A review of the evidence from U.S. Clinical Trials on Post-treatment Lyme Syndrome

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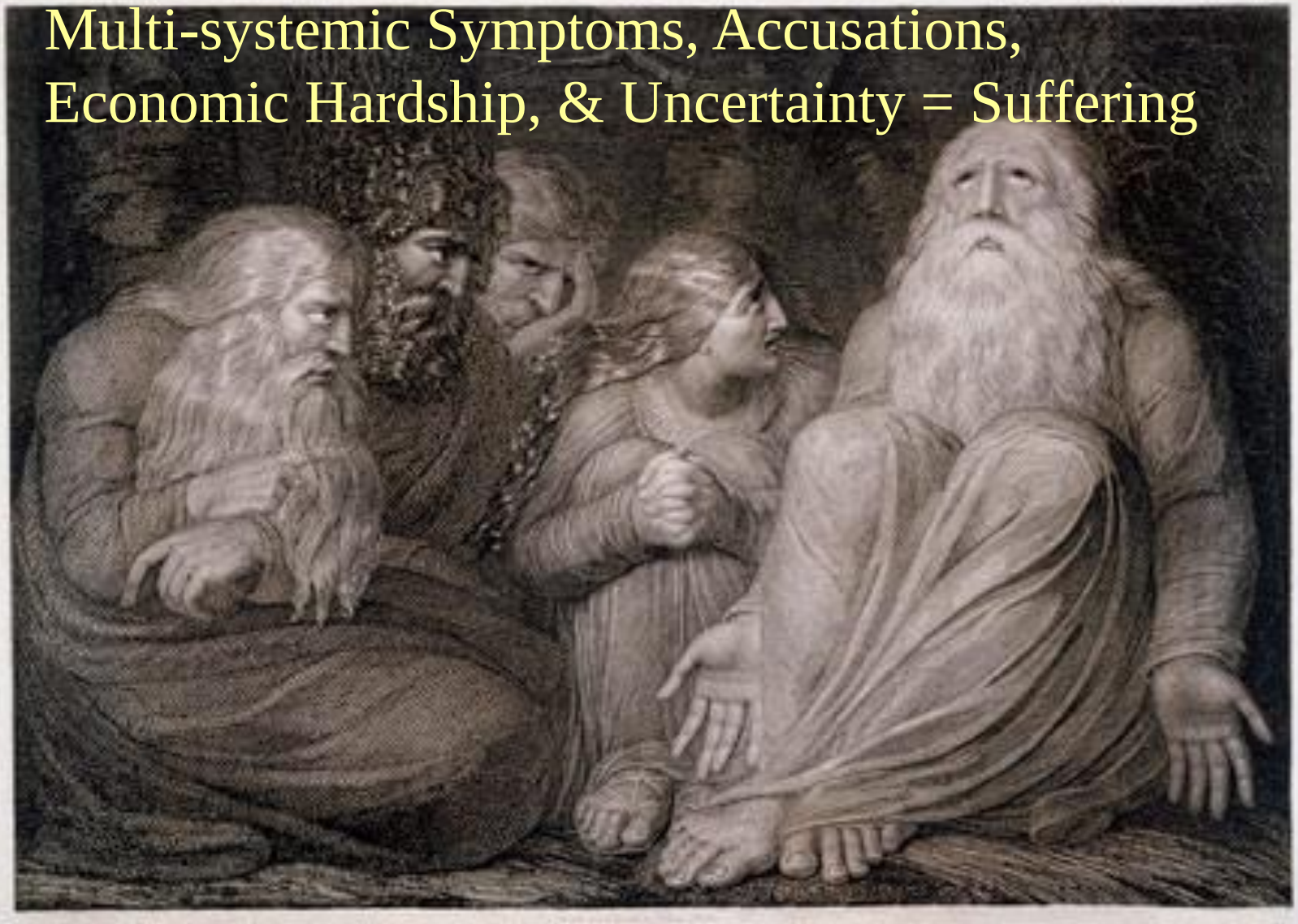
Thank you

- Public Health England. Dr. Tim Brooks.
- Patients who seek to help disseminate knowledge, raise awareness, and raise funds to support research
  - U.S.:
    - Lyme Disease Association
    - Lyme Research Alliance
    - Tick-borne Diseases Alliance

Preliminary Assumptions

- Some patients experience disabling chronic symptoms after Lyme disease
- The cause of these chronic symptoms is uncertain – likely heterogeneous
- Terms –
  - Post-treatment Lyme Disease
  - Post-treatment Lyme Syndrome
  - Chronic Lyme disease
- Relatively few studies have been done of patients with chronic persistent symptoms.

The bad news is...you have Lyme disease. The good news is, I don't believe in that disease so you're fine!



"The Book of Job" Illustrated by William Blake

Cost to Society of Lyme Disease Based on large samples

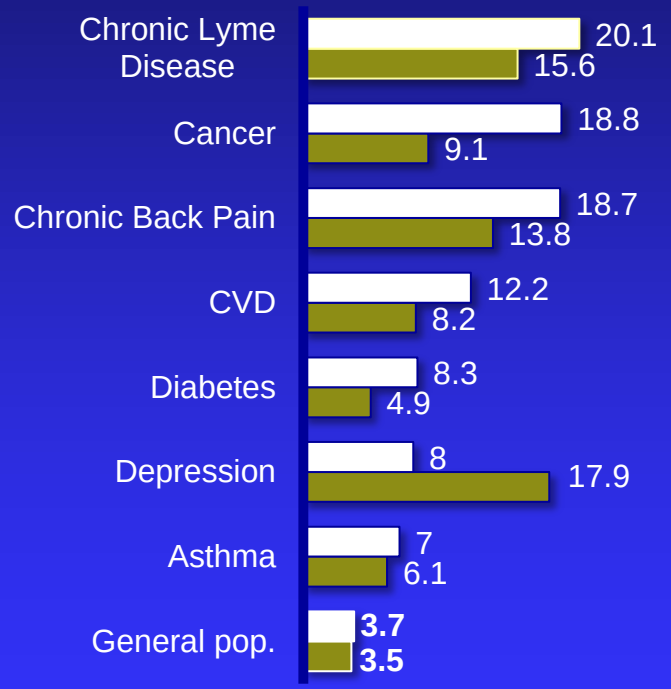
- Retrospective Medical Claim Study

  52,795 Lyme Patients vs 263,075 matched controls without Lyme test orders or Lyme diagnosis

  - Lyme Disease pts had $2,968 higher total health care costs and 87% more outpatient visits in a 12 month period.

(Adrion et al, PloS One 2015)

On-Line Survey: Number of poor physical (white) and mental (olive) days per month of patients by disease condition[1]



3,090 Patients with chronic symptoms after Lyme Disease report more days of feeling sick per month than those with many other common chronic diseases Johnson et al, Peer J , 2014.

Outline of Talk

- Columbia Lyme Encephalopathy Study
  - Other U.S. Clinical Trials
  - Post-Trial Investigations
- Laboratory Testing in Patients with PTLS
- New Research: Implications & Questions

GOALS
Lyme Encephalopathy Study (PTLE)

Post-treatment Lyme Encephalopathy – a more objective subcategory of PTLDS (Fallon et al, Neurology 2008)

- To assess brain structure (MRI)
- To assess brain functioning (PET)
  - Metabolism by FDG PET
  - Blood flow by O-15 PET (before & after hypercapnea)
- To assess improvement in response to 10 week of IV ceftriaxone vs IV placebo
  - Primary Measures – cognition/memory
  - Secondary Measures– fatigue, pain, physical functioning

Randomized, Double-Masked Study Design

- Random assignment to 10 weeks of IV ceftriaxone vs 10 weeks of IV placebo
- Because of repeated neurocognitive testing, a healthy control group was included to assess the practice effect
- Primary outcome at 12 weeks
  Sustainability assessed at 24 weeks

These patients met highly conservative criteria for Post-treatment Lyme Syndrome

- Age 18-65
- Lyme patients:
  - Historically well-documented Lyme disease based on CDC surveillance clinical and laboratory criteria
  - Prior treatment with at least 3 weeks of IV ceftriaxone
  - Memory deficit (-1SD) confirmed by cognitive testing
  - Current positive IgG Western blot at our reference lab
- Healthy Controls: age-, sex-, education-matched

Assessments

- Structural Imaging (MRI)
- Functional Imaging (PET)
  - FDG to assess metabolism
  - O-15 with hypercapnea to assess flow
- Primary Outcome: Neurocognitive Testing
- Secondary Outcomes (Self-reports):
  - Fatigue, Pain, Functional Status

PRIMARY OUTCOME - Cognition

Cognitive Index (avg score of Domains)

- **Memory***
- **Working memory**
- **Verbal Fluency**
- **Attention**
- **Motor**
- **Psychomotor**

Final Study Entry Sample Size

- Total: 55
  - **Lyme patients: 37**
    - 23 randomized to ceftriaxone
    - 14 randomized to placebo
  - **Healthy controls: 18**
- Of 3700 evaluated, 1% were enrolled
  - Why so few? Conservative criteria requiring cognitive impairment, current +IgG WB, at least 3 wks of IV Abx
  - Results may not be generalizable to larger sample of persistently symptomatic patients who do not meet these narrow study criteria

Enrolled Sample of 37 Patients with Post-treatment Lyme Encephalopathy

- Age: 45.1 yrs,
- Gender: 59% female
- Delay bet symptoms and treatment: **1.2 yrs**
- Amount of Prior Treatment: Considerable. <u>This is a particularly chronic sample – less likely to benefit from re-treatment</u>
  - **Mean IV: 2.3 months**
  - **Mean oral: 7.7 months**

Baseline CSF Laboratory Results

- CSF (n=33):
  - Few Routine abnormalities:
    - WBC: 2 mildly increased
    - Protein: 4 mildly increased
    - Oligoclonal bands: none
  - CSF Bb Antibodies:

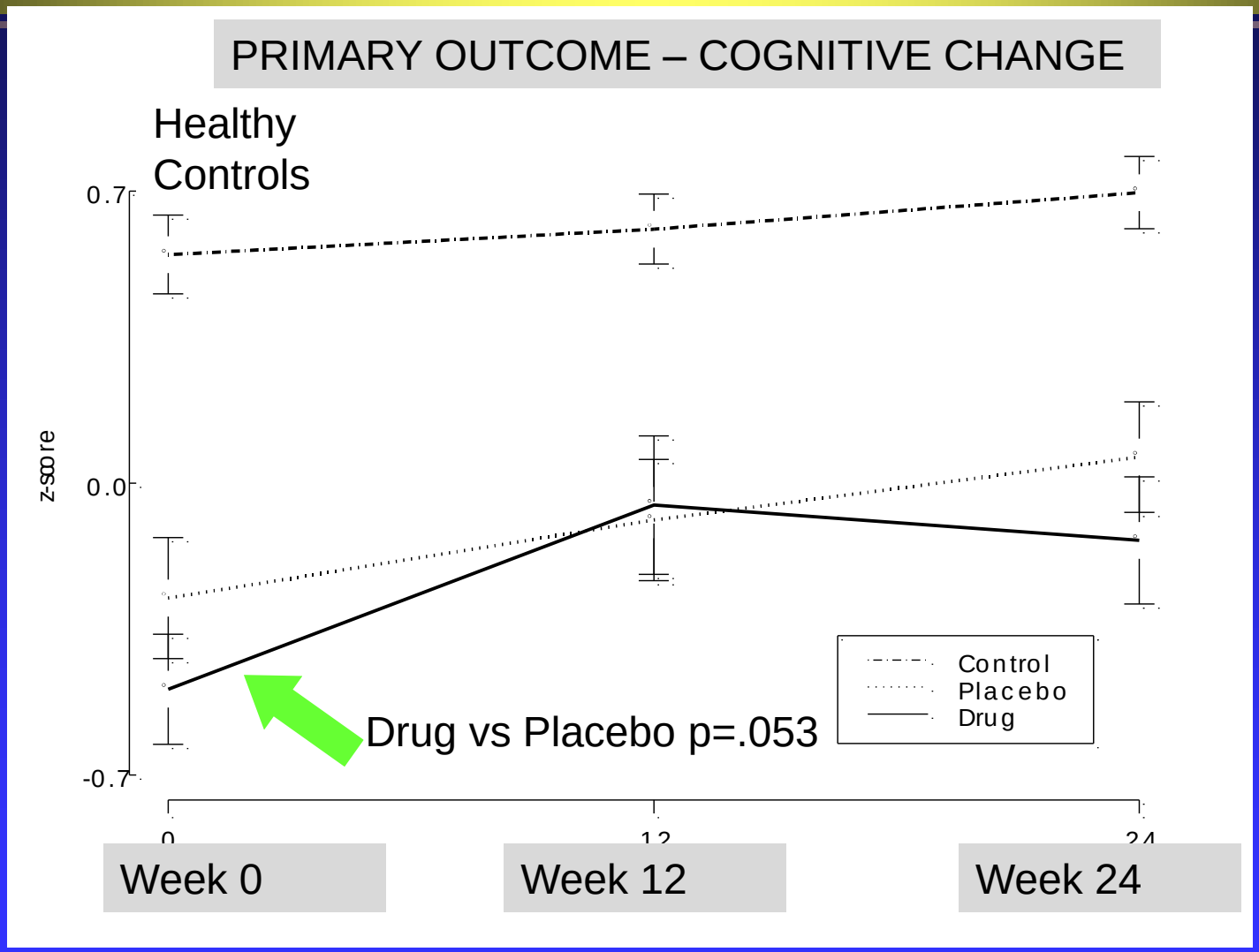
    WCS Intrathecal Ab Index positive: 12.1%

    C6 Peptide Intrathecal Ab Index Positive: 62.5%
  - PCR Positive: None using OspA based PCR
  - Culture Positive: 1 sample (false positive)

Clinical Profile of Post-treatment Lyme Encephalopathy

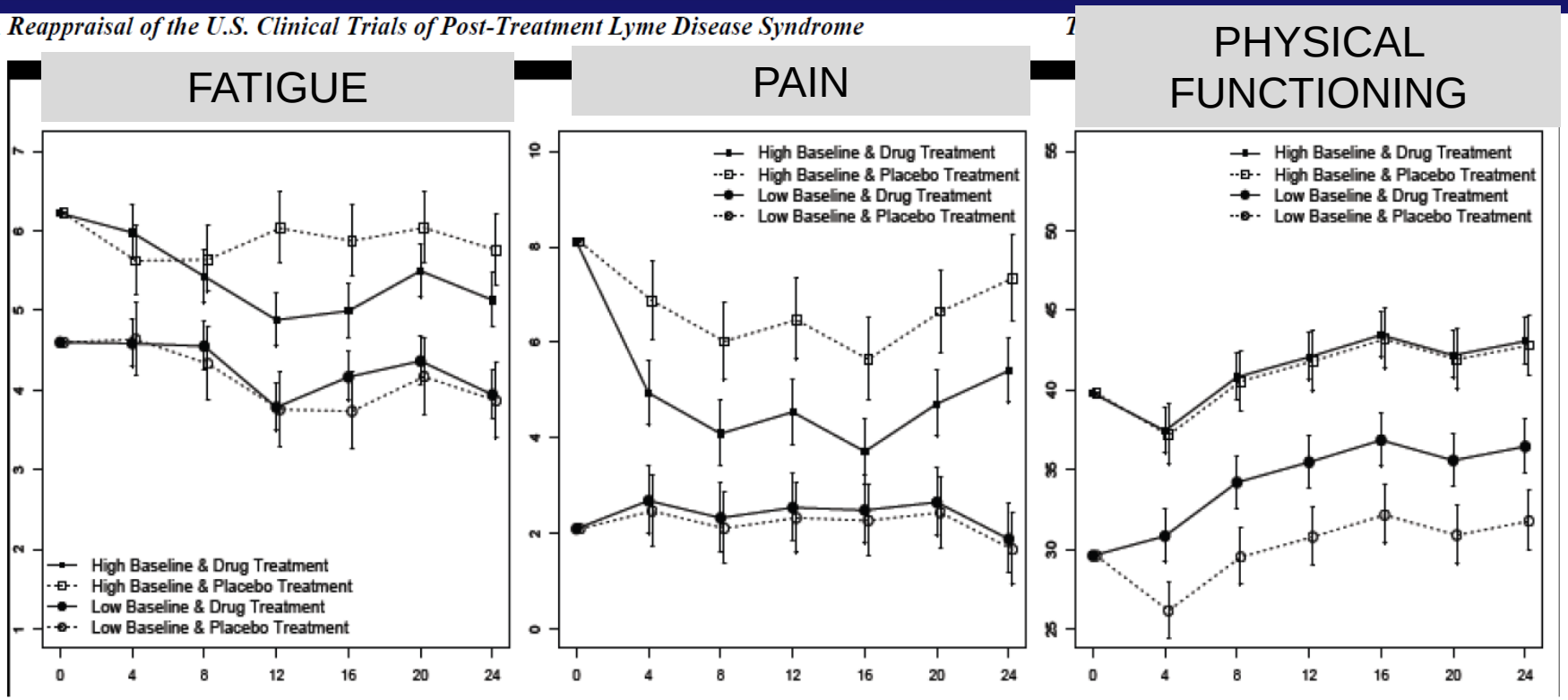
- Cognitive: mild-moderate deficits
  - Verbal Memory (z=-1.0),
  - Working memory (concentration) (z=-0.7)
  - Verbal Fluency (z=-0.8)
- **Psychiatric:** mild depression & anxiety
- **Systemic Symptoms are debilitating:**
  - **PAIN – comparable to post-surgical pain**
  - **FATIGUE – comparable to M.S. patients**
  - **PHYSICAL DISABILITY – comparable to congestive heart failure patients**

Significant Change in Cognition over time for 3 groups (p=.04): 10 wks of IV Ceftriaxone vs Placebo vs healthy controls- Response then Relapse when off antibiotics



SECONDARY OUTCOMES

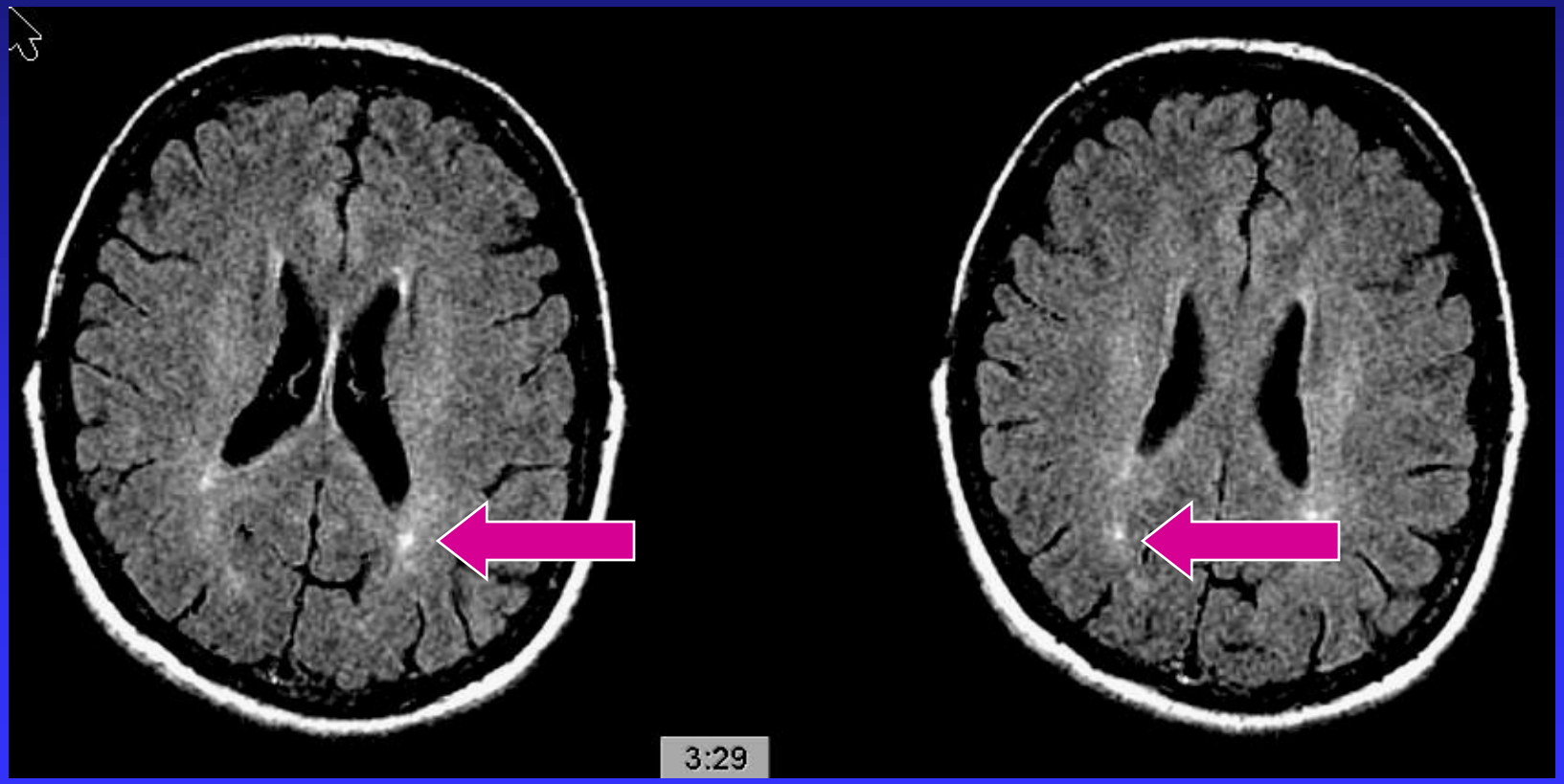
Baseline Severity is key to detecting a treatment effect. Treatment response is sustained



Note: High scores on fatigue and pain and low scores on physical functioning indicate greater severity.

Fig. (3). Model-based Illustrations from Lyme Encephalopathy Study: change over time as a function of baseline severity on measures of Fatigue, Pain, and Physical Functioning.

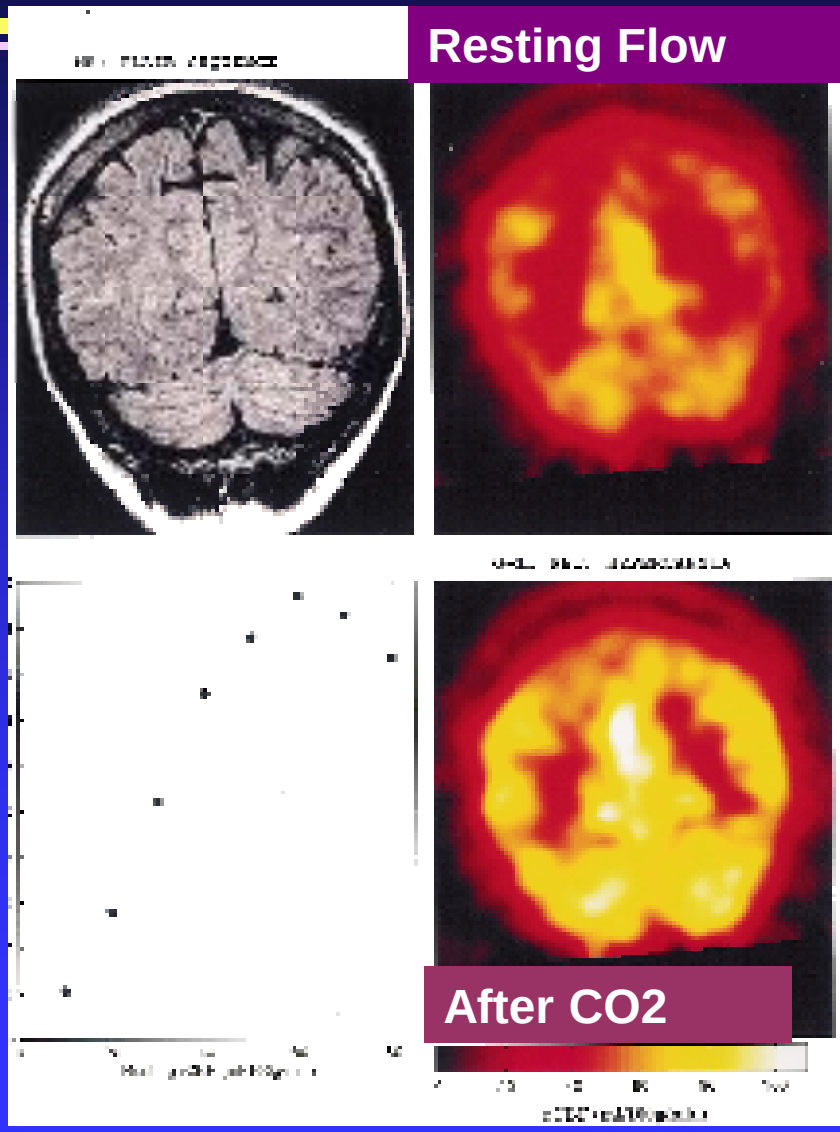
Baseline MRI:
Lyme patients did not have significantly more white matter hyper-intensities than matched controls



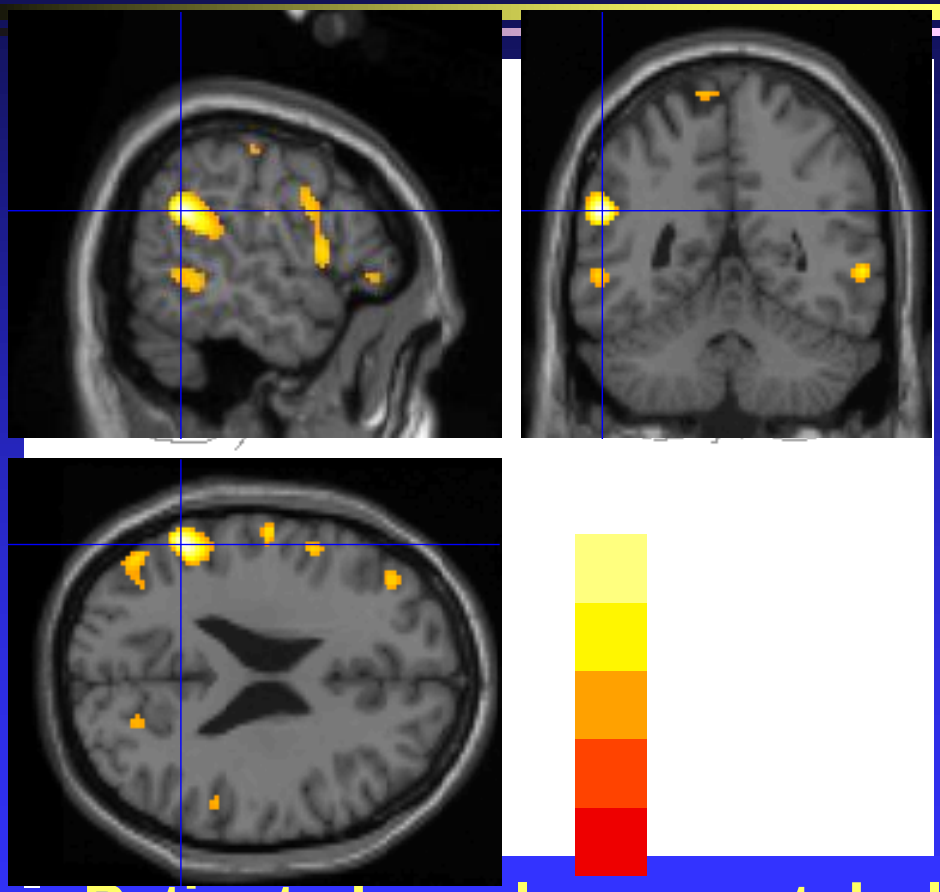
Scheltens scale of 5 for WMH

Global Cerebral Blood Flow: is there a difference in vasodilation capacity bet pts and controls?

- Yes, the response to hypercapnic CBF challenge differed:
  - The patient group showed a diminished ability to enhance blood flow compared to controls (8.2% v 28.1%, p<.02)
  - This finding suggests vascular compromise in the patient group (not attributable to CVD risk factors)



FDG Imaging: are there regional metabolic differences between patients & controls? Yes.



- Large clusters of specific areas of decreased metabolism
- This was not a random distribution…most abnl areas on metabolic scan were also abnl on the 3 blood flow scans.

Patients have lower metabolism in 12 clusters (3049 voxels)

Left Hemisphere: Temporal (1199v), Frontal (527v), claustrum (539v), Parietal (139v)

Right Hemisphere: Cingulate (487 v BA 24/30), Parietal (265 v), Insula (154v)

Patients have higher metabolism in 3 clusters (680 voxels)

Which variables were not associated with response to re-treatment?

- Presence of positive titers for other infections (Anaplasma, Babesia, Bartonella)
- Positive Lyme IgM Western blot

- Magnitude of NK CD 57
- Positive ANA, CRP, or ESR

- CSF abnormalities: protein, WBC, WCS ELISA, IgG WB

- Amount of prior IV or oral Abx was not associated with responsiveness to IV Ceftriaxone treatment

What was associated with greater likelihood of improvement?

Greater impairment at the start of treatment

- Worse Cognitive impairment
- Worse Pain, Fatigue, Physical Functioning
- Greater # of joints in pain on rheum exam
- Greater # of abnormal areas on neuro exam

Elevated C6 Peptide index (CSF/serum)

Intrathecal Ab Production in PTLS: Whole Cell Sonicate vs C6 ELISA

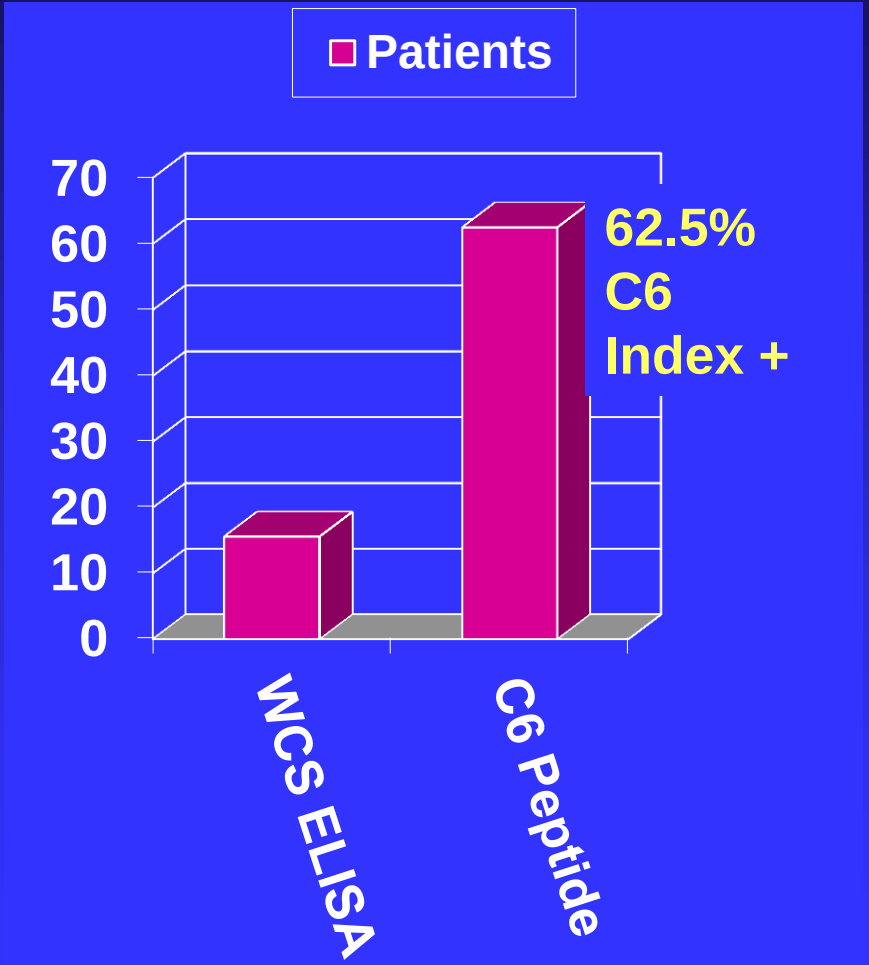
C6 ELISA Index:

4x more sensitive

- **Than Whole Cell Sonicate ELISA in identifying Bb-specific Intrathecal Ab Production (p<.001)**

Positively correlated with improvement in Physical functioning Index of SF 36 among drug-treated pts at wk 12 (r=.5, p=.04) & week 24 (r=.5, p=.02)

AI= [OD CSF/OD Serum]/
[Total IgG CSF/Total IgG Serum]



Summary

Cognition:

- Memory, Verbal fluency, & Processing Speed are deficient

Brain MRI:

- White Matter Hyperintensities do not distinguish Lyme vs controls

PET Imaging: Decreased perfusion and metabolism observed

Treatment with Ceftriaxone

- Although change in cognition was more favorable for the ceftriaxone group than the placebo and control group at 12 wks – by 24 weeks all gains were lost.
- Improvement was sustained over 24 weeks on secondary measures of pain & physical functioning when baseline severity was included as an interaction effect

Durability to Week 24:

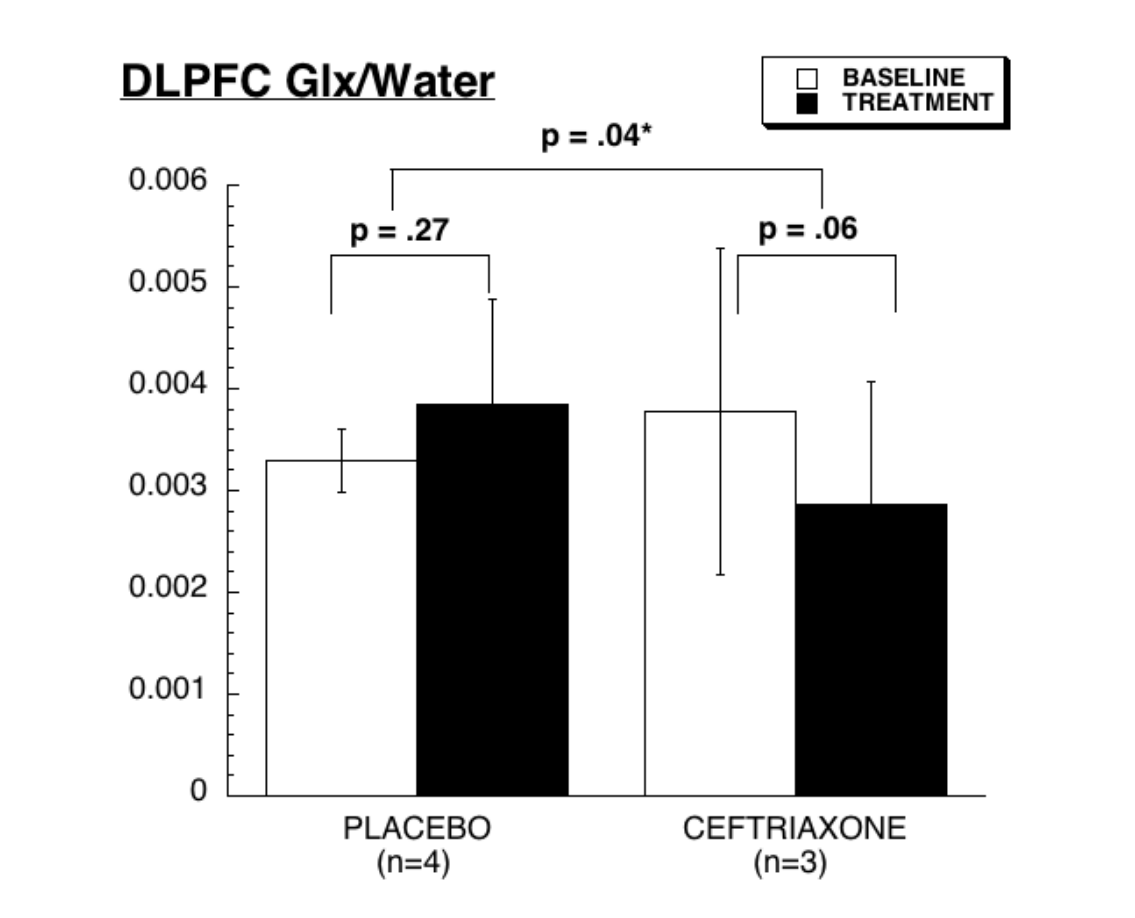
- Patients given antibiotic lose gains in cognition
- Improvement continues in pain & physical functioning related to baseline severity.

Other Studies from this Lyme Encephalopathy sample

- MR Spectroscopy study of Ceftriaxone's effect on CNS Glutamate

- Studies suggesting heightened immune activation
  - 1. Proteomic Study of CSF of PTLS
  - 2. Serum Anti-neuronal Ab
  - 3. Serum Interferon α-inducible genes

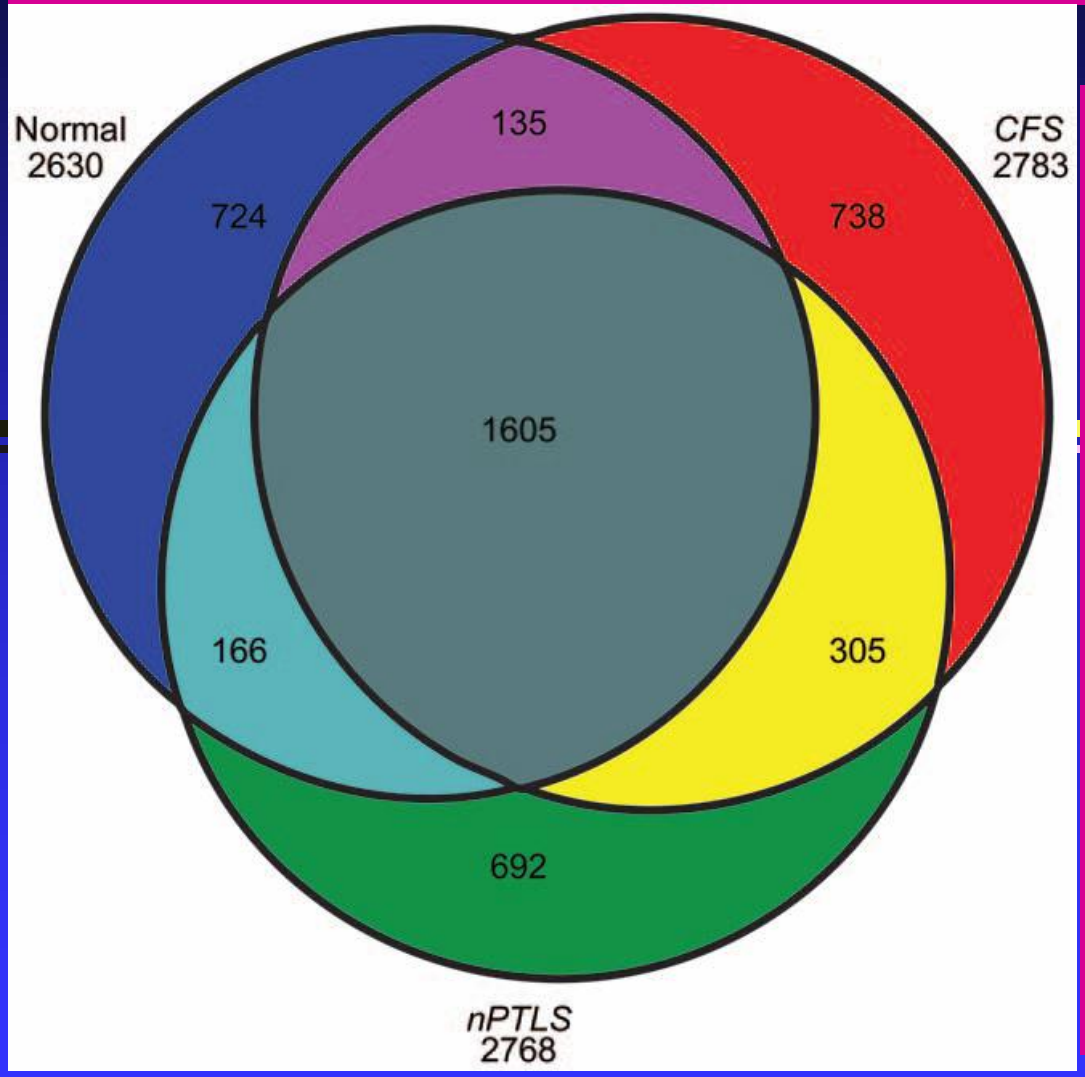
MR Spectroscopy before after 30 days of ceftriaxone or placebo



IV Ceftriaxone decreased the excitatory neurotransmitter glutumate

Fallon et al,
manuscript in prep.

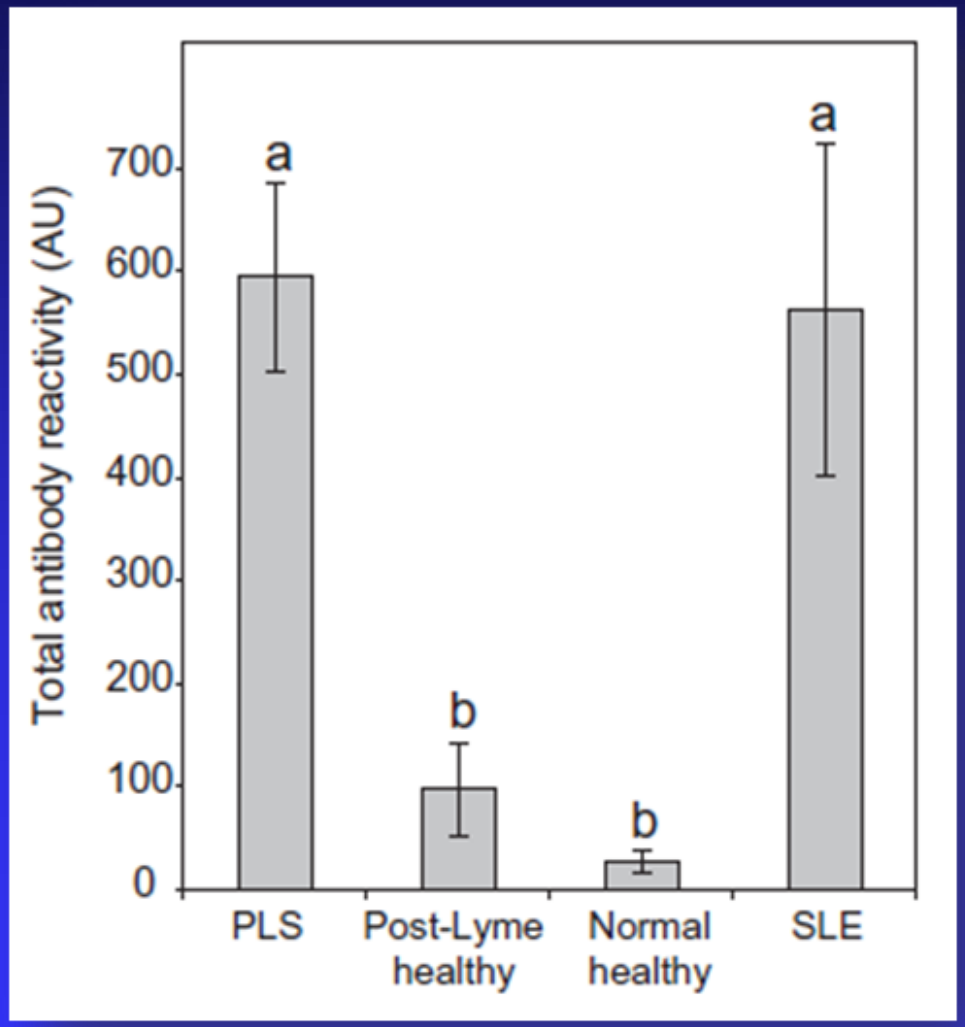
The CSF Proteome: unique proteins differentiate post-Lyme vs CFS vs Normal

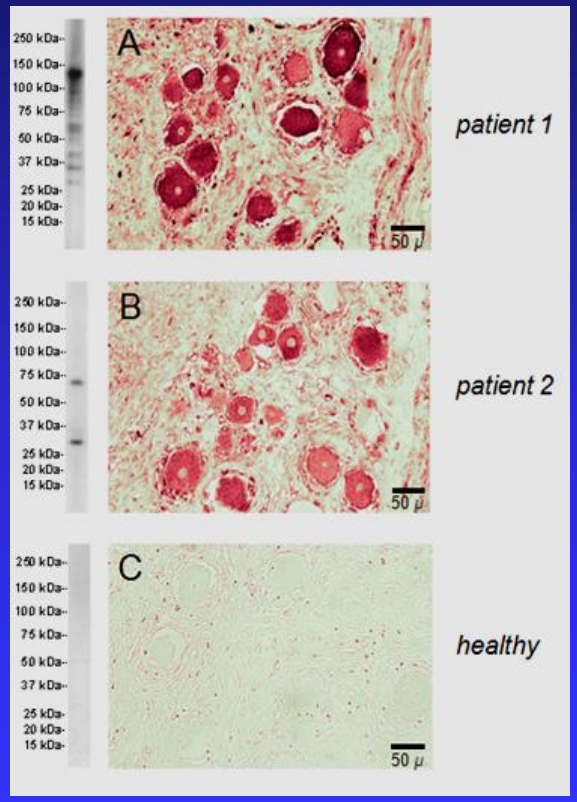


- Unique Proteins:
  - 738 in CFS
  - 692 in post-treatment Lyme
- Proteins in the complement cascade were elevated in abundance in Lyme and CFS.

Schutzer, Angel, Liu…Fallon, Natelson, PLoS-One, 2011

Serum Antineuronal Antibodies are increased in PTLDS…. but not in recovered Lyme.

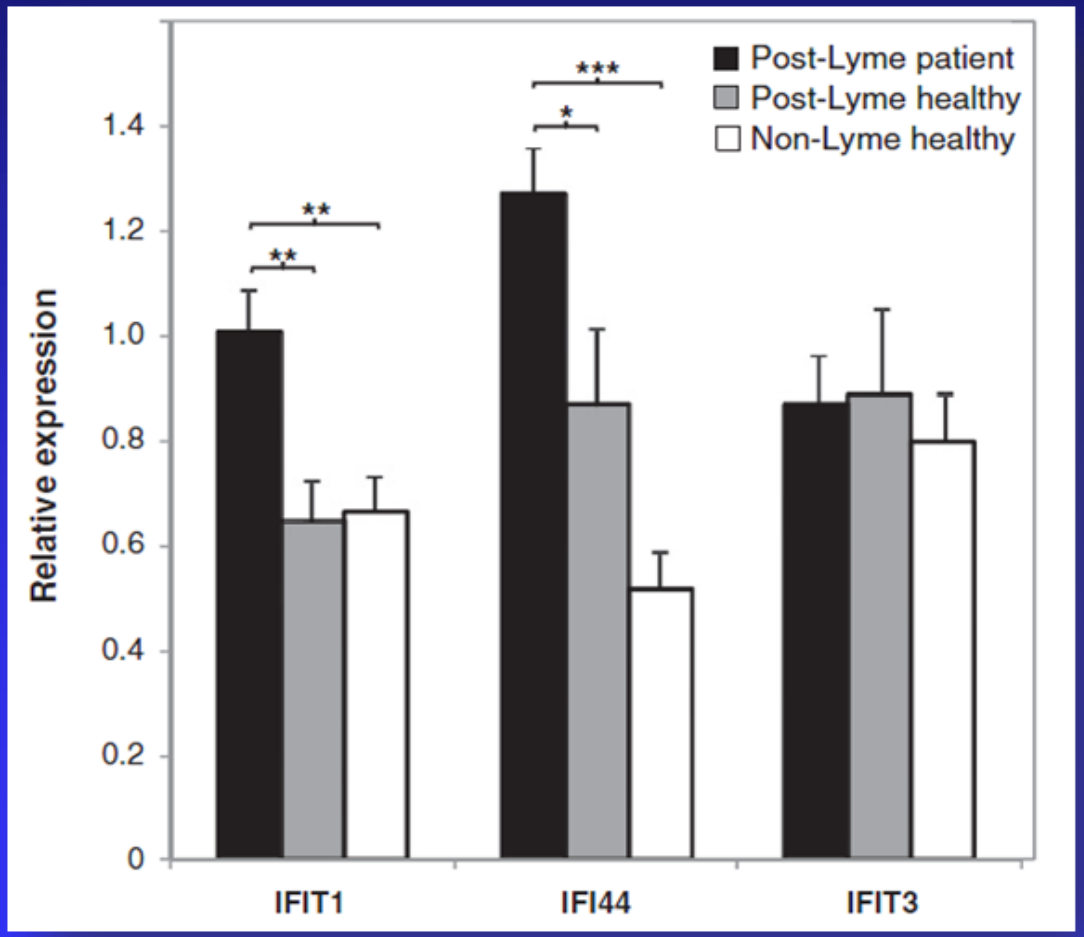




Chandra et al.,
Brain, Behavior, Immunology, 2010

Sera from PTLS patients induce higher expression of IFNα-inducible genes.

Jacek, Fallon, Chandra, Crown, Wormser, Alaedini, J Neuroimmunology 2013



Strengths of the NINDS Lyme Encephalopathy Study

- Rigorously Defined Patients
- Quantitative methods for assessment
- Excellent Study Retention
- Age-, Gender-, and Education-matched controls
- Excellent collaborations
- Stored samples in Columbia repository

Limitations of the Study

- Generalizability was limited
  - Enrollment criteria were so restricted
- Sample size was too small (n=37)
  - Due to difficulty finding eligible subjects
  - Markedly reduced the power to detect treatment effects
- DNA/RNA samples not stored

Adverse Effects & Suggestion for future studies

- IV Ceftriaxone confers risk – blood clots, blood stream infection, biliary stones/ cholecystitis, allergic reaction. Each of these occurred in our small study of 37 patients.

- Primary focus of future studies should not be on cognition…but on pain, fatigue, and functional impairment

Significance and Questions

Why do patients not sustain improvement in cognition?

Would a less treatment refractory sample have done better?

What alternative & safer non-antibiotic therapies may enhance patient response among those not responding to repeated antibiotics?

Can additional baseline predictors of response be identified?

What was the mechanism? Antimicrobial? Glutamate?

Other Randomized Placebo-Controlled U.S. Studies of PTLDS

STOP-LD Study (Krupp, Neurology 2003)

Klempner et al. (New England J Med 2001)

STOP-LD Study (Krupp et al, Neurology 2003)

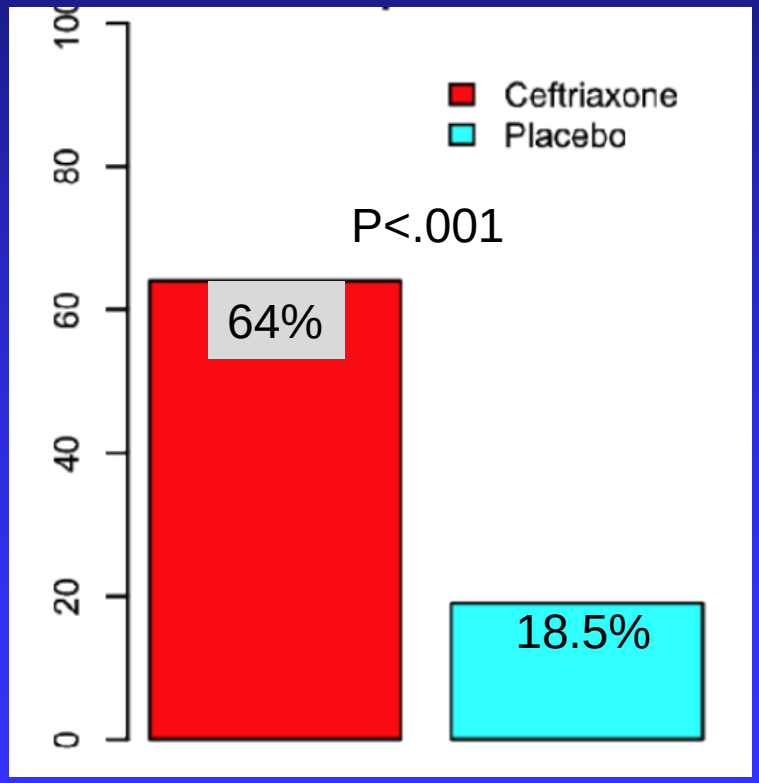
- 55 patients with persistent fatigue after treated LD who had had at least 3 weeks of antibiotic treatment previously
- Fatigue severity for all enrollees had to be at least “moderate” on the Fatigue Severity Scale

STOP-LD Study Design

- Three primary outcome measures (fatigue, reaction time, CSF OspA) but only on one – Fatigue – were patients uniformly impaired.
- Random assignment to 1 month of IV ceftriaxone or placebo followed by 5 months of no treatment.
- Primary end-point: 6 months

Stop-LD Primary Outcomes

Significant improvement on the primary outcome of Fatigue



% of Responders on Fatigue

- No improvement in reaction times, but "deficits were relatively mild which may have contributed to a lack of a treatment effect"
- No reduction in OspA antigen but "only 16% had this marker at baseline… it was not a useful measure of outcome"

STOP-LD Study (Krupp, Neurology 2003)

STOP-Lyme Fatigue Study: Improvement in Fatigue continued to occur during the 5 month drug-free interval after one month of IV ceftriaxone therapy.

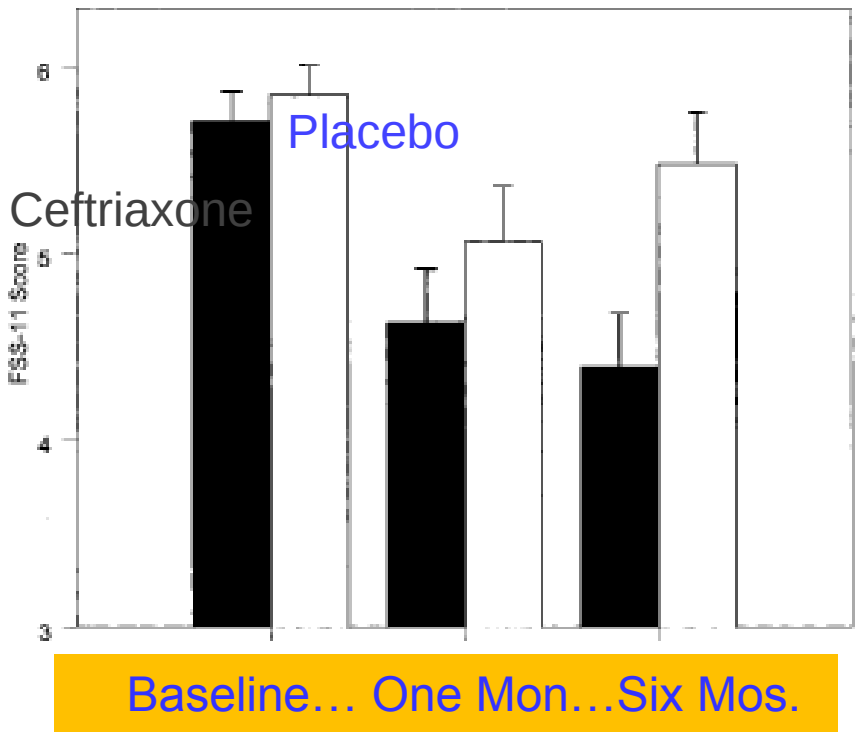
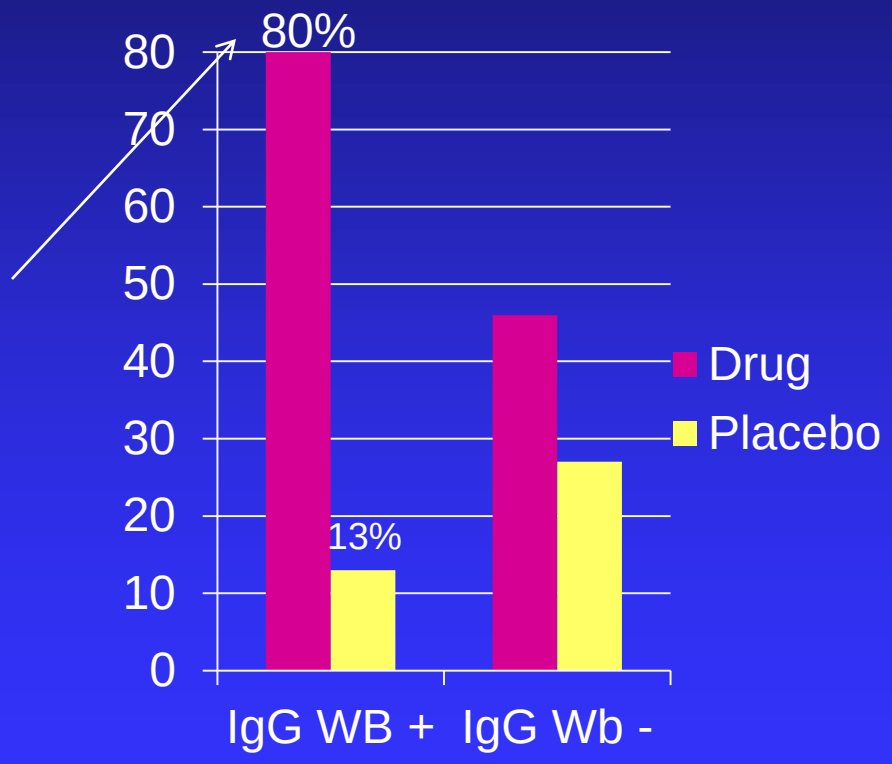


Figure 3. Mean (± SE) Fatigue Severity Scale (FSS-11) scores for study groups at baseline, 1 month, and 6 months during treatment with ceftriaxone (black columns; n = 26) and placebo (white columns; n = 22).

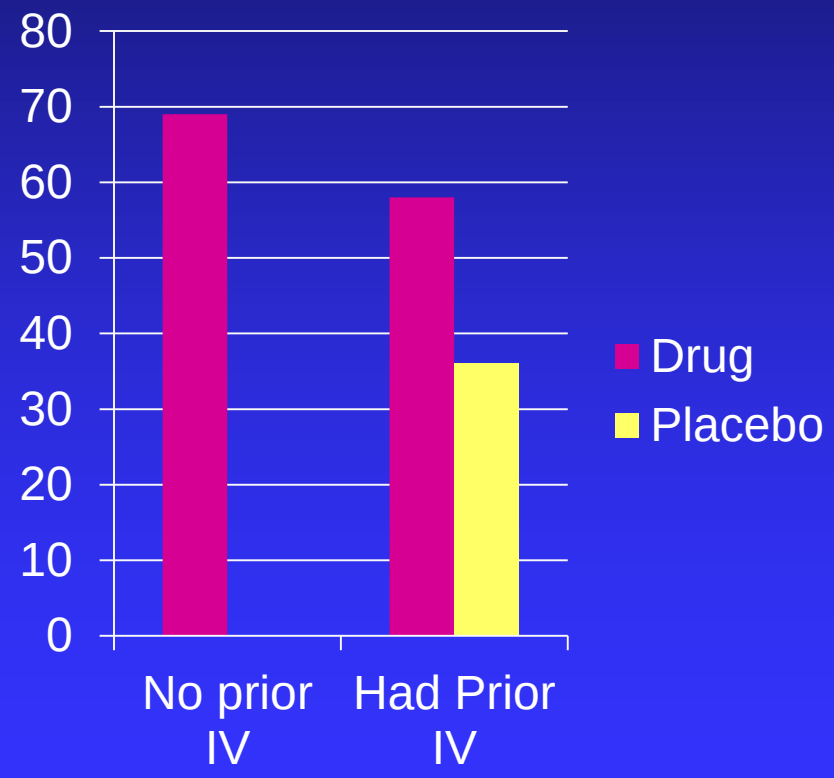
STOP-Lyme Study (Stonybrook, Krupp, Neurology 2003)

80% Responder Rate to Ceftriaxone among IgG WB+ patients

IgG WB+ Patients did better (p<.01)

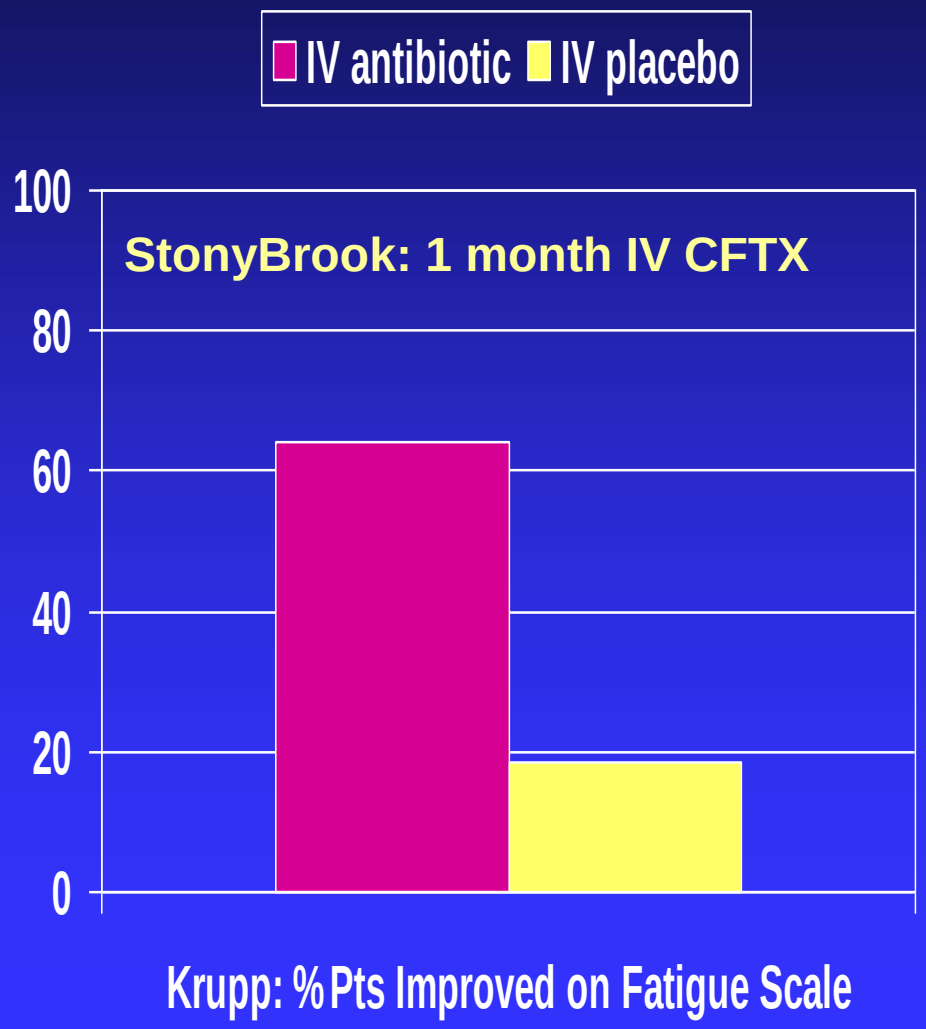


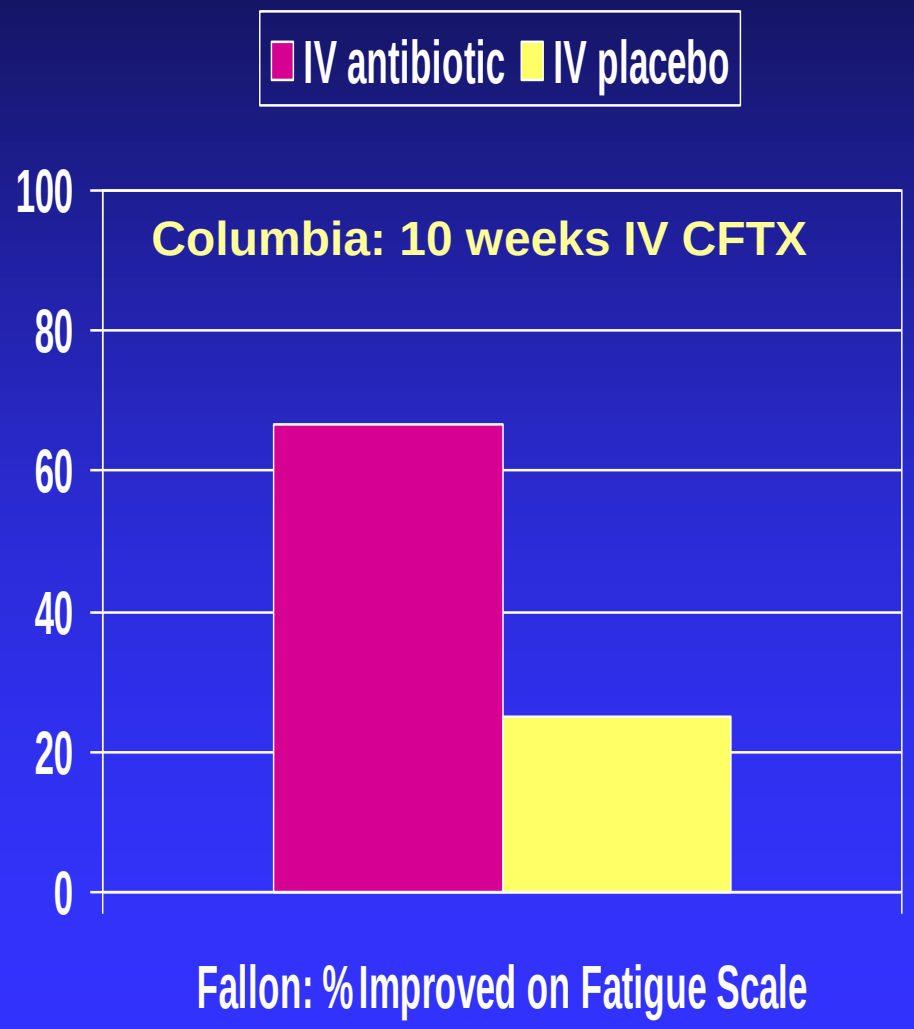
Prior IV therapy did not confer significantly worse outcome.



(STOP-Lyme Study, Stonybrook, Krupp, Neurology 2003)

Comparing Two PTLDS Studies – the Columbia results replicated the STOP-LD results





Prominent International Treatment Guidelines for PTLDS disagree with the prior summaries

- Infectious Disease Society of America:

  "antibiotic therapy has not proven to be useful"

- British Infection Association:

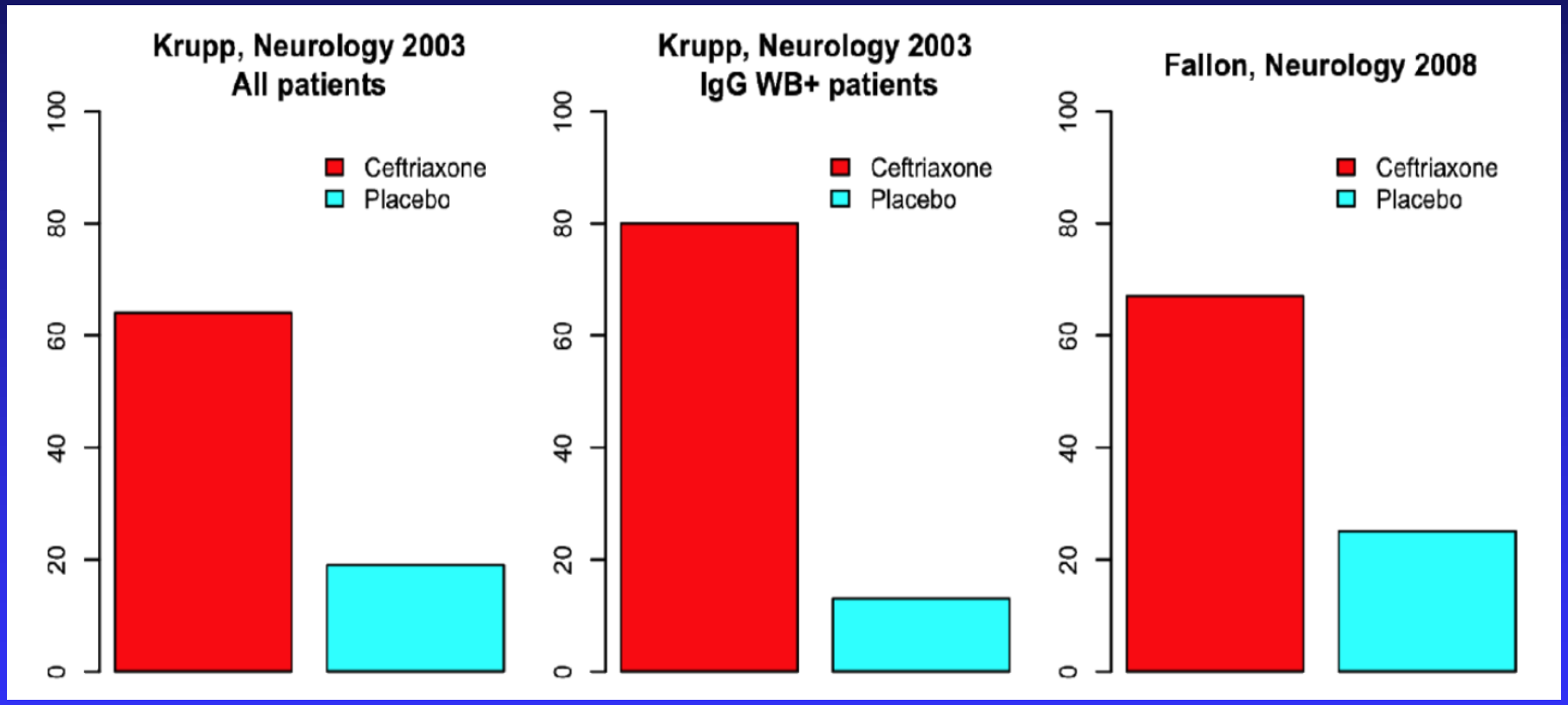
  "Studies of prolonged antimicrobial treatments of patients with Post Lyme Syndrome have not shown sustained benefit"

More Disagreement

- European Federation of Neurological Societies:

"American trials have demonstrated that additional prolonged antimicrobial treatment is ineffective in Post Lyme Disease Syndrome"

But these results are impressive & sustained. Why are they being ignored?



Effect Size: Moderate to Large

Fallon, Open Neurology, 2012

Klempner's 2 Studies of PTLDS

NEJM 2001

- 129 seronegative & seropositive patients
  - Enrolled based on report of functional impairment – but no severity cutoff or severity measure was used for enrollment.
  - Did not require documentation of classic Lyme signs in the seropositive group

- Randomly assigned to:
  - 30 days of IV CFTX + 60 days of oral doxy
  - 30 days of IV placebo + 60 days of oral placebo

Results at 6 months

Klempner et al, NEJM, 2001

- Primary Outcome:
  - No difference in functional outcome between drug and placebo as assessed by the SF-36 measure
- Secondary outcomes:
  - No difference in change for cognition or depression.
- Result: Study stopped early due to lack of benefit.

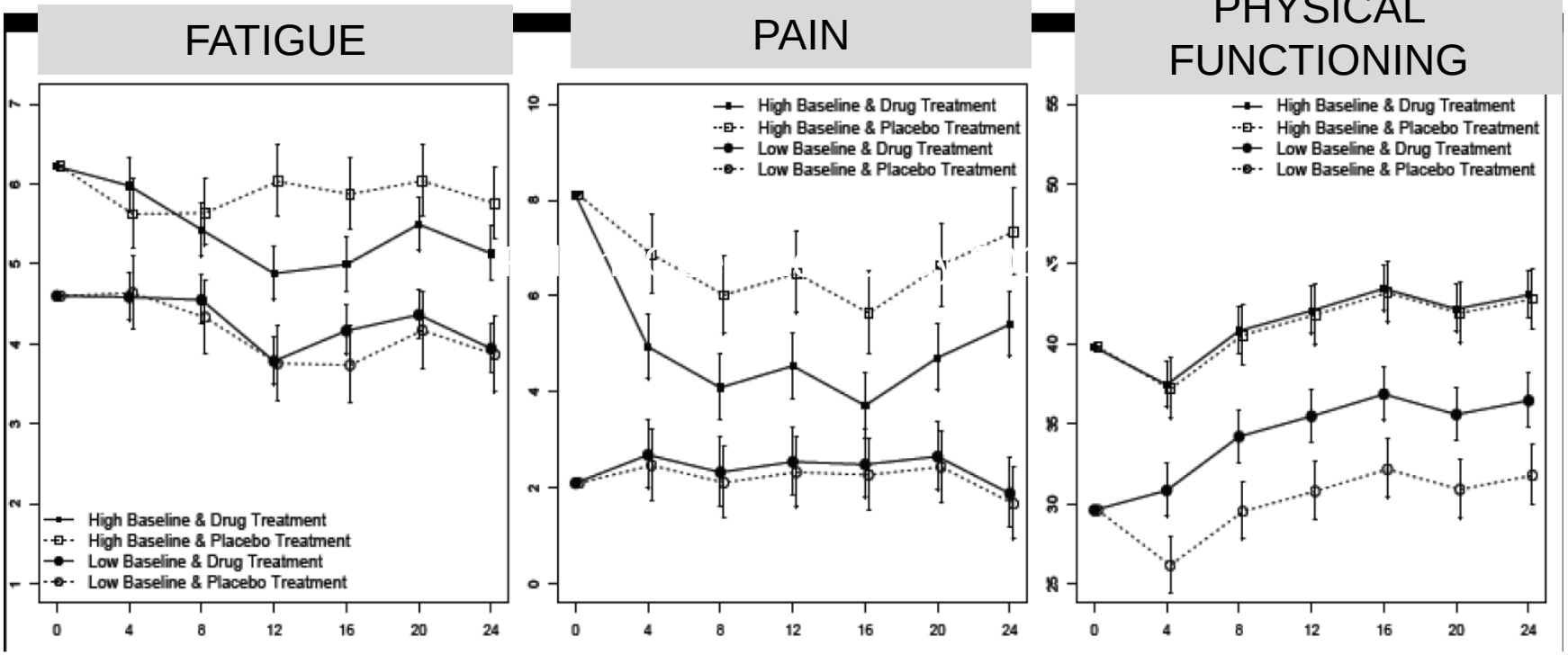
Why were these results so different from the earlier studies?

- Strength of this study: sample size
- Limitations of this study:
  - Heterogeneity of patient sample
    - Not enrolled based on a pre-selected "severity level of impairment"
    - Did not require documentation of clinical signs of Lyme in the "seropositive group"
  - Statistical analysis did not adjust for differences in baseline severity on functional impairment

Fallon, Open Neurology 2012
Delong, Cont Clinical trials 2012
Klempner, AmerJMed, 2013

SECONDARY OUTCOMES

Baseline Severity is key to detecting a treatment effect. Treatment response is then sustained



Note: High scores on fatigue and pain and low scores on physical functioning indicate greater severity.

Fig. (3). Model-based Illustrations from Lyme Encephalopathy Study: change over time as a function of baseline severity on measures of Fatigue, Pain, and Physical Functioning.

Fallon, Open Neurology 2012

“Efficacy” vs. “Clinically Recommended”

- Krupp Fatigue Study:
  - Efficacy for IV ceftriaxone was shown for fatigue but IV treatment was not recommended due to risks associated with IV antibiotics
- Fallon Encephalopathy Study:
  - results showed non-sustained benefit on the primary outcome measure of cognition and IV risks – so IV treatment was not recommended for sustained improvement in cognition

Conclusions

- Guidelines should revise their assessment of the U.S. clinical trials - as retreatment with IV ceftriaxone has been shown to reduce PTLDS – Fatigue
- Patients & Physicians should discuss together the risk/benefit ratio of retreatment.
- Alternative treatments are needed for those who are no longer benefiting from antibiotics
- Immune activation is present in many of these patients with PTLS – a potential clue

A Comparison of Lyme Disease Serologic Test Results From 4 Laboratories in Patients With Persistent Symptoms After Antibiotic Treatment

Brian A. Fallon,[1] Martina Pavlicova,[2] Samantha W. Coffino,[3] and Carl Brenner[4]

Clinical Infectious Diseases Advance Access published October 13, 2014

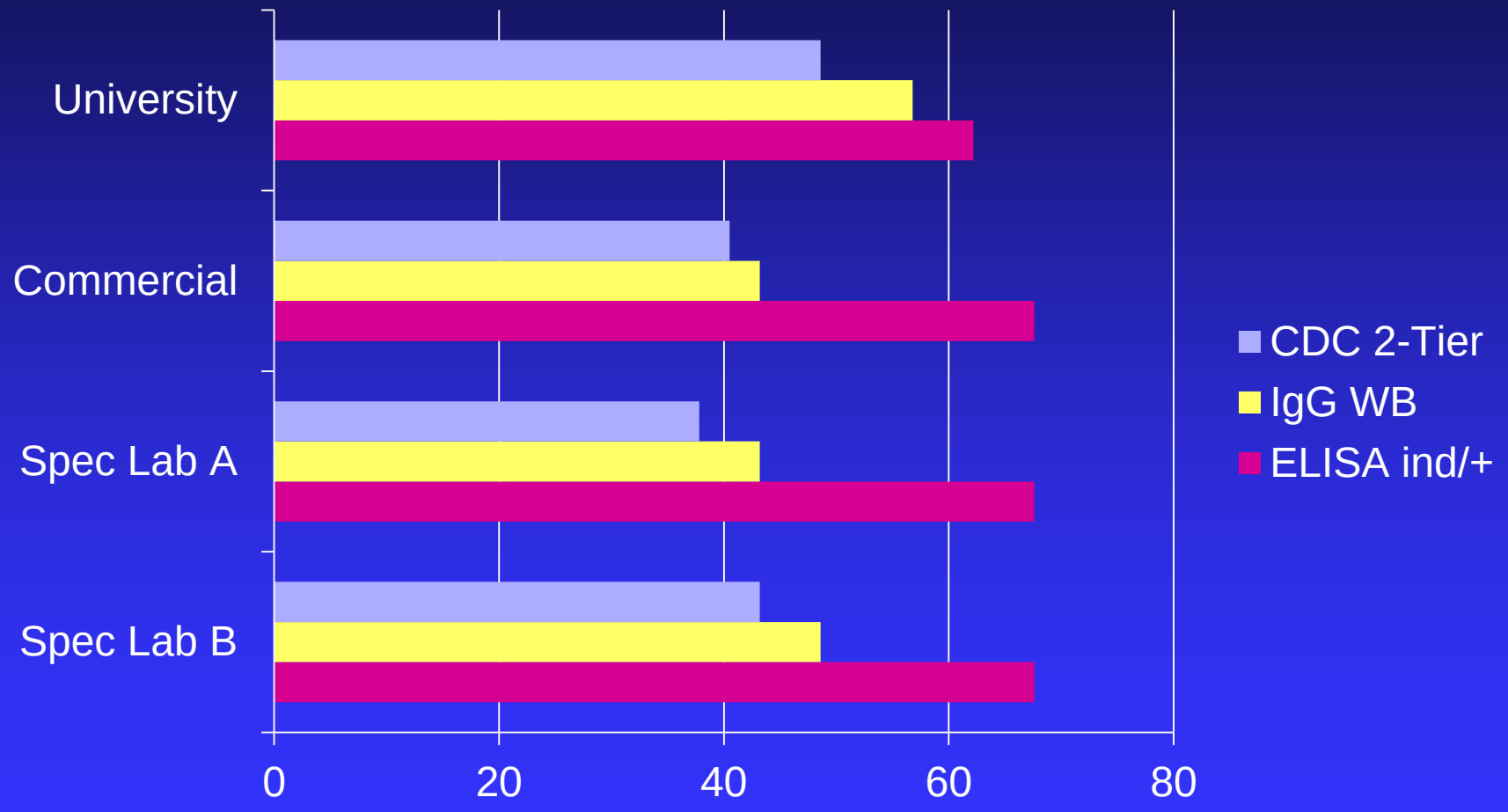
Five Questions

- Do labs differ in the likelihood of detecting a sample as positive?
  - Lyme specialty vs national commercial vs University lab
- Are the labs concordant with each other?
  - ie, will a positive result at one lab also be positive at the next lab.
- Is the % of False Positives comparably low at the different labs?
- Do the in-house lab criteria for interpretation of a Western blot provide an advantage over the CDC criteria?
- How does the IgM Western blot perform from PTLDS samples?

Methods

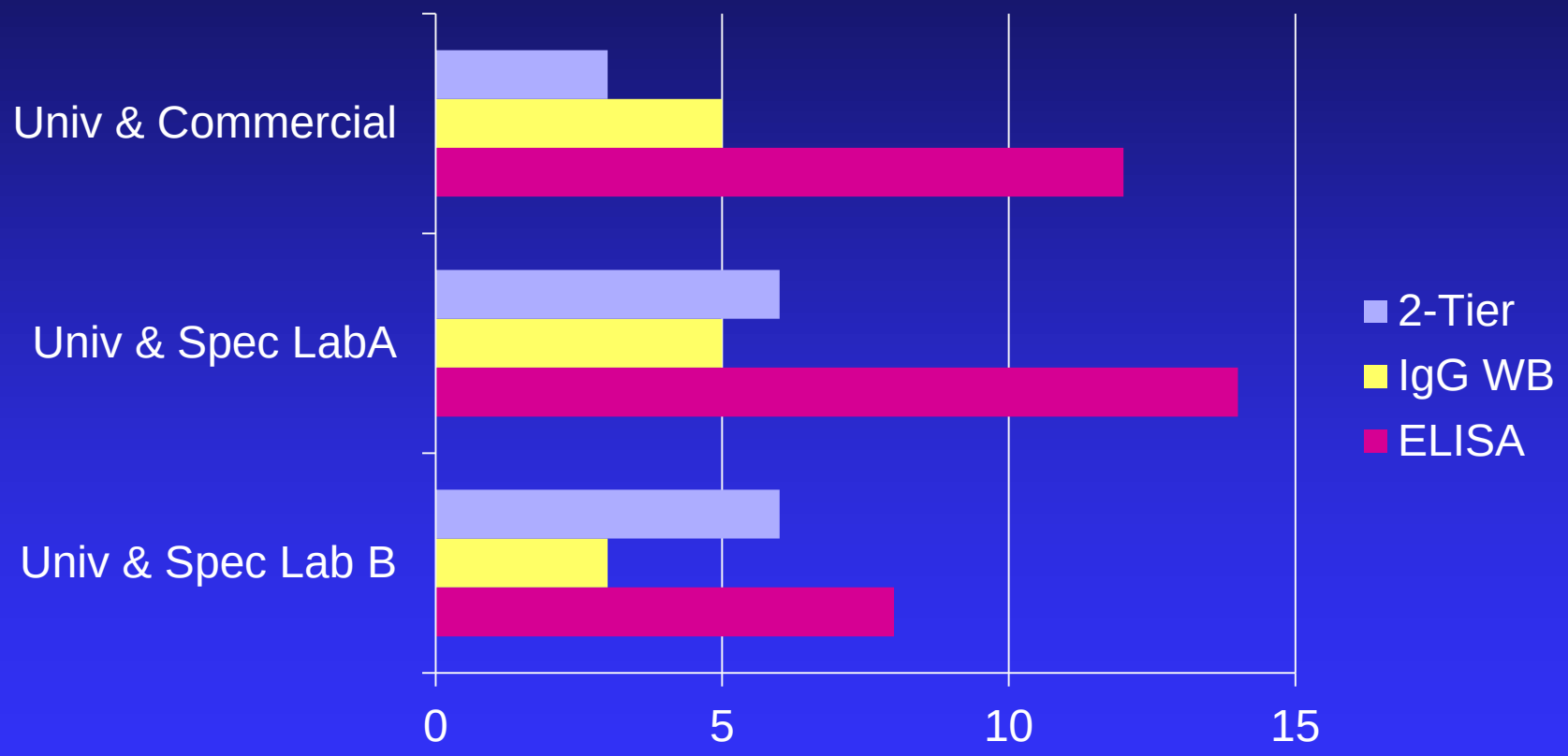
- Serum from two groups:
  - 37 persistently symptomatic treated patients with well-documented prior Lyme disease (PTLDS)
  - 40 asymptomatic medically healthy controls without a history of Lyme disease diagnosis or treatment
- Samples from each subject sent to 4 labs:
  - University-based Lyme lab
  - Commercial Lab
  - Two Lyme Specialty Labs

Is there a difference between labs in % Positives? – No, comparable performance



% of + Tests results among 37 Patients with Post-Treatment Lyme Syndrome

But discordance occurs – results vary depending on lab, especially with the ELISA



# of Discordant Pairs between 2 labs for 37 PTLS Patients –

ie, 1/4 to 1/3 of the time the ELISA is discordant

Uncommonly, when ELISA is negative the WB IgG can be positive (using CDC criteria)

	WB only +	IgG Specificity
Commercial lab:	1/37 (2.7%)	100%
University Lab:	3/37 (8.1%)	97.5%
Lyme Specialty Lab A:	2/37 (5.4%)	100%
Lyme Specialty Lab B:	2/37 (5.4%)	92.5%

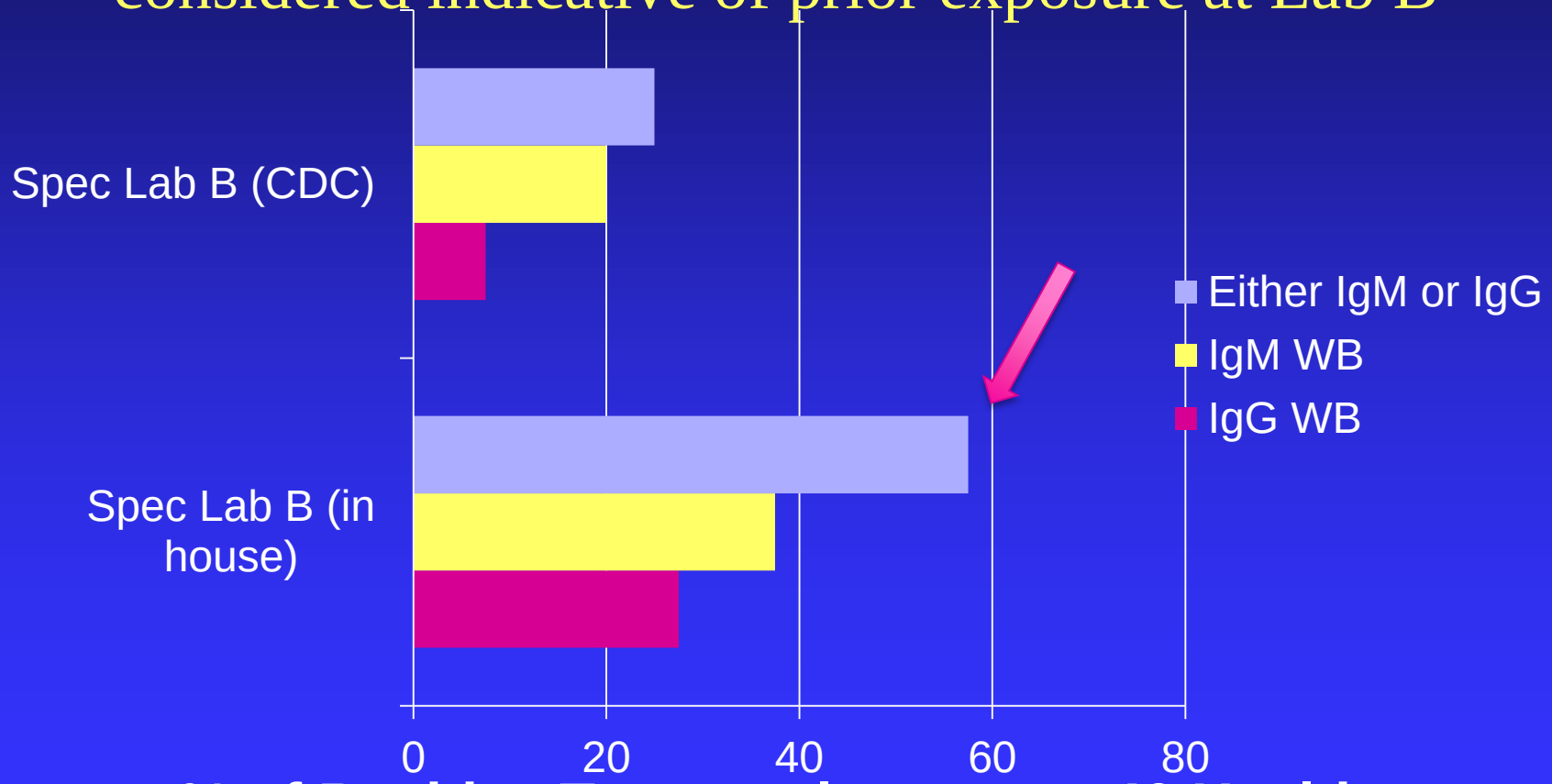
- Clinicians may consider requesting an IgG Western blot if the ELISA is negative and the clinical picture & exposure history are supportive.

For every test except the IgM WB when CDC criteria are used, % of False Positives (FP) are comparable across labs among 40 medically healthy controls

	ELISA (+/Ind)	IgG WB	2-Tier	C6 Peptide
University Lab	12.5%	2.5%	0%	--
Commercial Lab	7.5%	0%	0%	--
Specialty Lab A	2.5%	0%	0%	0%
Specialty Lab B	7.5%	7.5%	2.5%	0%

NOTE: The IgG WB, 2-Tier Assay, & the C6 Peptide ELISA performed well with few or no false positives

But # of False Positives among healthy controls increase when in-house WB criteria are used – up to 57% False Positive when either an IgM or IgG WB is considered indicative of prior exposure at Lab B



% of Positive Test results among 40 Healthy Controls

Summary

- Labs had comparable performance when CDC criteria were employed for ELISA & IgG WB
- "In-house" criteria for WB interpretation did not help, as risk of False Positives was high
- Discordance in results between labs suggest that using more than one lab for testing in highly suggestive cases may be a useful clinical practice if the labs have high specificity using the CDC criteria.

Limitations of the Study

- Were some of the medically healthy controls previously infected?
  - Possibly a small number
  - But these individuals felt healthy & had no prior diagnosis or treatment for Lyme disease.

  Had these same individuals been fatigued, a physician relying upon a positive IgM serologic test for diagnosis may have erroneously initiated treatment for Lyme.
- Antibody-based assays are problematic. We need a sensitive & specific marker of active infection.

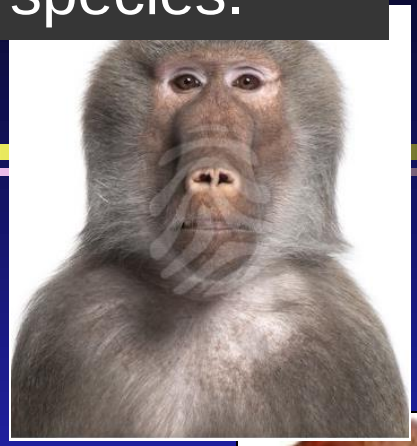
NEW RESEARCH DIRECTIONS

GOOD NEWS –
WHEN THE LYME RASH
LOOKS LIKE THIS.

Bad news - when it looks like a Spider bite. Serum turned positive for Lyme after 3 weeks. Also PCR+. Only 20% of Lyme rashes have the bull's eye appearance.

(Aucott & Schutzer, Emerging Infectious Disease, May 2013)

Bad News? Borrelia persist despite antibiotics. This has been shown in many species.



Often with minimal or No Disease

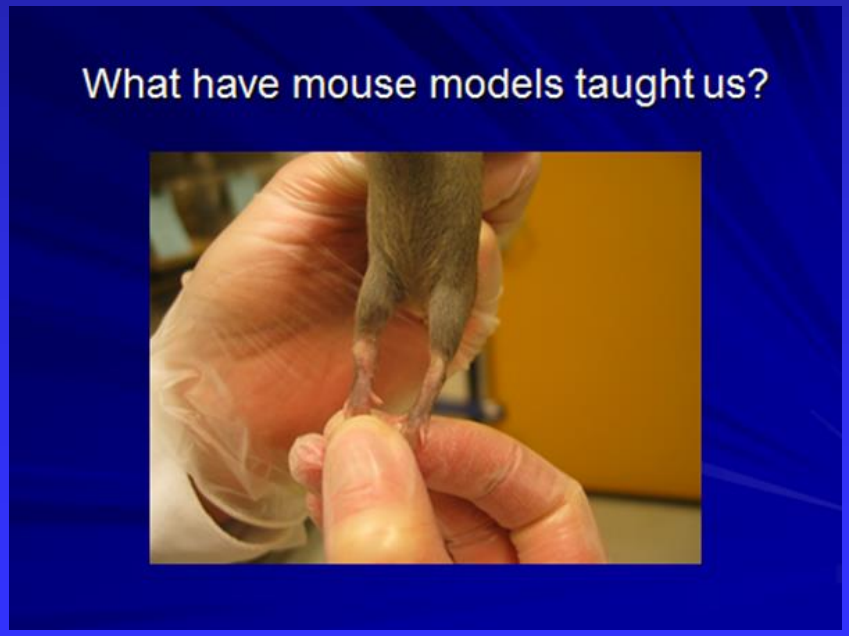
Slide courtesy of Stephen Barthold, UC Davis

Peer-reviewed articles demonstrating that Borrelia Persist – antibiotic tolerant

- Mouse Model:
  - Hodzic et al, 2008; UC Davis, California
  - Yrjänäinen, 2010; Univ Turku, Finland
  - Bockenstedt, 2002 & 2012; Yale, Connecticut
- Rhesus Macaques:
  - Embers et al, 2012; Tulane, Lousianna
- Dog Model:
  - Straubinger, 2000; Cornell, NY

Good news – there are special techniques to detect persistent spirochetes

- Borrelia spirochetes may not be detected unless the xenodiagnostic method is used



Human Xenodiagnosis Study

Larval Ticks (Marques, Hu, et al, CID, 2014)

Early results from the human xenodiagnostic study are intriguing

- 1 of 9 persons with PTLDS & C6+ tested positive , but only for DNA by PCR -- not by isolation of spirochete.
- Possible Explanations:
  - Persistent Infection
    - Fully viable, disease inducing
    - Reduced viability, disease inducing
    - Non-disease inducing
  - A new infection from an unseen tick bite

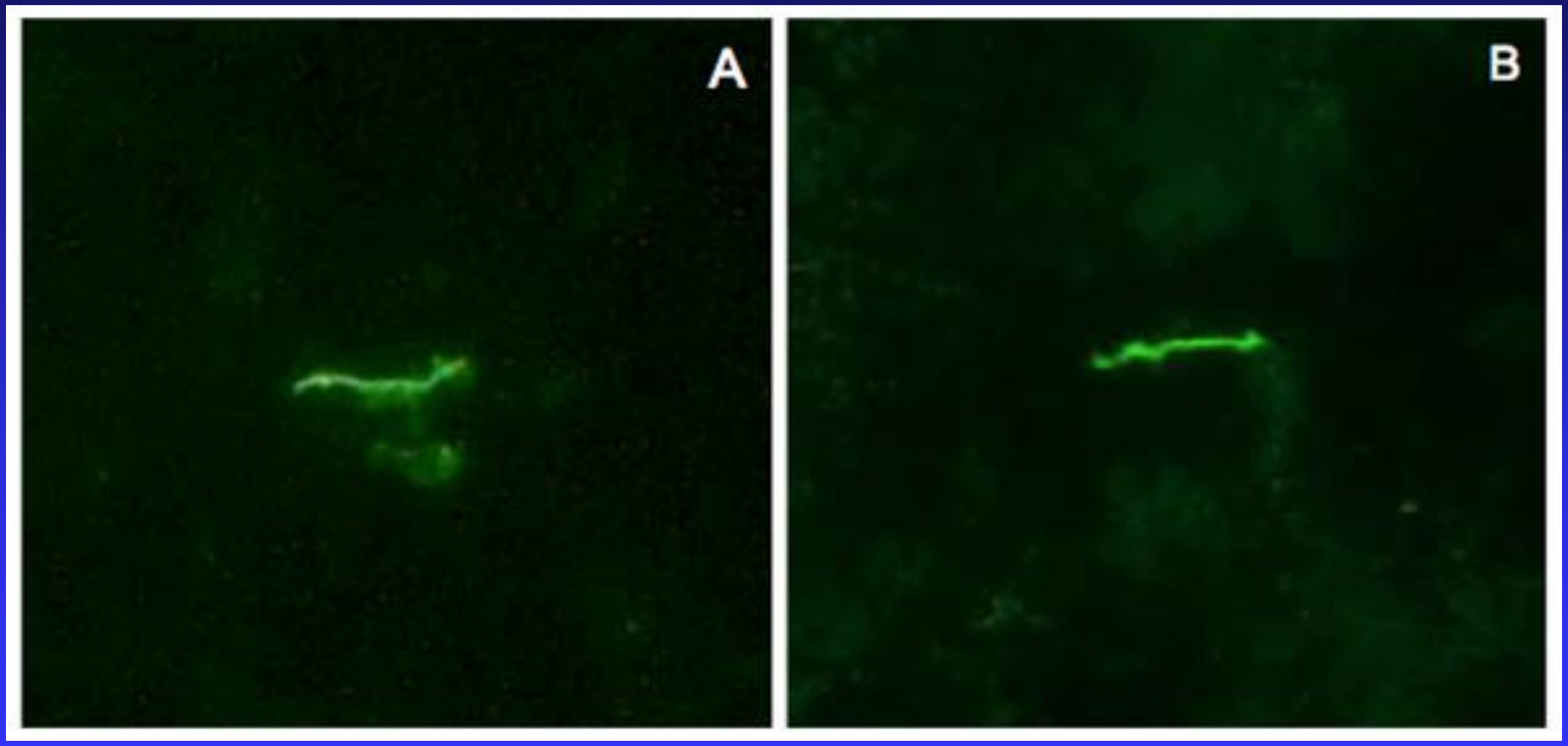
What is the long-term fate of persisting spirochetes? What do they do?

"Resurgence of Persisting Non-Cultivable Borrelia burgdorferi following Antibiotic Treatment in Mice"

Emir Hodzic, Denise Imai, Sunlian Feng, Stephen W. Barthold

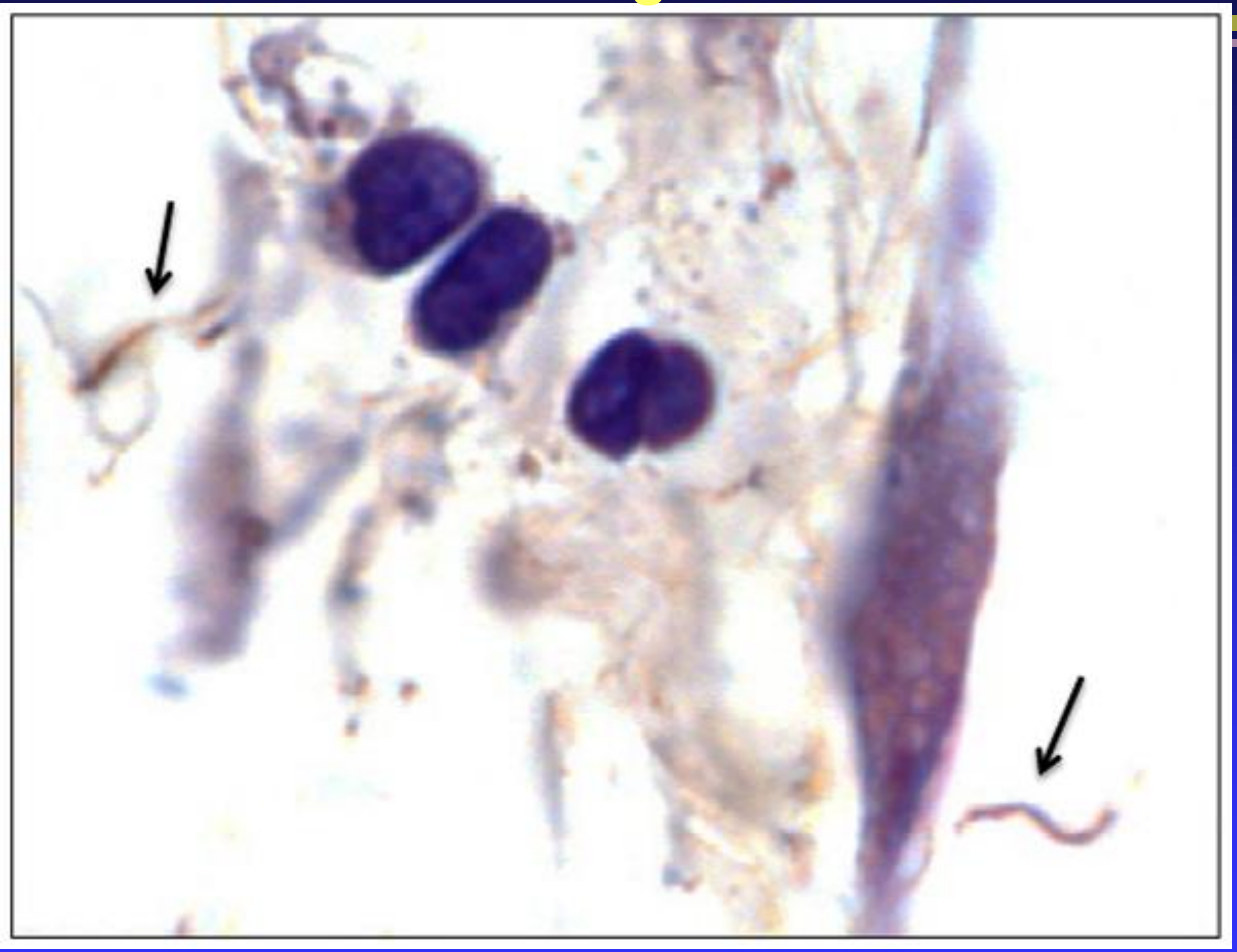
- PloS One January 23, 2014

Spirochetes can be found within xenodiagnostic ticks that fed upon saline-treated (A) - or antibiotic-treated (B) mice at 12 months after treatment.



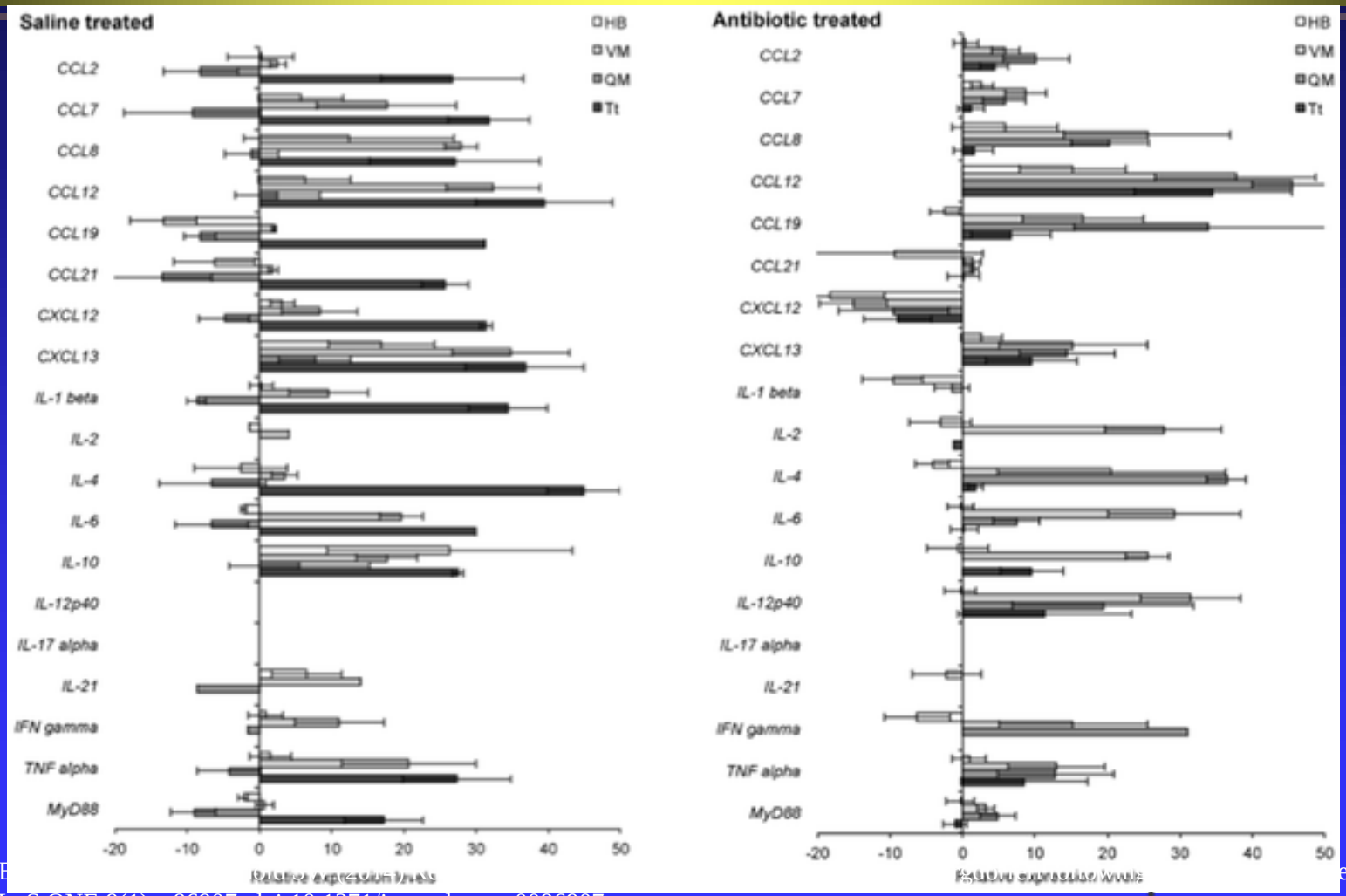
Hodzic E, Imai D, Feng S, Barthold SW (2014) Resurgence of Persisting Non-Cultivable Borrelia burgdorferi following Antibiotic Treatment in Mice. PLoS ONE 9(1): e86907. doi:10.1371/journal.pone.0086907
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0086907

Spirochetes can be visualized within tissue of a mouse at 12 months following antibiotic treatment.



Hodzic E, Imai D, Feng S, Barthold SW (2014) Resurgence of Persisting Non-Cultivable Borrelia burgdorferi following Antibiotic Treatment in Mice. PLoS ONE 9(1): e86907. doi:10.1371/journal.pone.0086907
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0086907

Persisting B. burgdorferi elicit host cytokine responses at 12 months following saline or antibiotic treatment.



Hodzic E ent in Mice. PLoS ONE 9(1): e86907. doi:10.1371/journal.pone.0086907
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0086907

What does this mouse research mean?

- Borrelia persist but often can’t be cultured
  - Documented by DNA with RNA transcription
  - Spirochetes visualized by immunoflouresence
  - Could resurgence at 12 months be due to declining antibody titers?
  - A “finger-print” of pro-inflammatory cytokine expression was seen – could these be related to persistent fatigue, pain, and cognitive problems?

Not all Tick-borne Infections cause Lyme disease

- Babesia
- Anaplasma
- Ehrlichia
- Powassan Virus
- Borrelia miyamotoi
- Tick-borne encephalitis virus

Research questions to pursue

- Borrelia persisters
  - Do the use of antibiotics shown to be effective against non-dividing stationery persisters (round forms) in vitro confer a better long term outcome in humans with later stage disease than doxycycline or ceftriaxone alone?
  - If so, what is the appropriate duration and what are the risks?

Research questions to pursue

- Immunomodulatory therapies
  - IV Ig –
    - what role does this play among PTLS patients with neuropathic symptoms suggestive of autoimmune neuropathy?
    - Pilot data from U.S. suggests that some patients with PTLS & neuropathic pain have an immune mediated neuropathy with abnormal nerve fiber density that is reversed by IVIg treatment.

Research Questions to Pursue

- Brain Imaging
  - Central Sensitization
    - Do post-Treatment LD patients with pain have abnormally activated brain circuits?
    - Can these neural circuits be down-regulated with pharmacologic or brain stimulation paradigms?
  - Central Inflammation
    - Is there evidence of microglial activation (inflammation) in the CNS of patients with PTLS using molecular imaging (PET)
    - Does this correlate with clinical symptoms & change with treatment?

Clinical question of some urgency – how to add psychiatric care to the treatment plan

- About 40% of patients with PTLS have psychiatric symptoms that persist
  - Some avoid psychiatric care
- Results:
  - Persistent Despair, depression, anxiety
  - Sleep disturbance exacerbating fatigue
  - Cognitive impairment due to depression
  - Interpersonal distress – loss of support
  - Suicidal thoughts in about 20% of PTLS pts

3-25
WAIT! THE TICKS! WE FORGOT THE TICKS!
WWW.CLOSETOHOME.COM E: CLOSETOHOME@MAC.COM
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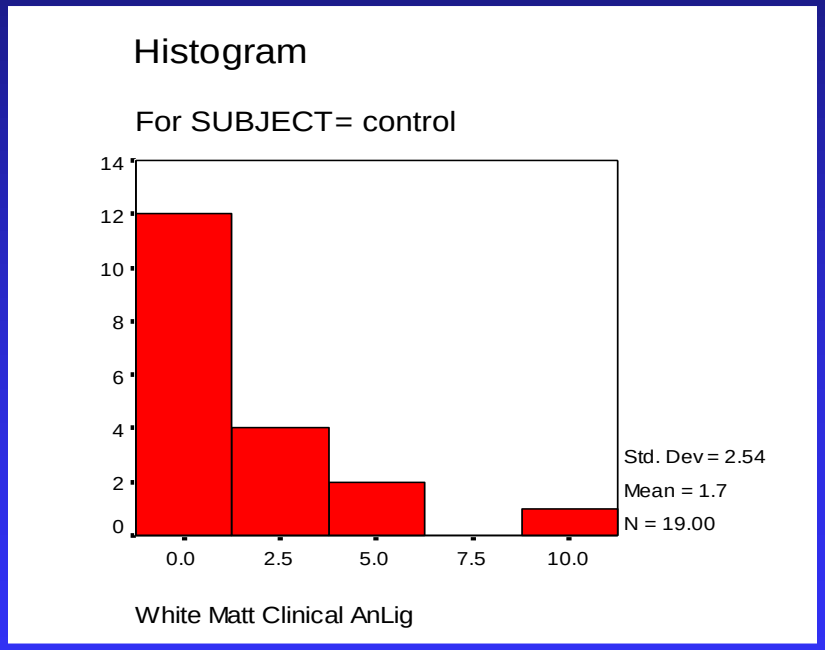
McPHERSON

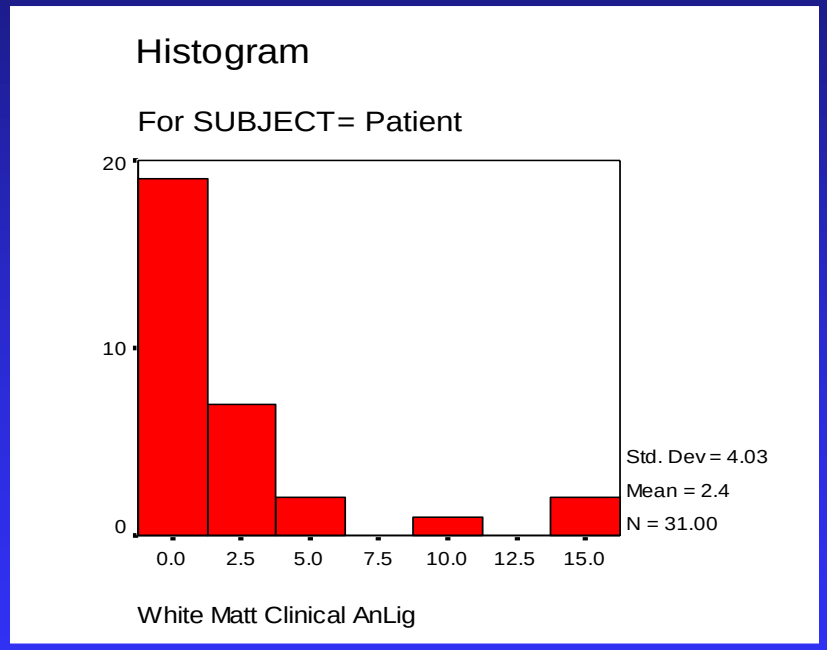
Prevention is key

Deep White Matter Hyperintensities in Brain of Controls vs Patients

DWMH in Controls



DWMH in Patients

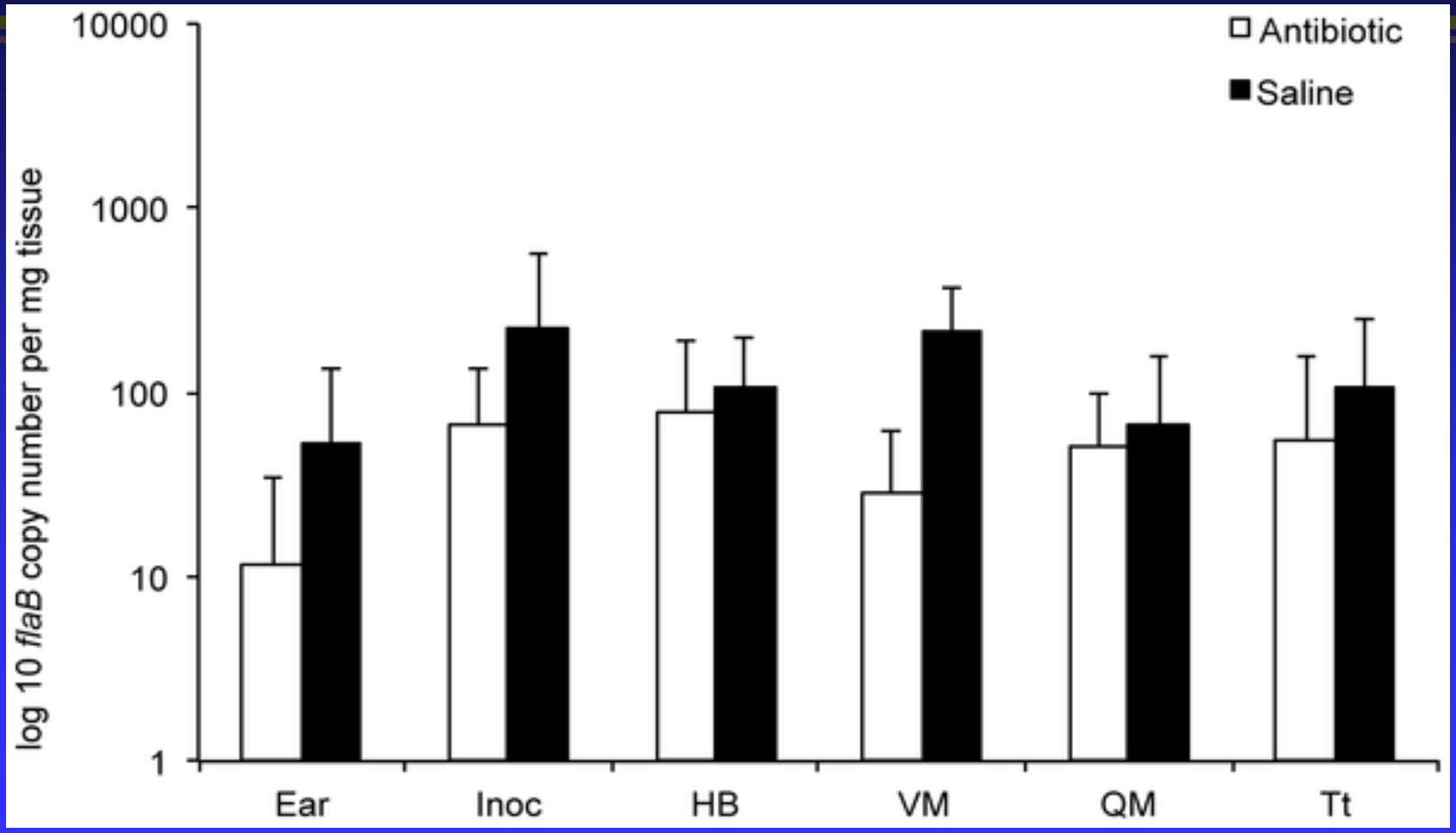


Note: No diff. in WMHI Score between 2 groups

What about new papers on morphology, Bb persisters, & differential treatment needs?

- Do the in vitro findings of “persisters” requiring different antibiotics for eradication have clinical relevance in vivo in humans?
- Would alternative treatments directed at these variants result in long-term improved clinical outcomes?

Borrelia burgdorferi levels (by PCR) resurge in tissues at 12 months after antibiotic treatment.



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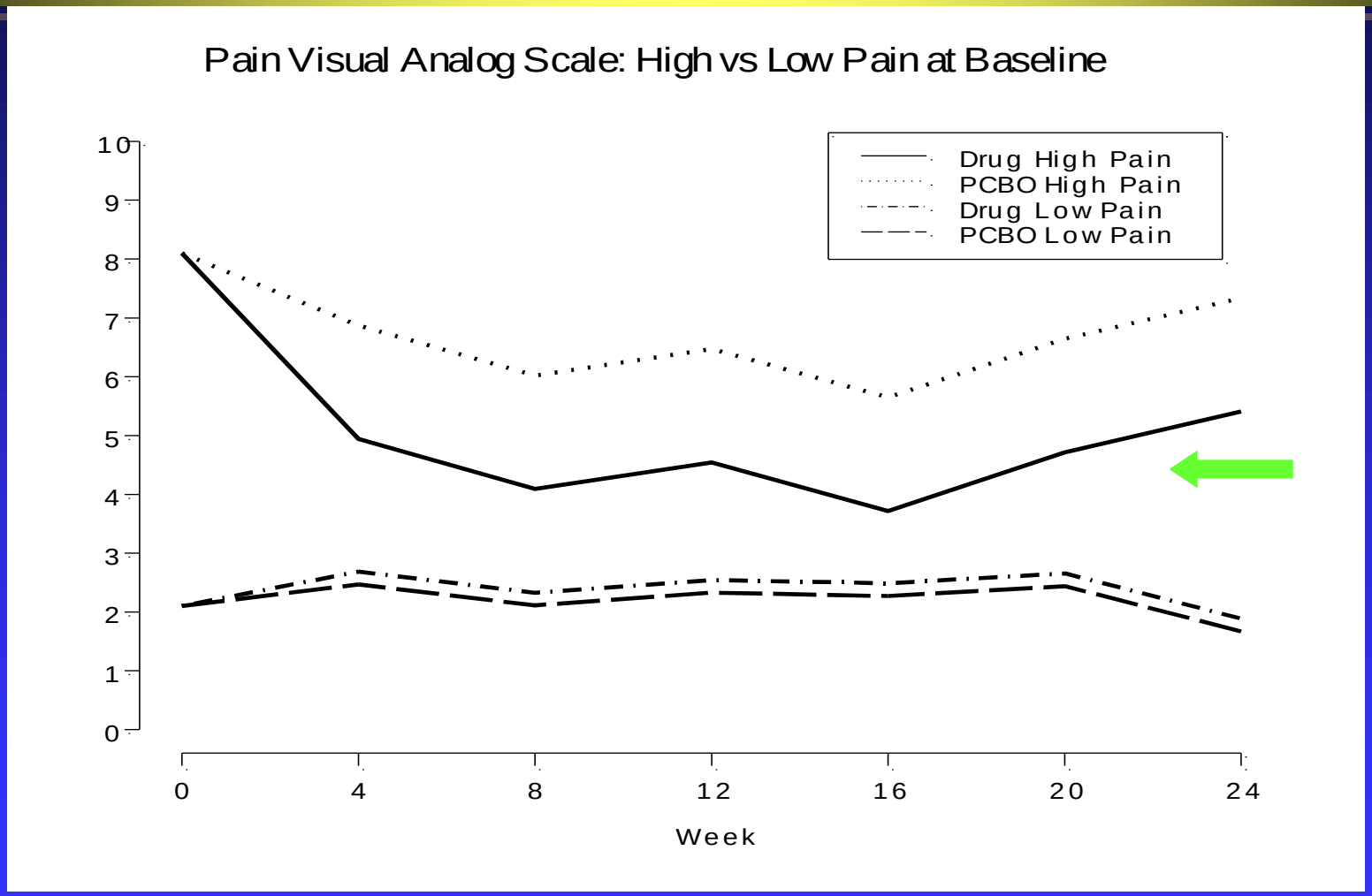
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0086907

Decline in Pain is Sustained over Time among more impaired given antibiotic

(Model-based Illustration from Lyme Encephalopathy Study, Fallon 2006)



MRI: Treatment Results

- Change in Hyperintensities was not different between patients on drug vs placebo

Proteomic: Experimental design

~20 ml of CSF

pooled

Immunoaffinity depletion

Tryptic Digestion (Flow through)

30 SCX fractionation

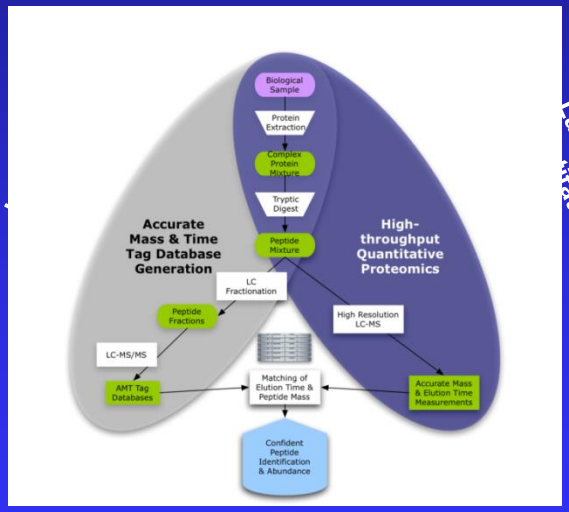
Proteome profiling by NanoLC-MS/MS

Tryptic Digest (Bound fraction)

Proteome profiling by NanoLC-MS/MS

In depth proteome analysis

~3K proteins



Label free

individuals

Tryptic digestion

Protein quantitation by NanoLC-MS

Individual patient sample analysis

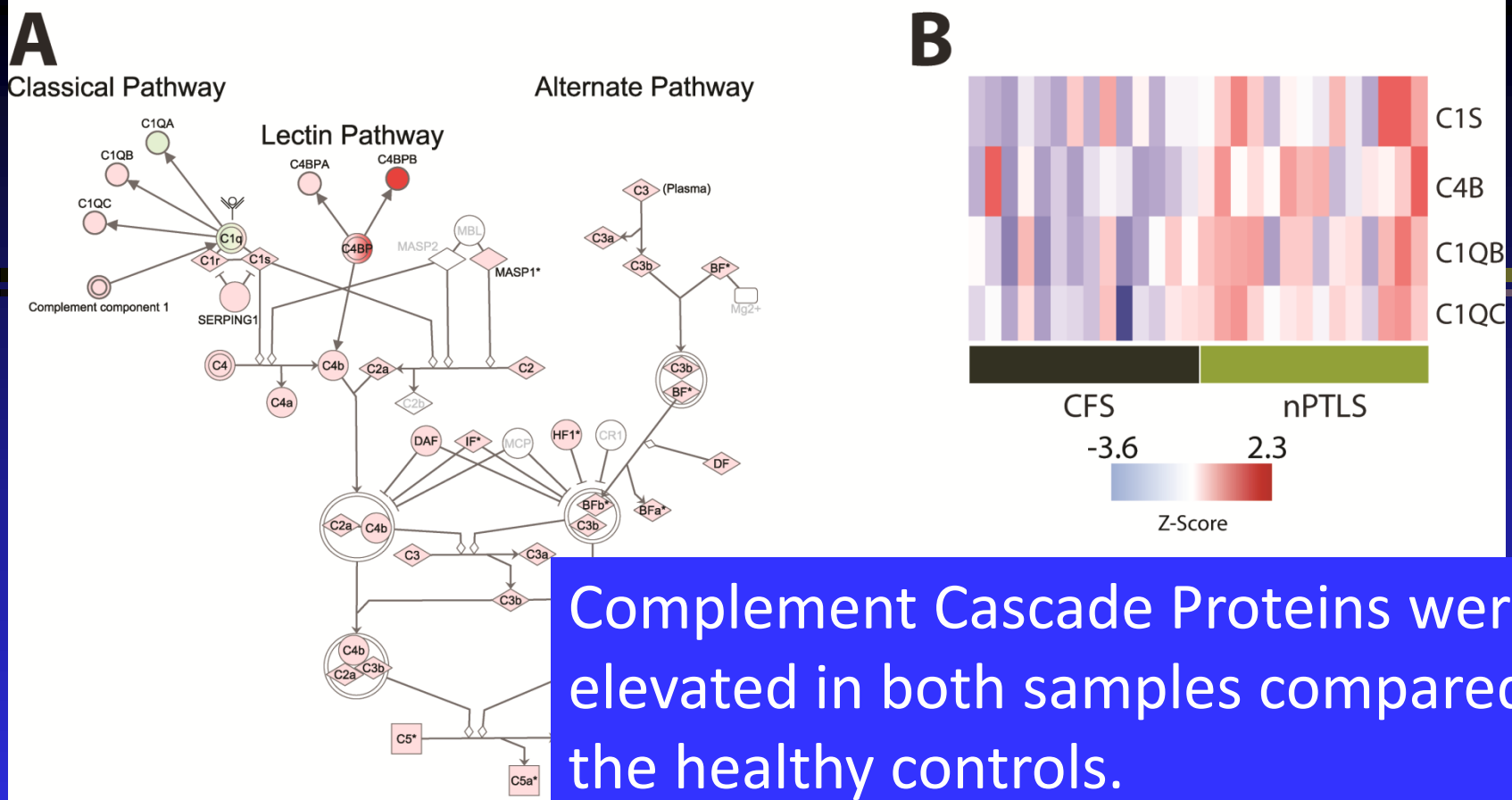
2 replicates per sample

~400 proteins per patient sample

Angel, PNNL

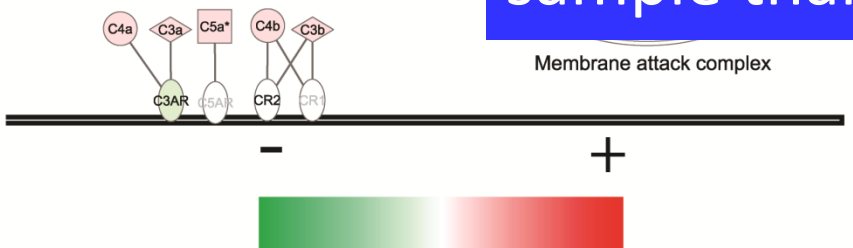
Functional Imaging: Are there global differences at rest bet pts & ctrls?

- No.
- Global Flow & Metabolism at Rest:
  - No differences in resting blood flow
  - No differences in resting metabolism
  - No differences also within gray matter regions or white matter regions



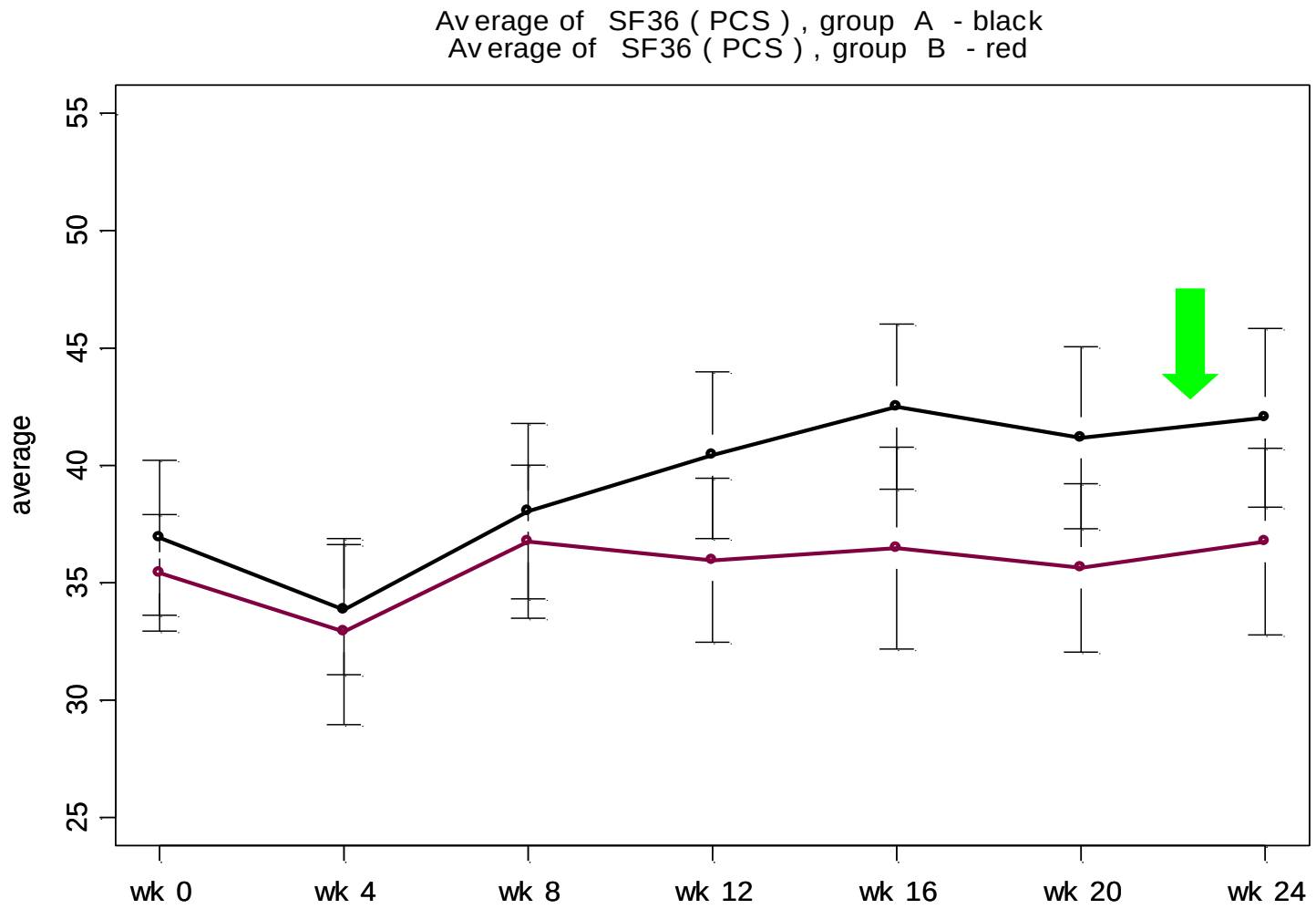
Complement Cascade Proteins were elevated in both samples compared to the healthy controls.

A greater abundance of these 4 proteins above were found in the nPTLS sample than the CFS sample.



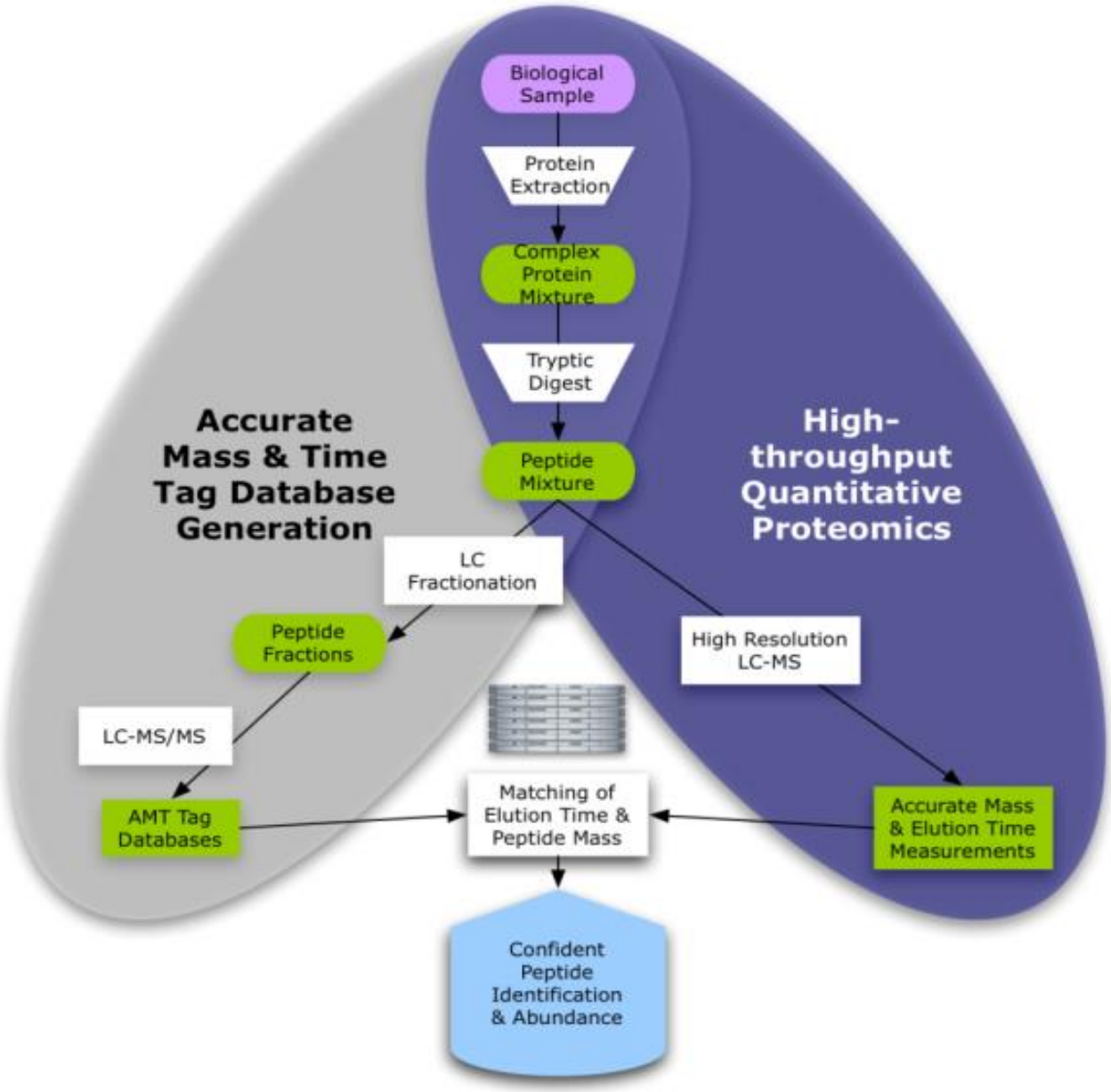
Improvement in Physical Functioning is greater in antibiotic group & sustained over time

(Data from Lyme Encephalopathy Study, Fallon et al., 2007)

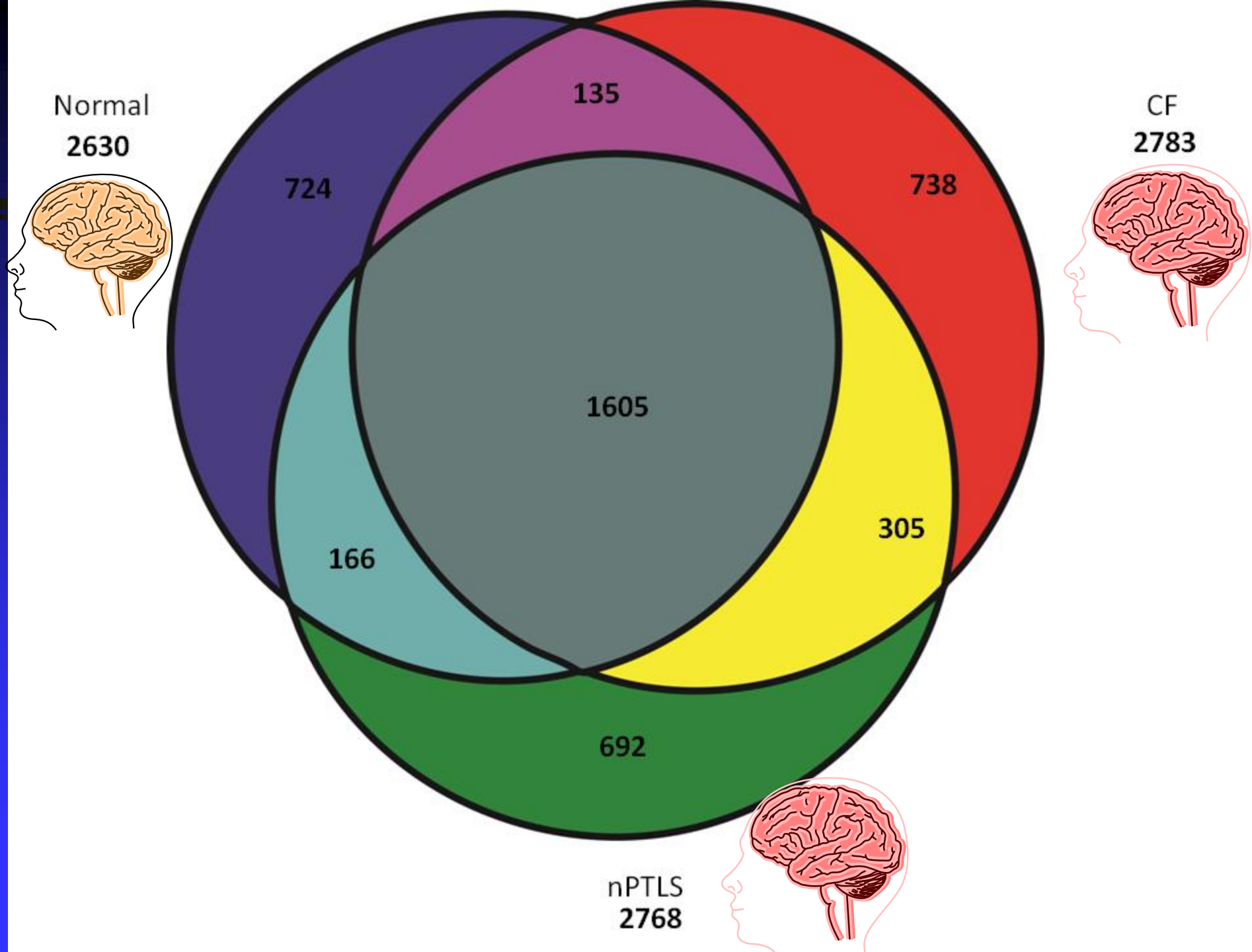


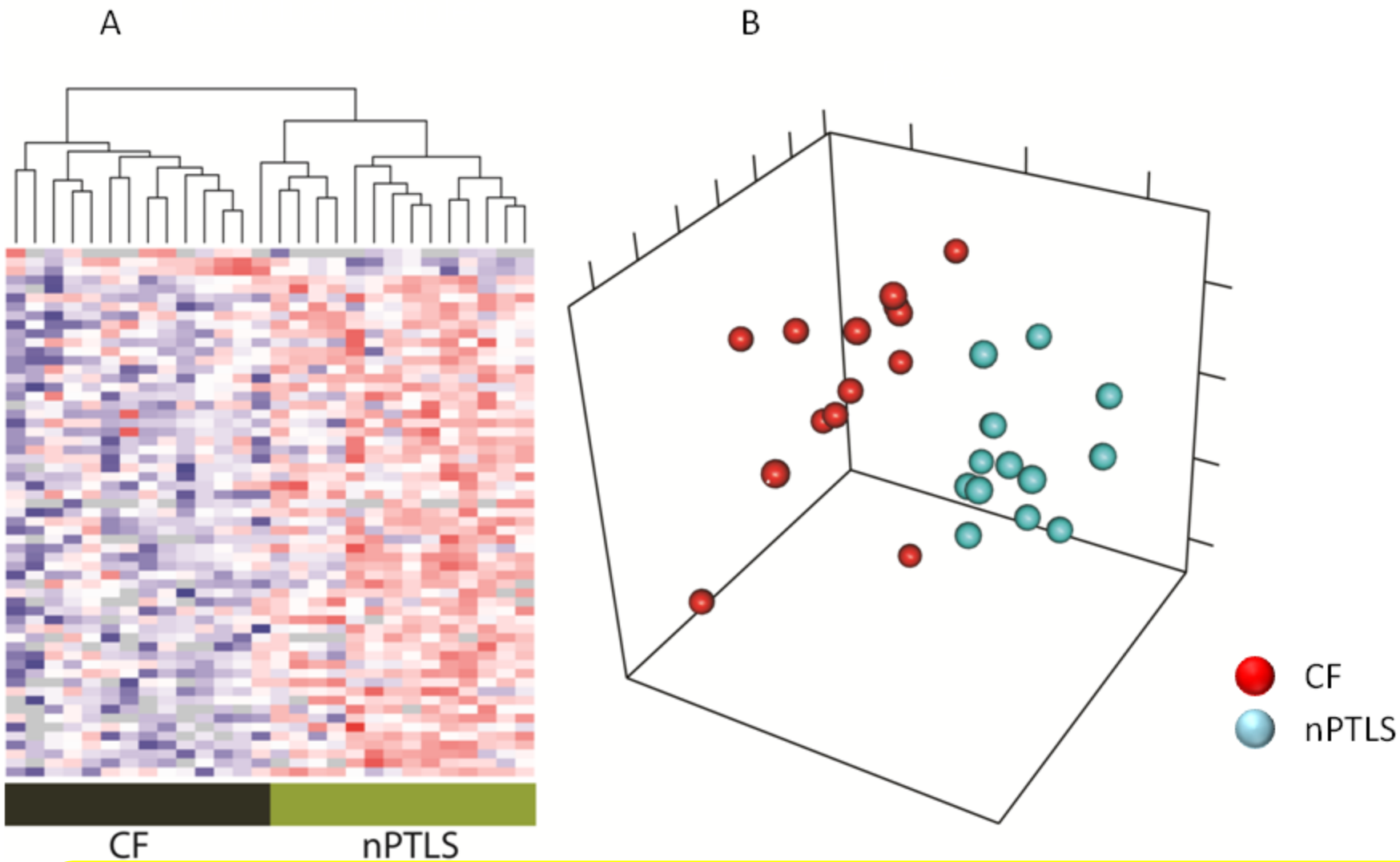
Limitations of Study

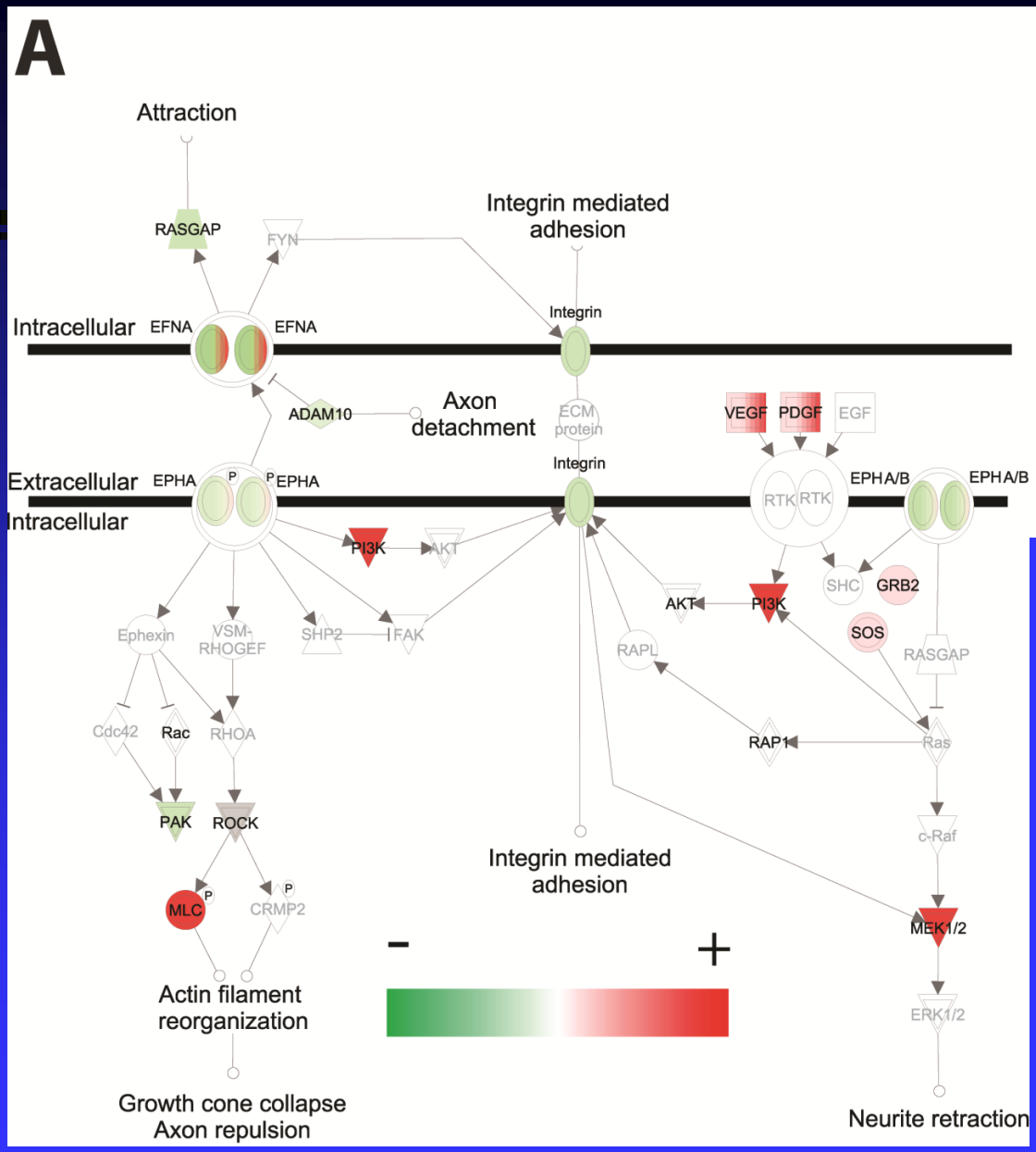
- Small sample size
- Limited generalizability secondary to narrow entry criteria
  - Would seronegative pts who have persistent symptoms benefit?
  - Would pts who have had less prior antibiotic treatment do better?
- Can we do better identifying biomarkers to help guide treatment selection?

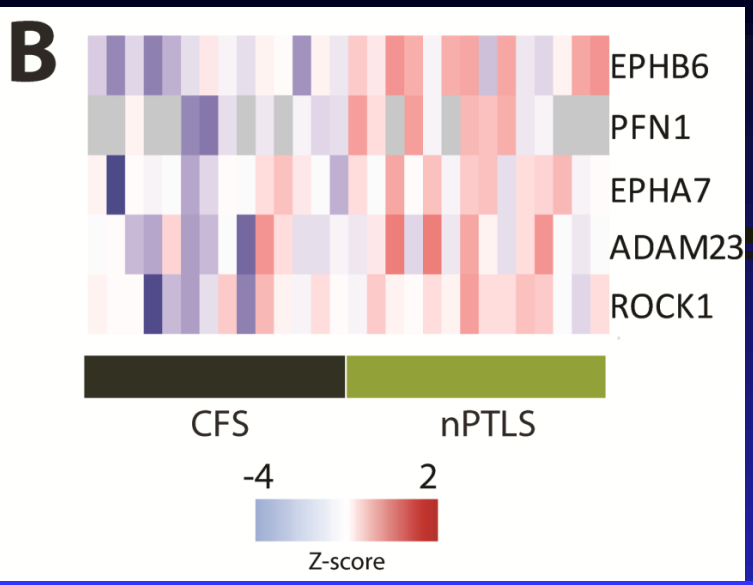

Biological Sample
Protein Extraction
Complex Protein Mixture
Tryptic Digest
Peptide Mixture
Accurate Mass & Time Tag Database Generation
High-throughput Quantitative Proteomics
LC Fractionation
Peptide Fractions
LC-MS/MS
AMT Tag Databases
High Resolution LC-MS
Accurate Mass & Elution Time Measurements
Matching of Elution Time & Peptide Mass
Confident Peptide Identification & Abundance

NL


Normal
2630
CF
2783
nPTLS
2768
724
135
738
1605
166
305
692


A
B
CF
nPTLS
CF
nPTLS



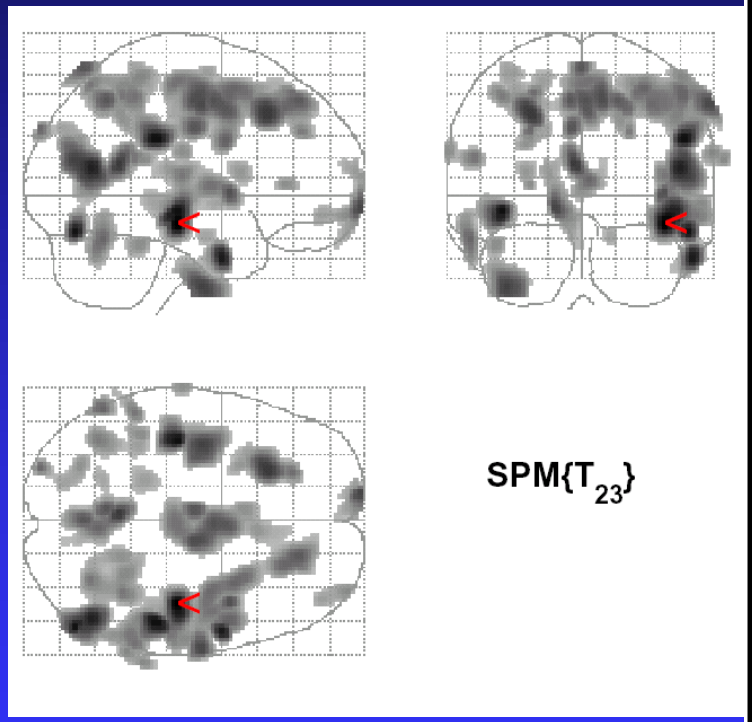


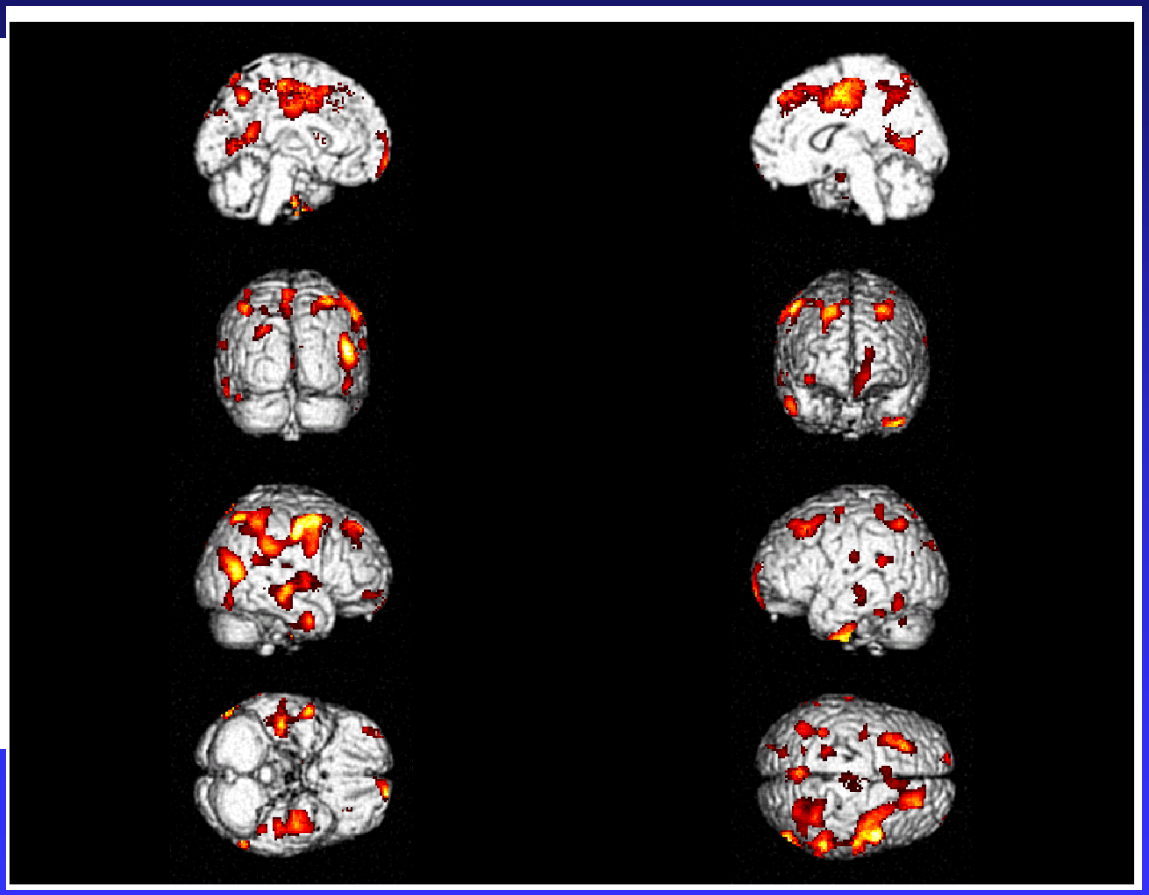
Neurologic function proteins were decreased in nPTLS and CFS compared to controls. These proteins effect dynamic changes in CNS cellular architecture, such as axon,neurite, and dendritic spine growth and organization.

Heightened Immune Response

Post-treatment patients have more blood flow & metabolic deficits than matched controls

(Fallon 2009)





Parahippocampal Gyrus
Inferior Parietal Lobe
Insula, Cingulate, Hippocampus

Summary of Background for Lyme Encephalopathy Study

- Symptoms may persist after standard courses of treatment for neurologic Lyme disease
- Functional Imaging SPECT scans show patchy hypoperfusion (similar to vasculitis): multiple areas of decreased blood flow – partially reversible. (Logigian et al). Objective measures of deficit are helpful.
- Structural imaging MR Scans show small white matter hyper-intensities
- Uncontrolled data suggested that re-treatment with IV Antibiotics may be helpful (Fallon 1999)

Columbia University Study of Chronic Lyme Encephalopathy

(Fallon et al, 2007)

Baseline	Week 12	Week 24
PET/MRI	PET/MRI	PET/MRI
Neuropsych tests	Neuropsych	Neuopsych
Self-report Neuro & Rheum exam	Self-report Rheum Exam	Self-report Rheum Exam
LP Blood work	Blood Work	Blood work
Start 10 wks IV Placebo or IV CFTX	No Antibiotics From wk 10-24	End of Double-Blind

Positron Emission Tomography

- **Cerebral Blood flow (O-15): CBF**
  - Condition #1: at rest at room air
  - Condition #2: With snorkel at room air
  - Condition #3: With vasodilation challenge (room air enhanced with 5% CO2).
- **Cerebral Metabolism (FDG): CMR**
  - **Condition #4: at rest**
- **Imaging Time Points:**
  - Patients & Controls at baseline, only Patients at weeks 12 and 24
- **Analysis: SPM (Statistical Parametric Mapping)**
  - Subtraction images patients vs controls
  - All significant at cluster size of 25 voxels & p<,01

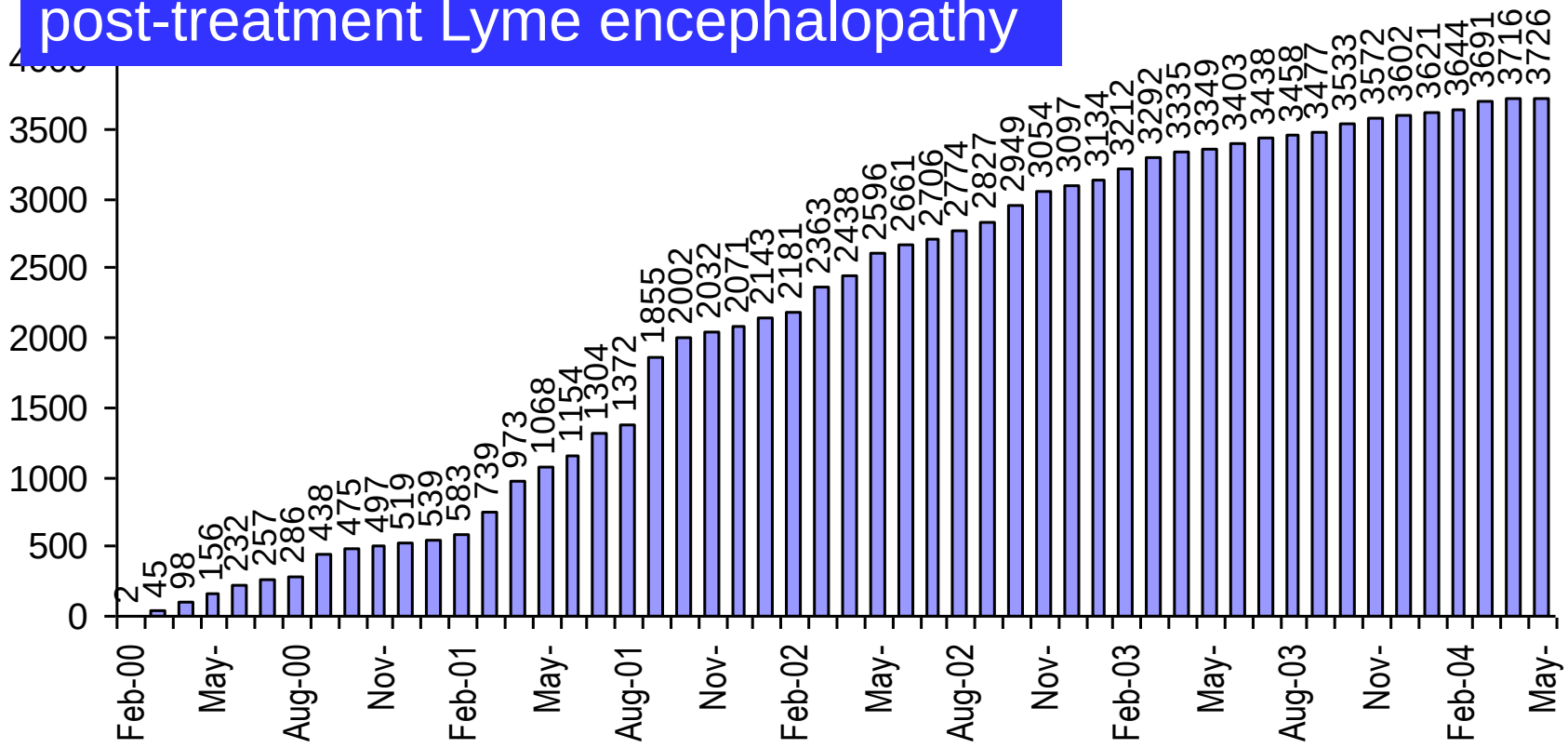
Other Clinical Measures

Measures

- **Functional Status Indices** (SF36)
- **Physical**: Fatigue (FSS), Pain (McGill)
- **Psychopathology** (BDI, Zung, SCL)
- **Rheumatologist's Joint Exam**

Patient Screenings for Encephalopathy Study

NB: Large number of patients with persistent symptoms & only 1% met our conservative diagnostic criteria for post-treatment Lyme encephalopathy



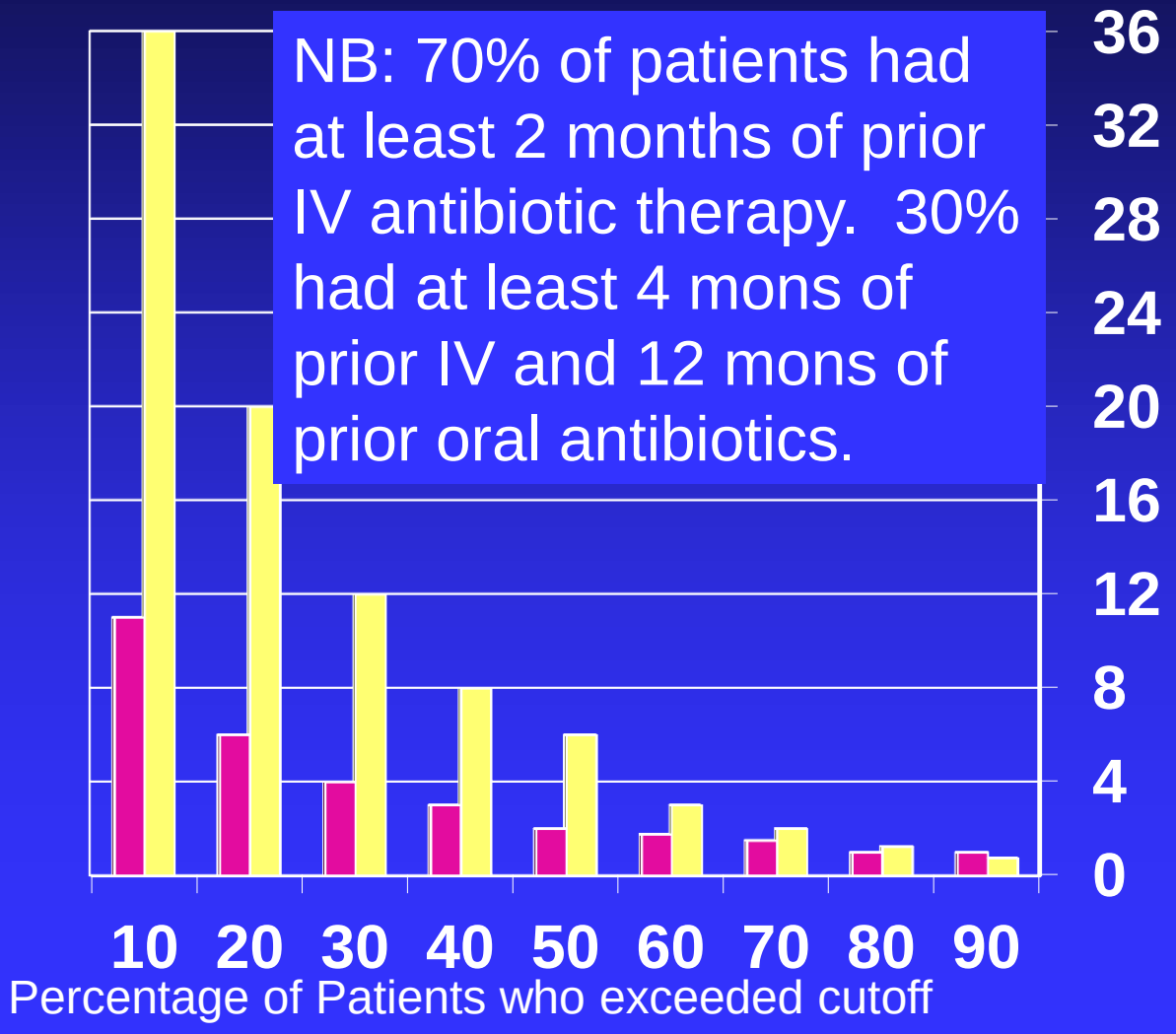
What was the cognitive profile among evaluated Patients with Post-Treatment Lyme Disease & cognitive complaints? (Keilp et al, 2006)

- 81 CDC-criteria pts vs 39 healthy controls
- Index Scores:
  - WAIS-III: Processing Speed slowed in LD (p<.01)
    - Subtests: Digit Symbol and Symbol Search
  - WMS-III: Memory is affected in CLD (Auditory immed & delayed)
    - Subtests: Logical story memory (auditory) & family pictures (visual mem). Place demands on context & need to organize material
- Discriminant Analysis:
  - **Logical memory subtest** correctly classified 73.7% of Lyme and 75% of controls.

Disclosures & Grant Support

- Disclosures:
  - Roche Pharmaceuticals (supplied Ceftriaxone free of charge for NIH Study)
  - Immunectics (conducted C6 ELISA assays free of charge)
- Grant Support:
  - Primary: NINDS/NIH
  - Secondary studies:
    - Lyme Disease Association
    - Lyme Research Alliance, Inc.
    - National Research Fund for Tick Borne Diseases

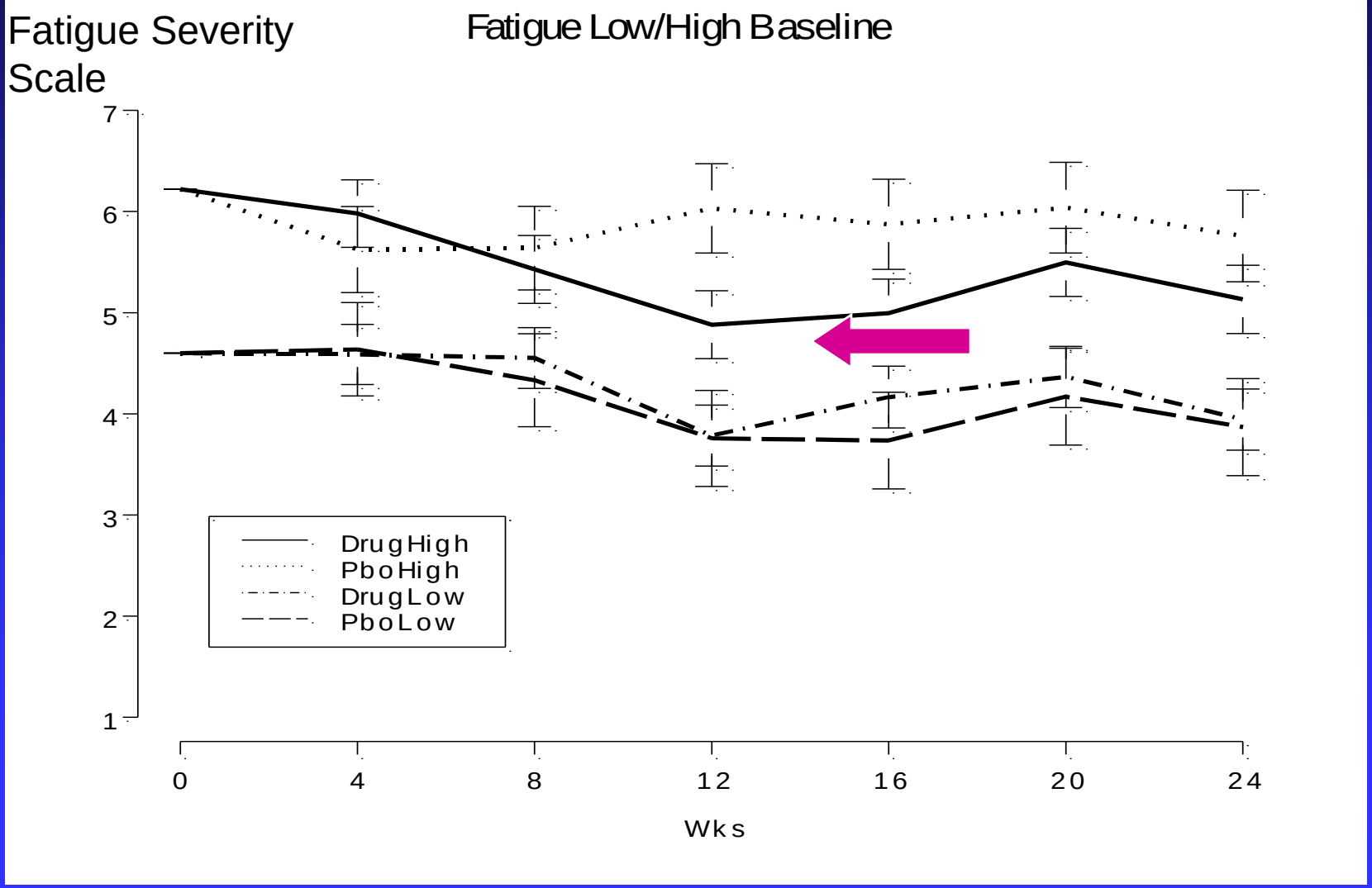
Total Amount of Prior Antibiotic Therapy among Patients with Chronic Lyme Disease: common to receive longer & repeated courses of therapy



NB: 70% of patients had at least 2 months of prior IV antibiotic therapy. 30% had at least 4 mons of prior IV and 12 mons of prior oral antibiotics.

Cutoff for Minimum # Months of Total Prior Antibiotic Treatment

Decline in Fatigue is significant at week 12 among the more impaired given antibiotic



Adverse Events leading to hospitalization or drug discontinuation

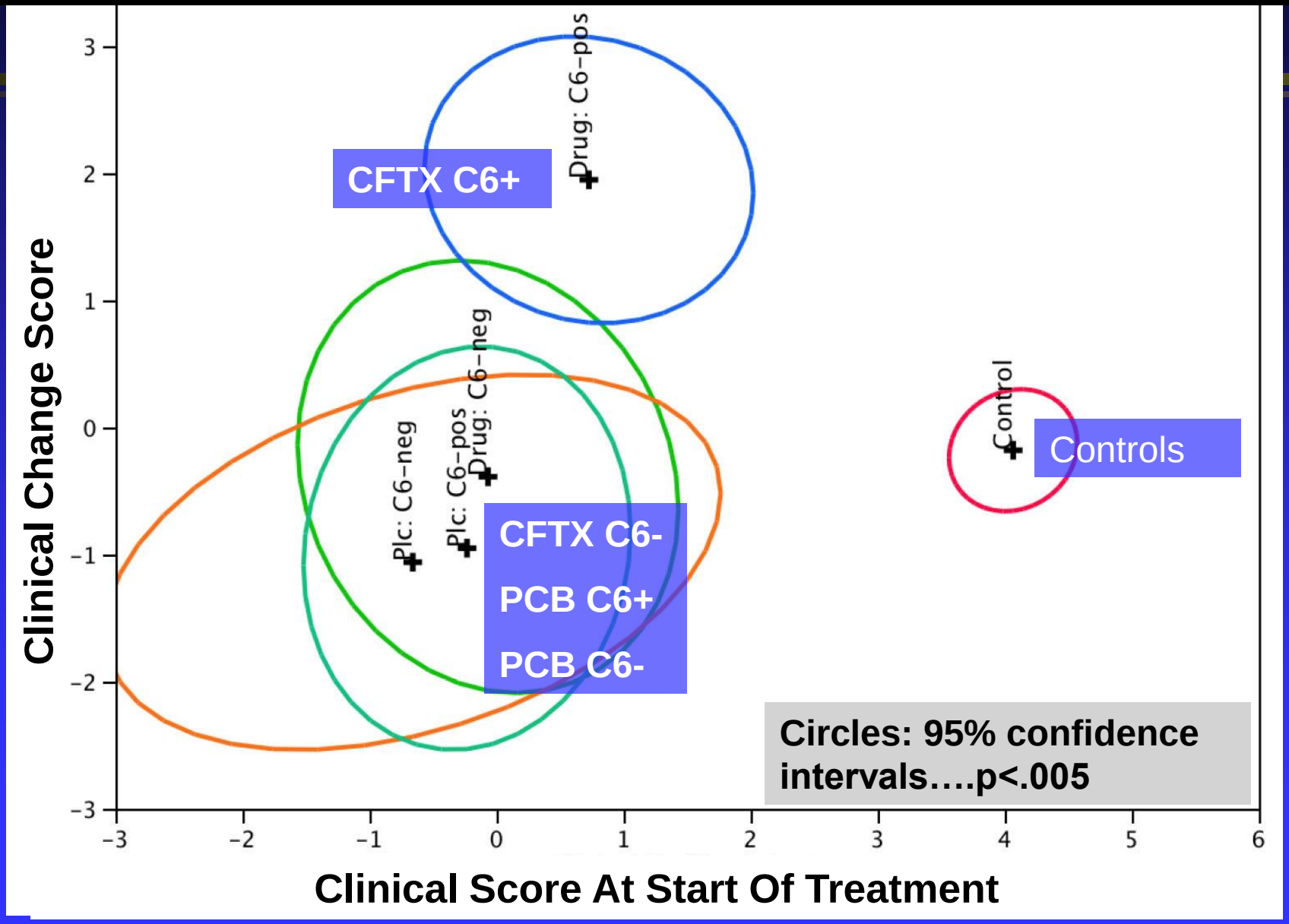
- 7 patients had treatment-related AE that led to drug-discontinuation or hospitalization
  - Distribution:
    - 1/14 (7.1%) on placebo vs 6/23 (26.1%) on drug
  - PICC line:
    - Thrombus: 2
    - Line Infection: 1
  - Drug-related:
    - Allergic reaction (hemolysis): 1
    - Allergic reaction (skin rash): 2
    - Biliary stones req cholecystectomy:

Predictors of Response?

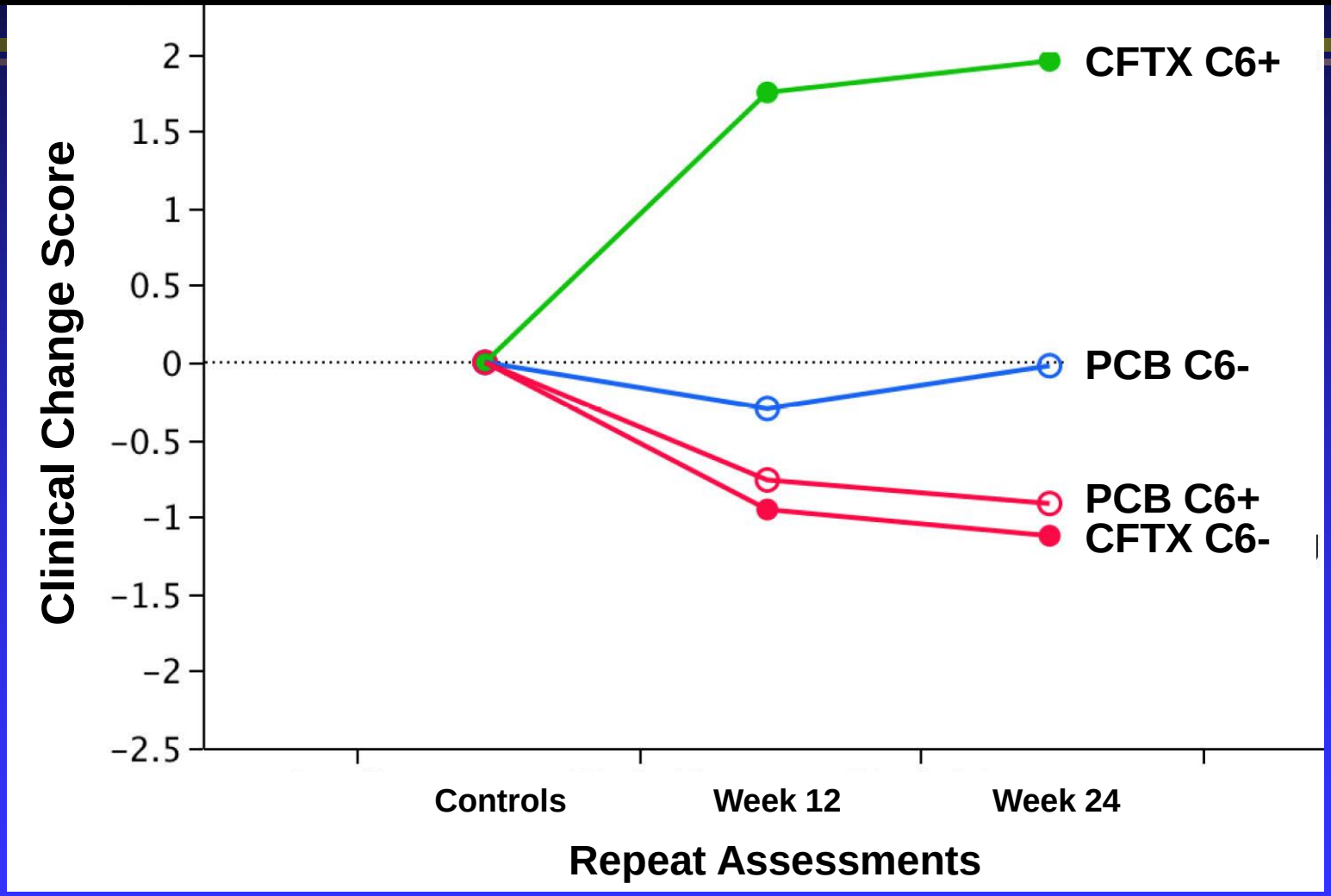
Does a positive C6 Ab Index have any clinical significance or any specific functional brain effects?

James Moeller, PhD

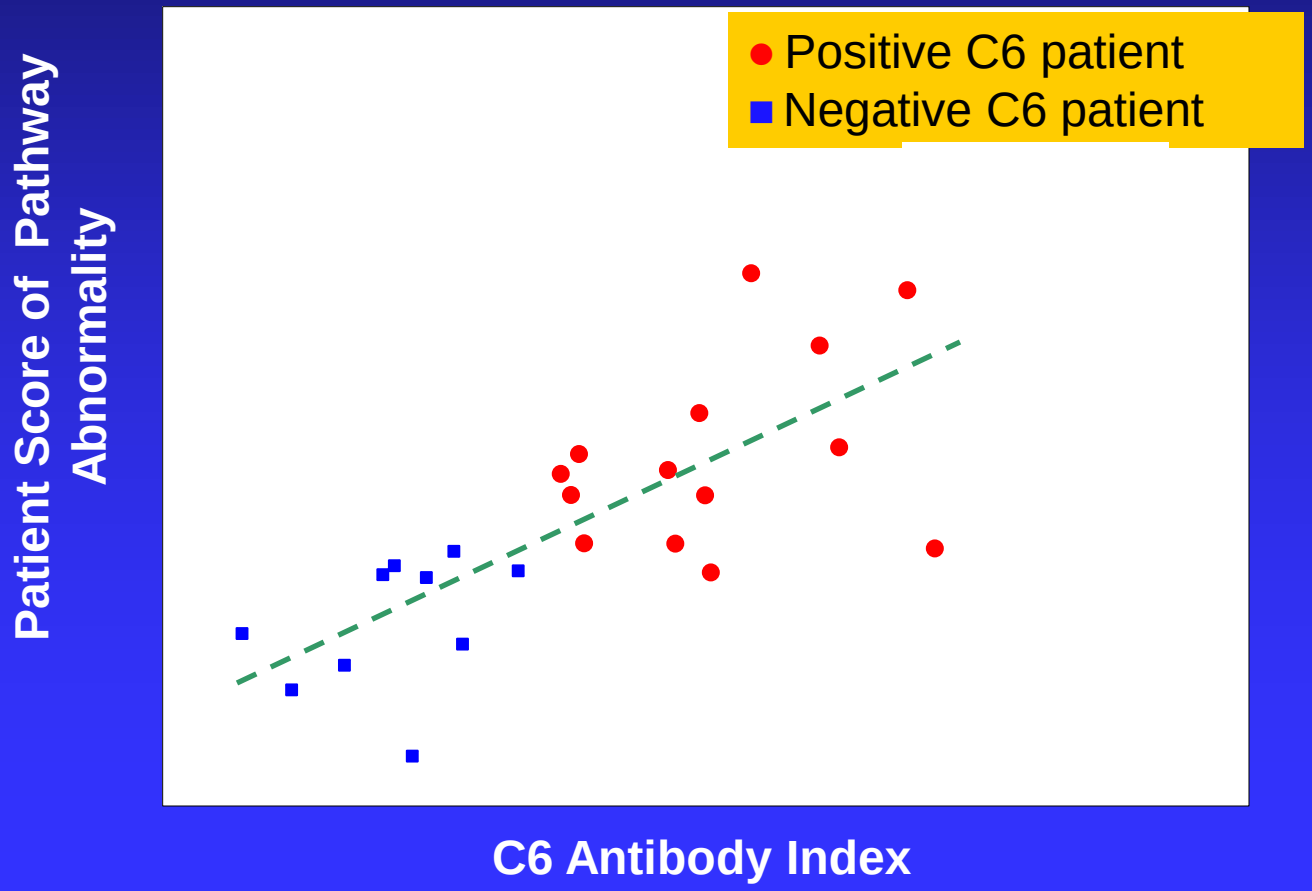
Only C6 AI Positive Patients Show Clear Clinical Reductions In Fatigue and Improvements in Physical Functioning After 10 Weeks of CFTX



Clinical reductions in fatigue & improvement in physical functioning are sustained to week 24

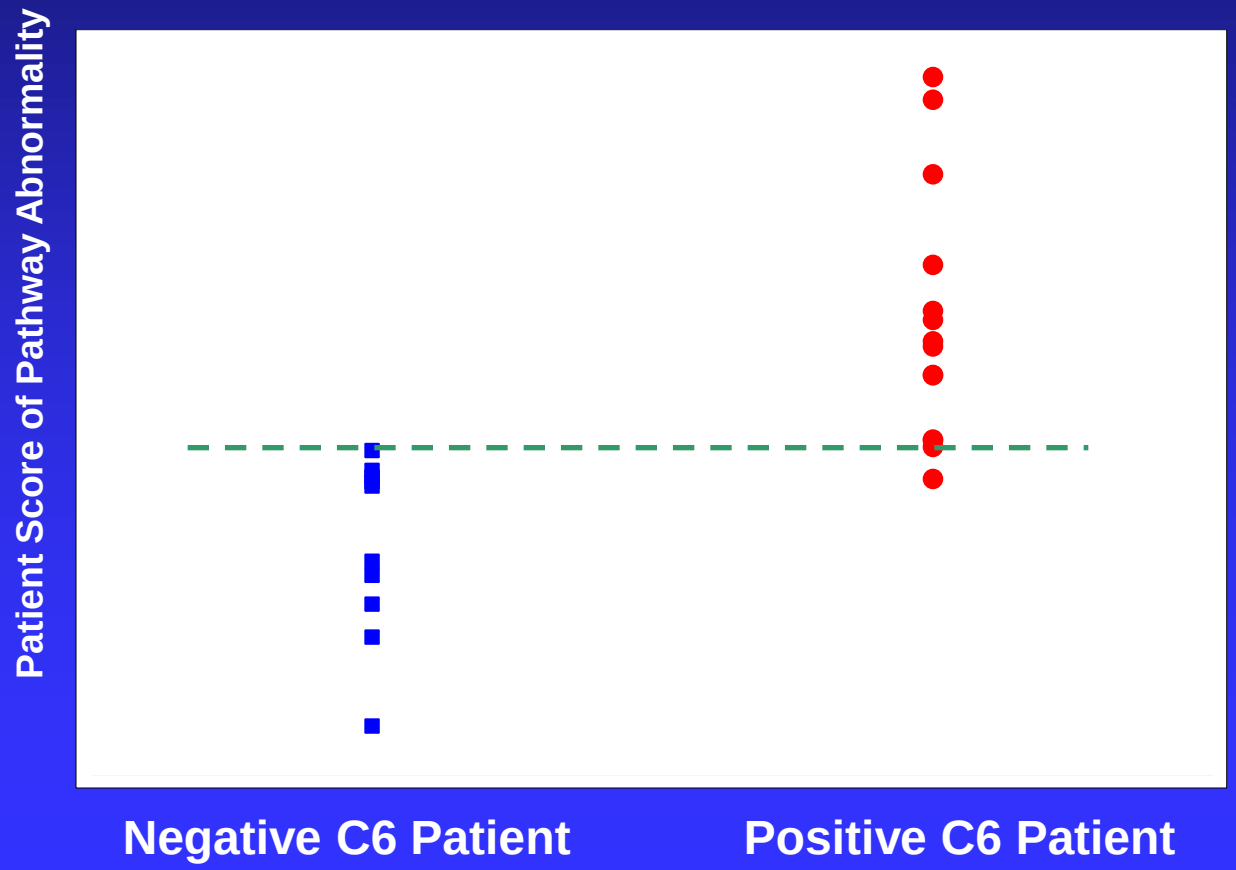


Using PET FDG, a brain pathway of abnormal activity was identified among all patients – a single score – and it was strongly correlated with C6 Antibody Index



- The PET FDG Brain MAP “Lyme Signature” score appears able to discriminate those who are C6 AI positive from those who are C6 AI negative.

Moeller et al, 2008, in prep.



Concluding Questions

- Can these findings be replicated?
  - C6 Index is more sensitive than WCS Index
  - C6 Index is correlated with a brain map
  - C6 Index positivity may identify patients who will benefit from repeated antibiotic treatment
- Why are those specific brain areas expressed to a greater extent as magnitude of the C6 index increases?

Recommendations for working with the patient with persistent symptoms who has been on antibiotics for prolonged periods

- Elicit the underlying fears
- Educate
  - Both antibiotics & healthy immune system help to fight

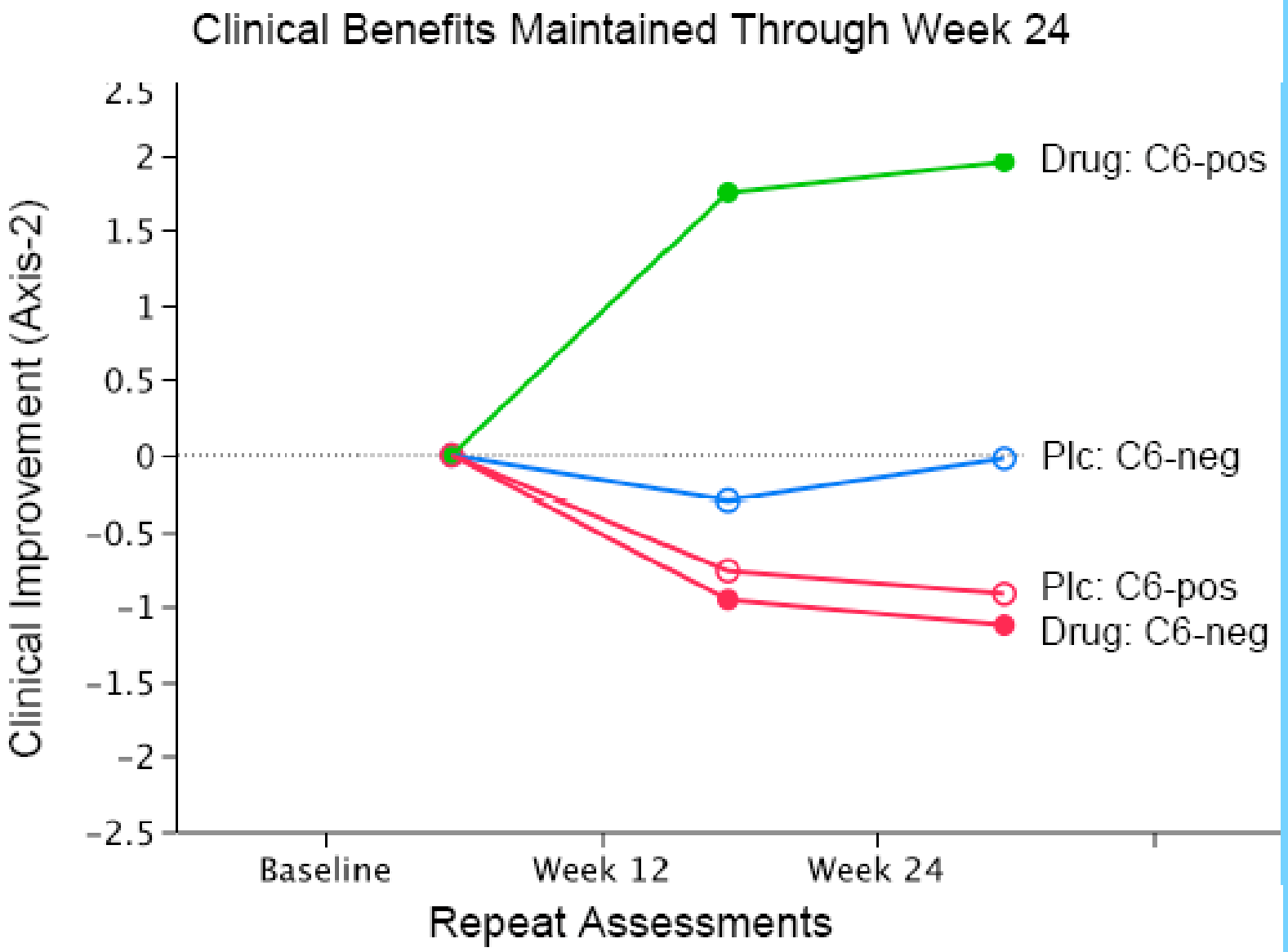
Additional Assessments

- Physical Exam: joints, neurologic
- Blood
  - Lyme: C6 Peptide ELISA, WCS ELISA, WB
  - Other infections (Babesia, Anaplasma)
  - Immune/inflammatory markers
    - CRP, ESR, ANA
    - Natural Kill cell CD57
    - Anti-neuronal antibodies
    - Interferon alpha activity

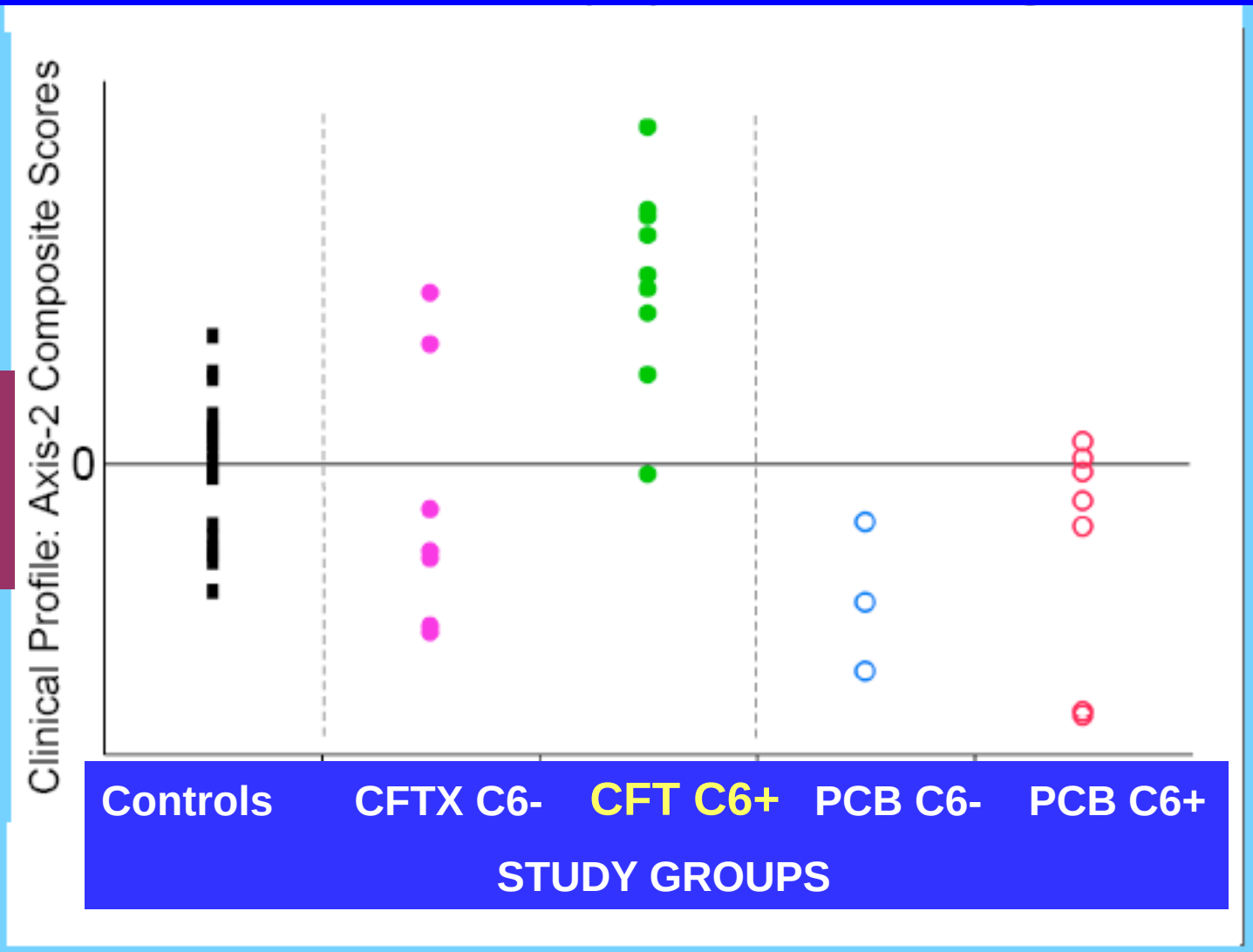
CSF Assessments

- WBC, Protein, Lyme Index
- Lyme PCR and Culture
- ELISA: Whole Cell Sonicate & C6 Peptide

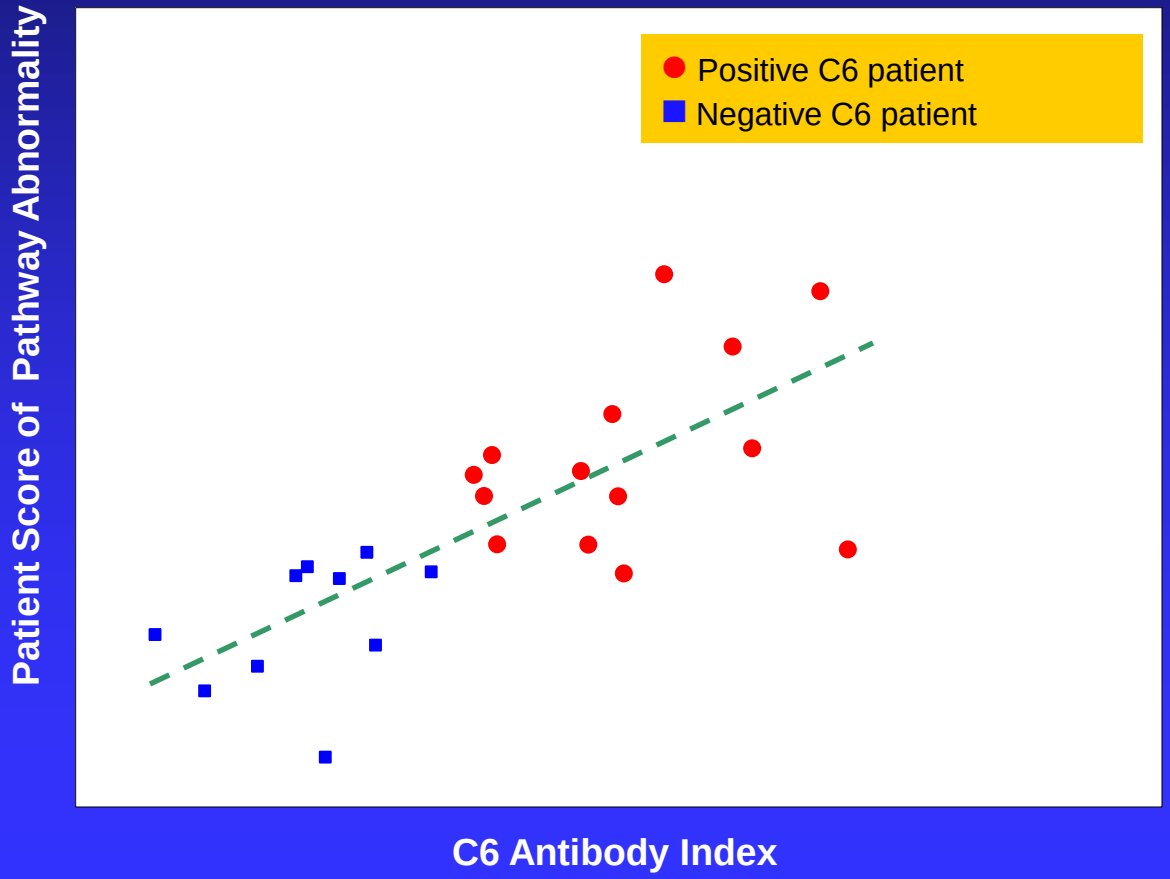
- Later using a sub-sample of 25 CSF samples:
  - Proteomic Investigation
  - Lead investigators: Steve Schutzer, Tao Liu
  - Pacific Northwest National Laboratory


Clinical Benefits Maintained Through Week 24
Clinical Improvement (Axis-2)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
Drug: C6-pos
Plc: C6-neg
Plc: C6-pos
Drug: C6-neg
Baseline
Week 12
Week 24
Repeat Assessments

Clinical Improvement Associated with Ceftriaxone Treatment and positive C6 Antibody Index

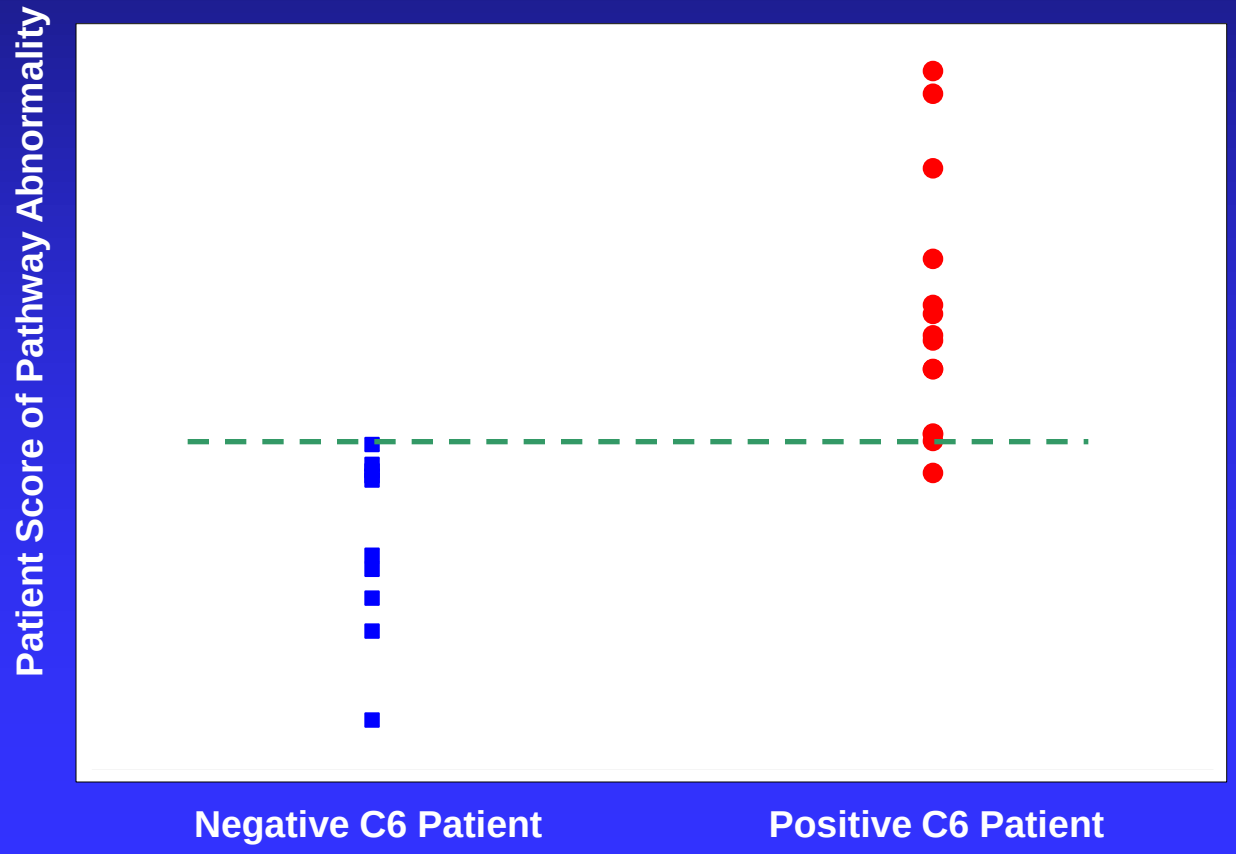


Using PET FDG, a brain pathway of abnormal activity was identified among all patients – a single score – and it was strongly correlated with C6 Antibody Index



- The PET FDG Brain MAP “Lyme Signature” score appears able to discriminate those who are C6 AI positive from those who are C6 AI negative.

Moeller et al, 2008, in prep.



Questions

- The C6 Brain pathway
  - Why is the magnitude of the C6 ELISA Index so tightly correlated with expression of a specific brain pathway?
  - Are the C6 Index AI positive patients truly those who benefit from antibiotic retreatment?
  - Does the expression of the C6 Brain map relate to treatment response?

Summary of Results at Wk 12

Did IV ceftriaxone result in specific improvement in memory?

- **No, the improvement was spread across multiple domains and not specific to memory.**

Did the IV Ceftriaxone show preferential response on other measures at wk12?

Yes, among the more impaired given IV ceftriaxone, greater improvement was noted in self-report measures of fatigue, pain, and physical functioning.

Summary of Efficacy Results

Was there a significant difference in cognitive change across the 3 groups (Lyme pts on drug, Lyme on placebo, healthy controls) over the 24 weeks?

- **Yes (p=.04). This was due to the greater improvement among the IV ceftriaxone treated patients noted at the primary efficacy time point of week 12.**

Was the drug-placebo difference at week 12 significant for cognition?

- **Only the drug group had within group improvement (p<.01).**
- **The between group improvement (Drug vs Placebo) fell at the margin of significance (p=.053)…needs replication.**
- **Magnitude of improvement was moderate.**

Was the improvement in the ceftriaxone group sustained to week 24 (after 14 wks of no treatment)?

No, for cognition.

By week 24, there was no difference between groups in cognitive improvement over time.

Yes, for pain and physical functioning.

At week 24, among the more impaired in severity at baseline, the improvement was sustained in pain and physical functioning (but not in fatigue).

Clinical Significance

- Adverse events associated with PICC lines and IV ceftriaxone are of concern
- For sustained cognitive improvement to week 24, given the risks & loss of benefit, it is not recommended to treat with 10 weeks of IV ceftriaxone followed by 14 weeks of no treatment
- For sustained improvement in pain and physical dysfunction, among the more impaired, repeated treatment with IV ceftriaxone may have a role in well-documented LD….but careful discussion with the patient about risks needs to occur.

Were clinical markers of Treatment Response suggested by this study of Persistent Lyme Encephalopathy?

Greater severity associated with improvement:

- Cognition
- Pain, Fatigue, Physical Functioning
- # of joints in pain on rheum exam
- # of areas with abnormality on a neuro exam

CSF

- Positive C6 Antibody index associated with acute & long-term improvement in physical functioning/fatigue

Neuro-imaging

- Expression of Lyme Brain Map on PET FDG

CSF C6 AI and Brain Map together provided best predictor of who will benefit clinically (r=0.91)

Research Questions

- Why do patients relapse? What is the mechanism of action of ceftriaxone? an antimicrobial or a glutamate effect?
- What alternative treatments could be provided that might be safer and result in a more sustained response in cognition?
- Is the etiology of the persistent cognitive problem different from the etiology of the persistent pain, fatigue, physical dysfunction?

Additional Research Questions

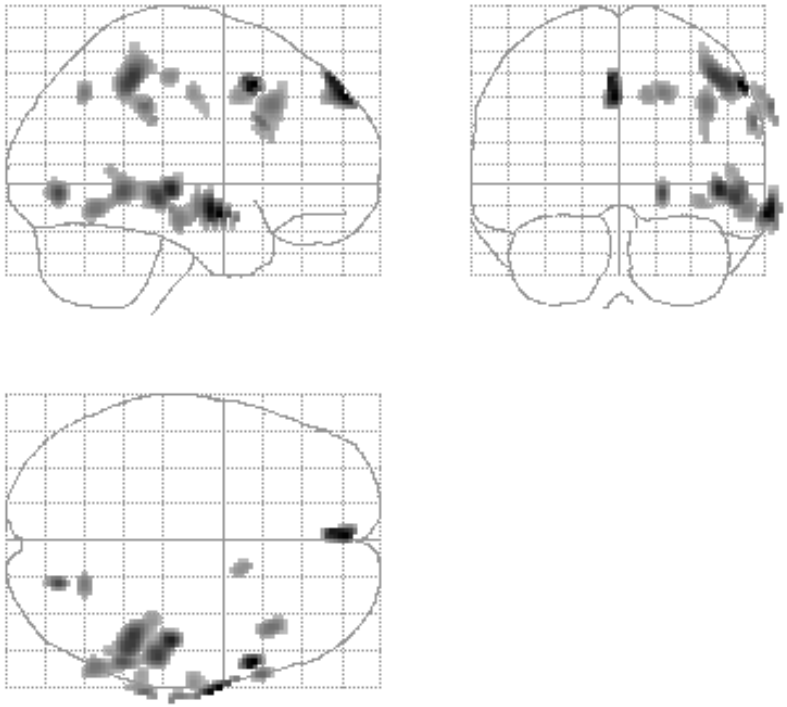
- Is the C6 antibody index able to prospectively identify patients who will benefit from repeated treatment?
- Does expression of the Lyme brain map also help to prospectively identify patients more likely to respond to repeated treatment?

Limbic System Mediation

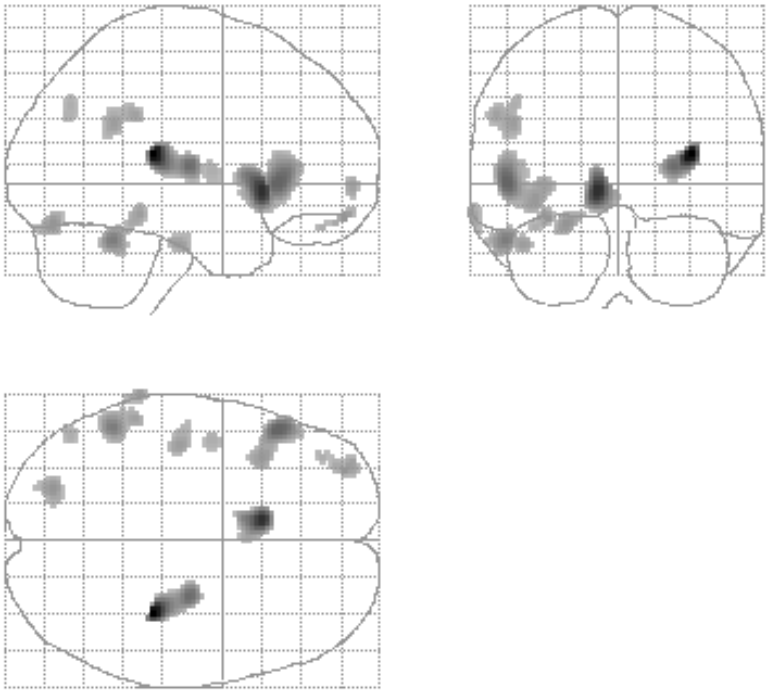
- Parahippocampal gyrus
  - Receives sensory input from outside world, integrates it, and projects it to the hippocampus (memory) and amygdala (fear, aggression, mood)
- Insula
  - Receives limbic input from amygdala.
  - Relays somatosensory info & pain reactions
  - Relays between Wernicke's and Broca's area (dysfunction contributes to aphasias & articulation deficits)

FDG PET: Metabolic Changes

Drug (T2-T1) Greater than Placebo (T2-T1)

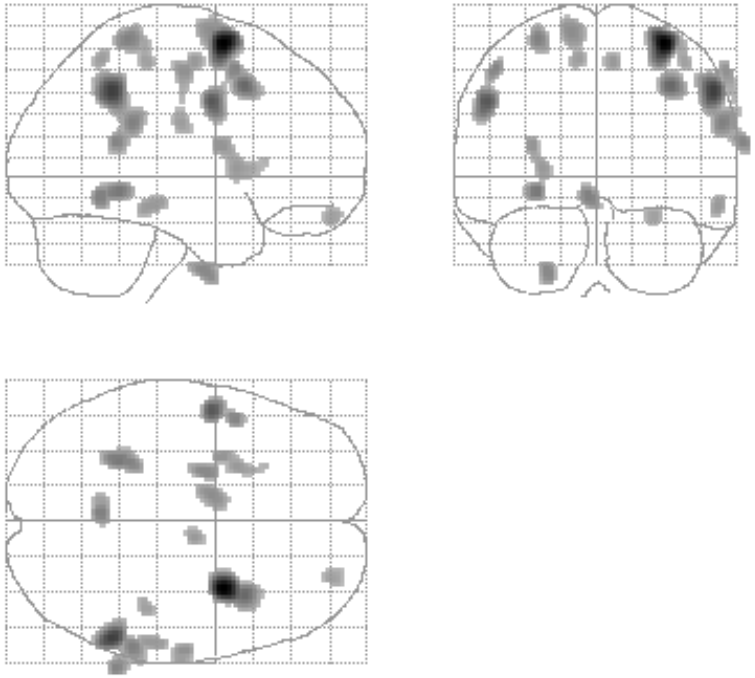


Placebo (T2-T1) Greater than Drug (T2-T1)

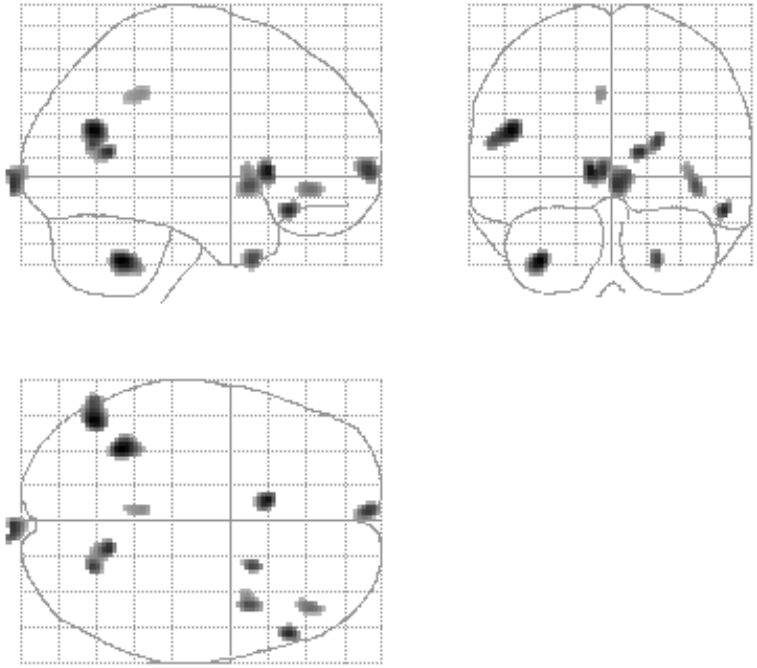


Resting Regional Cerebal Blood Flow: Drug vs Placebo

Drug (T2-T1) > Placebo (T2-T1)

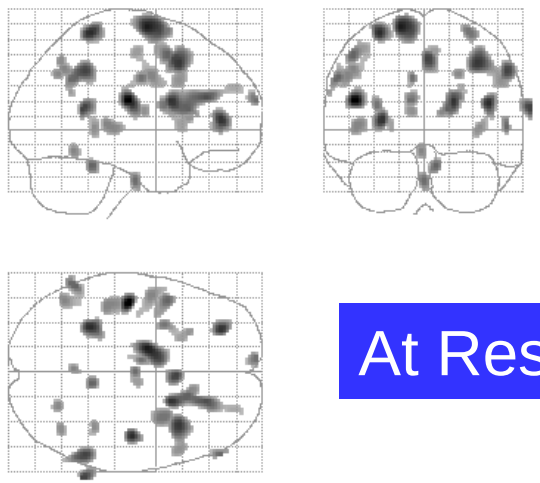


Placebo (T2-T1) > Drug (T2-T1)

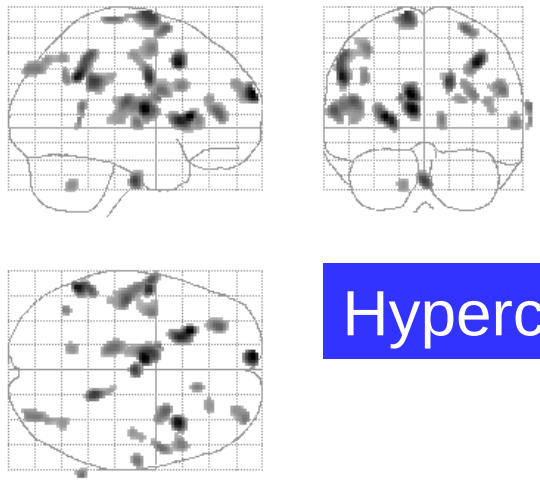


Week 12 improvement over Baseline for Drug-treated patients

Cerebral Blood flow improvement

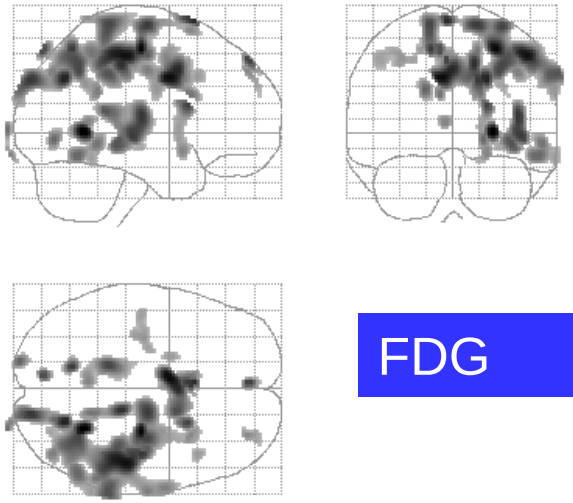


At Rest



Hypercapnia

Metabolic Improvement



FDG

Predictors of Response?

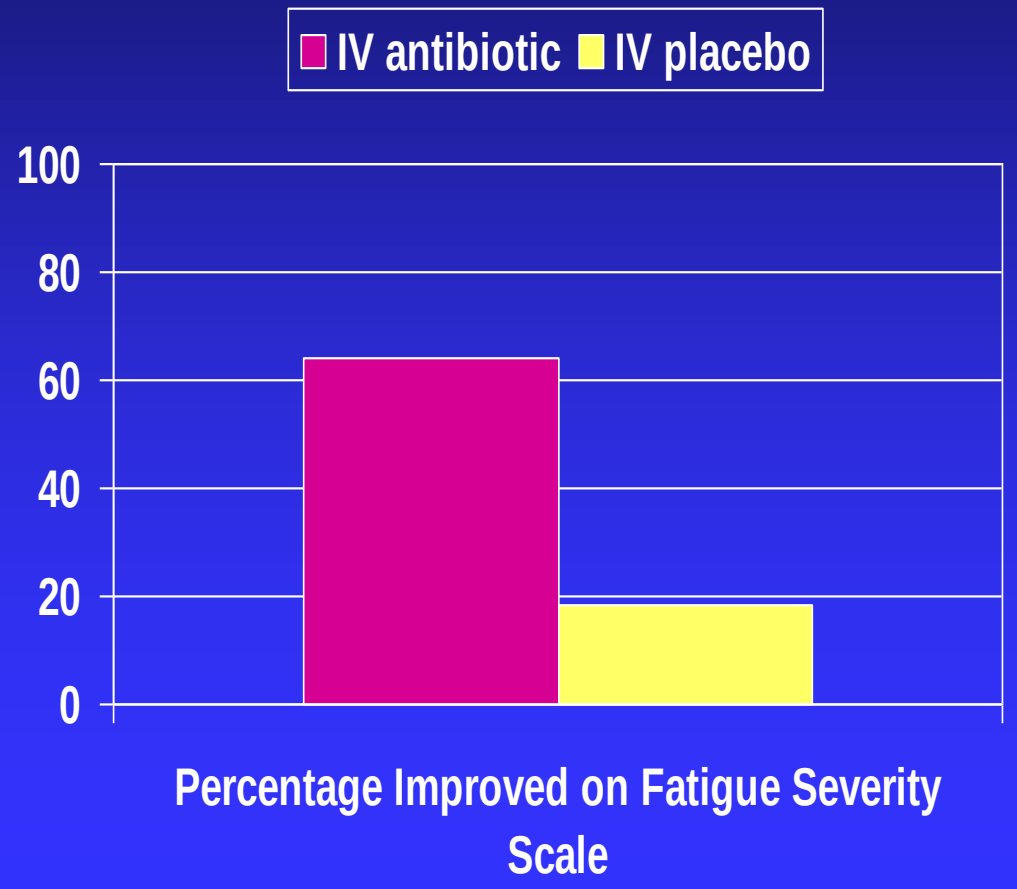
Pathophysiology of CNS Lyme Disease

- Direct Damage by spirochete
  - Invasion & attachment. Invades brain (rabbits, rhesus macaques) by penetrating CNS vascular endothelium and adhering to glial cells. In rat culture, Bb adheres to brain astrocytes, oligodendroglial cells & microglia, resulting in demyelination and neural cell cytotoxicity
- Indirect damage
  - Cytokines from neurons. Exposure to Bb causes neurons to produce interleukin 6, TNF, nitric oxide. N.O. is cytotoxic and proinflamatory. Elevated IL6 can cause sx of fatigue & malaise. These Bb may be dormant or active.
  - Immunologic damage. Bb's surface lipoproteins are pro-inflammatory and Bb glycolipids may elicit cross-reactive Ab. Quinolinic acid (NMDA excitotoxin) in LD encephalitis

Controlled Studies of the treatment of Chronic Lyme Disease

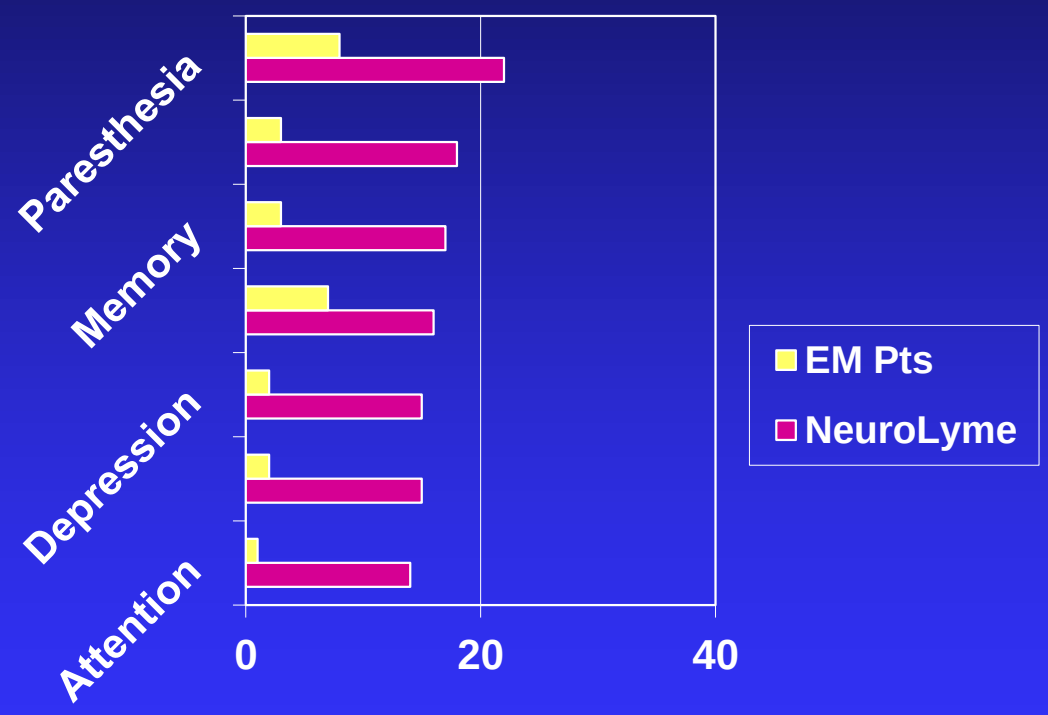
- **Chronic Lyme: all types (Klempner, NEJM)**
  - no difference between placebo & antibiotic at 6 months using a disability scale (SF-36)
- **Study of Fatigue in chronic Lyme (Krupp)**
  - 3.5X more pts benefited from IV antibiotic at 6 months on main outcome measure of fatigue
  - **(64% vs 19%,p<.001)**

Krupp's Study of Post-treatment Lyme Fatigue (Neurology, 2003)



Chronic Symptoms occur: Follow-up Study of adults with Neuro-Lyme vs EM (Act Neurol Scand 2002)

- 106 pts with neuroborreliosis vs 123 pts with EM.
- Mean Follow-up interval: 3 years
- 50% of NB pts had persistent neuro-psychiatric sx vs 16% of EM pts (p<.0001)



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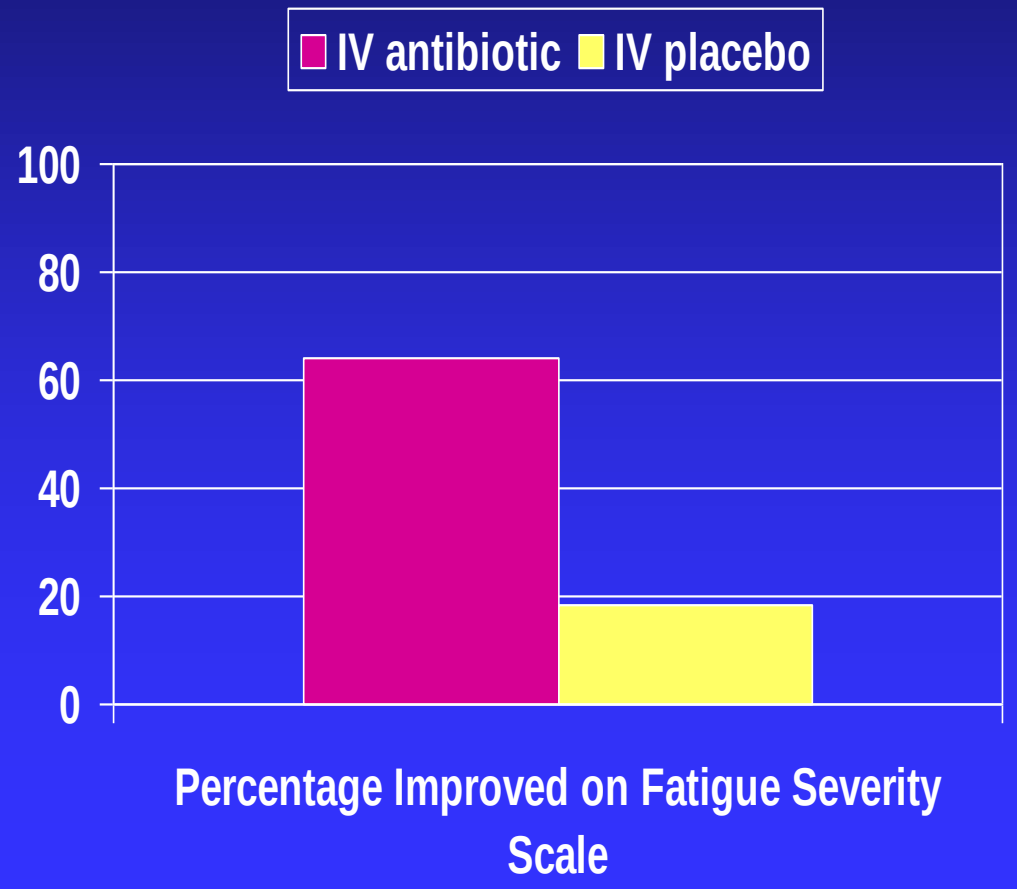

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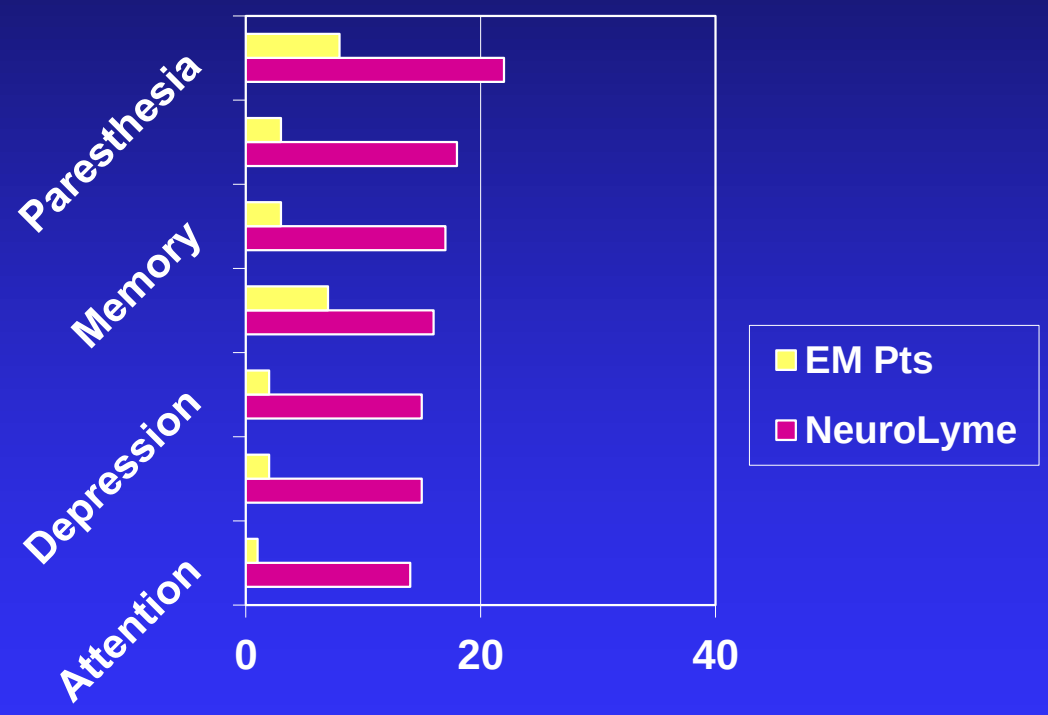


Baseline Clinical

- Cognitive: mild-moderate deficits
  - Verbal Memory (z=-1.0), Working memory (-0.7), Verbal Fluency (z=-0.8)
- Psychopathology: mild
  - mild depression & anxiety
- Neurologic exam:
  - Mild sensory findings (73% Ly vs 22.2% C, $p<.001$)
- Rheumatologic Exam:
  - Much joint pain (6.1 jts Ly vs 0.6 jts C, $p<.01$)
  - Very few trigger points (1.8 Ly vs 0 C)

Hypoperfusion on SPECT is common: "Reversible Cerebral Hypoperfusion in Lyme Encephalopathy"

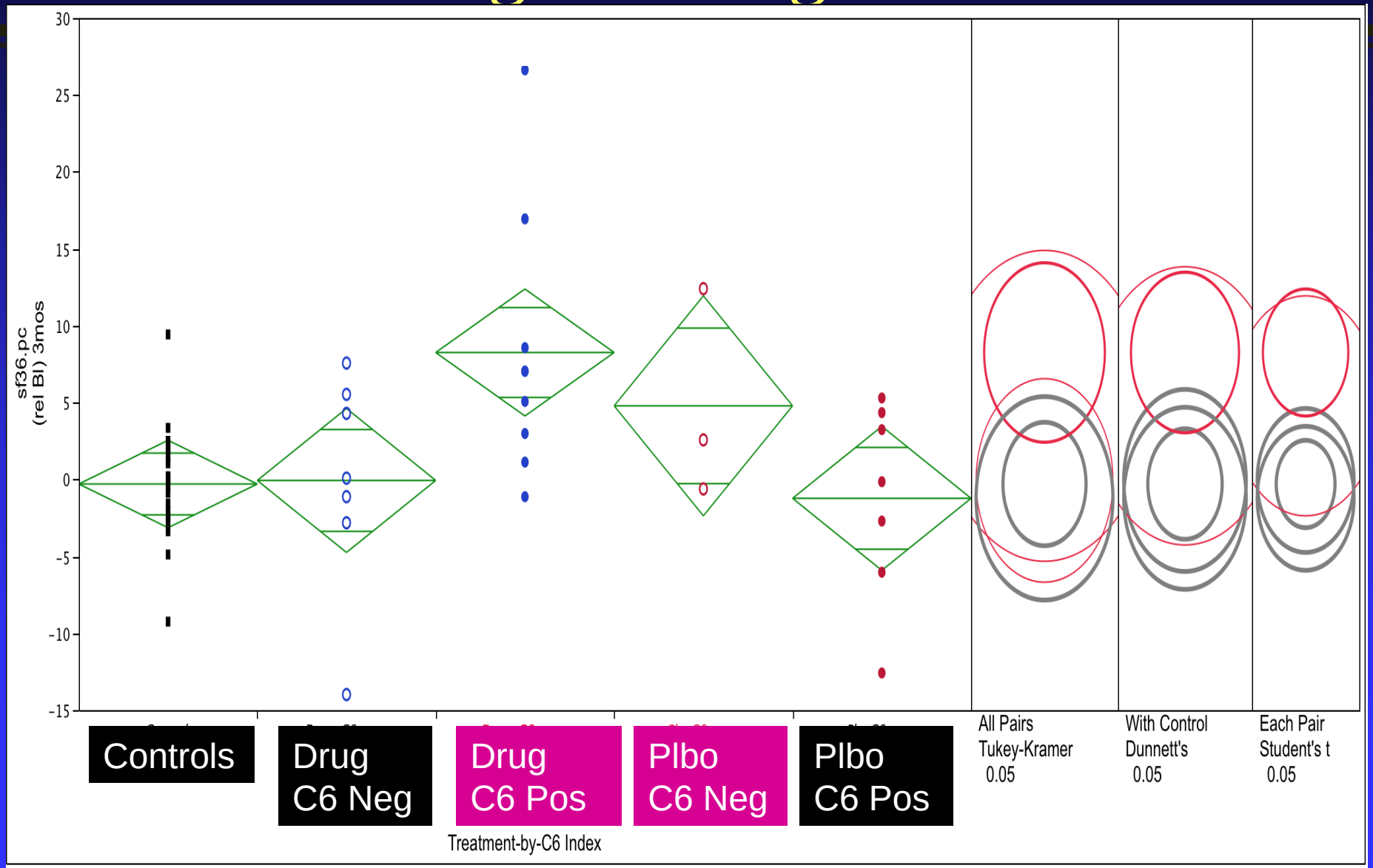
(Logigian et al, 1996)

- Definite LE (13 pts) vs Possible LE (9 pts) vs 26 normals
- Results:

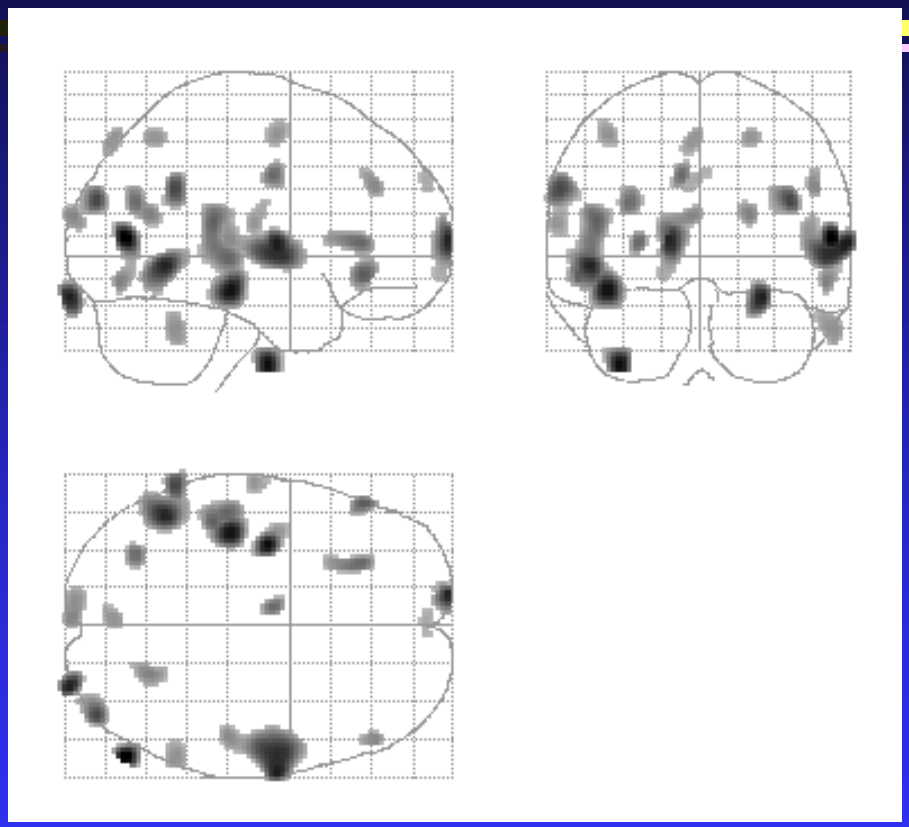
  A. Significantly more deficits: LE>Possible LE> Normals

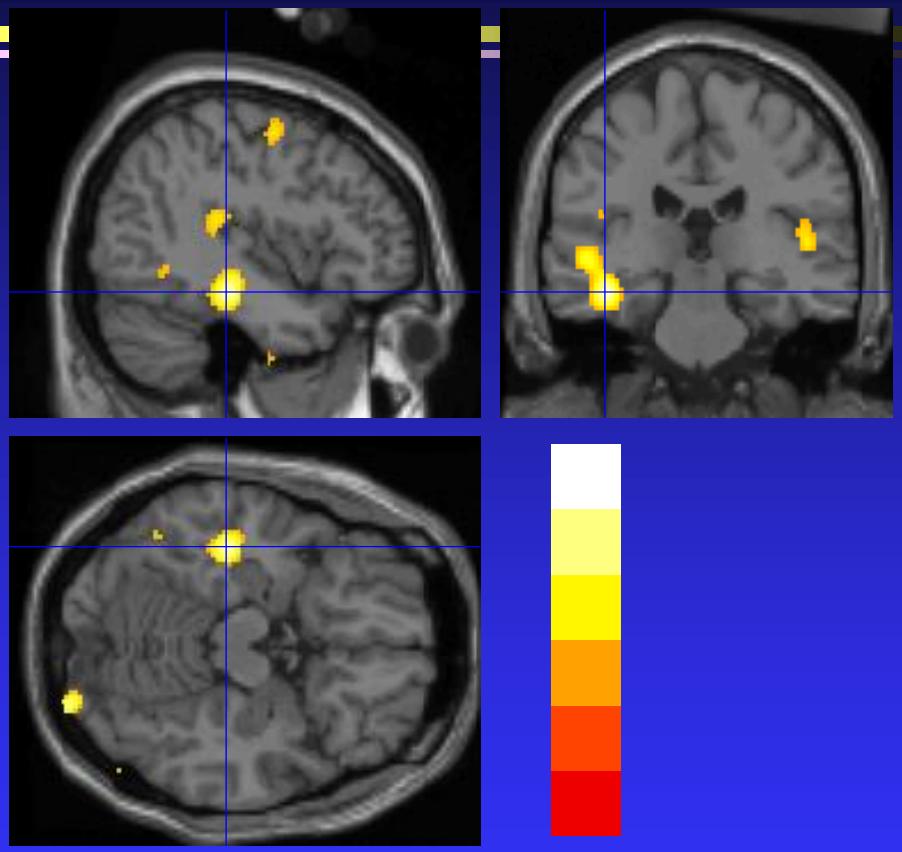
  B. Of 13 Pts with Definite LE treated with IV Ceftriaxone
  - 6 mons later: a partial reversal of perfusion deficits
- Conclusions:
  - **SPECT deficits are <u>worse with more severe disease</u>**
  - **<u>SPECT deficits may be seen even in absence of objective neuropsychological deficits</u>**
  - **Perfusion deficits are <u>at least partially reversible</u>**

Change from Baseline is significant for C6+ given drug vs others



O-15 Imaging: are there regional blood flow differences between pts and controls? Yes. (p<.001)





Patients have lower CBF in 9 clusters (2828 voxels).
Patients had higher CBF in only 1 cluster (222 voxels).

Areas of lower CBF in Lyme pts: L limbic parahippocampal, L&R temporal, R temporal, L & R occipital, L cingulate

- Co-investigators:
  - Columbia: Carolyn Britton, MD, Ted Dwyer, MD, Jay Dobkin, MD, Ronald Van Heertum, MD, Robert Delapaz, MD
  - NYSPI: Harold Sackeim, PhD, John Keilp, PhD, James Moeller, PhD, Mitch Nobler, MD, Shan Yu, PhD
- Study Coordinator: Kathy Corbera, MD
- Biostatisticians/Imaging Analysts: Eva Petkova, PhD, Iordan Slavov, PhD, Jianfeng Cheng, PhD, Shan Yu, PhD, Brett Mensh, MD, Angela Lignelli
- Other Columbia: K Marder,Y Stern, D Bloomfield
- Facilities: Irving Center for Clinical Research, PET Center, NYSPI, Home Care Services (NJ), Roche Pharm. , IRBs
- NINDS (A. Kerza, M. Nunn) & DSMB

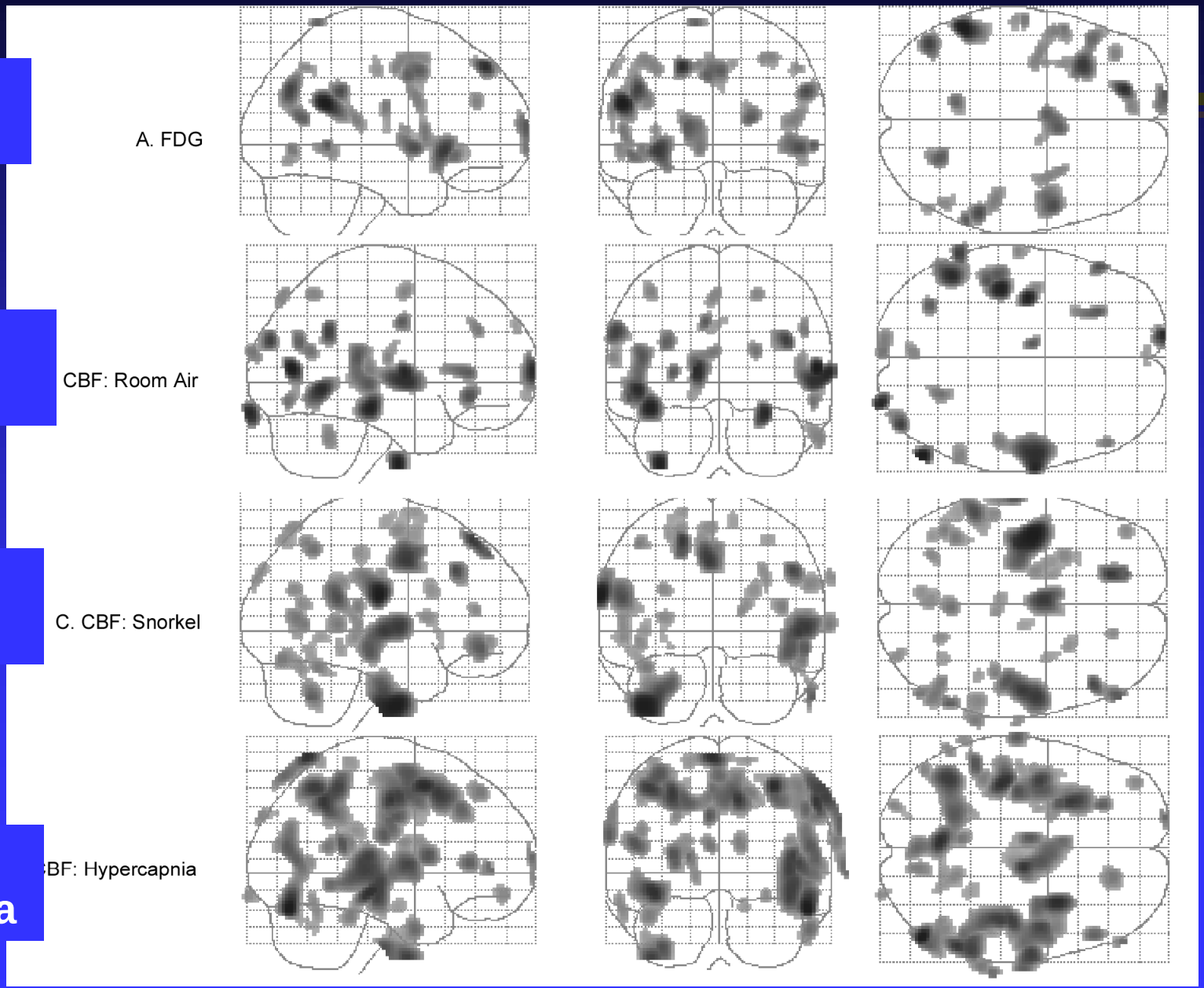
Baseline: Patients lower flow and metabolism than Controls at rest, accentuated with 5% CO2 air

Metabolism at rest

Flow at room air.

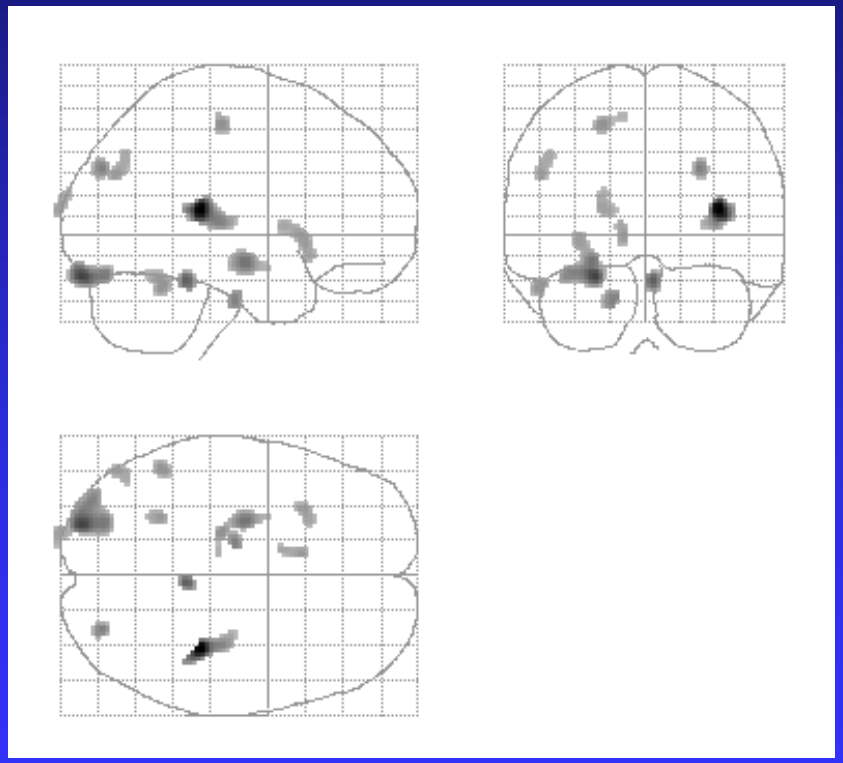
Flow w Snorkel

Flow w hypercapnia



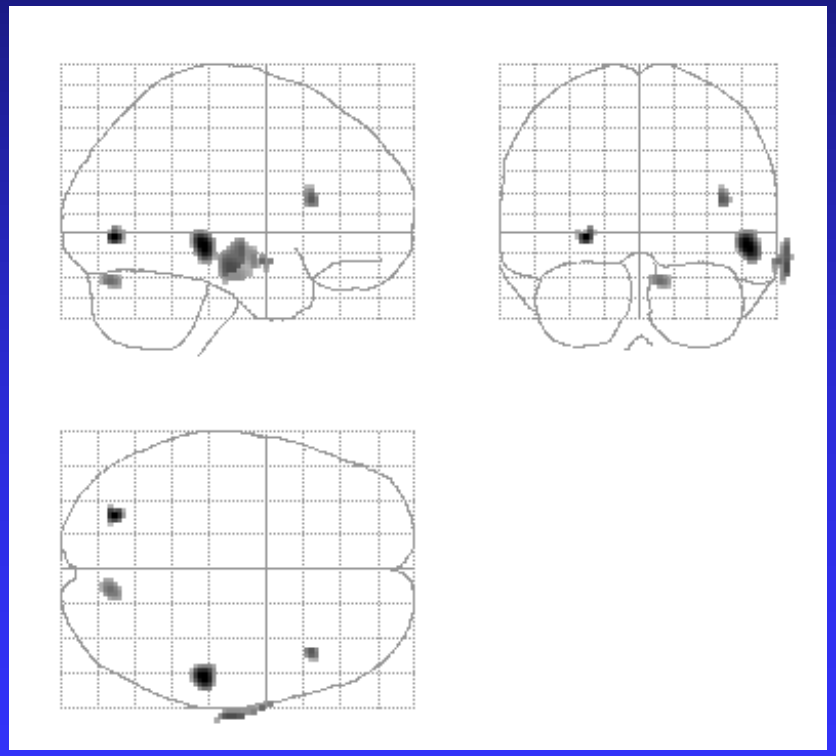
PET FDG: Few areas of metabolic change for patients given placebo (p<.001)

Week 12 metabolism greater than baseline



3 clusters, 470 voxels

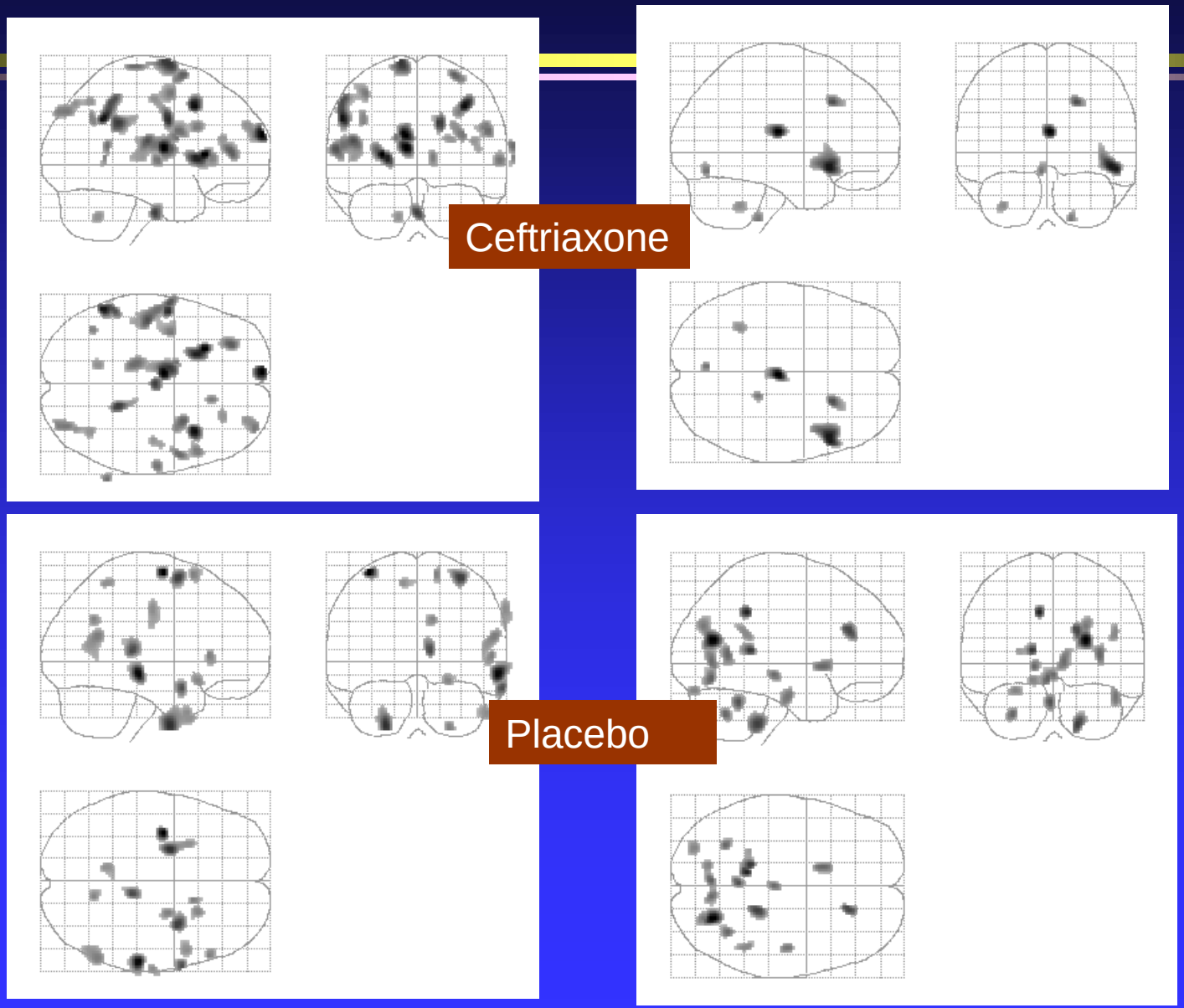
Week 12 metabolism less than baseline



2 clusters, 170 voxels

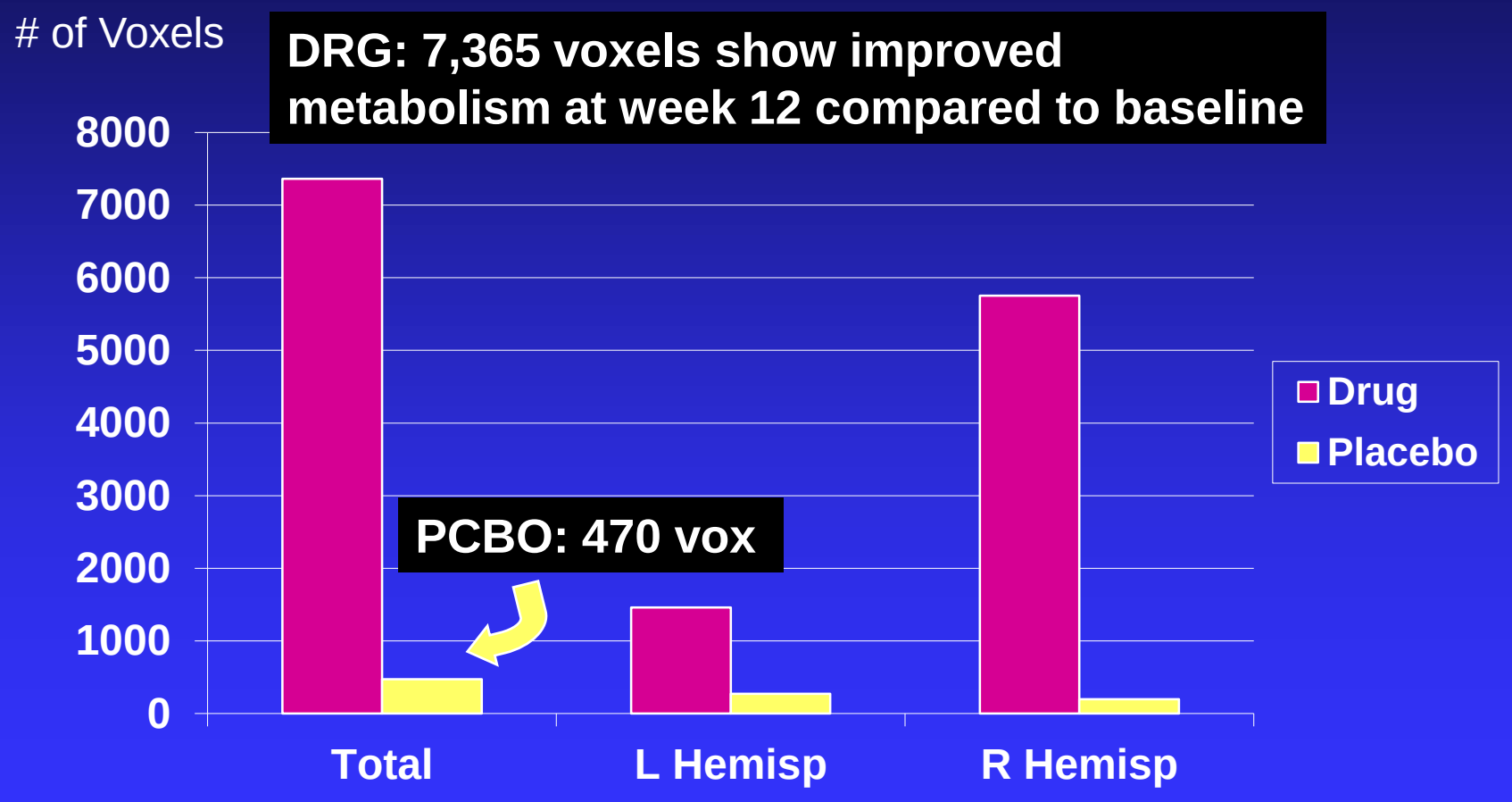
RCBF Hypercapnia Drug vs Placebo

Wk 12 Flow Greater than baseline Wk 12 Flow less than baseline



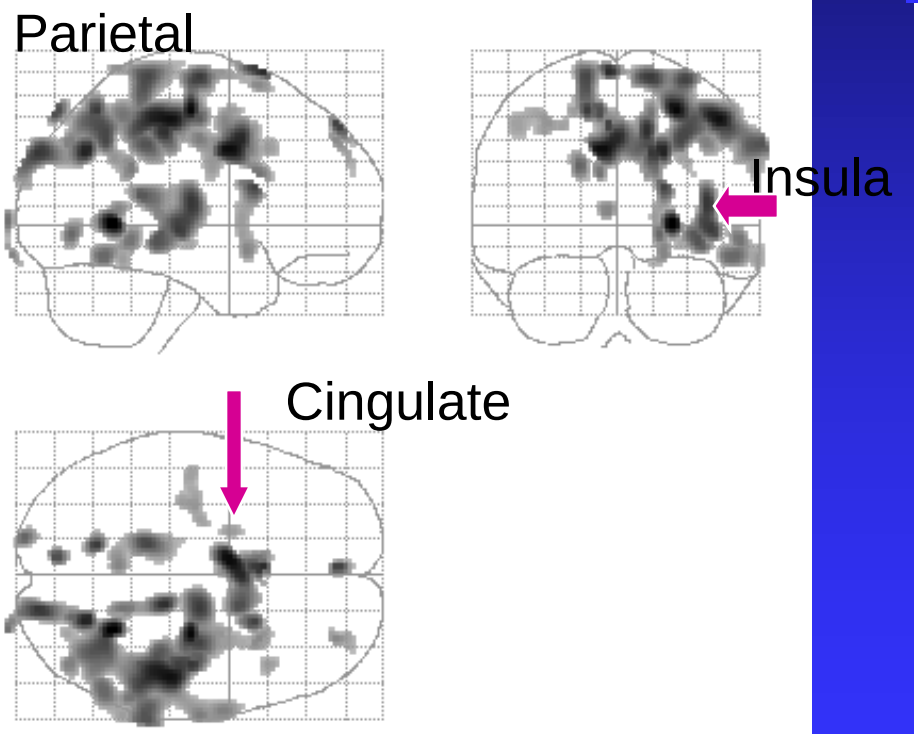
Large areas of improvement in metabolism from drug occur - in the Right Hemisphere (wk 12>wk0)

(significance p<.001, z>3)

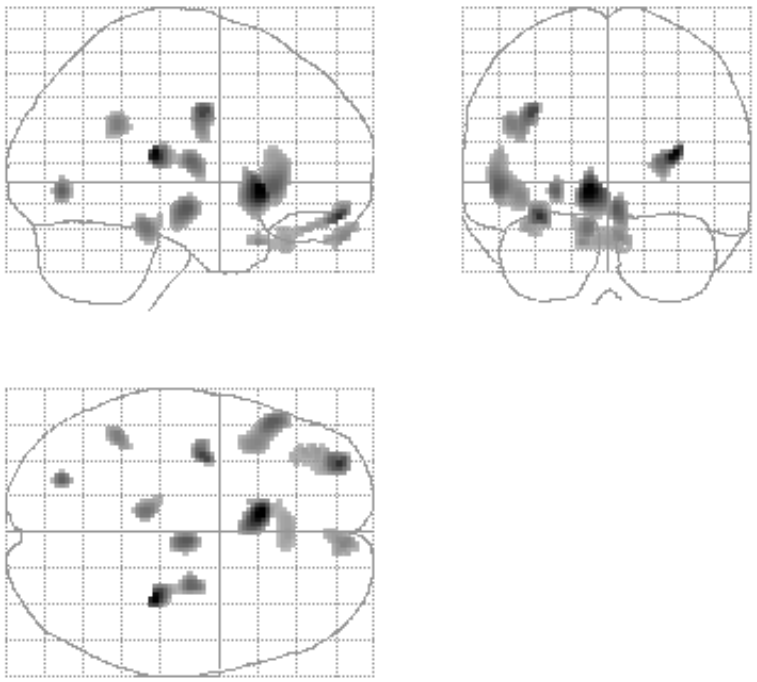


PET FDG: large metabolic increases for Patients given ceftriaxone (p<.001)

Week 12 Metabolism Greater than Baseline



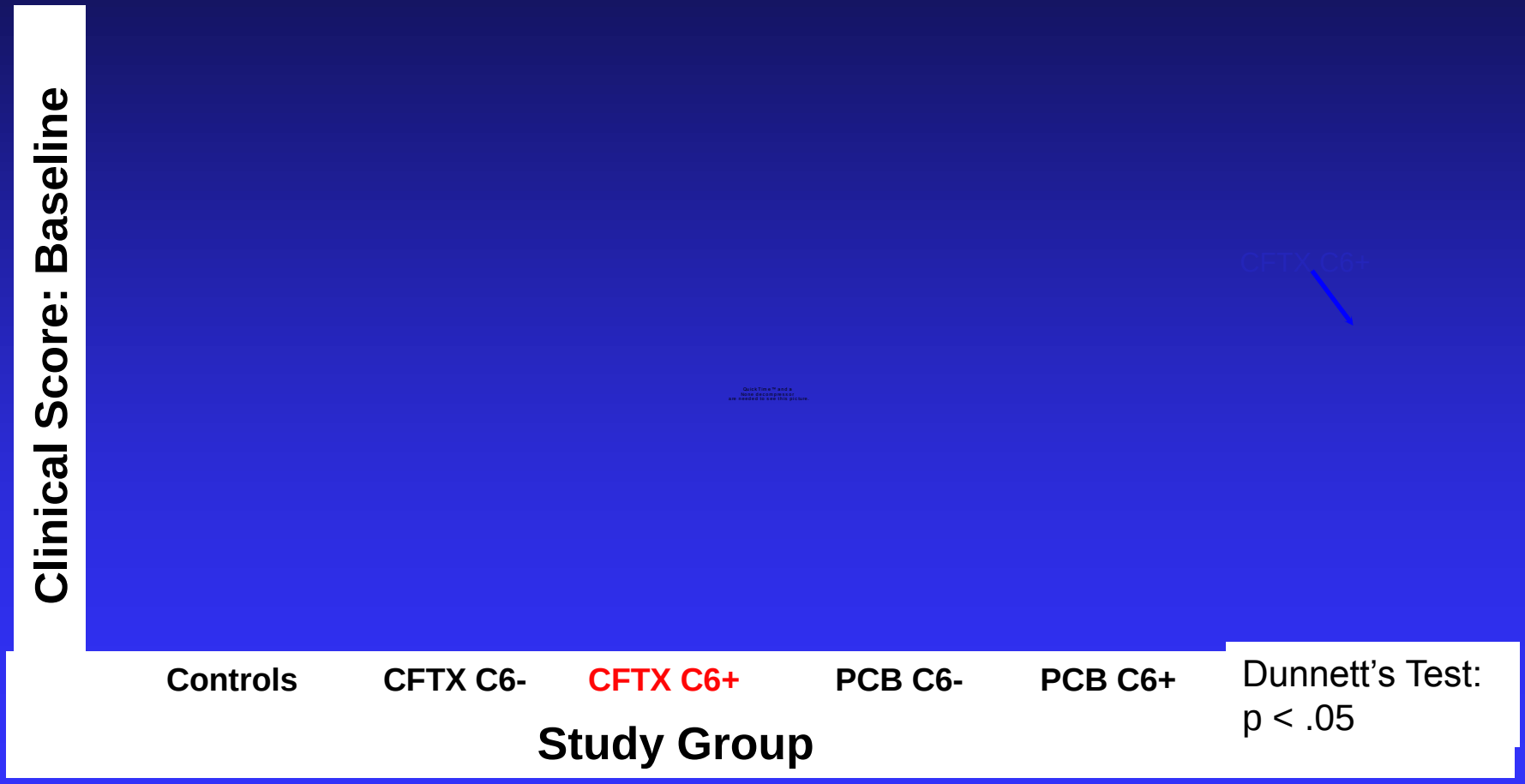
Week 12 Metabolism Less than Baseline



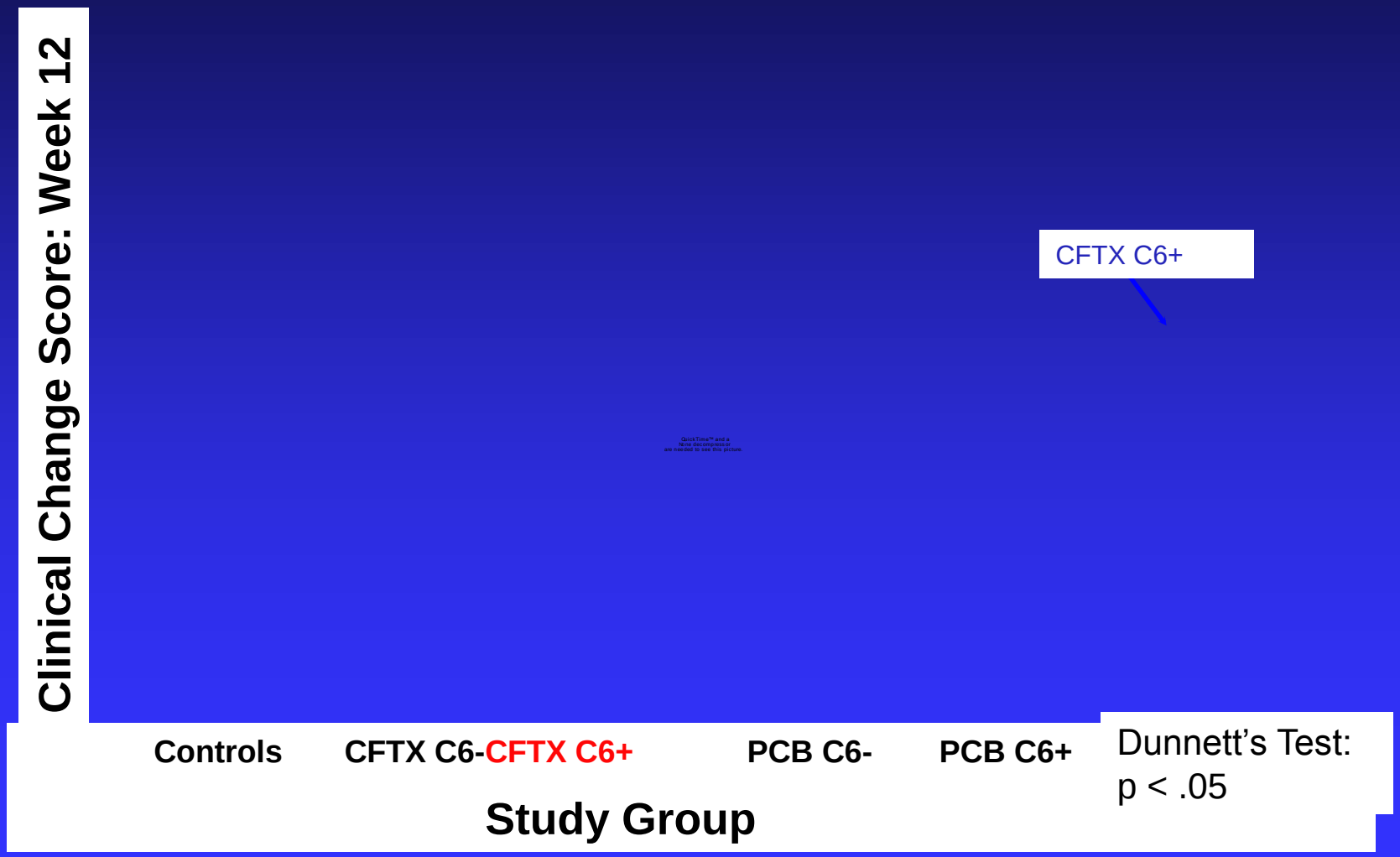
11 clusters, 7365 voxels

7 clusters, 1170 voxels

Baseline Values For SF-36 PCS: No Difference Between C6+ And C6- Patients



Week 12 Change Score For SF-36 PCS: Significant Difference Between CFTX C6+ And CFTX C6-



Clinical Improvement Associated with Ceftriaxone Treatment and positive C6 Antibody Index

Clinical Change Score

QuickTime™ and a
None decompressor
are needed to see this picture.

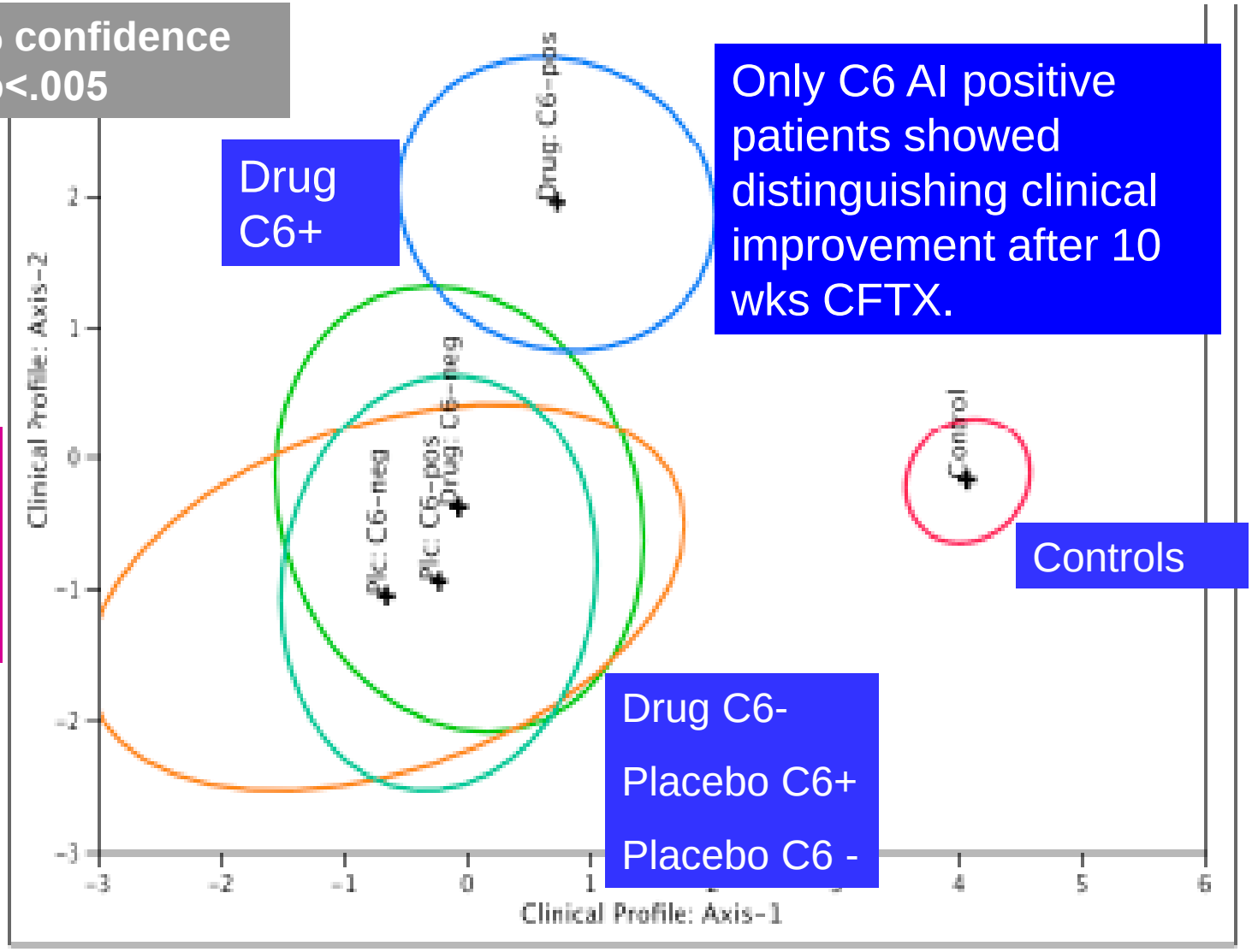
Controls | CFTX C6- | CFTX C6+ | PCB C6- | PCB C6+

STUDY GROUPS

Profile Analysis Signature

For C6 association with positive treatment outcomes

Circles: 95% confidence intervals….p<.005



Drug C6+

Only C6 AI positive patients showed distinguishing clinical improvement after 10 wks CFTX.

Controls

Drug C6-

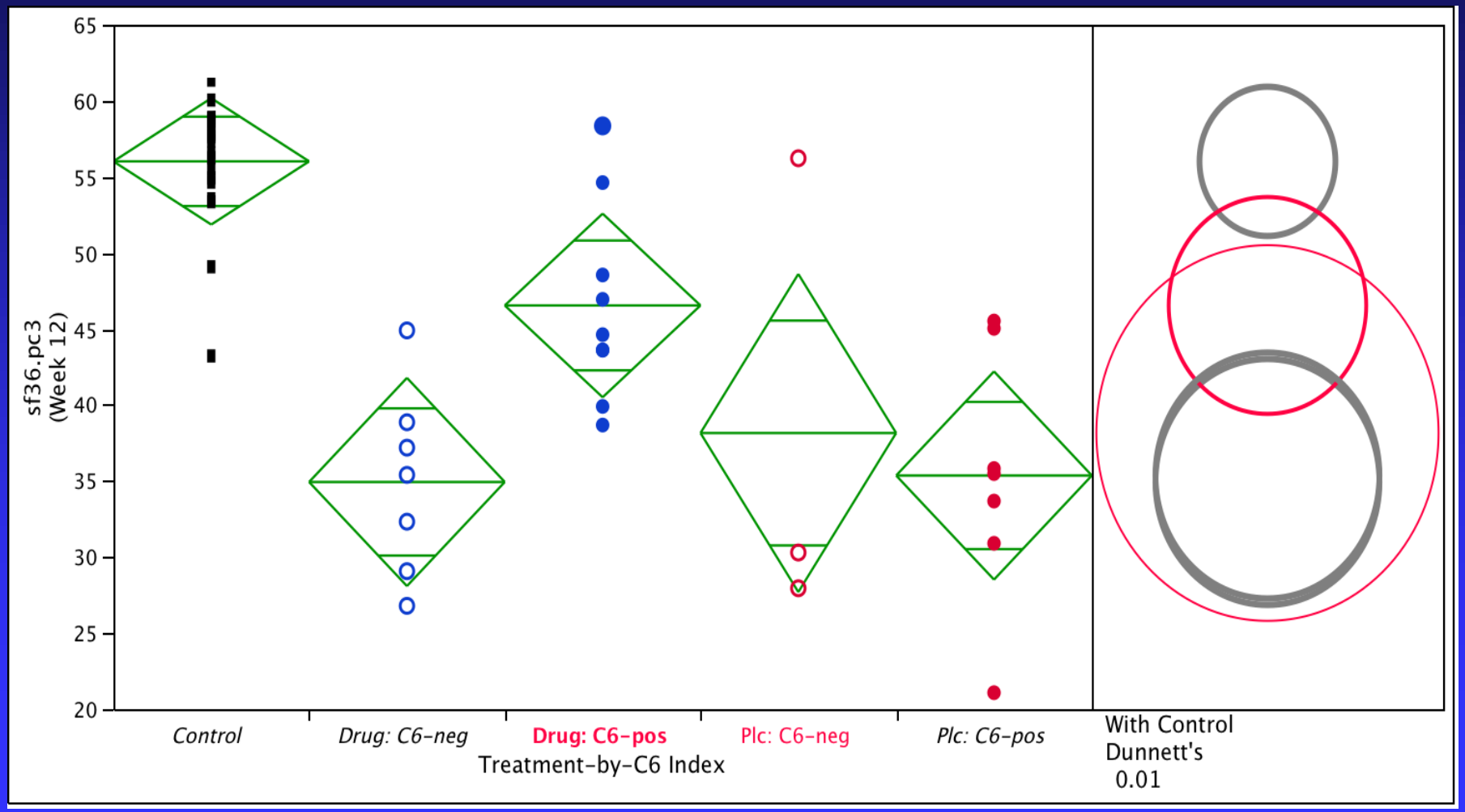
Placebo C6+

Placebo C6 -

Clinical Change Score

Clinical Profile score at start of treatment

Week 12 Values for SF-36 PCS: sig difference between Drug C6+ & Drug C6-



Community practice in use of ELISA & WB tests varies

- Strict: apply CDC recommended lab criteria to avoid false positives
- Broad: apply a modified method to avoid false negatives
  - Positive IgM or IgG Western blot (without ELISA)
  - Laboratory-adopted criteria for WB vs CDC
  - Consider the serologic diagnosis of “possible” Lyme disease if the IgG Western blot is suggestive with 3 or 4 bands….attend to the 31 & 34 kd bands as these may be expressed in late infection

Testing (Aguero-Rosenfeld et al, 2005)

- Indirect assays:
  - good sensitivity and specificity for the more acute inflammatory phases of Lyme disease such as arthritis (100%)
  - lesser sensitivity for neurologic Lyme (64-87%) (Dressler 1993, Bacon 2003) and for convalescent phase after EM treatment (2 tier: 29-78%)
- Bb antibodies (IgM and IgG) may persist for many years after successful treatment of LB

Sample Size Screened

- Lyme (patients samples from 2 studies):
  - A. Lab/SPECT study - screened 194 patients to find 13 (6.7%)
  - B. Screenings from prior NIH Study – added stored samples from pts with well documented LD - all of whom had also had prior IV antibiotic therapy
- Healthy controls
  - A. Screened 315 people of whom 26 were interested and eligible to participate (8.3%)
  - B. Stored serum from prior NIH Study – added 7

Preliminary results from 3 Labs – expanded sample

- Across 3 Labs: (n=33 HC, n=20 Ly)

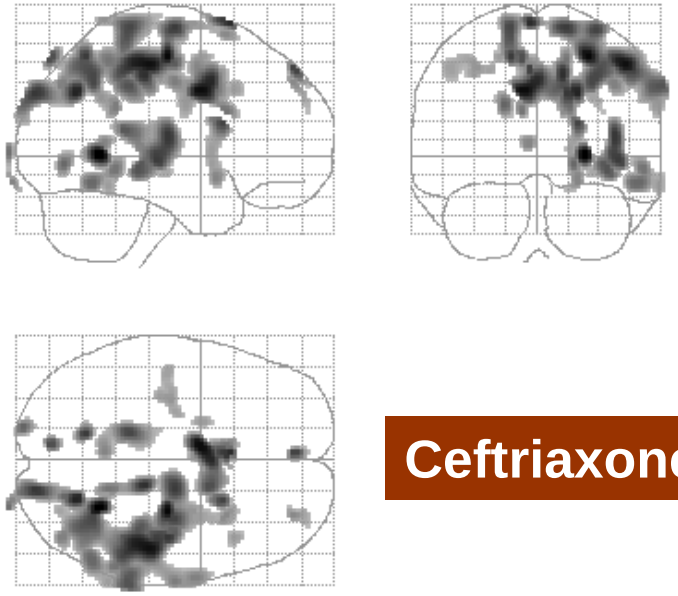
	Commercial		2 Lyme-specialty Labs	
	Sens	Spec	Sens	Specificity
ELISA:	55%	91%	50-55%	82-94%
C6 ELISA:			25%	100%
IgM WB (CDC)	20%	100%	5-20%	91-97%
IgM WB (Lab)			5%	96%
IgG WB (CDC)	25%	100%	20-30%	97-100%
IgG WB (Lab)			20%	100%
CDC (2 tier IgG)	25%	100%	20-25%	100%

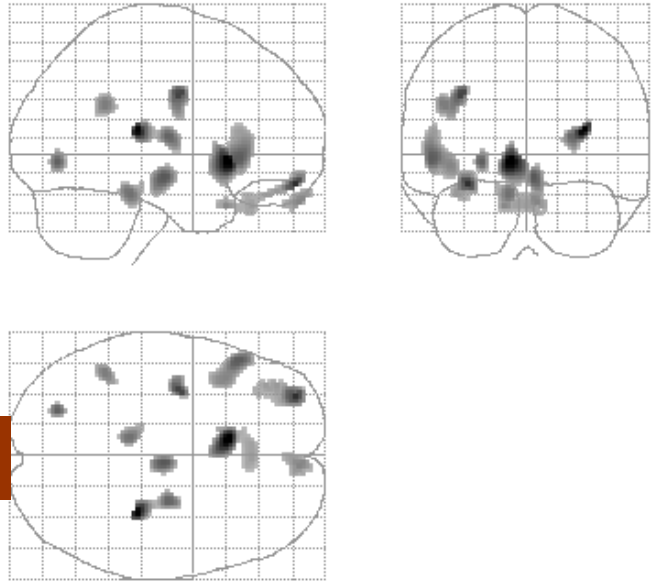
Note: Very Similar performance across Laboratories.

Note: C6 ELISA is comparable to IgG WB

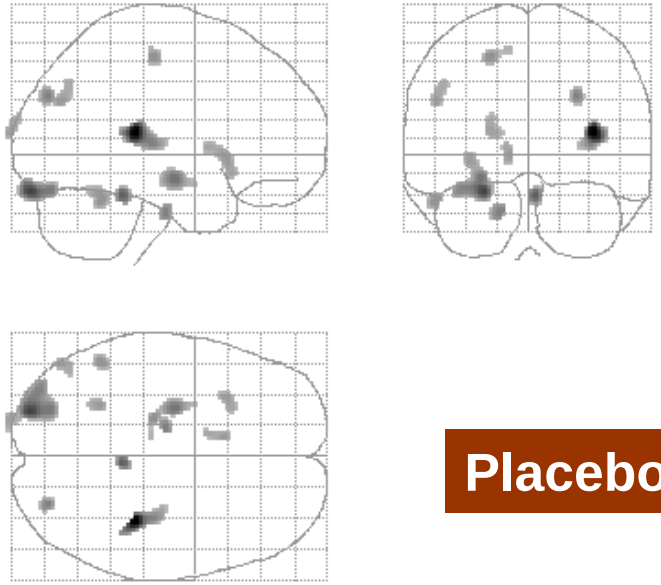
Wk 12 Metabolism increased vs Base

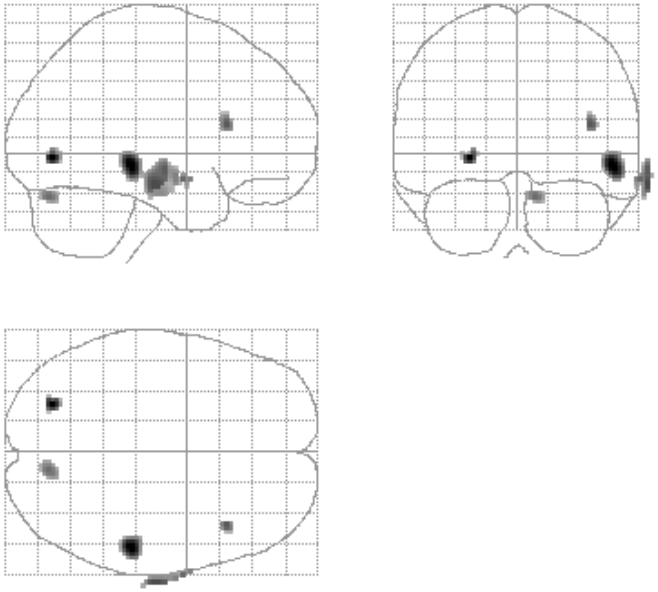
Wk 12 metabolism decreased vs Base





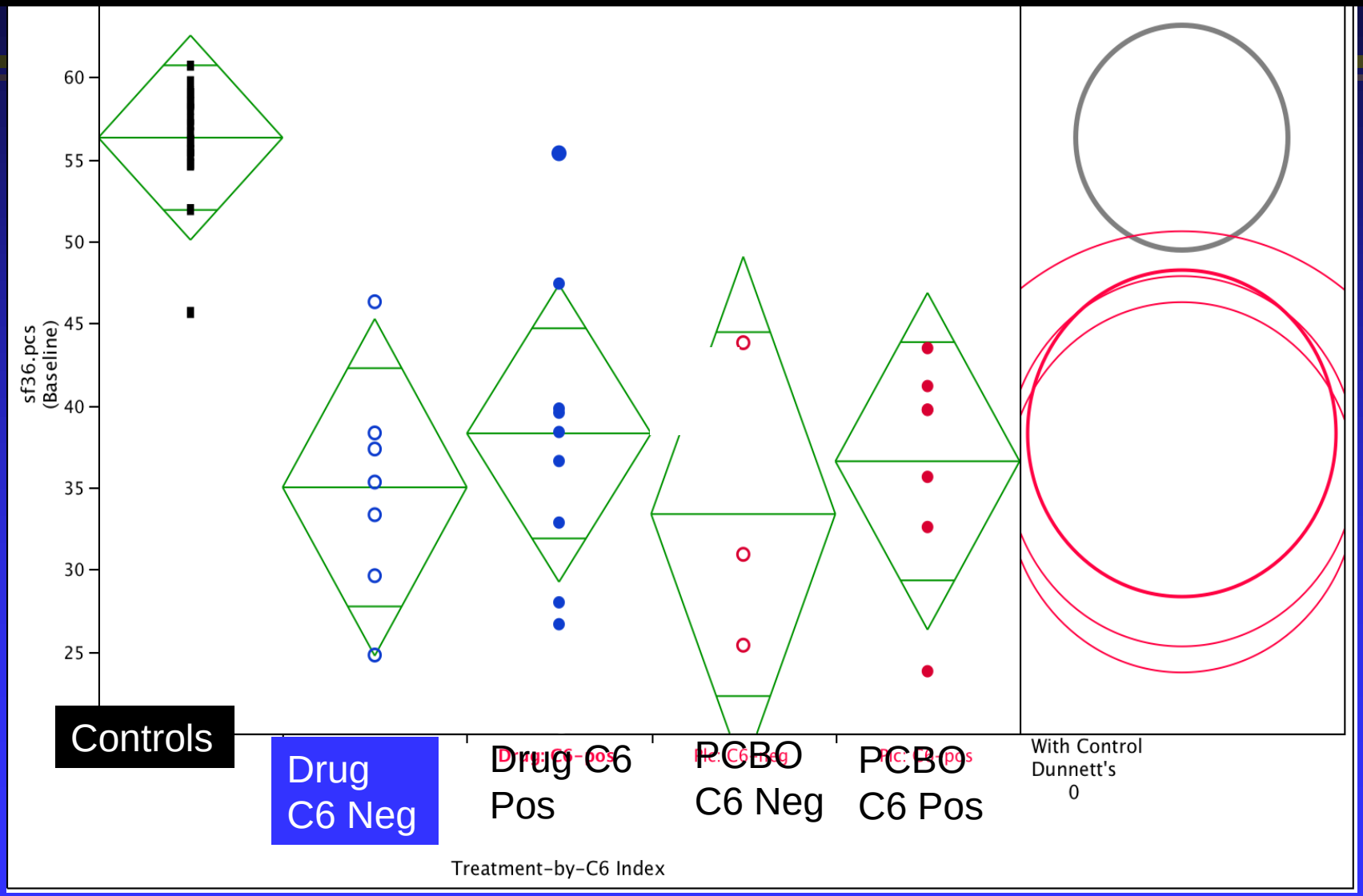
Ceftriaxone Treated



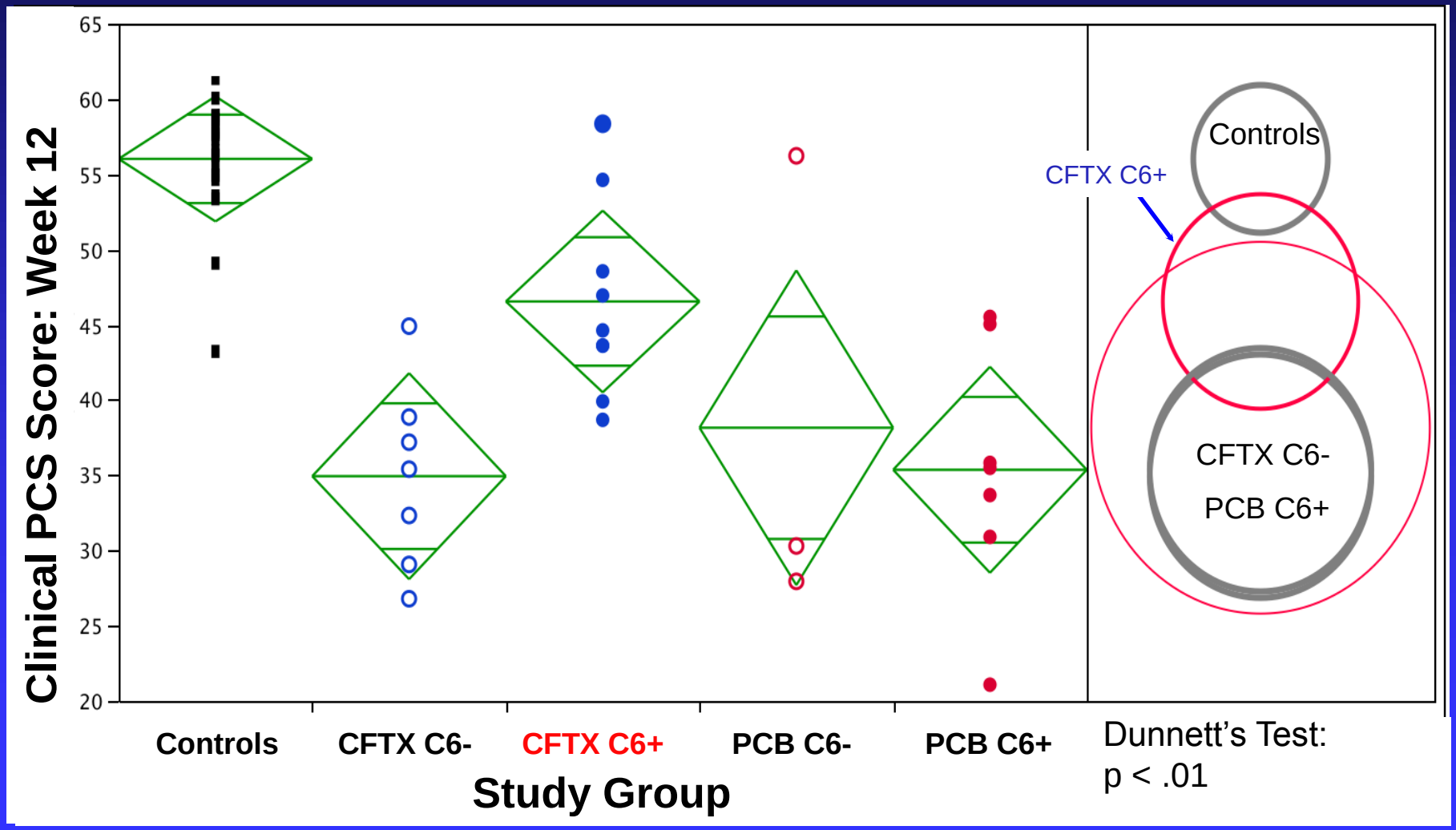


Placebo Treated

Baseline SF-36 Physical Component Scale (PCS): C6 Index – no baseline clinical differences bet patient groups



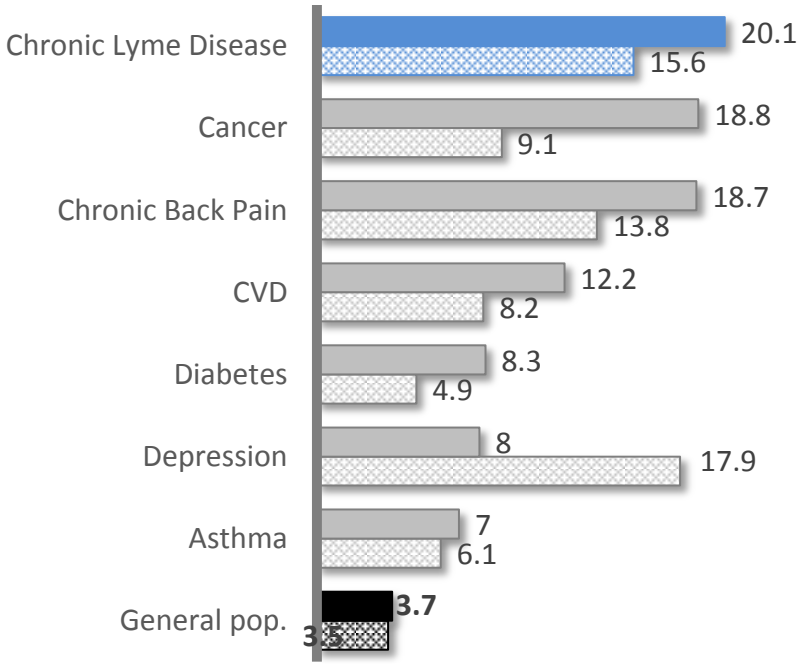
Week 12: Significantly better clinical improvement for C6+ Pts given Ceftriaxone vs C6- Pts given CFTX



Impact of Lyme disease

- Delayed diagnosis & treatment can lead to:
  - Chronic Lyme Arthritis with severe joint pain and swelling
  - Chronic conditions (neuropathy, encephalopathy, chronic fatigue)
- About 5-15% develop Post-treatment Lyme Syndrome
  - Physical impairment comparable to Congestive Heart Failure
  - Fatigue comparable to patients with Multiple Sclerosis

Number of poor physical and mental days per month of patients by disease condition[1]



Patients with chronic symptoms after Lyme Disease patients report more days of feeling sick per month than those with many other common chronic diseases

1. PeerJ. 2:e322, 2014.