University of California
San Francisco

Current Diagnostic Approaches and Emerging Technologies

Charles Chiu, MD / PhD

Professor, Department of Laboratory Medicine and Medicine / Infectious Diseases

Associate Director, UCSF Clinical Microbiology Laboratory

Co-Director, UCSF Lyme Clinical Trials Center

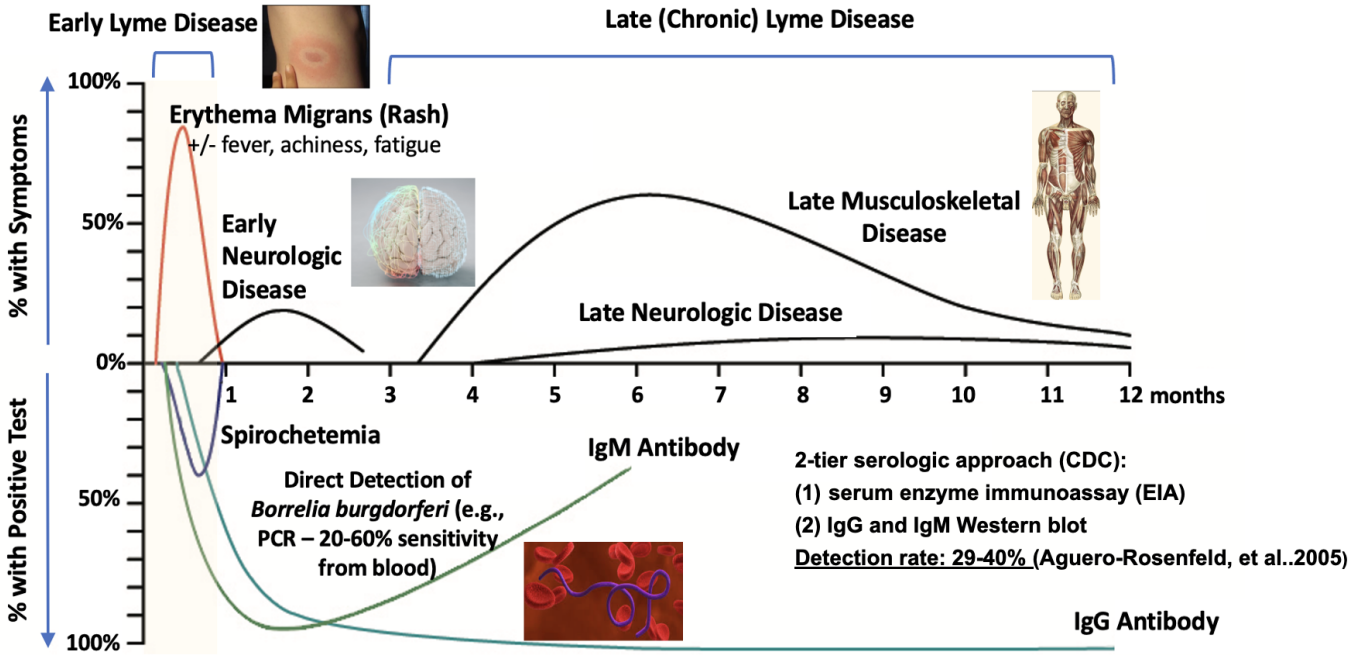
University of California, San Francisco

Disclosures

- Scientific Advisory Board Member for Bay Area Lyme Disease Foundation, Global Lyme Alliance, and Steven and Alexandra Cohen Foundation

- Scientific Advisory Board for Flightpath Biosciences, Delve Bio, and Co-founder of Delve Bio

Challenges in Diagnosis of Lyme IACI



Diagnostic Lab Testing

Lyme*

- Two-tiered serology
- Other serology (EIA)
- *Borrelia* PCR

LC/PASC

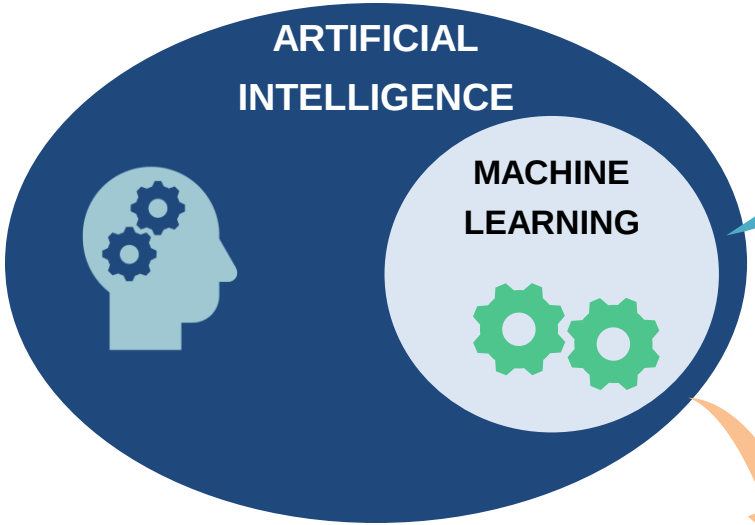
- Spike protein level
- Spike protein IgG Ab

ME/CFS

- none

*early Lyme, no tests available for PTLDS

Host Response Based Diagnostics for IACIs



PRECISION MEDICINE (**'OMICS: RNA transcriptomics, metagenomics, proteomics, metabolomics, pan-serology profiling**

DIAGNOSTICS

- CANCER
- RARE DISEASE GENETICS
- HOST IMMUNE RESPONSE TO INFECTION (

chronic

LYME DISEASE

- ❖ 476,000 cases/year (CDC Data and Surveillance)
- ❖ difficult to diagnose
- ❖ lack of accurate diagnostic assays early Lyme disease

ME/CFS AND LONG COVID

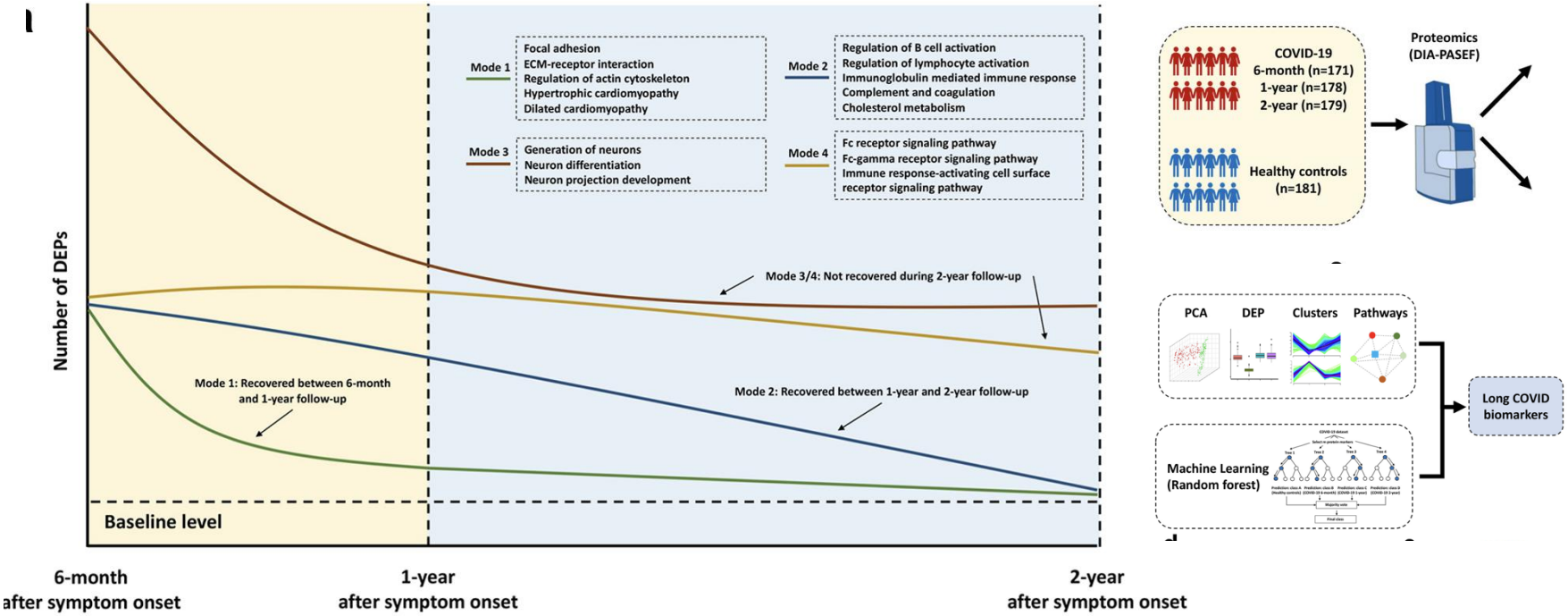
- ❖ Diagnostic tests not available

acute

THERAPEUTICS

Neurologic, bloodborne, and respiratory infections

Proteomics for Long COVID



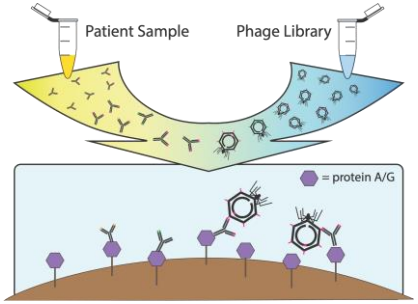
Four distinct recovery modes from acute COVID-19 identified in a longitudinal 2-year cohort study of proteomic biomarkers
<u>Conclusion</u>: the IACI patient population, including Lyme IACI, is heterogeneous

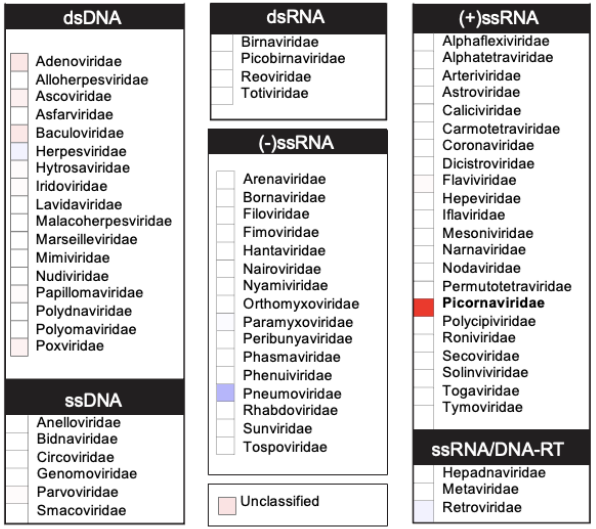
Gu, et al., *eBioMedicine*, 2023, 98:104851.

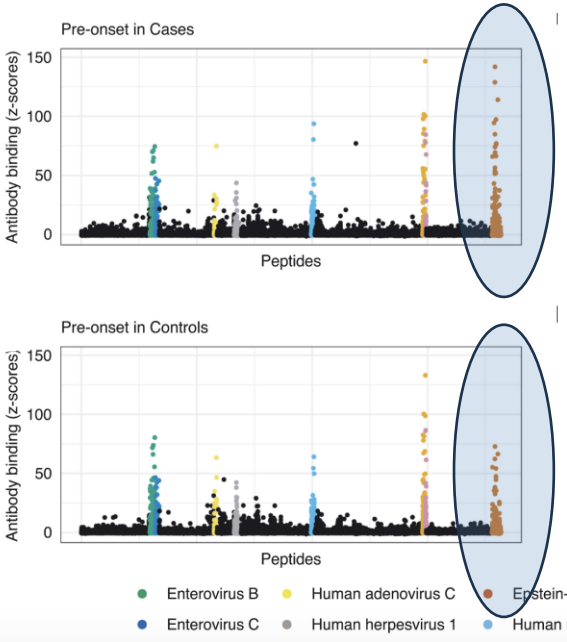
Pan-Pathogen Serology

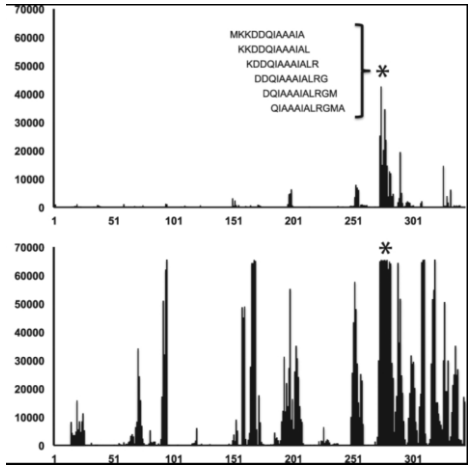
(VirScan, Tickborne Disease (TBD)-Serochip)

Bjornevik, et al., *Science*, 2022, 375:6578
Schubert, et al., *Nature Medicine*, 2019, 25(11):1748-1752
Tokartz, et al. *Scientific Reports*, 2018, 8(1).
Xu, et al., *Science*, 2015, 348:6239.



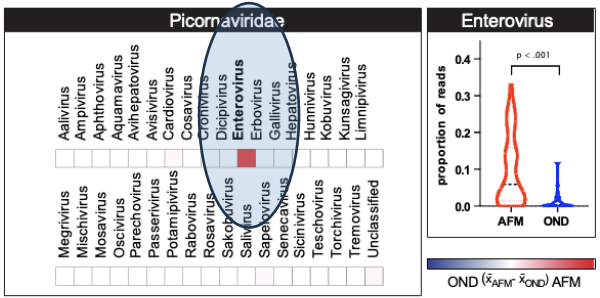






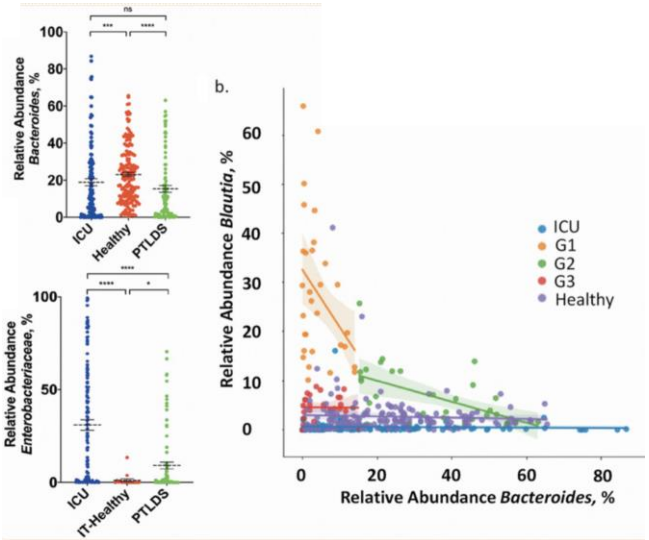
Antibodies to *Borrelia burgdorferi* VlsE antigen

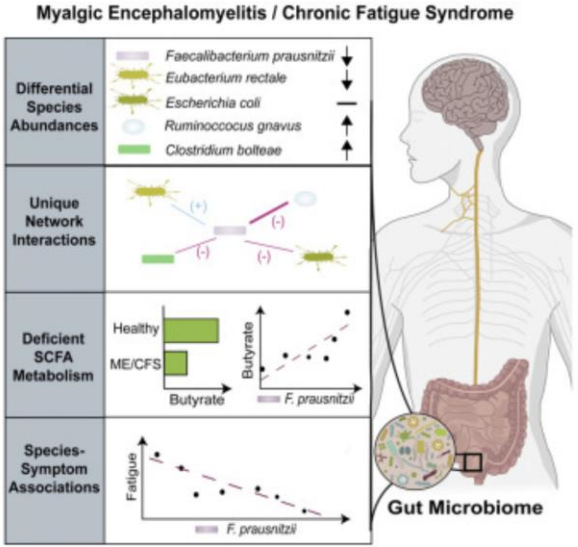
Association of EBV with multiple sclerosis



69% (29/42) of pediatric acute flaccid myelitis cases versus 7% (4/58) of controls positive for *Enterovirus* by VirScan

Microbiome and IACI

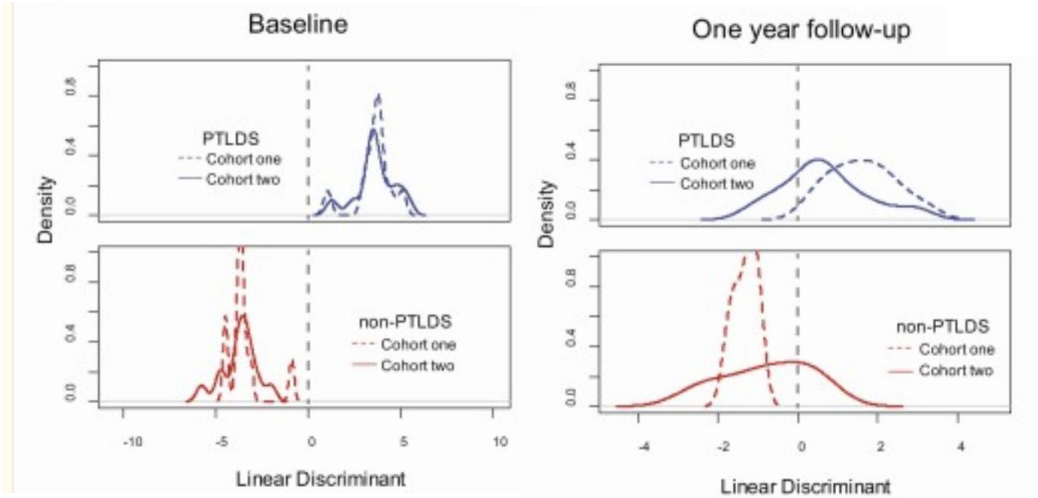




- Long COVID: Randomized, double-blind placebo control trial of 463 patients showed that treatment with synbiotic preparation versus placebo had significant improvements in fatigue ($p<0.0001$), memory loss ($p=0.0024$), and concentration ($p<0.0001$)
- PTLDS: Distinct microbiome signature in PTLDS that enabled ~80% classification accuracy
- ME/CFS: Microbiome disturbances is a hallmark signature of ME/CFS related to deficient butyrate-producing capacity
- Mechanisms thought related to normalization of gut dysbiosis and gut-immune axis

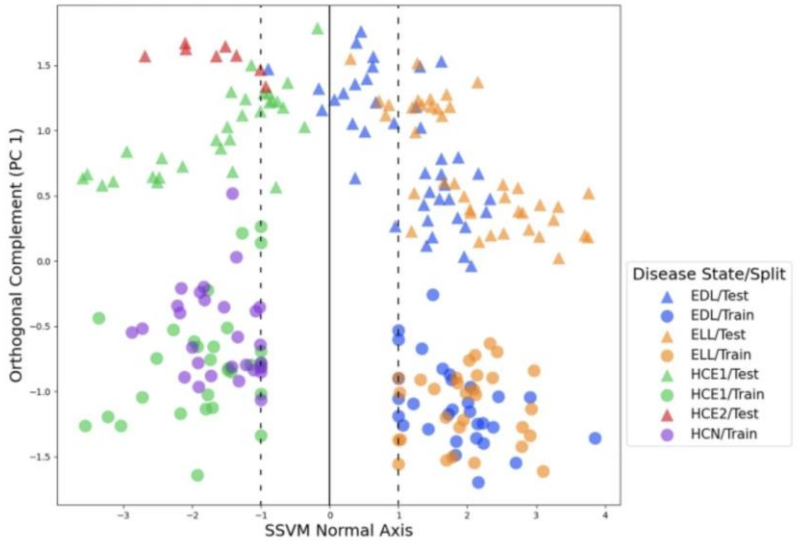
Lau, et al., 2023, *Lancet Infectious Diseases*; Morrissette, et al., 2020, *mBio* 11(5):e02310-20; Guo, et al., 2023, *Cell Host & Microbe,* 31(2):288-304.

Metabolomics for PTLDS



39 SMMs

13 SMMs

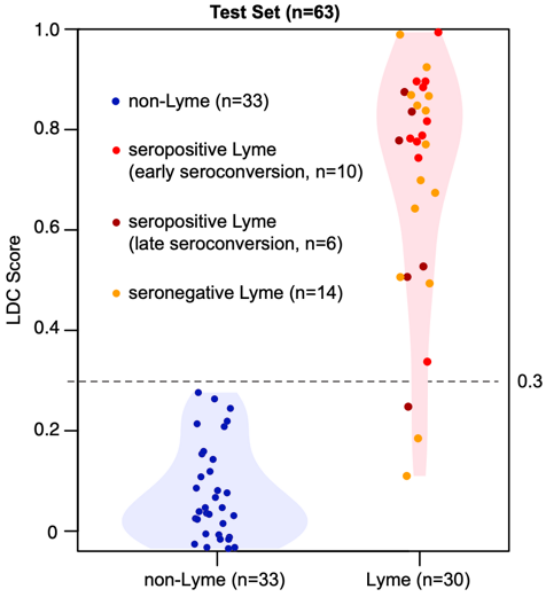


- non-PTLDS = Lyme disease patients with resolved infection
- Markers of glycerophospholipid, bile acid, and acylcarnitine metabolism;
- Increased prevalence of lipid disorders in PTLDS (Chung, et al., *Lancet,* 2023, 90:10424).

ELL = early localized Lyme disease;
EDL = early disseminated Lyme disease

Fitzgerald, et al., *Clinical Infectious Diseases*, 2021, 73(7); Kehoe, et al., *Scientific Reports*, 2022, 12:1478.

RNA-Seq for Early Lyme and PTLDS



Performance (Overall)	Lyme	non-Lyme	
LDC +	27	0	sensitivity = 90%
LDC−	3	33	specificity = 100%
			accuracy = 95.2%

Performance (Seropositive Lyme)	Lyme	non-Lyme	
LDC +	15	0	sensitivity = 93.7%
LDC−	1	33	specificity = 100%
			accuracy = 98.0%

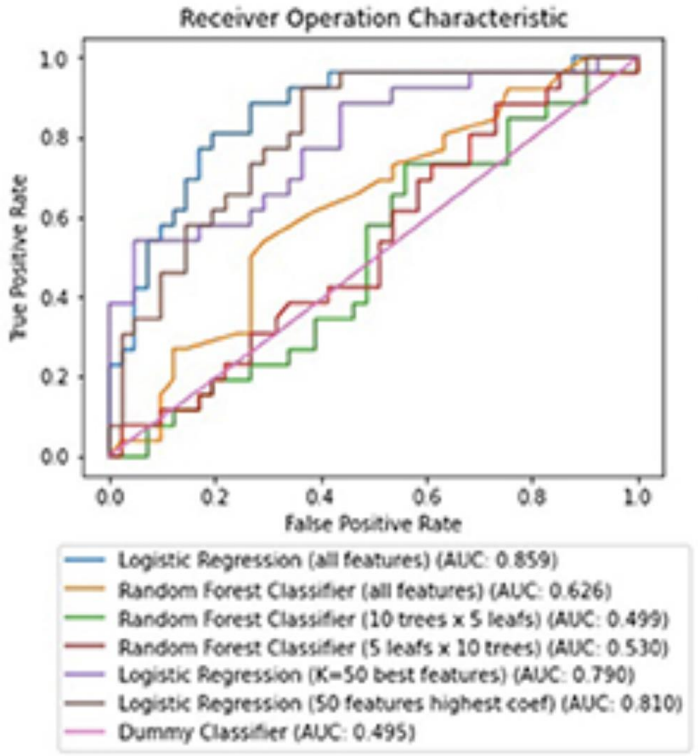
Performance (Seronegative Lyme)	Lyme	non-Lyme	
LDC +	12	0	sensitivity = 85.7%
LDC−	2	33	specificity = 100%
			accuracy = 95.7%

Lyme versus Controls (influenza, bacterial sepsis, MTB, healthy)

90% sensitivity

100% specificity

95% accuracy

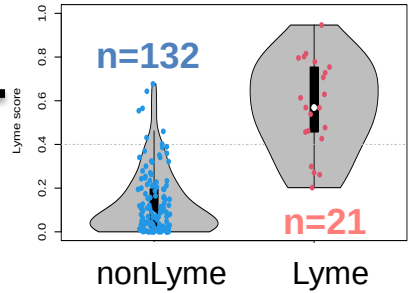


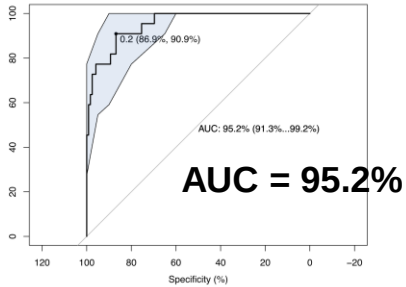
PTLDS vs. Resolved Lyme (AUC=0.86)

Clarke, et al., *Frontiers in Immunology*, 2021, 12; Servellita, et al., *Communications Medicine*, 2022,

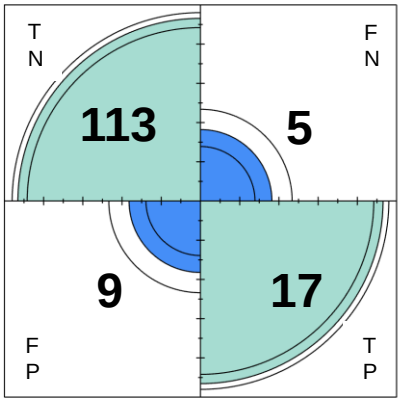
RNA-Seq Classifiers for Early Lyme and Babesiosis from Whole Blood

Lyme: 21
sepsis: 12
influenza: 6
covid: 11
asympBab: 26
sympBab: 2
anaplasma: 4
ill_controls: 7
donor_controls: 71





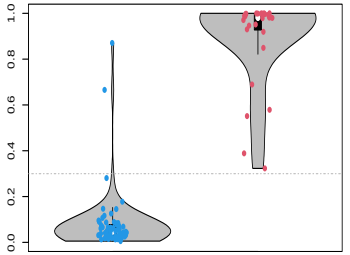
AUC = 95.2%

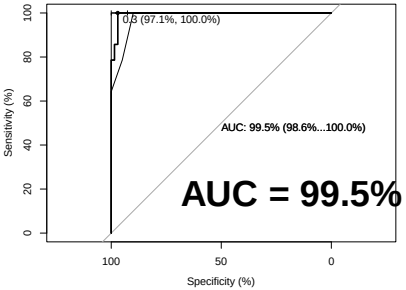


Sensitivity : 86.9%
Specificity : 90.9%

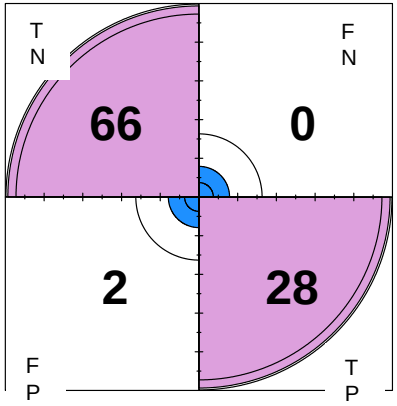
Early Lyme

Babesia: 28
Lyme: 21
sepsis: 12
influenza: 6
covid: 11
anaplasma: 2
ill_controls: 2
donor_controls: 14





AUC = 99.5%



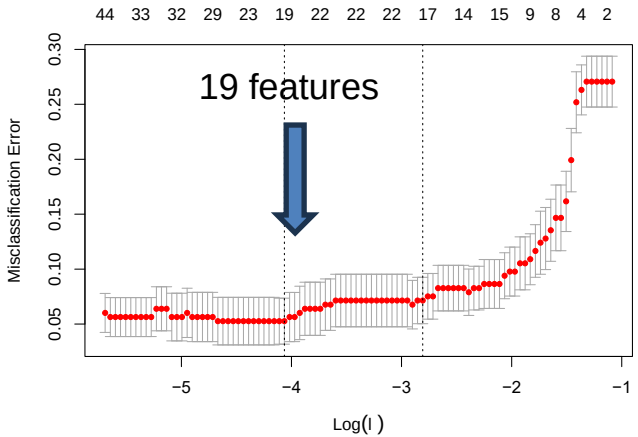
Accuracy: 97.9%
95% CI : (0.9268, 0.9975)
Sensitivity : 100%
Specificity : 97.1%

Babesiosis

Insights into Pathogenesis?

union of top DEGs from pairwise comparisons

727 genes



Babesiosis-Associated Genes

ML-selected genes

- IL21
- CCNL1
- SPECC1
- GUSBP11
- OR2W3
- PPCDC
- PTPRC
- ERVK13-1
- ZNF700
- TIGD1
- TRIM22
- MAL
- IFI44
- LIMK2
- PARP12
- ADGRG3
- MIR22HG
- SMCHD1
- ZEB2

IL21 is a pleiotropic cytokine; functions in protection and immunopathology during parasitic diseases (Djokic et al. 2018); significant induction observed following *Babesia microti* infection (Yi et al. 2018)

PTPRC is an essential regulator of T- and B-cell antigen receptor signaling;

ERVK13-1 mediates receptor recognition and membrane fusion during early infection

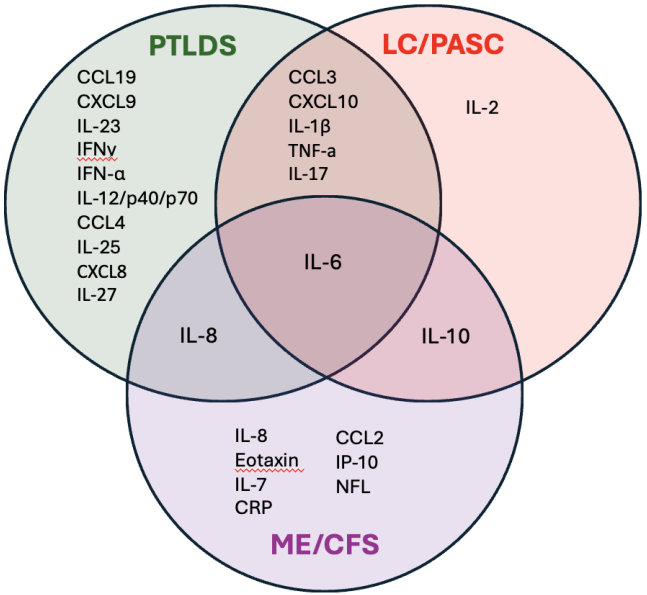
TRIM22 Interferon-induced antiviral protein involved in cell innate immunity

In *B. microti*-infected mice, the spleen initiates an immune response, with immune-related proteins playing an important role, including IFI44 (Xue et al. 2021)

ADGRG3 is abundant in granulocyte precursors and terminally differentiated neutrophilic, eosinophilic, and basophilic granulocytes (Hsiao et al. 2018)

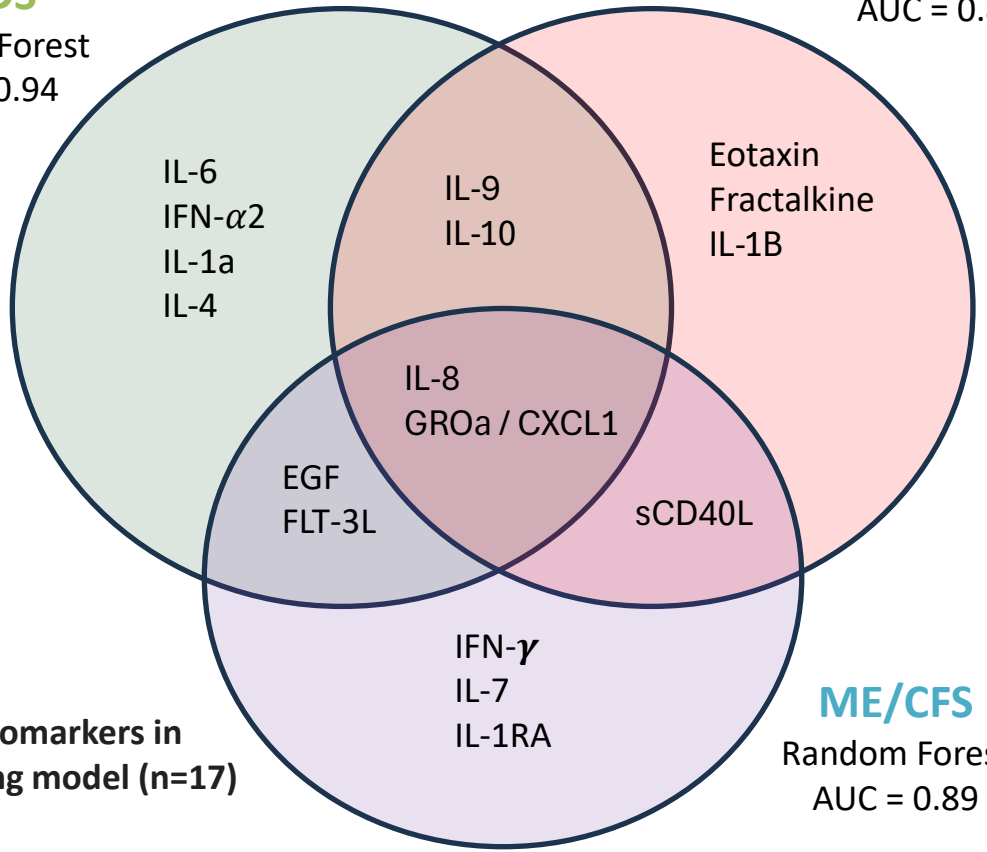
Cytokine Profiling for IACIs

Targeted cytokine biomarkers from literature (n=26)



PTLDS
Random Forest
AUC = 0.94

PASC
Random Forest
AUC = 0.88



Selected biomarkers in best performing model (n=17)

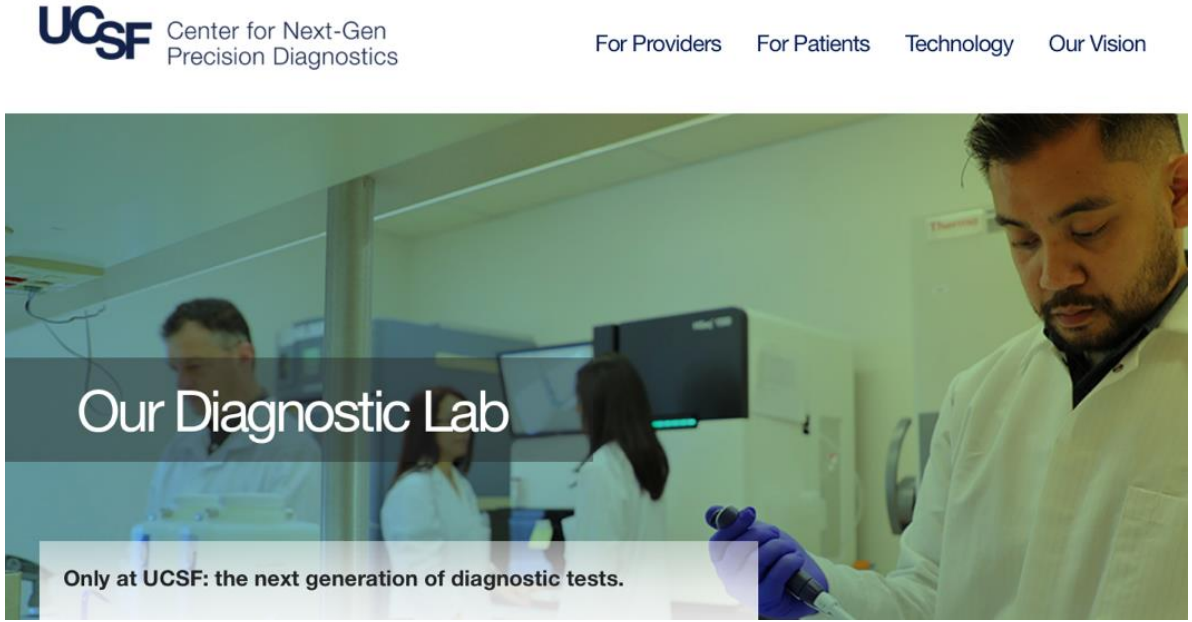
ME/CFS
Random Forest
AUC = 0.89

Servellita and Madison, et al., 2024, in preparation.

Clinical Metagenomic Next-Generation Sequencing Assays at University of California, San Francisco

- CSF mNGS*#
- Plasma mNGS* (re-validation in progress with launch in summer)
- Viral Respiratory mNGS*#
- Body fluids mNGS*

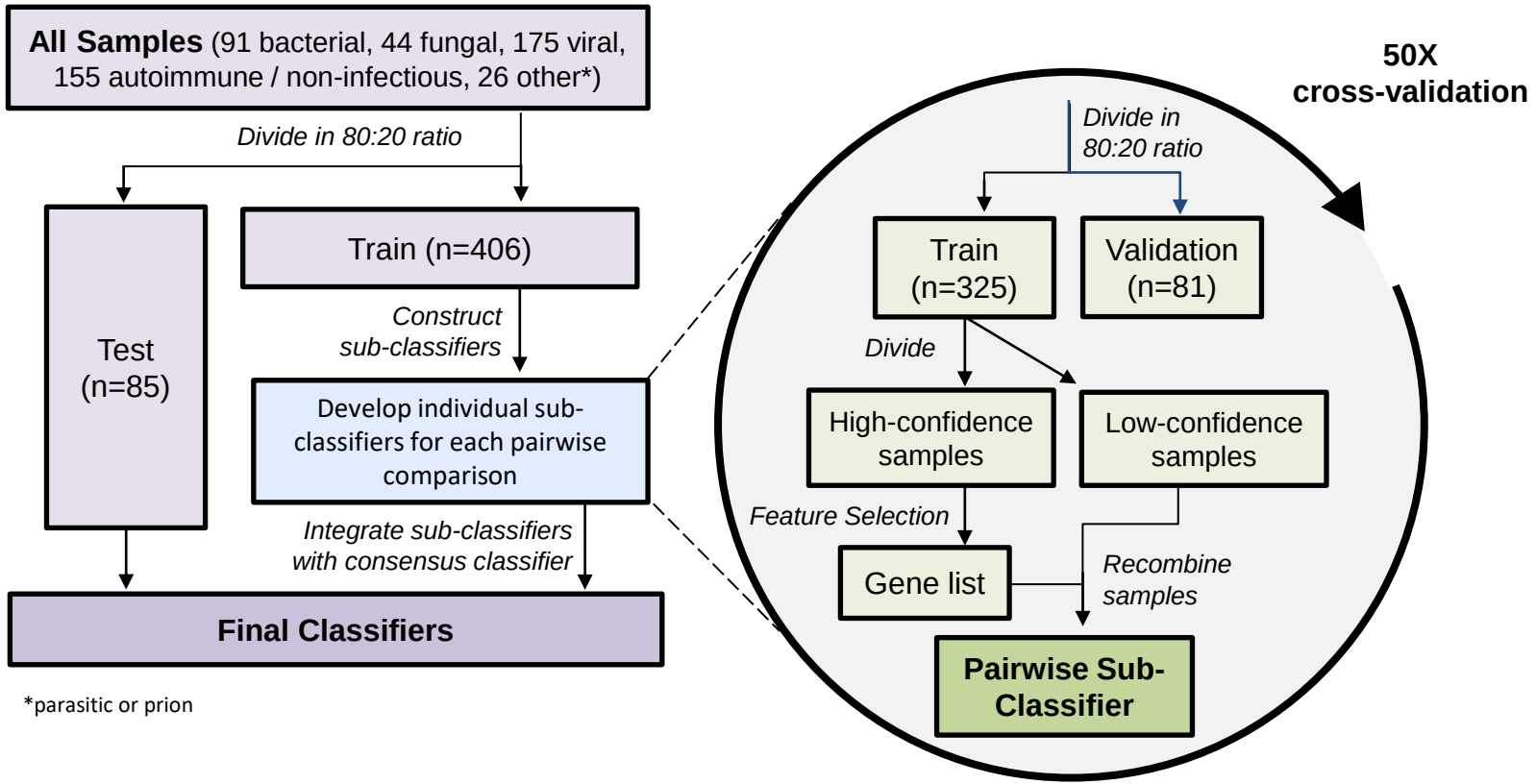
**all tests are LDTs and not FDA-approved IVDs; #granted breakthrough device designation by the FDA*



1. Miller, et al., *Genome Research*, 29(5): 831-842.
2. Wilson, et al., *NEJM*, 380(24):2327-2340.
3. Gu, et al., *Nature Medicine*, 27(1):115-124.

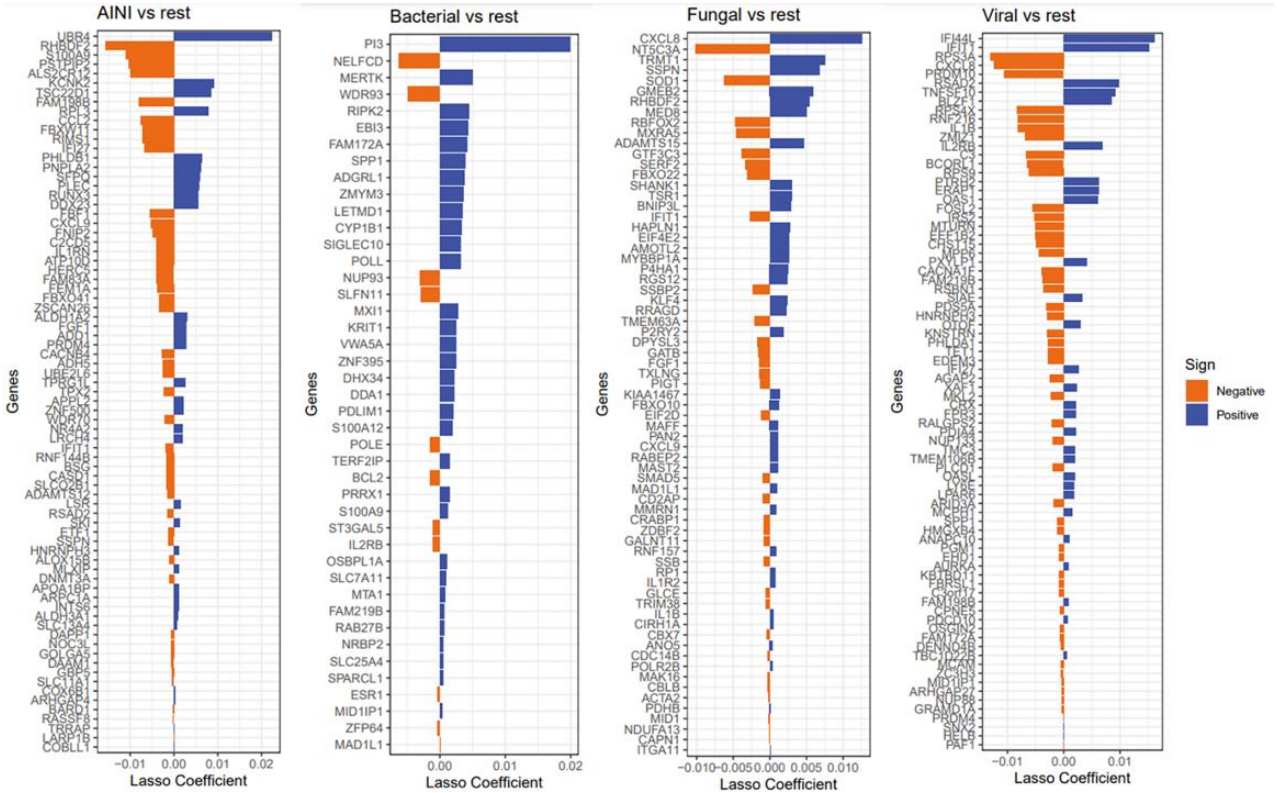
http://nextgendiagnostics.ucsf.edu

CSF RNA-Seq and Machine Learning for Differential Diagnosis of CNS Syndromes (meningitis, encephalitis, and myelitis)



Omura and Chiu, et al, 2024, manuscript in preparation

Differential CSF Biomarkers of Infectious/Non-Infectious Syndromes



AUTOIMMUNE / NON-INFECTIOUS

- UBR4 – ubiquitin-ligase enzyme, cancer neoantigen
- RHDBF2 – cancer biomarker
- S100A9 – calcium-binding protein (cancer, neurodegenerative disorder, autoimmune)
- PSTPIP2 – autoinflammatory diseases
- ALS2CR12/CASP8 – neurodegenerative disease

BACTERIAL

- PI3 – antimicrobial peptide
- NELFCD – macrophage-associated inflammation
- MERTK – ,macrophage apoptotic cell recognition
- WDR93 – oxidoreductase activity
- RIPK2 – induced by bacterial infection

FUNGAL

- CXCL8 – neutrophil-associated chemokine
- NT5C3A – negative cytokine signaling regulator
- TRMT1 – dimethyltransferase
- SSPN – dystrophin-associated gene
- SOD1 – superoxide dismutase

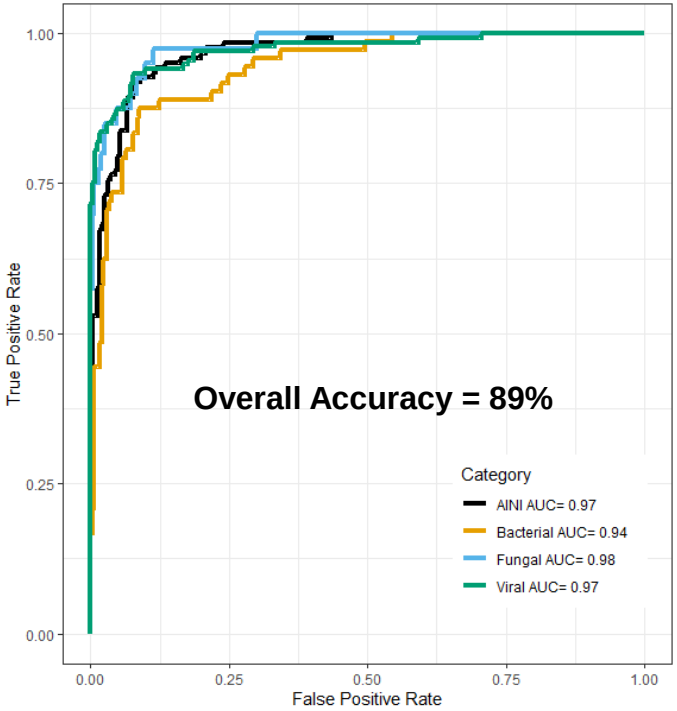
VIRAL

- IFI44L – antiviral gene (interferon-associated)
- IFIT1 – antiviral gene (interferon-induced)
- IRPS3A – interferon-stimulated ribosomal gene
- CXCL8 – neutrophil-associated chemokine
- PRDM!0 – histone deactylation

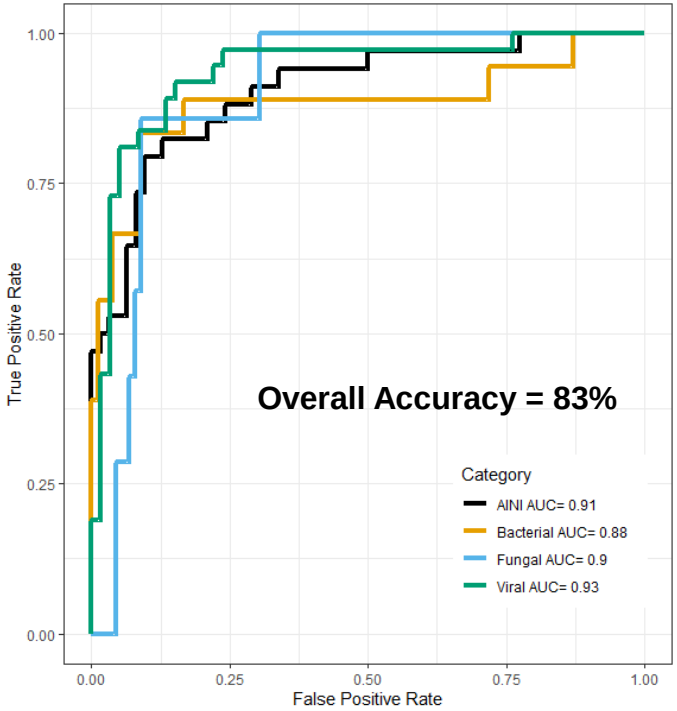
Omura and Chiu, et al, 2024, manuscript in preparation

Multinomial Classifier Performance for Infection Diagnosis

Training Set



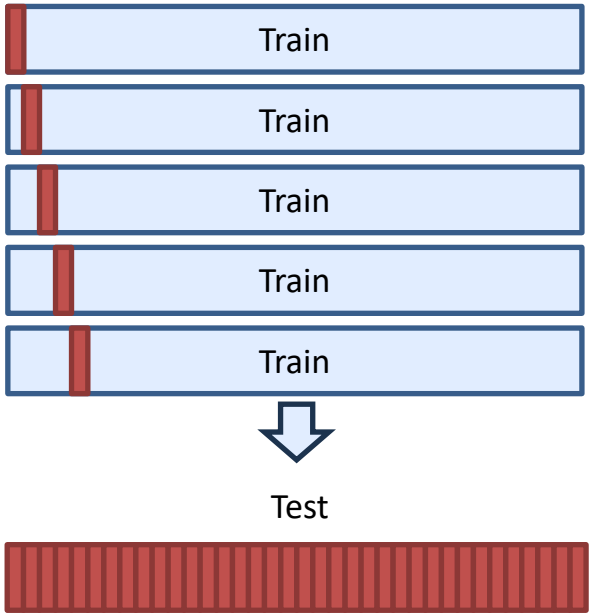
Test Set



Omura and Chiu, et al, 2024, manuscript in preparation

Subcategory Classification for Non-Infectious Syndromes

"Leave-One-Out" Approach



Category	LOO AUC	n
WBC cancer	0.74829	22
amyloid	0.790746	8
autoantibody	0.590167	40
brain cancer	0.576503	8
hereditary	0.776087	7
lupus	0.861365	7
MS	0.533951	11
neurosarcoidosis	0.853823	15
other_autoimmune	0.510366	29
solid organ cancer	0.800694	9
structural	0.569121	18
toxic-metabolic	0.865714	16
vascular	0.643735	11
vasculitis	0.7723	20

Omura and Chiu, et al, 2024, manuscript in preparation

CSF Host Response Signature for CNS Amyloidosis

MNC_5932	Score	Signature
Autoimmune/Noninfectious	10	Strong
Bacterial (typical)	1	Very Weak
Bacterial (atypical)	1	Very Weak
Fungal	0	No Signature
Viral	1	Very Weak
Parasitic	1%	Unlikely
Worm	0%	Unlikely
Flavivirus	2%	Possible
Mycobacterium	0%	Unlikely
Dimorphic Fungi	1%	Unlikely
Mold vs rest	2%	Possible
Enterovirus- AFM	3%	Possible
Amyloid	21%	Possible
Lupus	0%	Unlikely
Solid Organ Cancer	0%	Unlikely

CNS amyloidosis

Hospitalized with altered mental status, encephalopathy, fatigue, and neutrophilic pleocytosis; brain biopsy performed after discharge consistent with cerebral amyloid angiopathy

Omura and Chiu, et al, 2024, manuscript in preparation

CSF Host Response Signature for Neuroborreliosis

4 cases of neuroborreliosis

CLCF1	2.784069	4.236764	3.20767	3.626301	IL-6 family cytokine	*
ADCK2	1.808971	2.54256	3.470445	2.424954	unknown function	
SLC39A13	1.717214	1.749701	3.280119	2.311277	zinc transporter	
TMEM243	2.180132	3.727614	3.329168	-0.361985	transmembrane protein	
S1PR2	1.661798	1.690055	2.575797	2.665008	mast cell receptor	*
ST6GALNAC4	1.533716	1.963451	2.217012	2.496924	sialic acid transfer	*
KIF26A	1.462193	2.267583	2.073608	2.00224	kinesin	*
LILRA4	-0.445988	2.774495	3.027446	2.339625	plasmacytoid dendritic cell sig	*
SLC25A16	1.843911	1.878132	1.459621	2.469678	solute carrier transporter	
KCNN4	2.206551	2.244962	-0.352212	3.417366	potassium channel	*
RELB	1.226078	2.097651	2.113863	1.731392	NFkB pathway	*
IL15RA	1.260931	2.133217	1.833491	1.766658	interleukin receptor	*
WWOX	1.296417	2.271688	1.191076	2.173951	spinocerebellar ataxia	
RELT	1.279794	1.886893	1.688061	1.789566	NFkB pathway	*

*evidence from literature supporting involving in *Borrelia* cell culture infections, mouse models, or Lyme neuroborreliosis patients

Take-Home Messages

- Both direct detection (e.g.,*Borrelia burgdorferi* for LD) and indirect detection diagnostic approaches (e.g., host response ‘omics testing) will likely be necessary for diagnosis of Lyme IACI
- A variety of diagnostic test modalities are in development; multiple test modalities are needed as we do not understand the pathogenesis of PTLDS
- Objective diagnostic biomarkers are urgently needed to support clinical trials of drugs and vaccines
- Given overlapping symptoms, prospective clinical studies must consider including samples from different IACIs, ideally from matched biobank collections
- Precise definitions of Lyme IACI subsets are needed to guide clinical trials, perhaps obtained by emerging ‘omics technologies (population is not heterogeneous)
- Host response ‘omics tests will enable not only diagnosis and discrimination, but have the potential to monitor patients and their response to experimental treatments longitudinally

Acknowledgements

UCSF Chiu Lab and VDDC
Candace Wang, BS
Jerome Bouquet, PhD
Dianna Ng, MD
Dustin Glasner, PhD
Miriam Oseguera, BS
Venice Servellita, BS/CLS
Benjamin Briggs, MD
Jenny Nguyen, BS
Alicia Sotomayor-Gonzalez, PhD
Charles Omura, BS/MS
Maia Madison, BS

UCSF Long COVID investigators
Michael Peluso, MD
Steven Deeks, MD

Johns Hopkins University
John Aucott, MD
Mark Soloski, MD

Harvard / BCH
Lise Nigrovic, MD

UCSF Lyme Disease Clinical Trials Center (a node of the Lyme Clinical Trials Network)
Felicia Chow, MD
Margaret Wilson, BS
Cassandra Recchioni, BS/NP
Venice Servellita, BS/CLS
Miriam Oseguera, BS

American Red Cross
Laura Tonetti, PhD
Sue Stramer, PhD

Lyme Disease Biobank
Liz Horn, PhD

Columbia University
Brian Fallon, MD

ME/CFS Biobank
Eliana Lacerda, MD/PhD

Funding
- Steven and Alexandra Cohen Foundation
- Bay Area Lyme Disease Foundation
- Marc Benioff Foundation
- NIH R33 AI120977 and R01 HL105701-01
- US Centers for Disease Control and Prevention
- DoD Tickborne Disease Research Program
- Mammoth Biosciences, Inc.
- Abbott Pathogen Discovery Award
- California Initiative to Advance Precision Medicine
- Charles and Helen Schwab Foundation
- George and Judy Marcus Innovation Fund
- Chan-Zuckerberg Biohub
- Delve Bio

Venice Servellita

UCSF Lyme Disease Clinical Trials Center

Web: https://chiulab.ucsf.edu

https://lyme.ucsf.edu