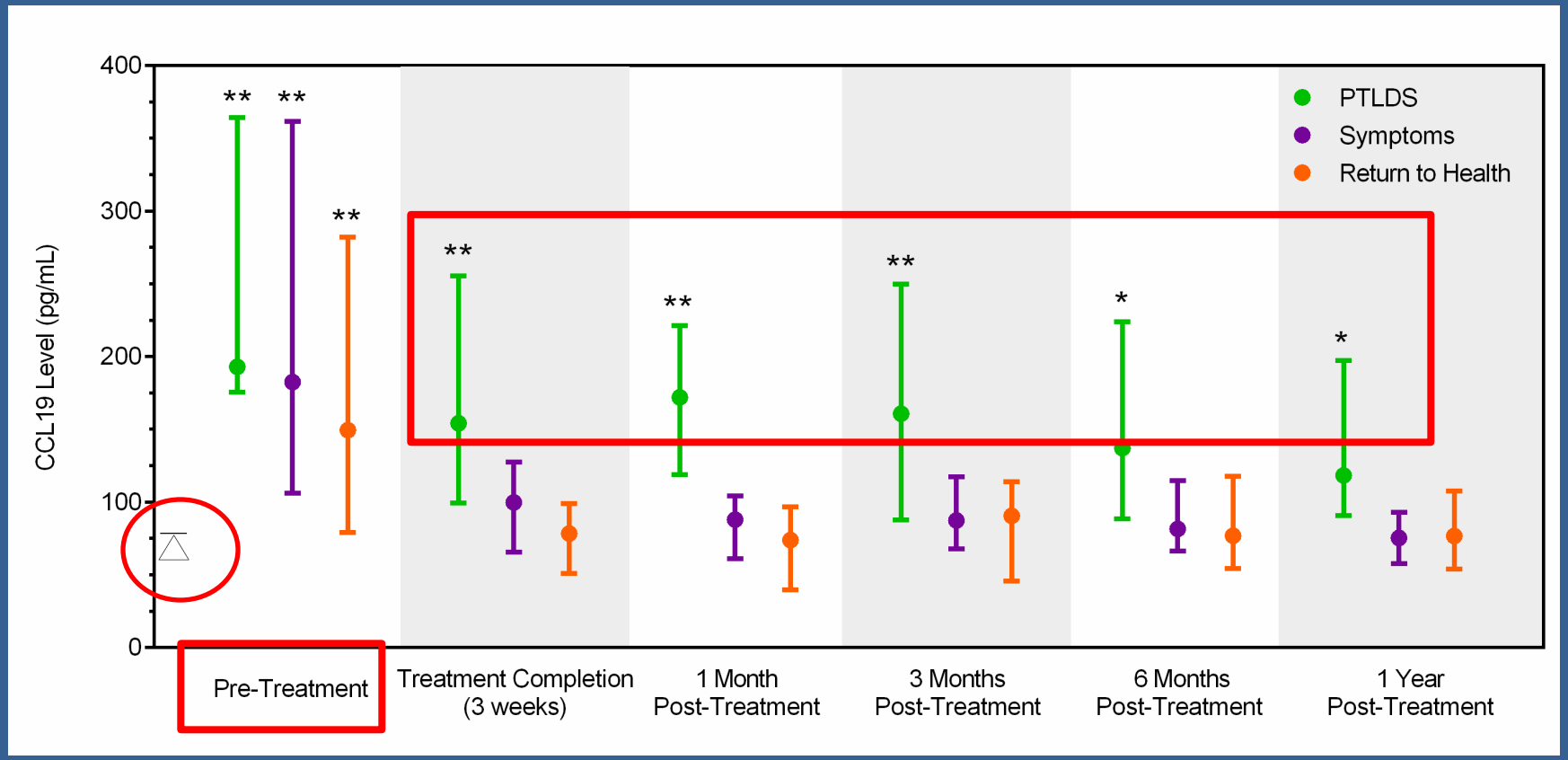
Elevated Levels of IL-23 in a Subset of Patients With Post–Lyme Disease Symptoms Following Erythema Migrans

Klemen Strle,[1] Daša Stupica,[2] Elise E. Drouin,[1] Allen C. Steere,[1] and Franc Strle[2]

Clinical Infectious Diseases 2014;58(3):372–80



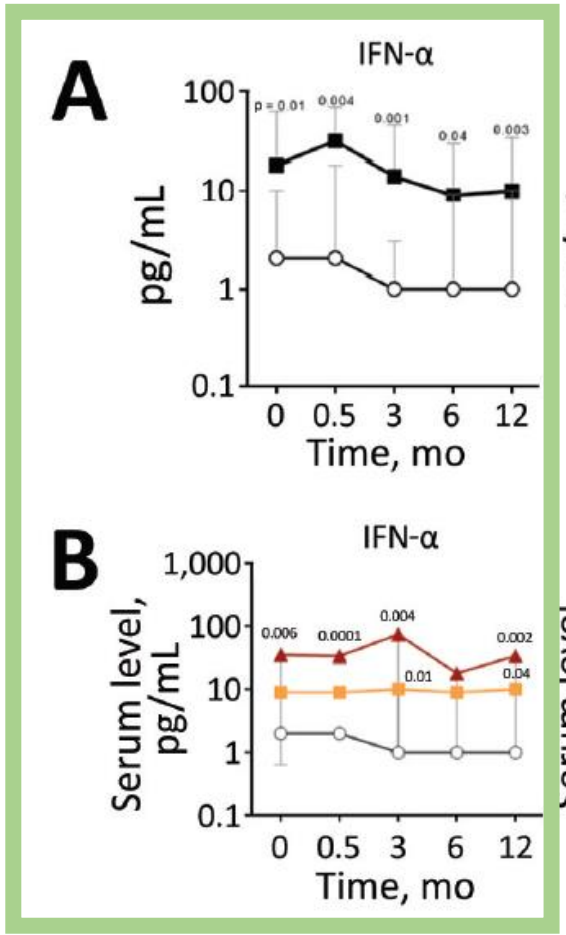
CCL19 levels over time compared to control group levels by clinical outcome status



The median control values (79.3 pg/mL for CCL19) is represented by a triangle in the graph; * p≤0.05, **p≤0.01, ***p<0.001 for comparison of each group to controls

Association of Persistent Symptoms after Lyme Neuroborreliosis and Increased Levels of Interferon-α in Blood

Sergio A. Hernández, Katarina Ogrinc, Miša Korva, Andrej Kastrin, Petra Bogovič, Tereza Rojko, Keith W. Kelley, Janis J. Weis, Franc Strle, Klemen Strle



frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 06 June 2022
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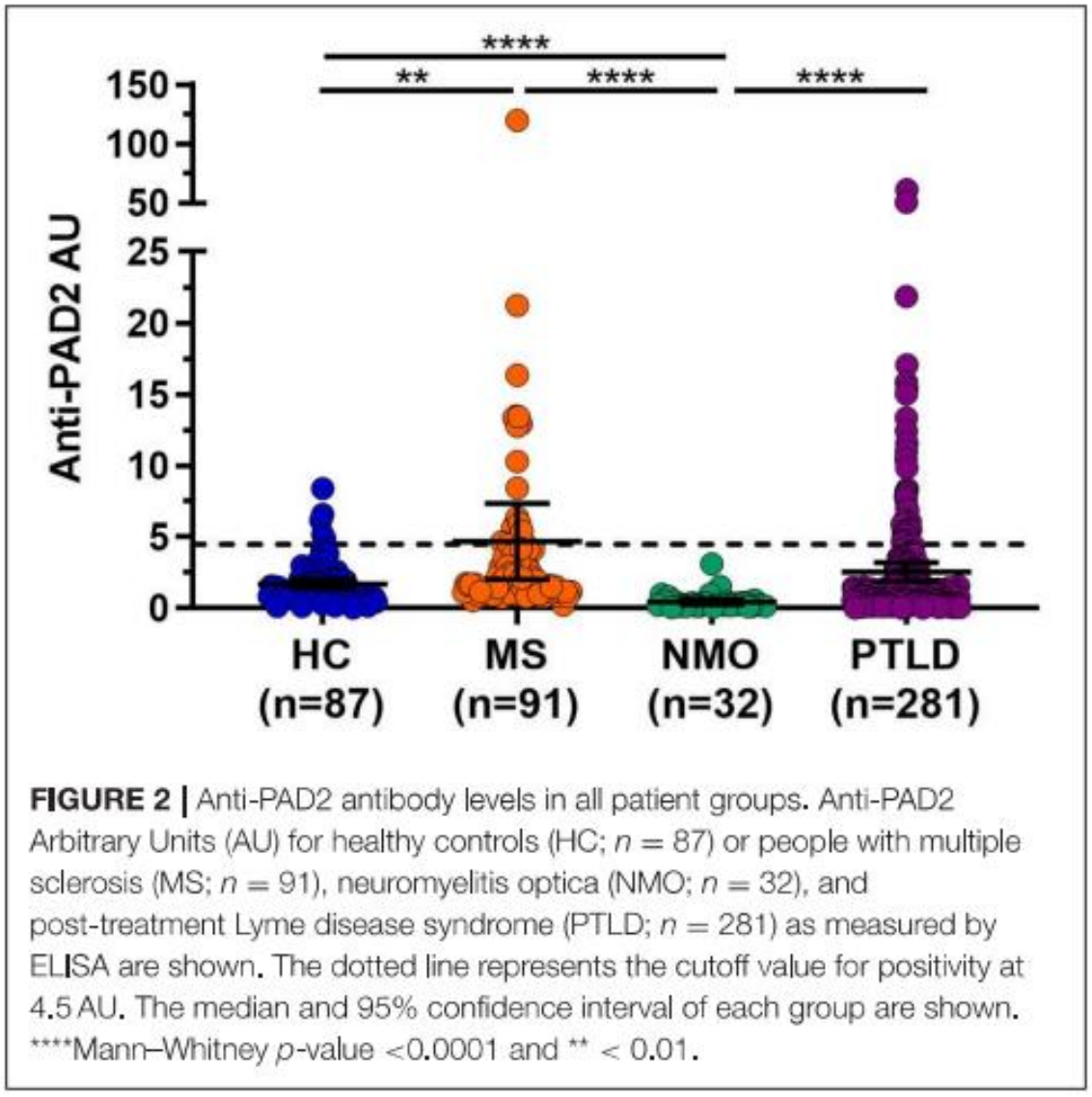
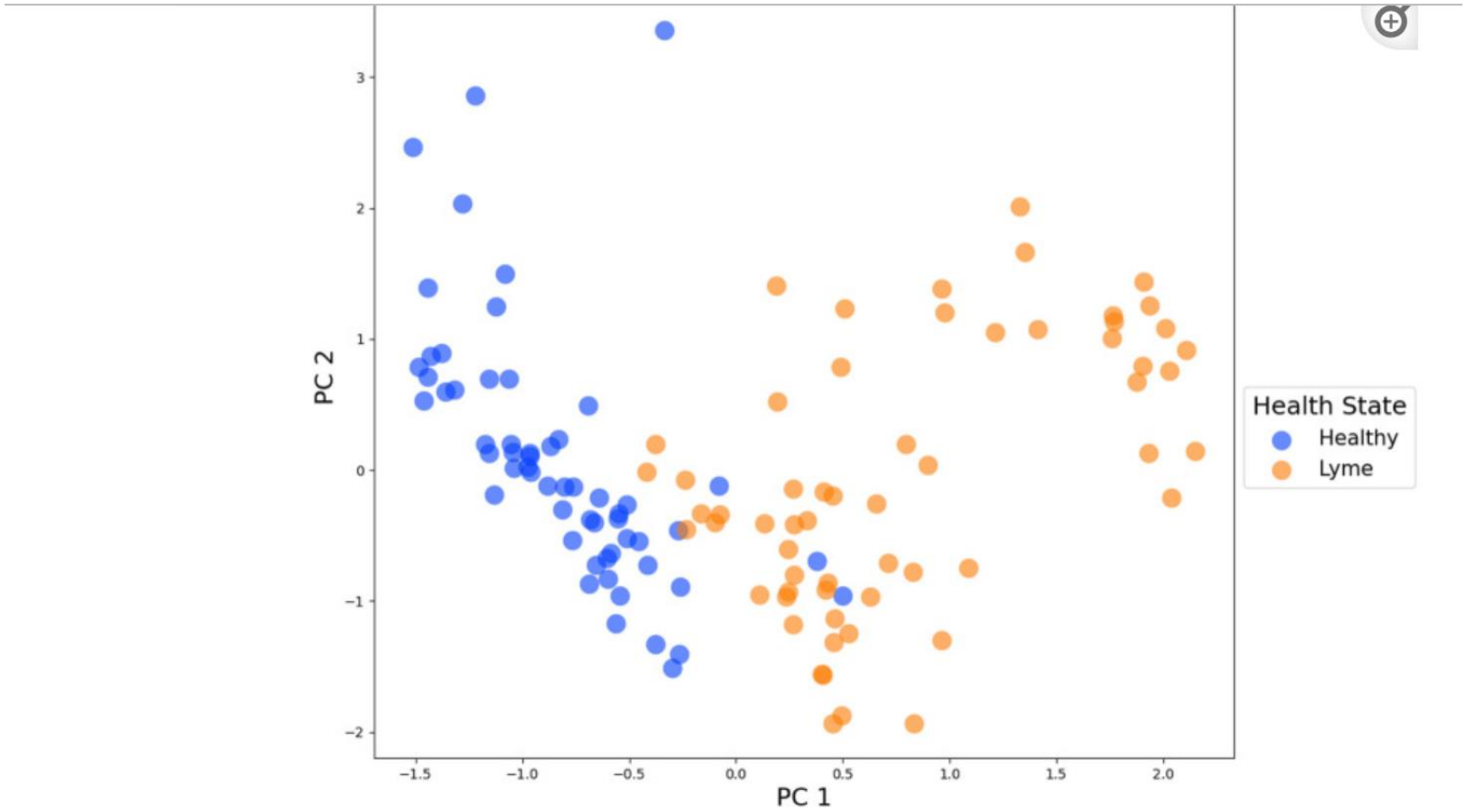


FIGURE 2 | Anti-PAD2 antibody levels in all patient groups. Anti-PAD2 Arbitrary Units (AU) for healthy controls (HC; $n = 87$) or people with multiple sclerosis (MS; $n = 91$), neuromyelitis optica (NMO; $n = 32$), and post-treatment Lyme disease syndrome (PTLD; $n = 281$) as measured by ELISA are shown. The dotted line represents the cutoff value for positivity at 4.5 AU. The median and 95% confidence interval of each group are shown. ****Mann–Whitney p-value <0.0001 and ** < 0.01.

Peptidylarginine Deiminase 2 Autoantibodies Are Linked to Less Severe Disease in Multiple Sclerosis and Post-treatment Lyme Disease

Yaewon Kim[1], Alison W. Rebman[1,2], Tory P. Johnson[3], Hong Wang[1], Ting Yang[1,2], Carlo Colantuoni[3,4,5], Pavan Bhargava[3], Michael Levy[3†], Peter A. Calabresi[3], John N. Aucott[1,2], Mark J. Soloski[1,2] and Erika Darrah[1,2]*

Metabolome-based Diagnosis of Lyme Disease

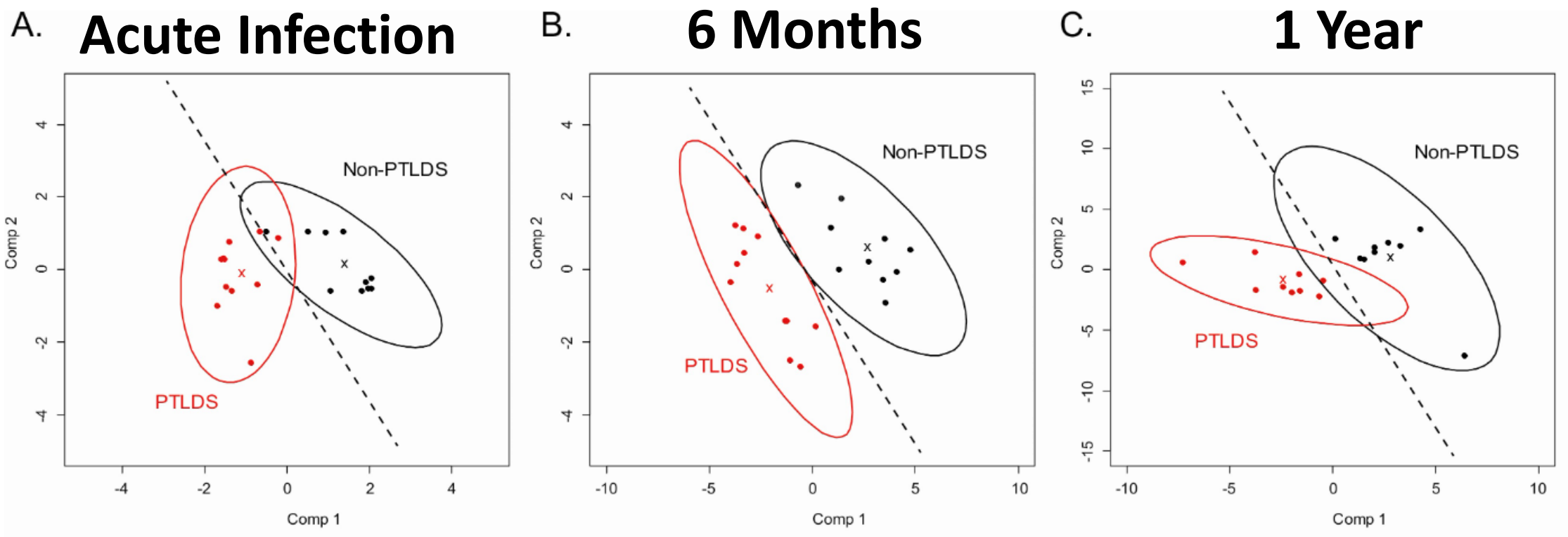


PCA visualization of log transformed and KNN imputed LC-MS data from training samples restricted to the optimal 45 features found by *k*FFS.

- *Kehoe ER, Fitzgerald BL, Graham B, Islam MN, Sharma K, Wormser GP, Belisle JT, Kirby MJ. Biomarker selection and a prospective metabolite-based machine learning diagnostic for lyme disease. Sci Rep. 2022 Jan 27;12(1):1478. doi: 10.1038/s41598-022-05451-0. PMID: 35087163; PMCID: PMC8795431*

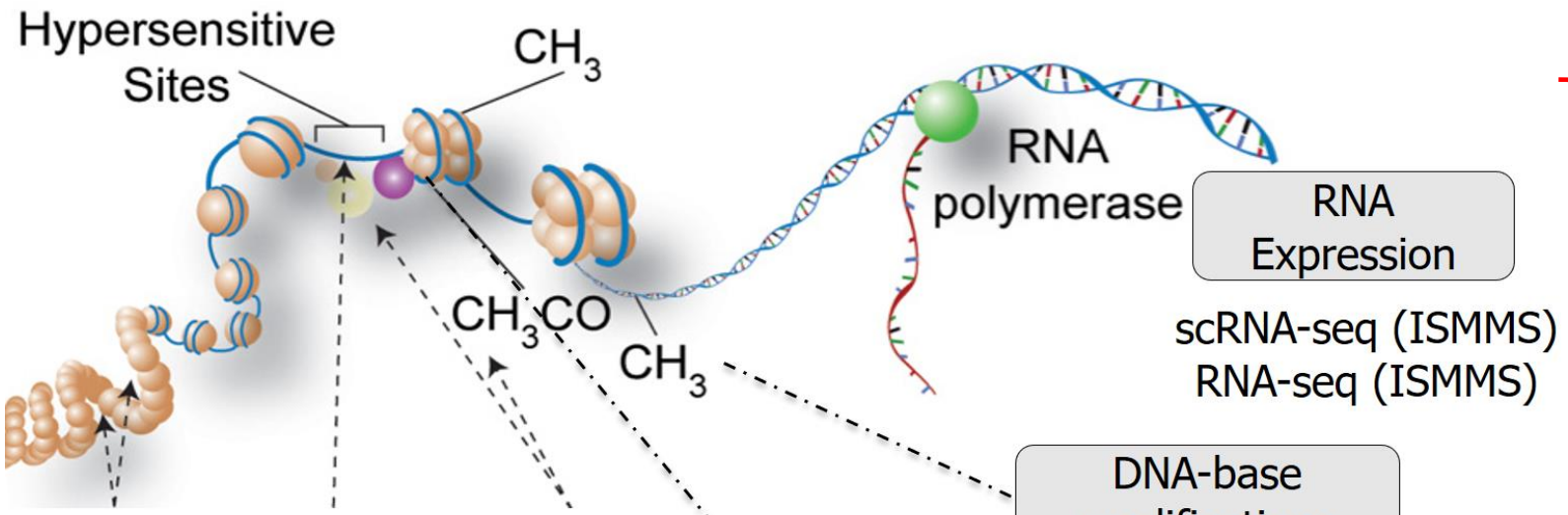
Metabolism

- Blood metabolomics from SLICE participants has found signatures which discriminate PTLD patients



Fitzgerald et al, Clin. Inf. Diseases 2021

ECHO Epigenetic Signatures: Developing Multilayer Modalities



RNA Expression

scRNA-seq (ISMMS)
RNA-seq (ISMMS)

DNA-base modifications

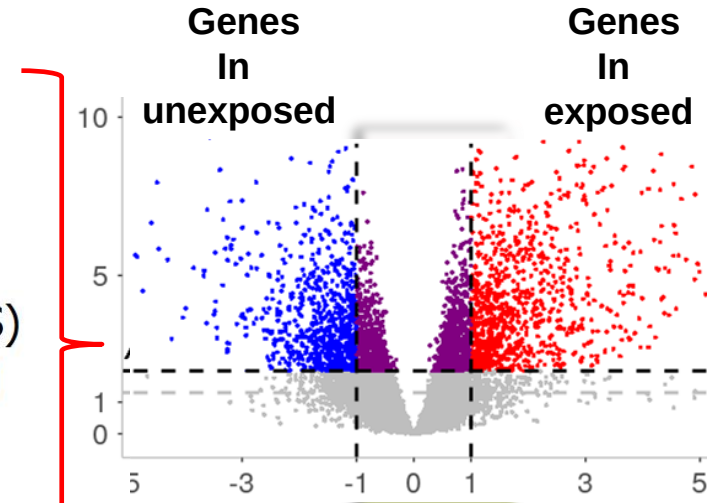
sn5mC-seq (Salk)
cf5mC-seq (ISMMS)

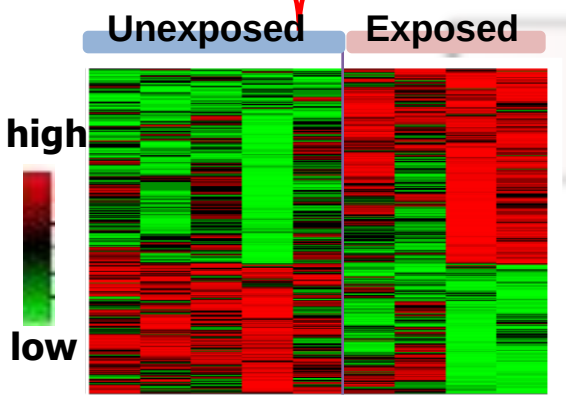
Chromatin Accessibility

snATAC-seq (Salk)
scATAC-seq (ISMMS)

Histone Modifications

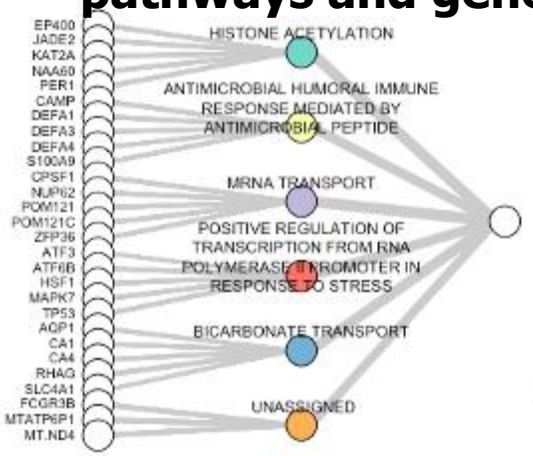
MINT-ChIP (Duke)

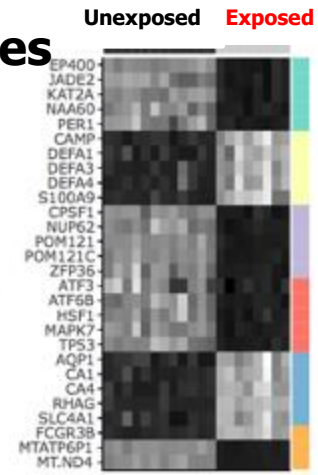




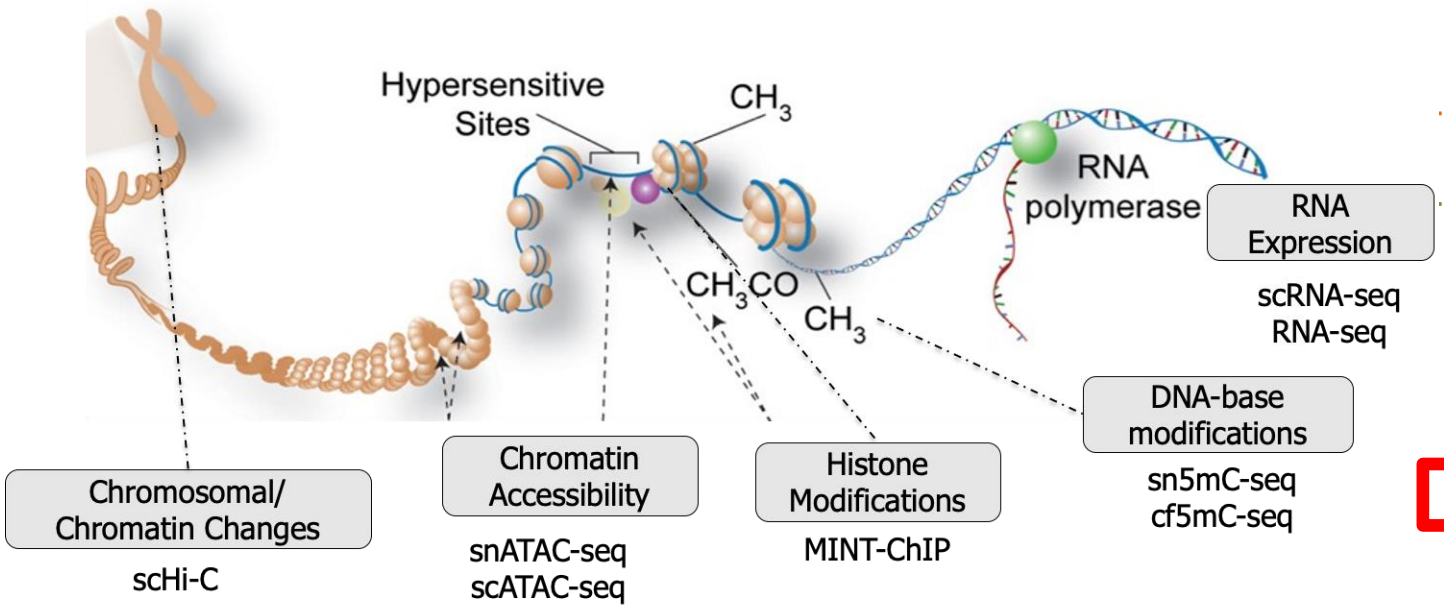
Diagnosis Based on Host Response Pathways

Exposure Specific pathways and genes





ECHO: Combining Molecular Methods for Epigenetic Diagnosis



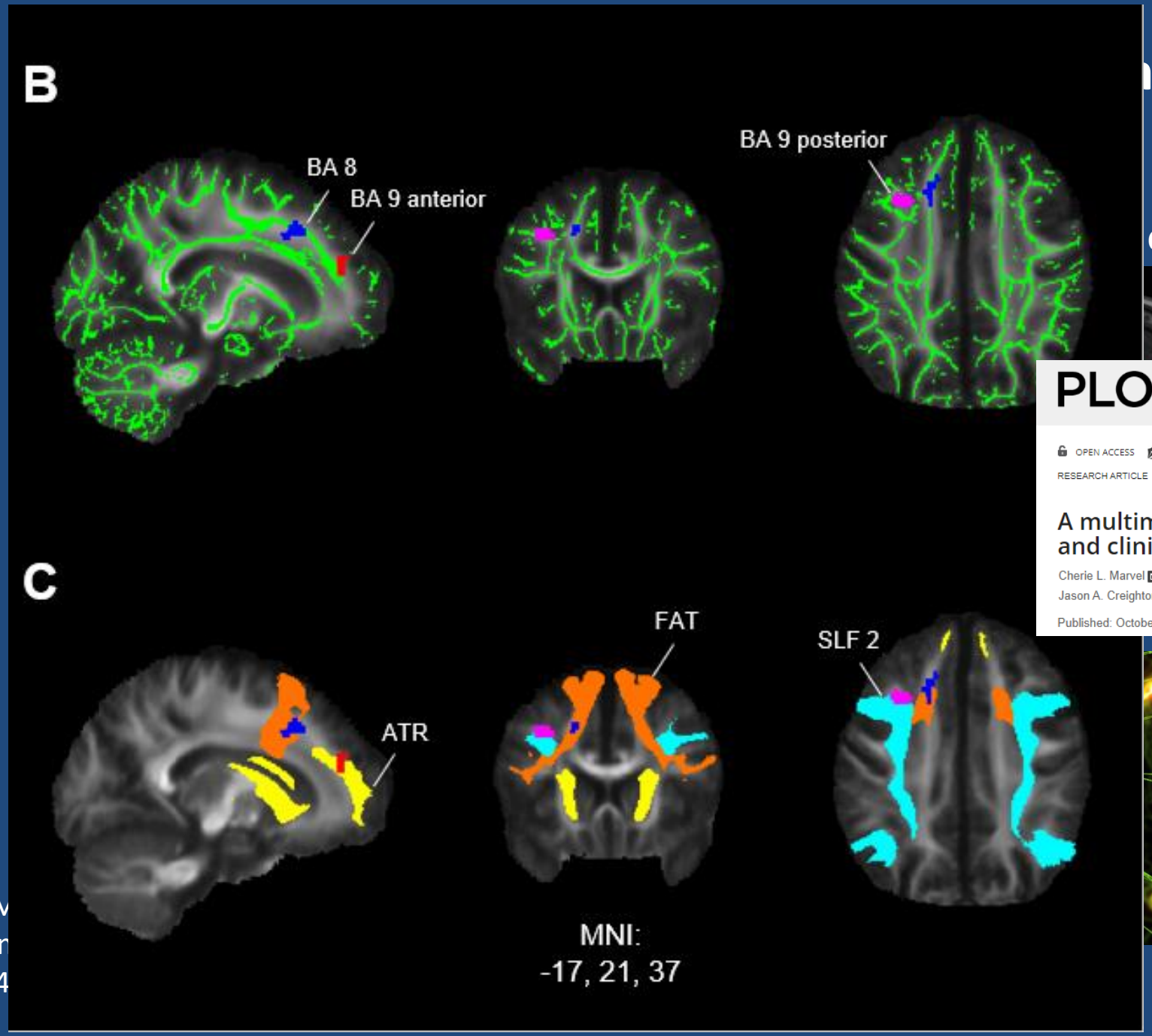
Exp #	Exposure	Source	
1	HIV ✓	GOV'T (MHRP) & DUKE	
2	MRSA ✓	DUKE	
3	Bacillus anthracis vaccination ✓	BATTELLE	First 6 months
4	Organophosphates (TCPY) ✓	DUKE	
5	SARS-CoV-2 ✓	ISMMS-DUKE-GOV'T	Months 6-12
	Influenza H3N2 ✓	GOV'T (BARDA)	
6	Influenza H3N2 vaccination ✓	GOV'T (BARDA)	
	Fentanyl	ISMMS	
7	Fentanyl	GOV'T (ECHO SKID)	
8	Explosives (ANFO and PETN)	GOV'T (SHELL SHOCK)	
9	Radiation (gamma)	ISMMS	
	Ebola-convalescent	DUKE	
10	Ebola-vaccine	DUKE	
11	B. pseudomallei	DUKE	
12	B. burgdorferi	JHU	Phase 2

ECHO human exposure samples (Excluding CHARM)

- Total number of samples committed to ECHO: **6,424**
- Total number of samples collected/ collecting/ on-hand: **4,542**
- **Longitudinal** collections have been given priority
- Matched geographically and ethnically **diverse control samples**
- Utilizing human samples from **other programs and research efforts** to compare against for **confounds**

ECHO sample groups

5
3
2
2



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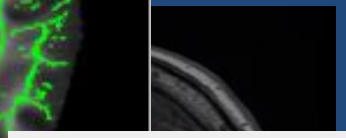

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

A multimodal neuroimaging study of brain abnormalities and clinical correlates in post treatment Lyme disease

Cherie L. Marvel, Kylie H. Alm, Deeya Bhattacharya, Alison W. Rebman, Arnold Bakker, Owen P. Morgan, Jason A. Creighton, Erica A. Kozero, Arun Venkatesan, Prianca A. Nadkarni, John N. Aucott

Published: October 26, 2022 • https://doi.org/10.1371/journal.pone.0271425

Coughlin JM
Neuroinflam
19;15(1):34

Neuroinflammation ?

Action Items and Key Findings

- Improved diagnostics are urgently needed for diagnosis of acute Lyme disease and its infection-associated chronic illness
- Direct diagnostic tests for microbial nucleic acid and proteins are promising alternatives for indirect serologic tests
- Measurement of host responses including inflammatory processes and its gene regulation, metabolic changes and epigenetic signatures are future opportunities for diagnostics

More Information at HopkinsLyme.org

Lyme Disease Clinical Research Center:

John Aucott, Alison Rebman, John Miller
Brit Adler, Ting Yang, Marzieh Keshtkarjahromi
Cheryl Novak, Verna Scheeler, Erica Mihm
Susan Joseph, Cindi Crews

Applied Physics Lab Whiting School of Engineering

Philippe Burlina
Yinzhi Cao, Haolin Yuan

Johns Hopkins Psychology/Neuropsychology

Pegah Touradji

Neurology & Neuro-Imaging

Cherie Marvel, Arun Venkatesan
Mary Pomper , Jennifer Coughlin

Bloomberg School of Public Health

Nicole Baumgarth
Jonathan Weiner
Brian Schwartz, Katherine Moon
Frank Curriero, Cara Wychgram

http://www.hopkinslymetracker.org/

Johns Hopkins Rheumatology Bayview Laboratories

Erika Darrah, Mark Soloski, Alex Girgis
Daniella Villegas de Flores,
Won Seok Song