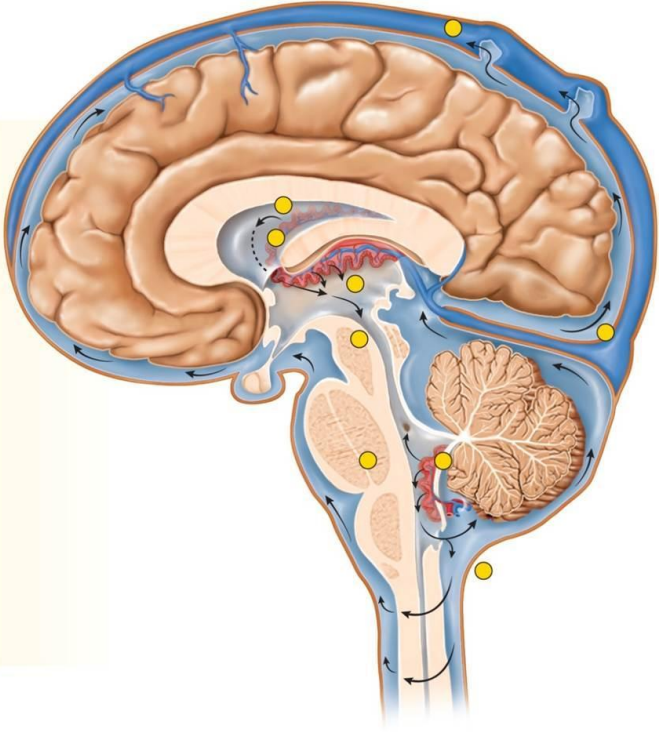


Clinical usage of CSF biomarkers in HIV

Magnus Gisslén

REGION VÄSTRA GÖTALAND
SAHLGRENSKA UNIVERSITY HOSPITAL

UNIVERSITY OF GOTHENBURG

Case 1

- 43 y old man
- HIV+
- CD4-cells 460 /μL
- P HIV-RNA 21 400 copies/mL = 4.3 log

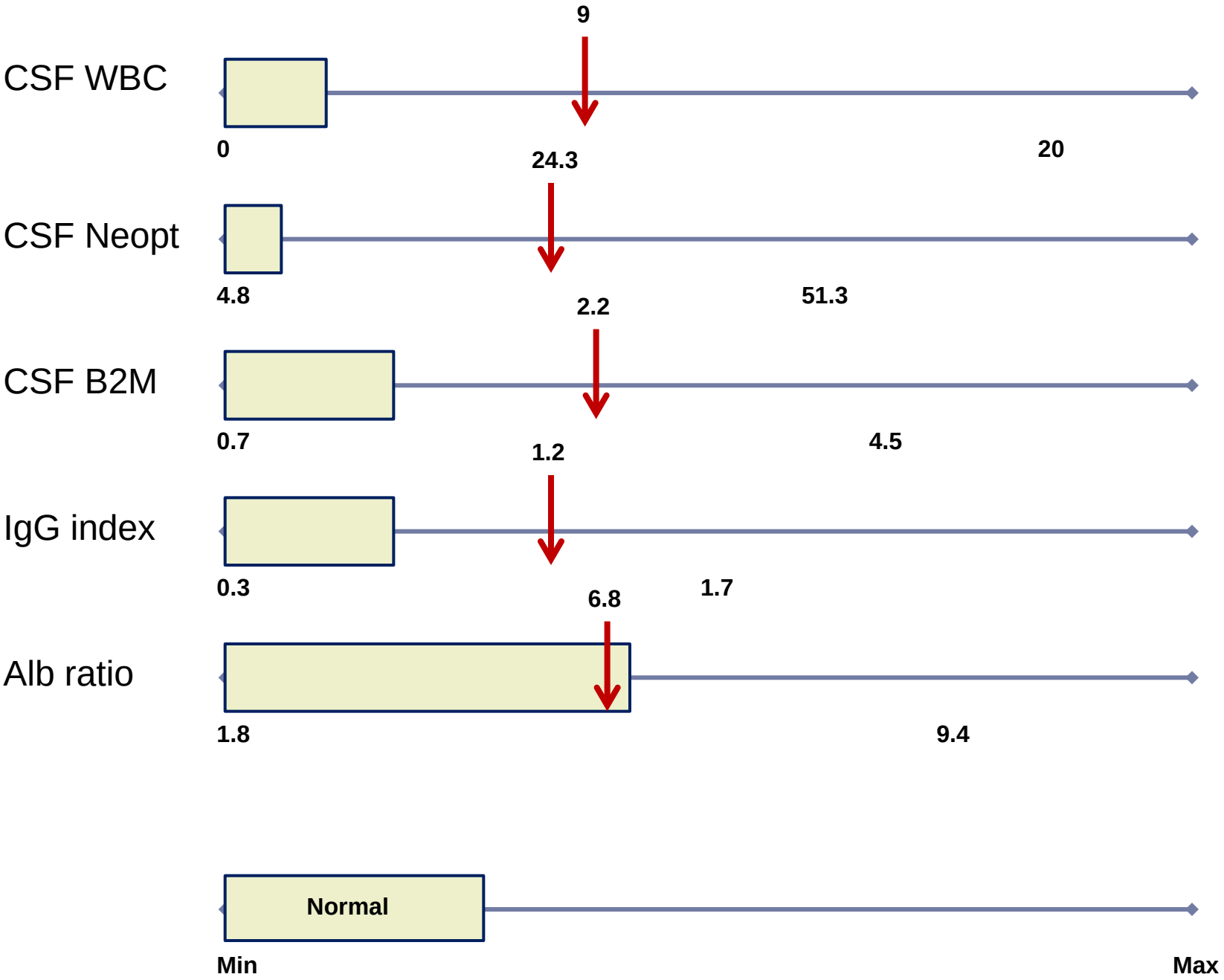
- Chronic headache
- Problem with concentration

- NP-testing (Cogstate): normal

Case 1

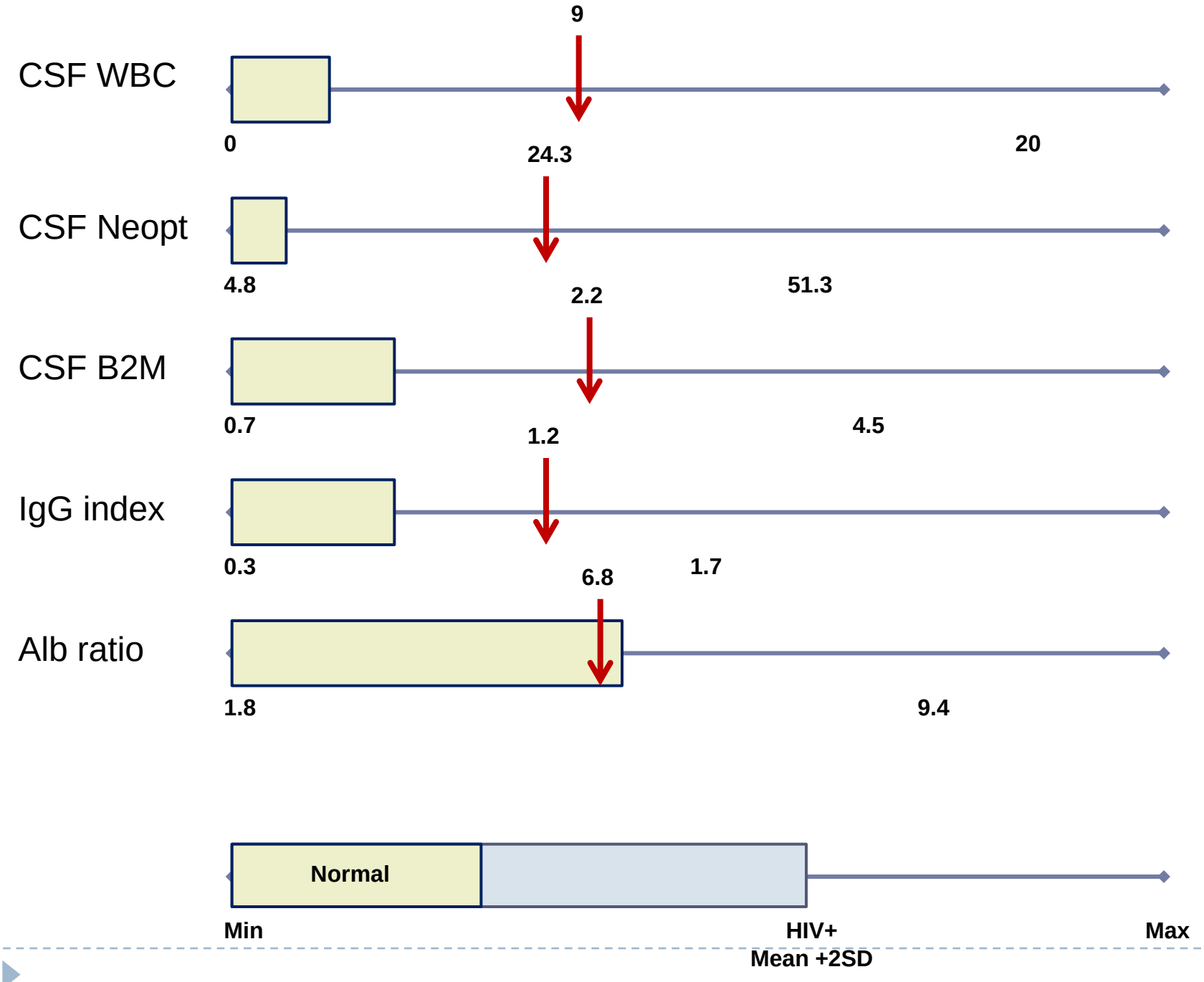
- LP

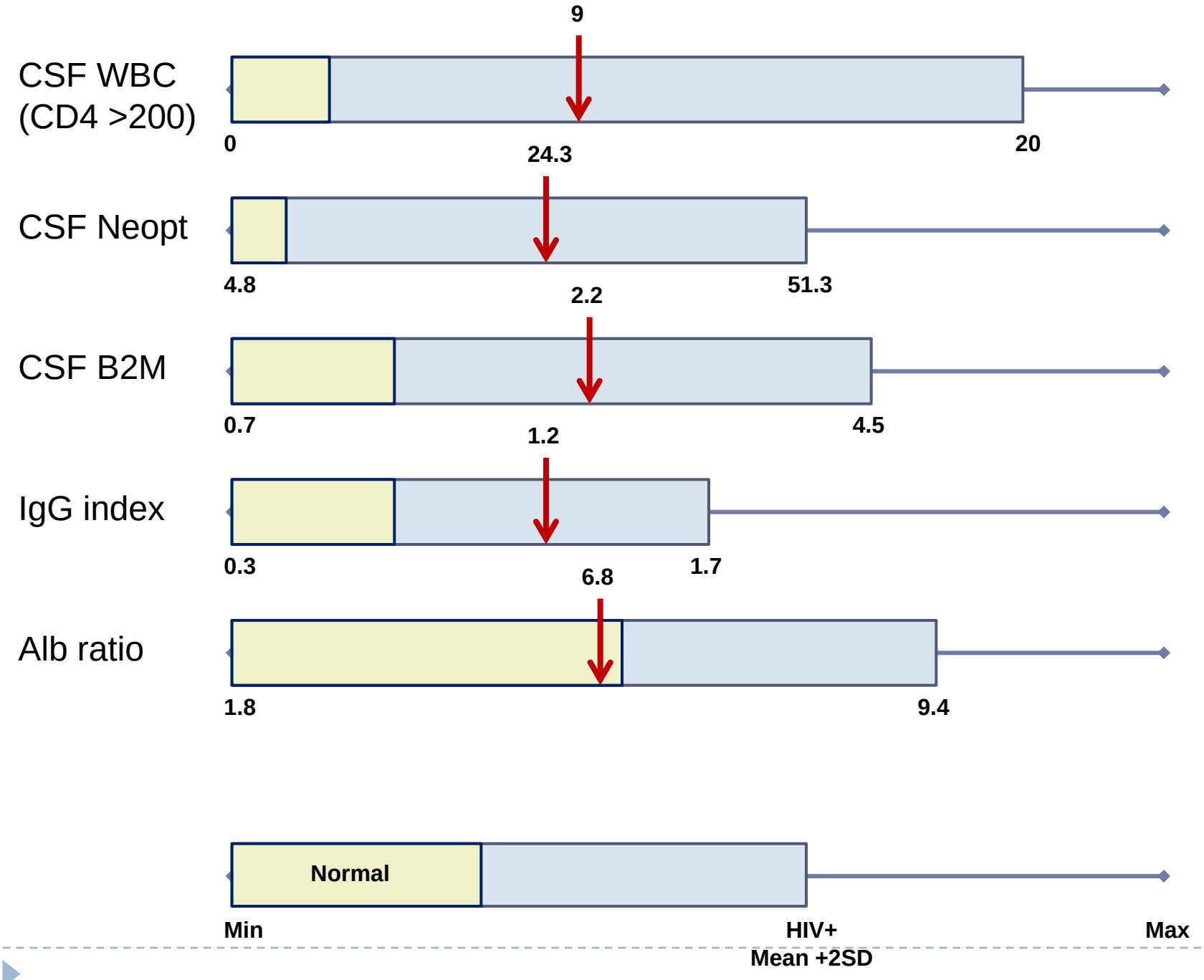
CSF HIV RNA	39 500 copies/mL = 4.6 log	
CSF WBC	9 /μL	(≤ 3)
IgG index	1.2	(<0.7)
CSF beta2-microglob	2.2 mg/L	(<1.2)
CSF neopterin	24.3 nmol/L	(<5.8)
CSF/P Albumin ratio	6.8	(<7.0)


CSF WBC
9
0
20
CSF Neopt
24.3
4.8
51.3
CSF B2M
2.2
0.7
4.5
IgG index
1.2
0.3
1.7
Alb ratio
6.8
1.8
9.4
Normal
Min
Max

CSF Biomarkers

- Not only important with knowledge about normal levels of CSF biomarkers, but also about common levels in asymptomatic HIV


CSF WBC
9
0
24.3
20
CSF Neopt
4.8
51.3
CSF B2M
2.2
0.7
4.5
IgG index
1.2
0.3
1.7
Alb ratio
6.8
1.8
9.4
Normal
Min
HIV+
Mean +2SD
Max

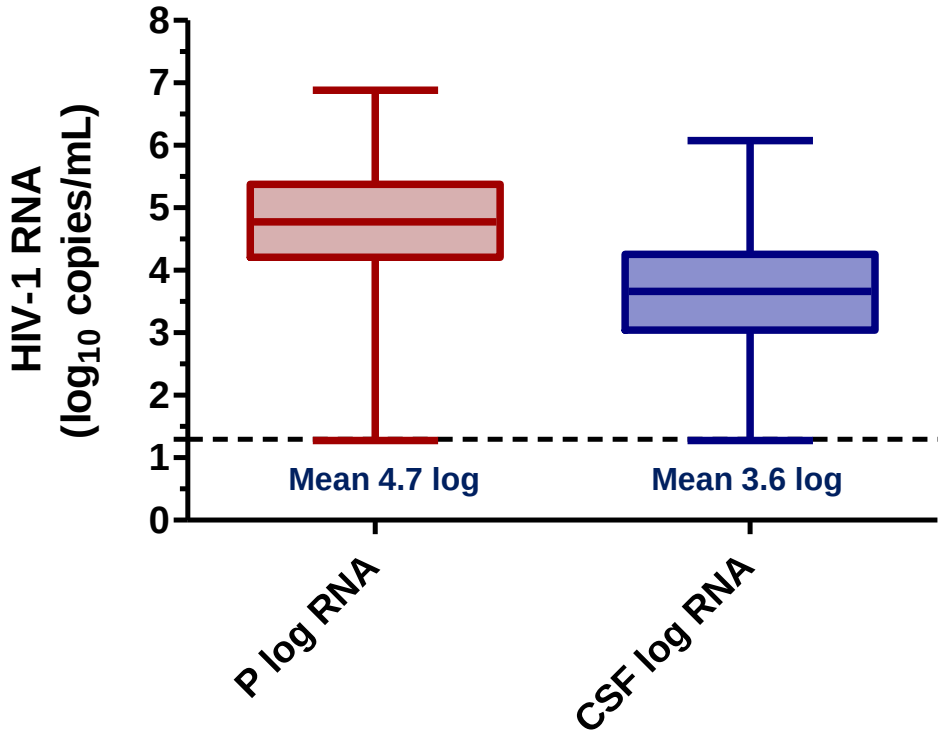

CSF WBC
(CD4 >200)
9
0
20
CSF Neopt
24.3
4.8
51.3
CSF B2M
2.2
0.7
4.5
IgG index
1.2
0.3
1.7
Alb ratio
6.8
1.8
9.4
Normal
Min
HIV+
Mean +2SD
Max

CSF Biomarkers

- **Viral**
- Immunoactivation
- Blood-brain barrier integrity
- Neuronal injury

CSF findings in HIV

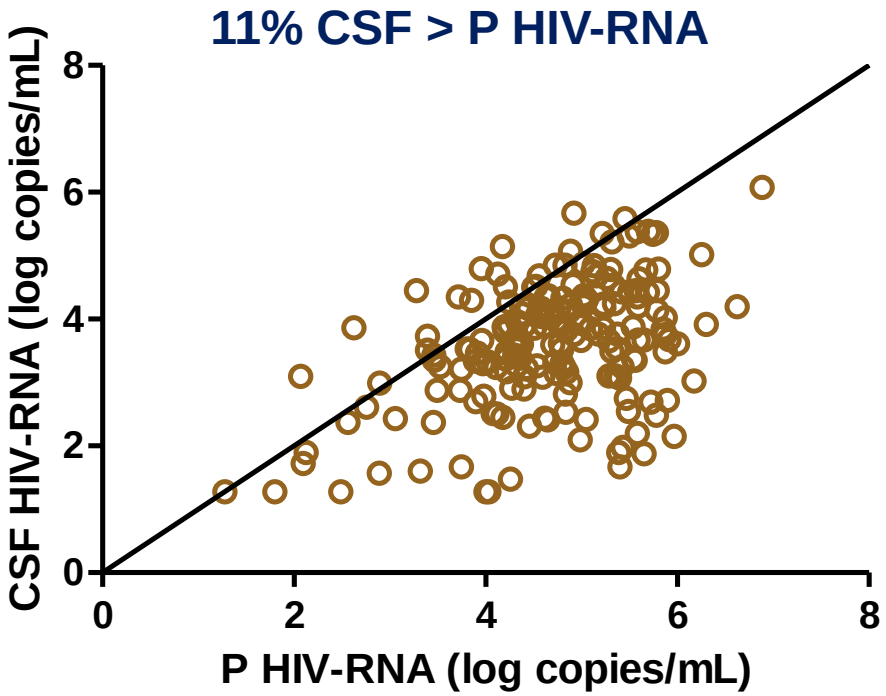
- 1. Untreated HIV - neuroasymptomatic
  - HIV-RNA



Data on file (n = 198)

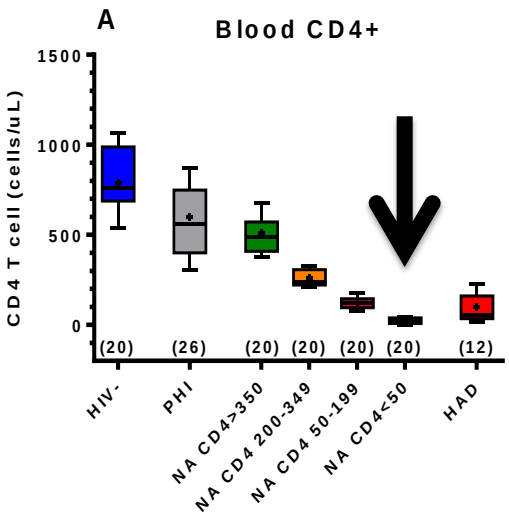
CSF findings in HIV

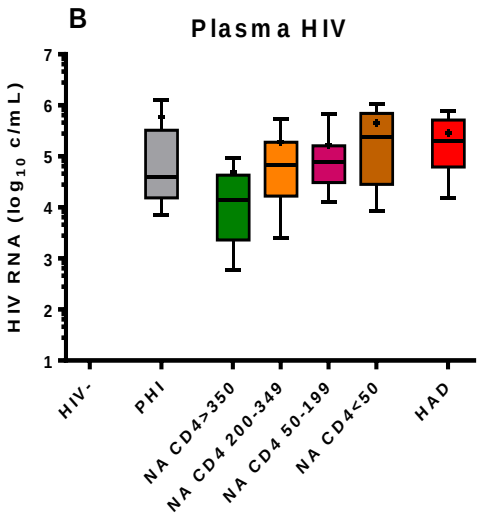
- 1. Untreated HIV - neuroasymptomatic
  - HIV-RNA

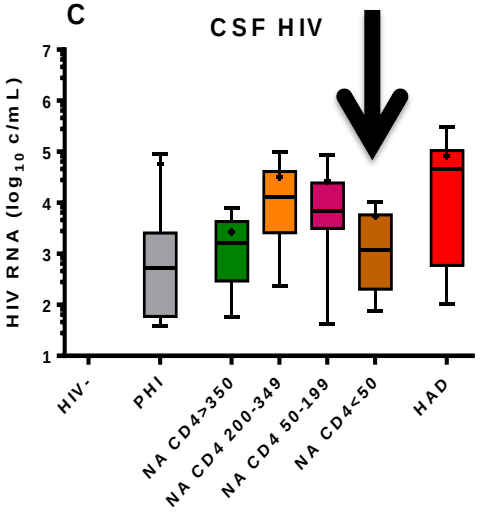


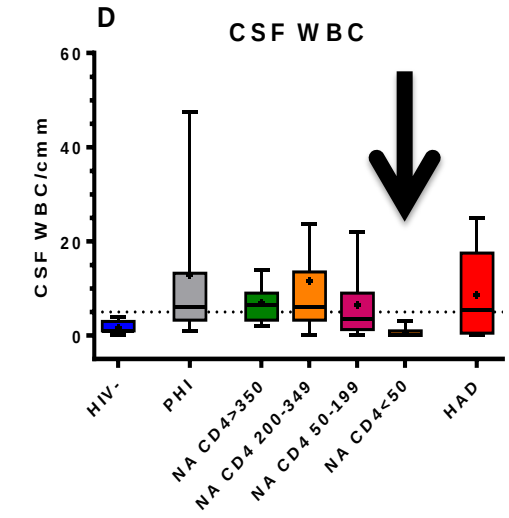
Data on file (n = 198)

Biomarkers Over the Entire Range of Infection



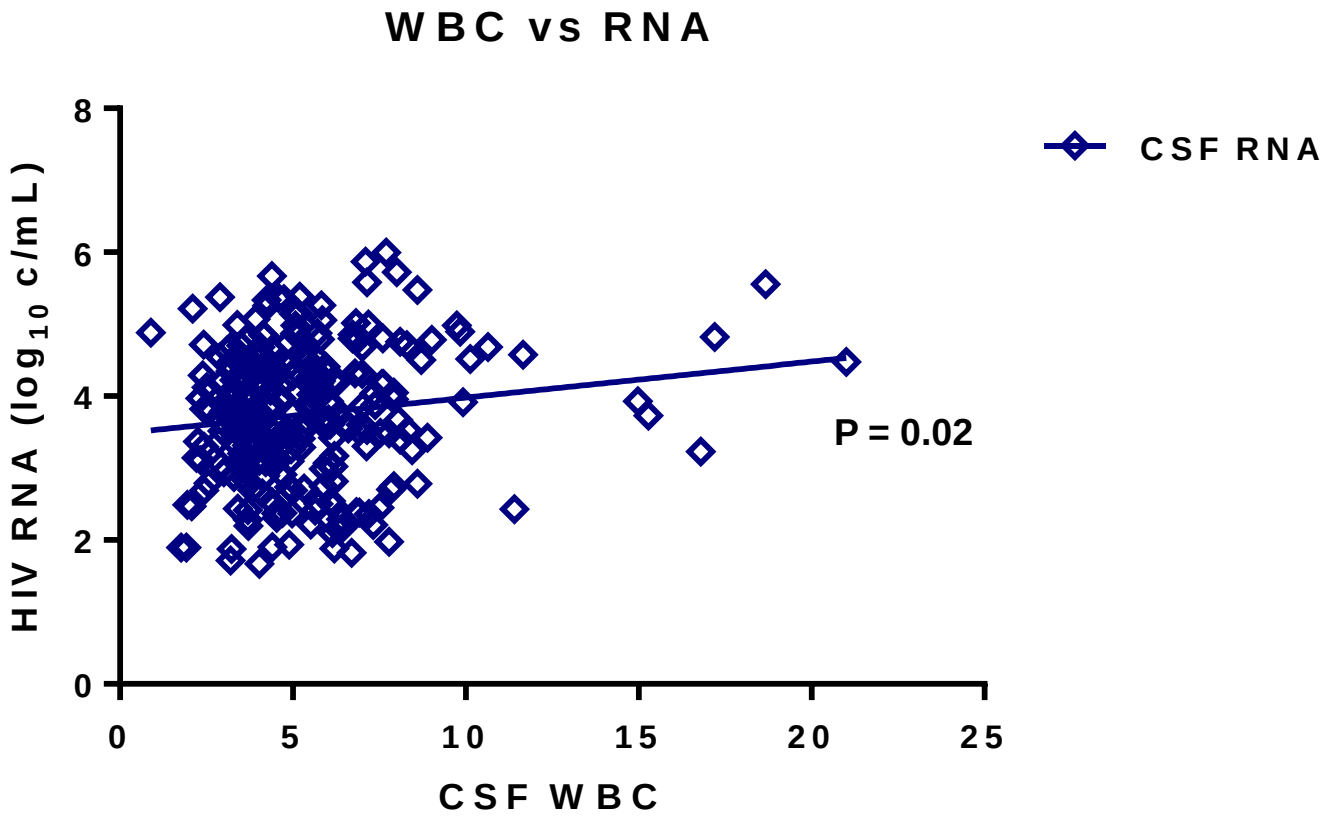






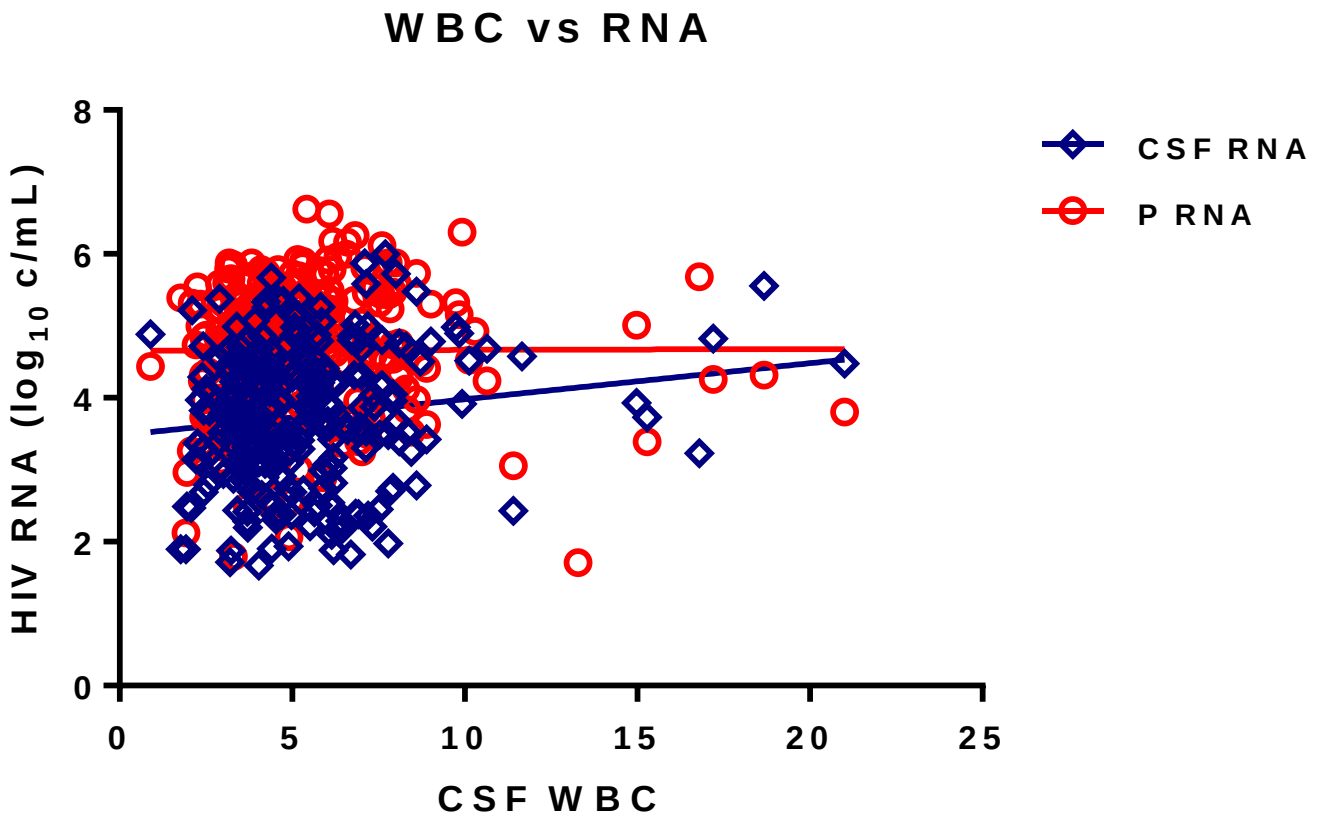
R. Price, UCSF

CSF cells correlates with CSF RNA



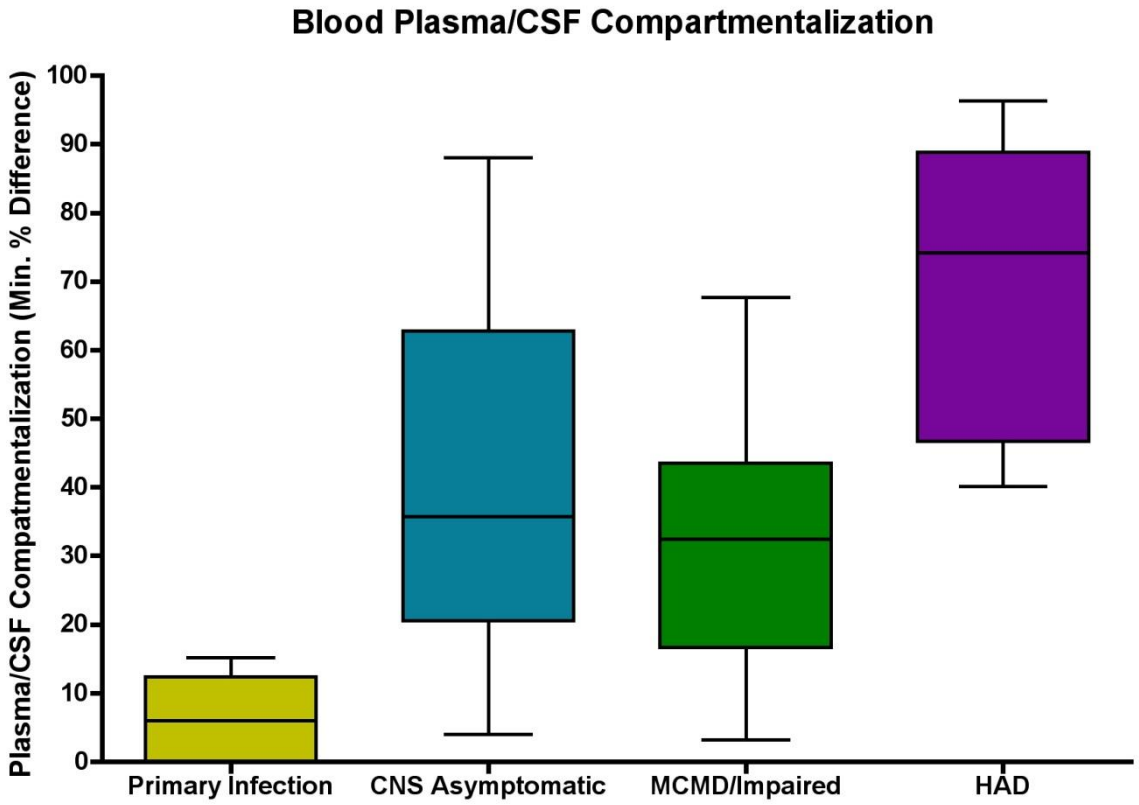
Data on file (n = 301)

CSF cells correlates with CSF RNA



Data on file (n = 301)

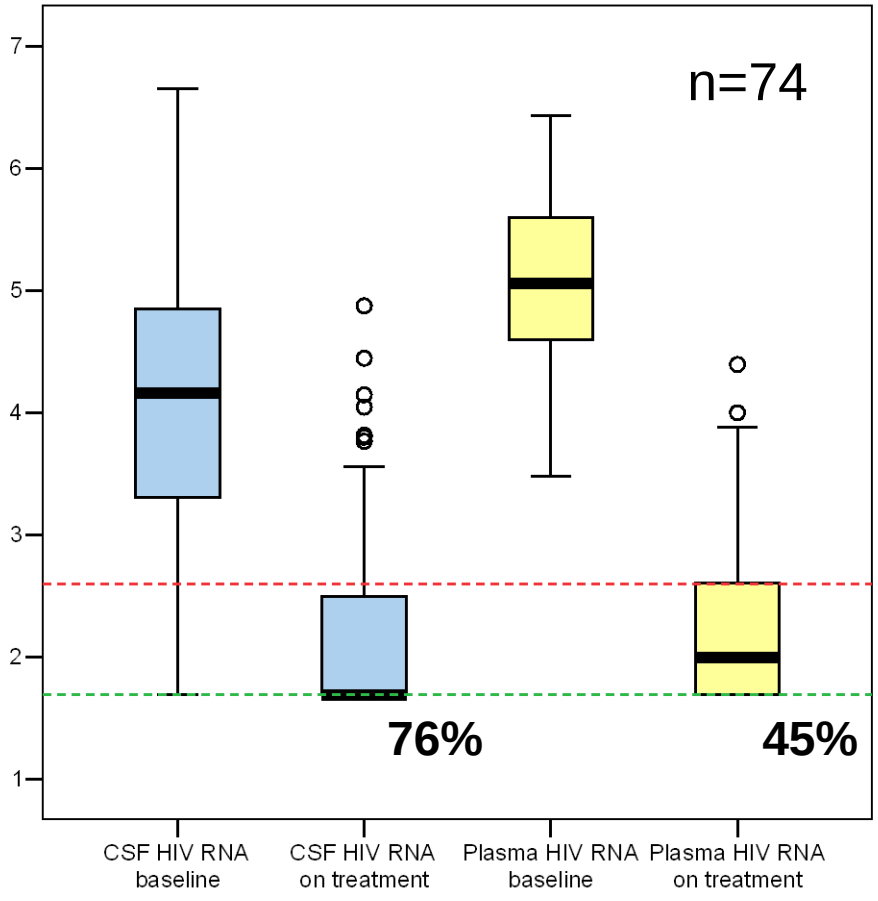
Analysis of CSF HIV-1 Compartmentalization across Spectrum of Disease by env HTA



PI vs. Asx p=0.002
Asx vs. MCMD/Imp p=0.2787
MCMD/Imp vs. HAD p=0.0004

Harrington PR et al. AIDS 2009; 23: 907

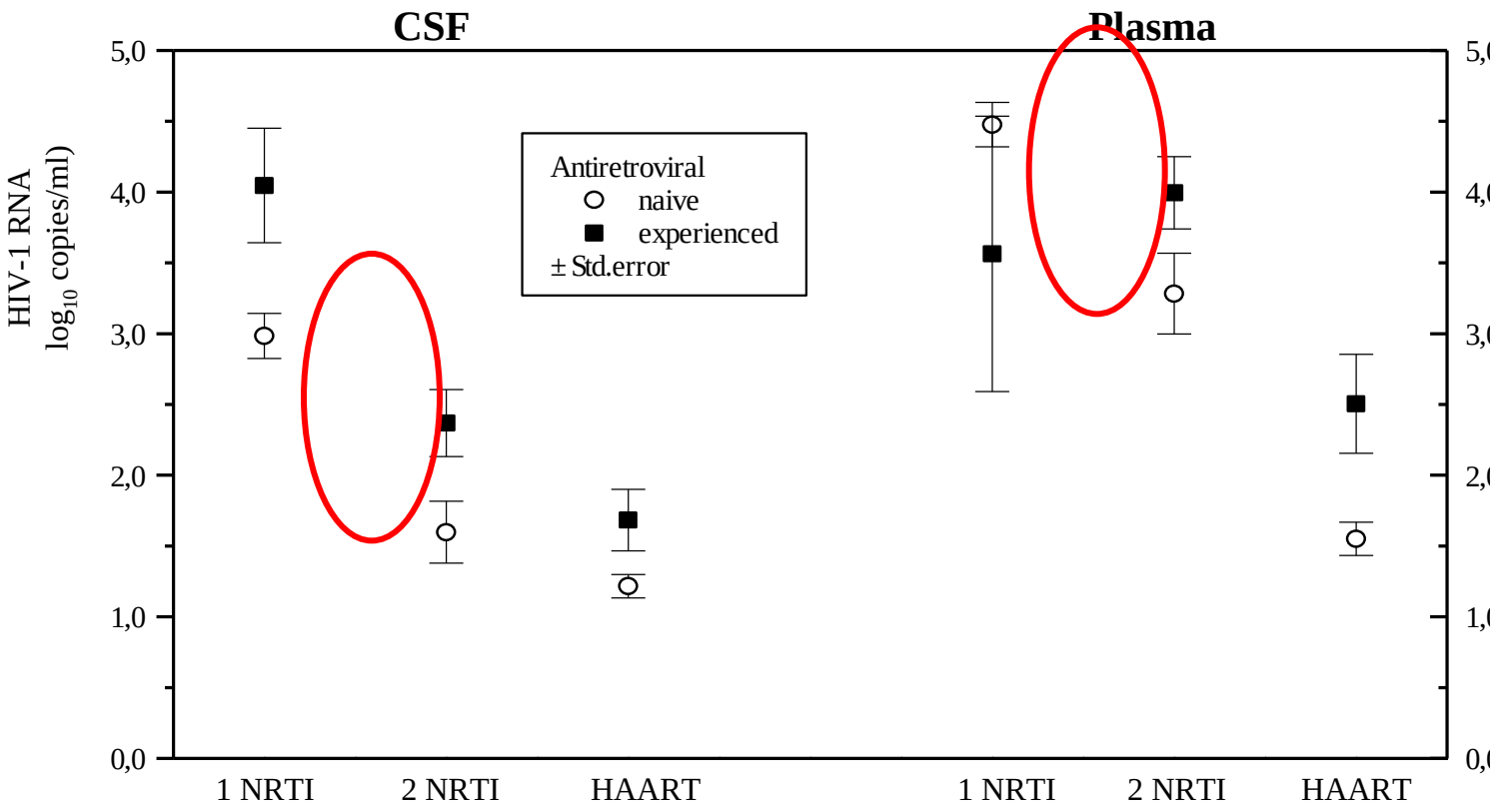
Normally excellent effect on CSF HIV RNA by cART



CSF and plasma HIV RNA before and after three months on HAART. Bars show median values and boxes show IQR. Hinges show range. Outliers are indicated as circles. Red dotted line shows detection limit of 400 copies/mL and green dotted line shows detection limit of 50 copies/mL.

Mellgren Å et al. *Antiviral Therapy* 2005; 10: 701

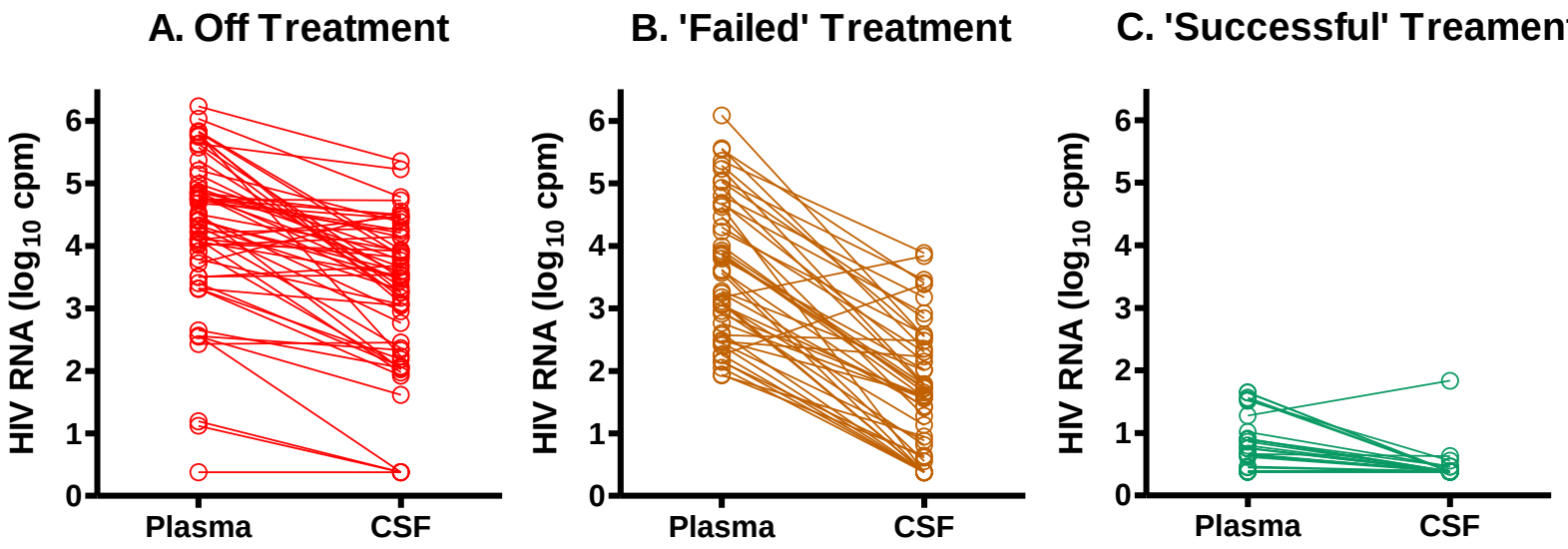
Better effect in CSF than in blood?



Scand J Infect Dis 2000; 32: 365

CSF HIV Responses to Treatment:

Relation of CSF to Plasma HIV-1 RNA in Individual Subjects



Price RW & Spudich S. JID 2008; 197 Suppl 3:S294

Symptomatic CSF viral escape

Cognitive and/or neurological symptoms

Undetectable (or low) plasma viral load

High CSF viral load with drug resistance mutations

- Canestri A et al. Clin Infect Dis 2010;50:773–77
  - 11 cases
- Peluso M et al. AIDS 2012;26(14):1765-74
  - 10 cases
- Several case reports

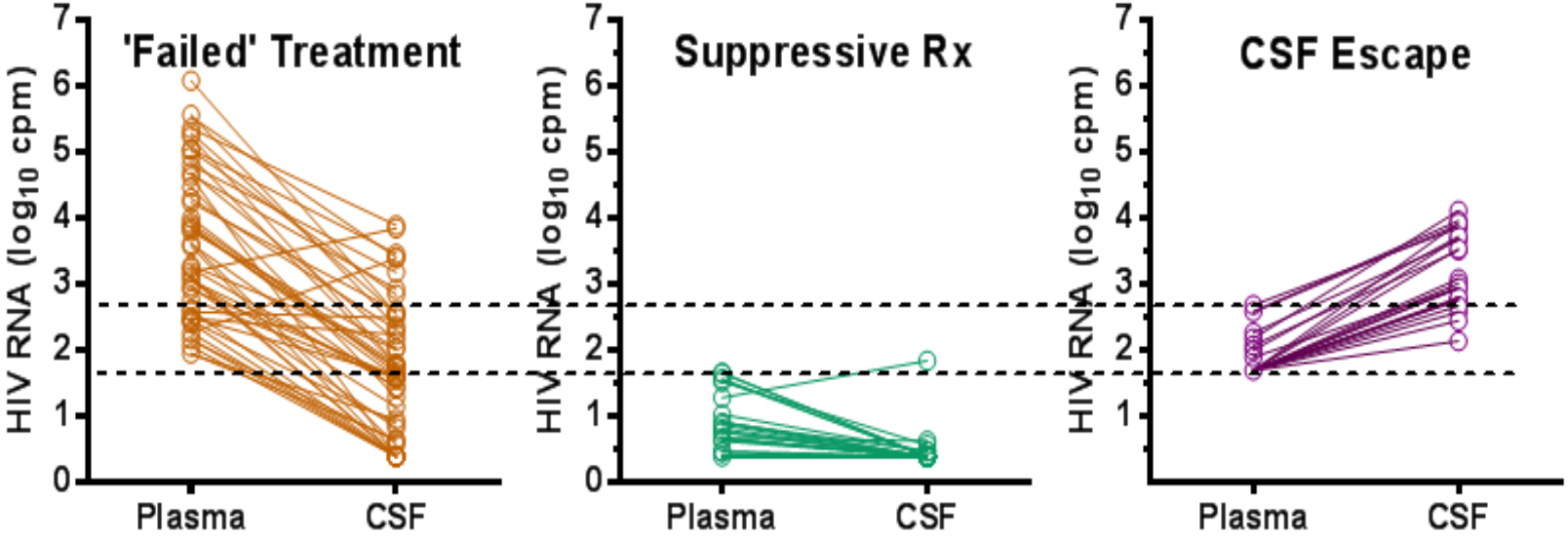
CSF Escape: Canestri & Peluso Series

Variable	Median (IQR)	Range
Blood CD4 (mean, SD, range)	520 (308 -592)	107 - 660
Nadir Blood CD4 (mean, SD)	55 (12 - 145)	2 - 250
*CSF WBC (median, range)**	22 (10 - 55)	0 - 200
Plasma HIV (log_{10}; median, IQR)	1.69 (1.69 - 2.68)	1.69 - 2.68
CSF HIV (log_{10}; median, IQR)	3.01 (2.76 - 3.72)	2.13 - 4.11
CSF:Plasma Difference (log_{10}; median, IQR)	1.25 (1.06 - 1.44)	0.44 - 2.23
Significant Resistance (% tested)	14/16 (70.6%)	

* Peluso only; Canestri median 31, range 6-270

Canestri A et al. Clin Infect Dis. 2010;50:773-778
Peluso MJ et al. AIDS 2012;26:1765-1774

CSF Escape: Canestri & Peluso Series



Canestri A et al. Clin Infect Dis. 2010;50:773-778
Peluso MJ et al. AIDS 2012;26:1765-1774

CSF HIV RNA >50 in 10% of asymptomatic subjects with plasma RNA <50 copies/mL

Efavirenz
Lopinavir/r
Atazanavir/r

+

Tenofovir
Abacavir
Zidovudine

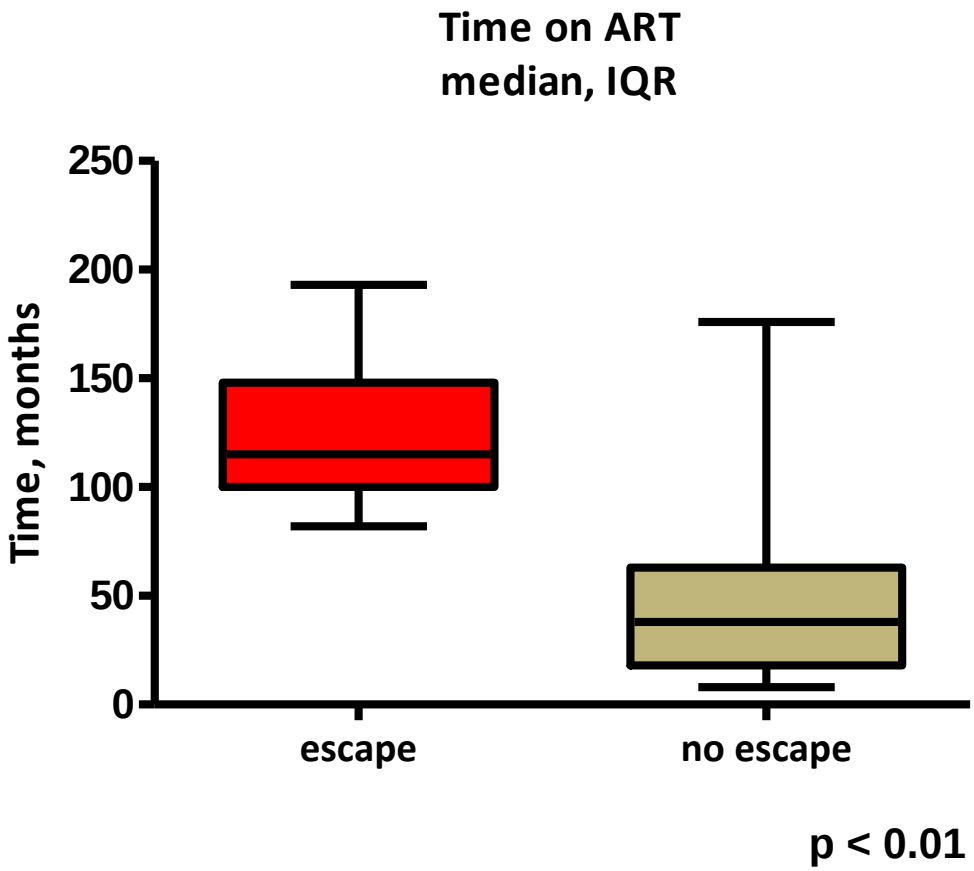
+

Lamivudine
Emtricitabine

Characteristic	CSF escape (n=7)	CSF suppressed (n=60)
Age, years median (range)	46 (36-64)	45 (22-71)
CD4 cell count, x106 cells/l median (IQR)	620 (400-810)	525 (393-633)
CD4 nadir, x106 cells/l median (IQR)	125 (33-213)	146 (54-200)
CSF HIV-1 RNA, copies/ml median (IQR)	121 (52-860)	<40
CPE-rank median (IQR)	2 (1-2)	1.75 (1-2)
CSF-neopterin, nmol/l median (IQR)	9.2 (6.6-16.2)*	4.9 (4.4-8.3)
P-neopterin, nmol/l median (IQR)	7.2 (6.1-8.0)	7.6 (4.9-9.7)
WBC, x106 cells/l median (IQR)	2.5 (1-9.5)	1 (1-3)
Total time on ART, months median (IQR)	131 (96-159)***	39 (18-64)

Edén A et al. J Infect Dis 2010; 202:1819

Patients with CSF escape had been longer time on ART



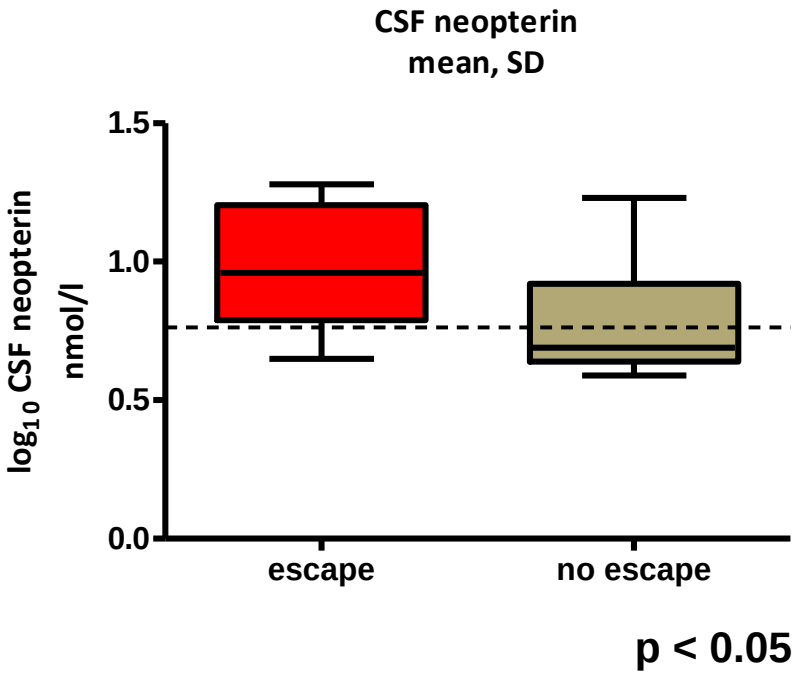
Edén A et al. J Infect Dis 2010; 202:1819

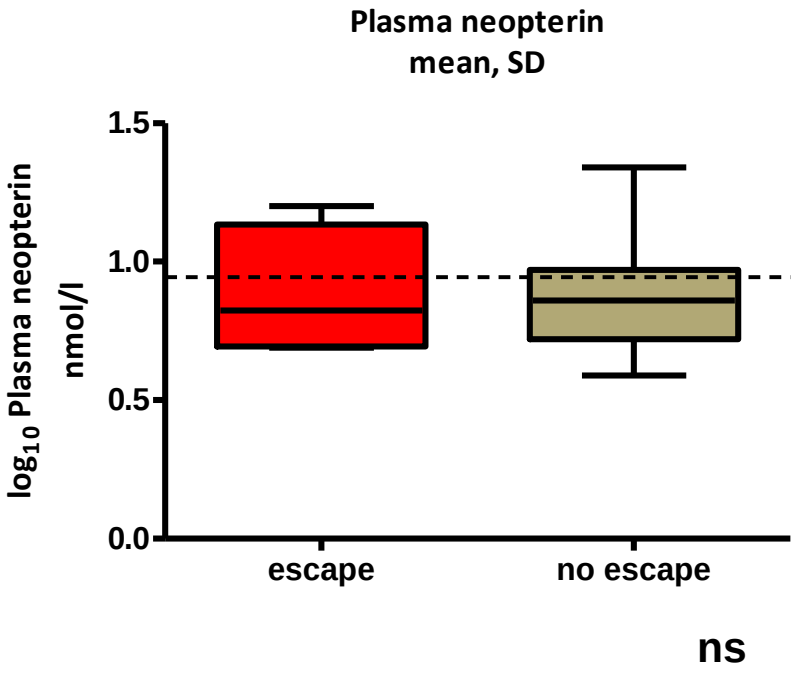
and had a history of more plasma viral blips and periods with treatment interruptions

	CSF escape	No escape	Sign
Viral blips plasma			
median (IQR)	2.5 (1-4)	0 (0-1)	p = 0.001
History of treatment interruptions	71%	15%	p < 0.01

Edén A et al. J Infect Dis 2010; 202:1819

CSF escape associated with intrathecal immunoactivation





Edén A et al. J Infect Dis 2010; 202:1819

Cerebrospinal Fluid Viral Blips in HIV Infected Patients on ART

Study design

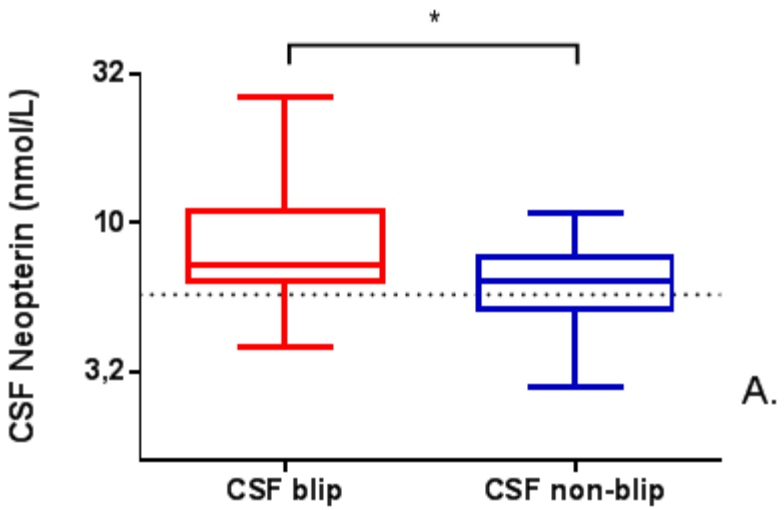
- **75 subjects on effective ART >6 months, plasma HIV-1 RNA <50 c/mL**
- **Longitudinal follow up: ≥2 (median 5) lumbar punctures**
- **HIV-1 RNA in CSF and plasma analyzed by *real time* PCR (Cobas TaqMan v2, Roche)**

Results

- **35 % CSF RNA >20 c/mL on ≥1 time point (23 % >50 c/mL)**
- **8 % >20 c/mL in consecutive samples (3 % >50 c/mL)**
- **Of all 373 CSF samples, 10 % had >20 c/mL (5 % >50 c/mL)**

Edén A et al. *CROI* 2014 Abstract #445

Cerebrospinal Fluid Viral Blips in HIV Infected Patients on ART



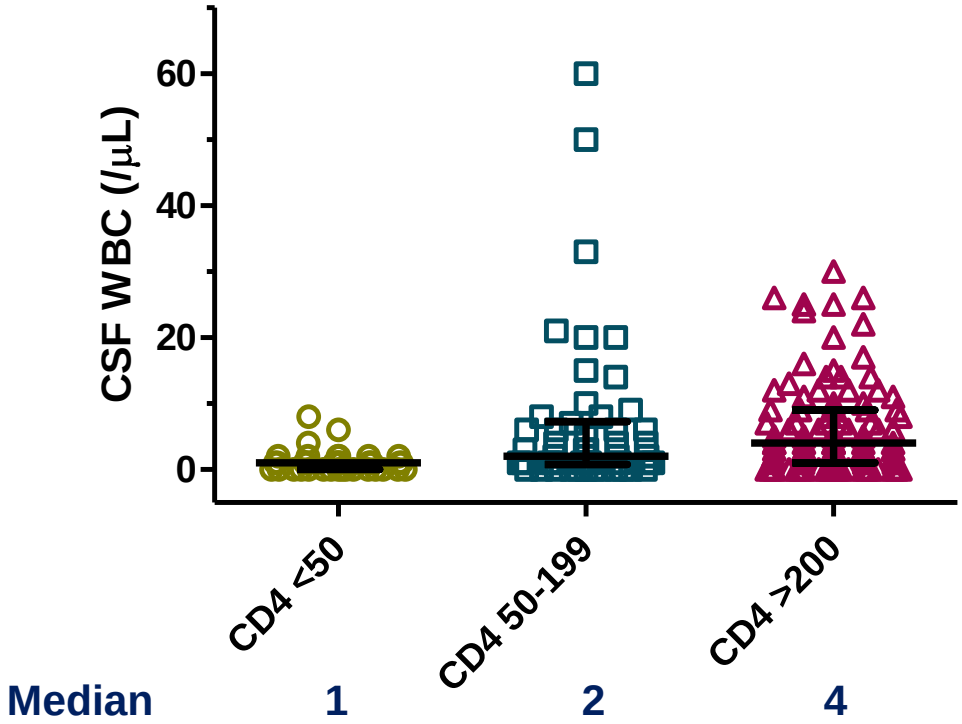
Edén A et al. *CROI* 2014 Abstract #445

CSF Biomarkers

- Viral
- **Immunoactivation**
- Blood-brain barrier integrity
- Neuronal injury

CSF findings in HIV

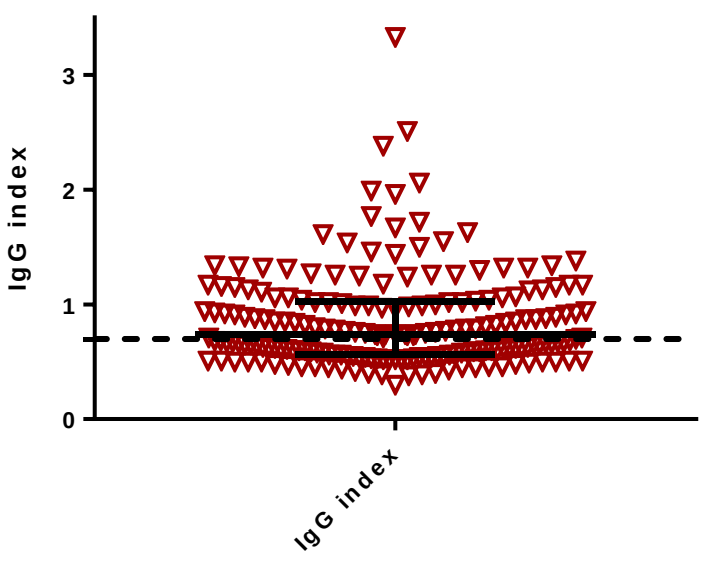
- 1. Untreated HIV - neuroasymptomatic
  - CSF WBC

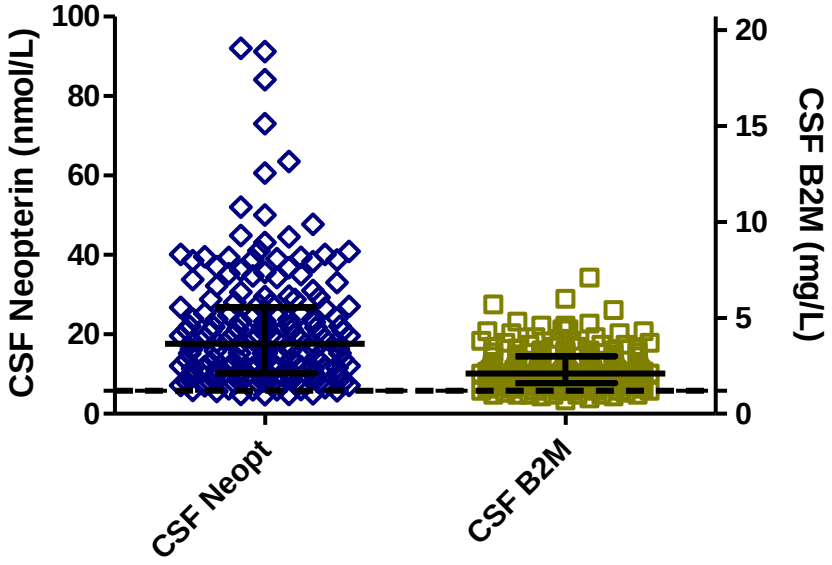


Data on file (n = 198)

CSF findings in HIV

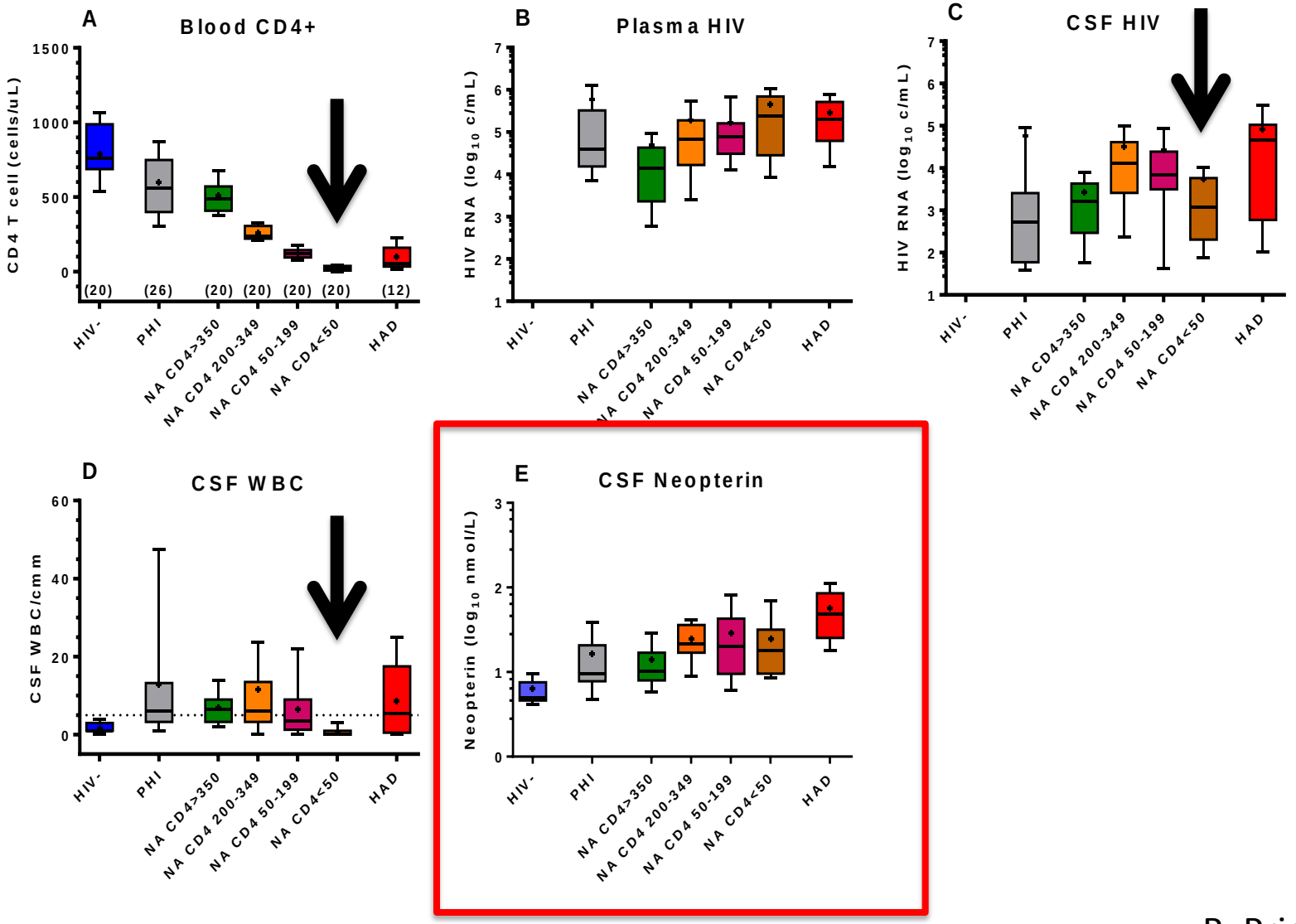
- 1. Untreated HIV - neuroasymptomatic
  - Markers of immunoactivation





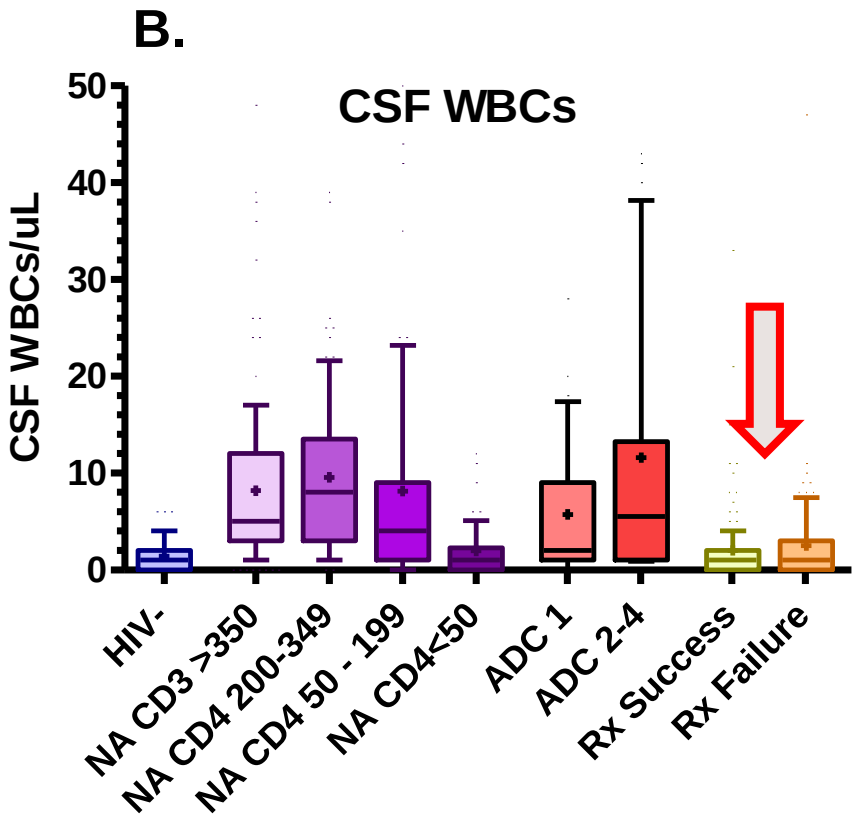
Data on file (n = 198)

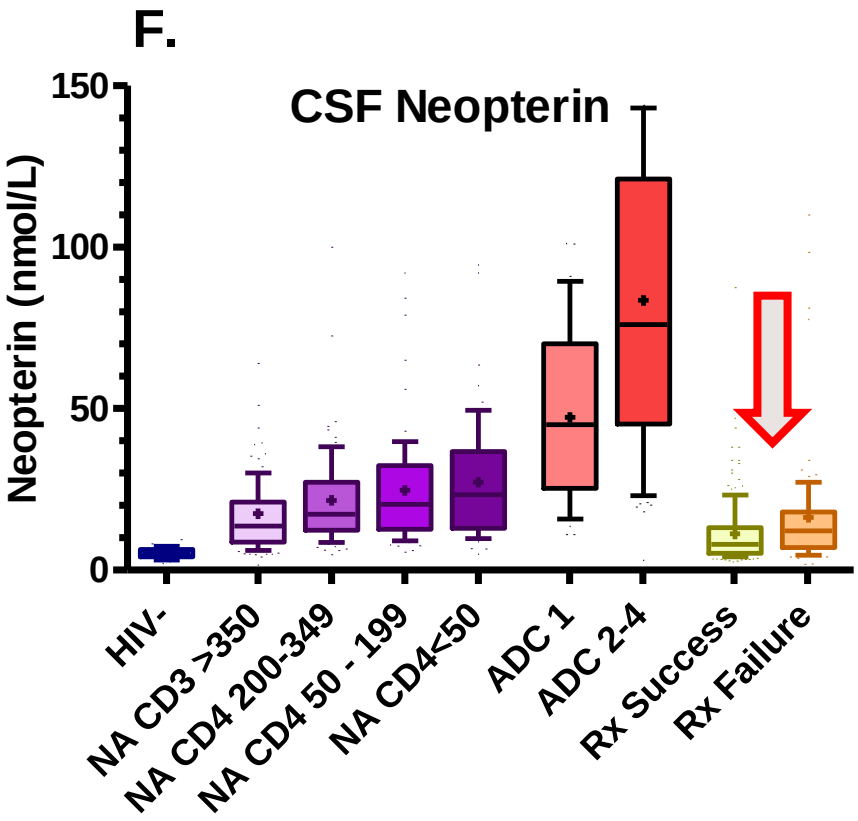
Biomarkers Over the Entire Range of Infection



R. Price, UCSF

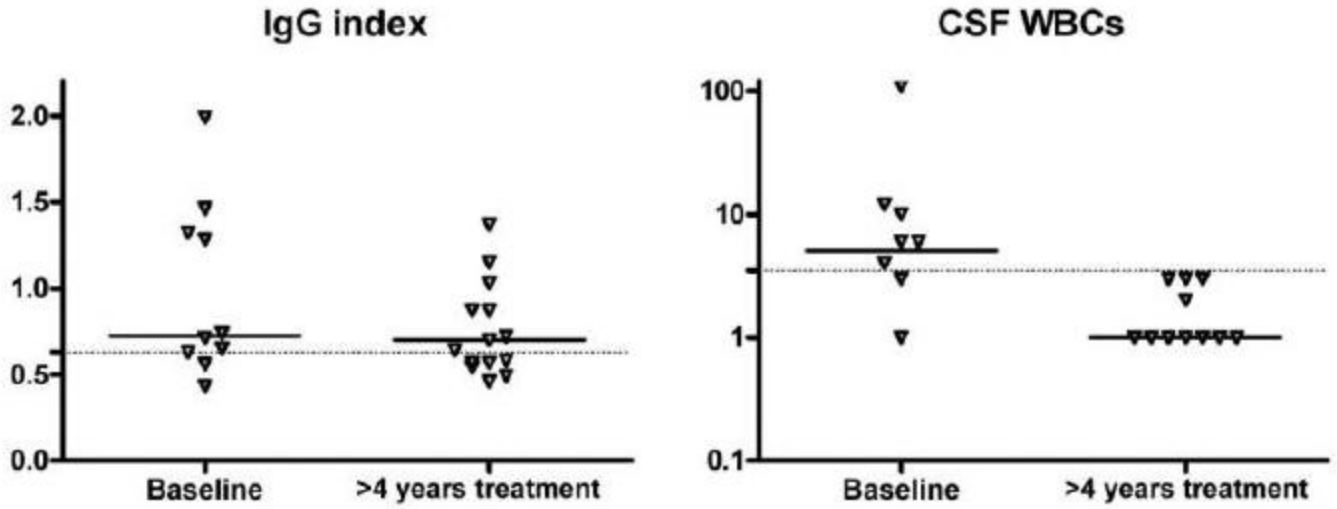
Treatment effects





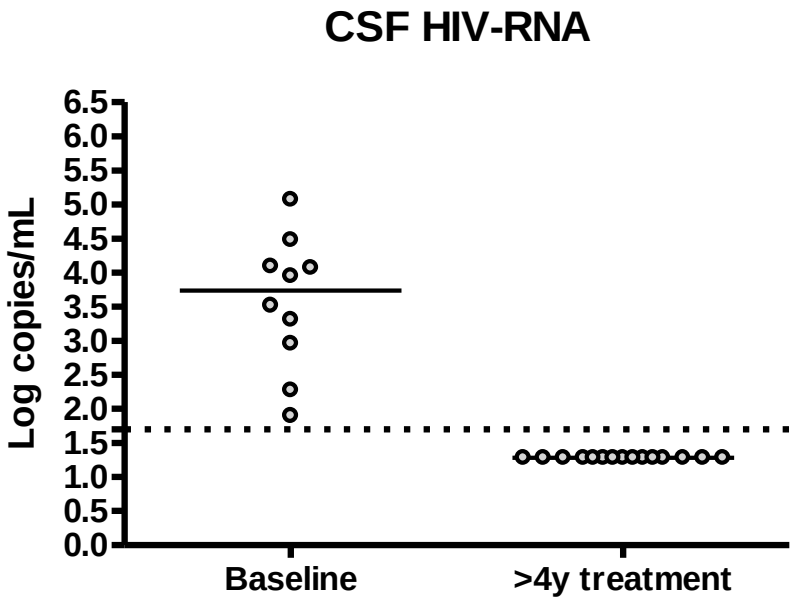
Hagberg L et al. *AIDS Res Ther.* 2010; 7: 15

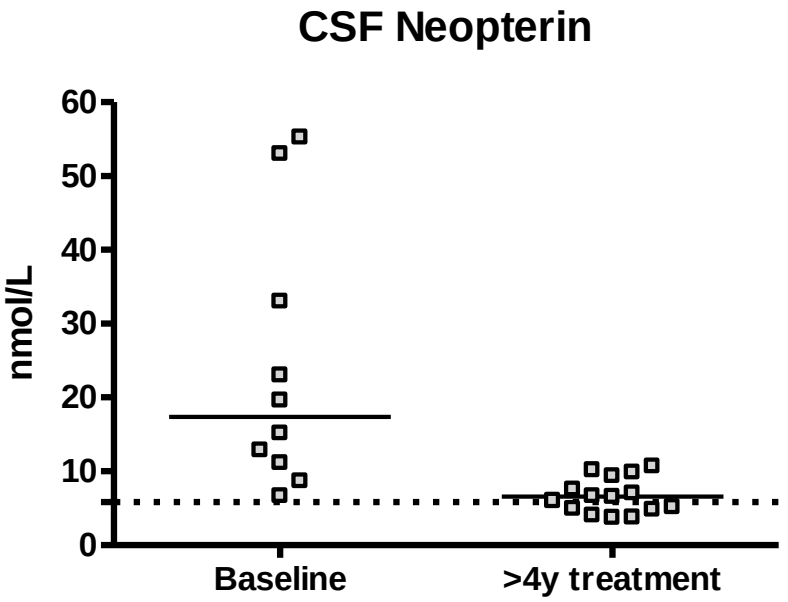
ART normalizes CSF WBC but not intrathecal immunoglobulin production



Edén A et al. *J Infect Dis* 2007; 196: 1779

Increased CSF neopterin during treatment

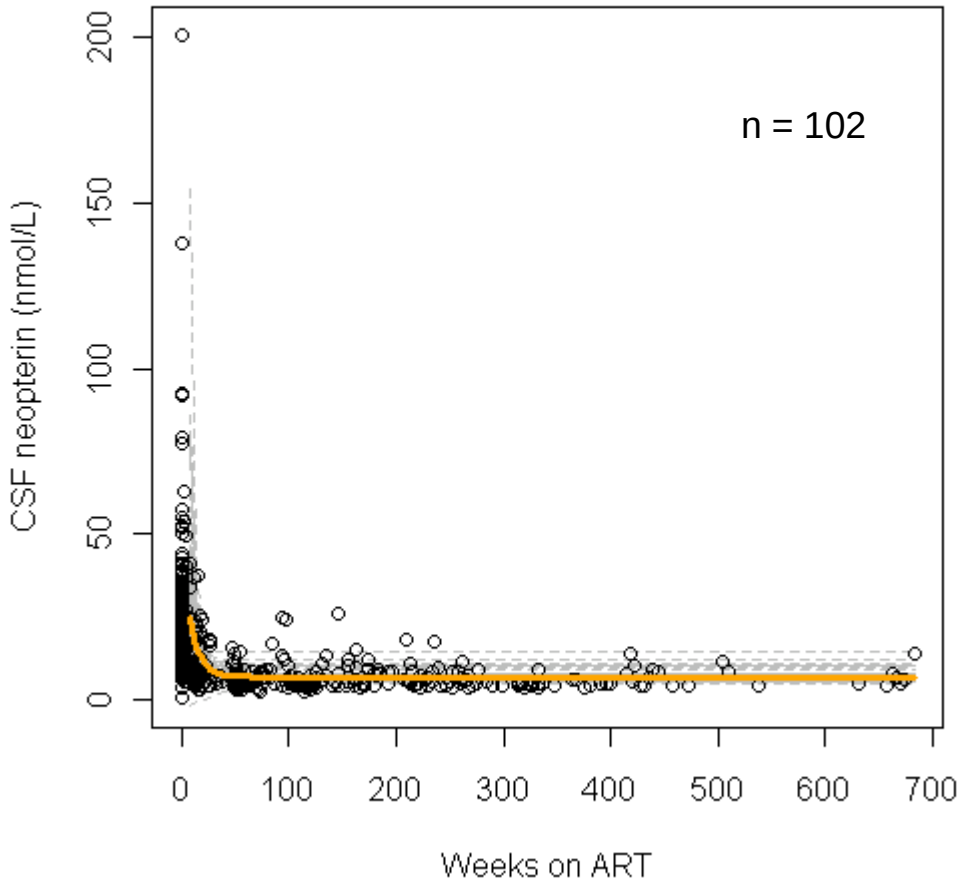




Edén A et al. *J Infect Dis* 2007; 196: 1779

CSF neopterin decay characteristics after initiation of ART

ART-naïve asymptomatic patients starting cART



Non-linear model to estimate neopterin decay in response to ART and a stable neopterin "set-point" attained after prolonged ART

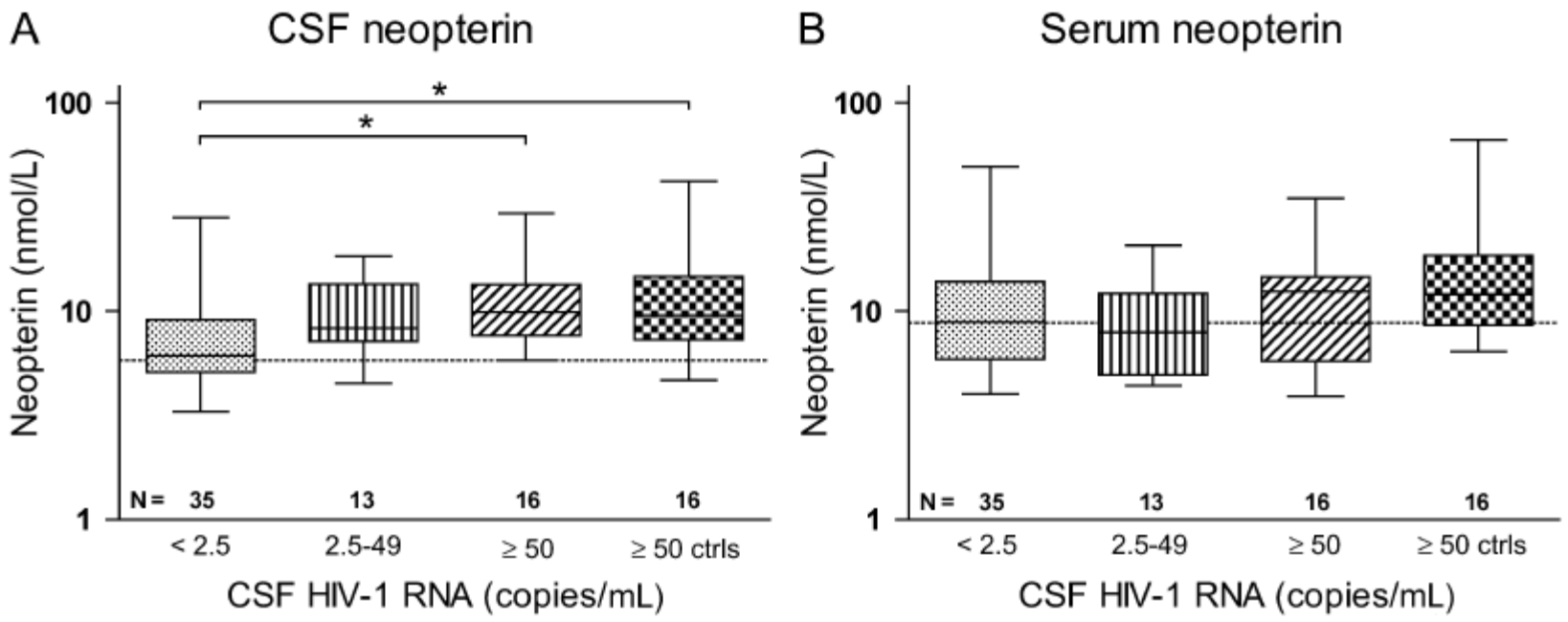
Results:

41% end up with stable elevated levels of CSF neopterin on suppressive long-term ART

Yilmaz A et al. J Neuroinflammation 2013

Neopterin and CSF HIV RNA

Subjects on HAART with plasma VL <50 copies/mL.
CSF-RNA measured by sensitive PCR

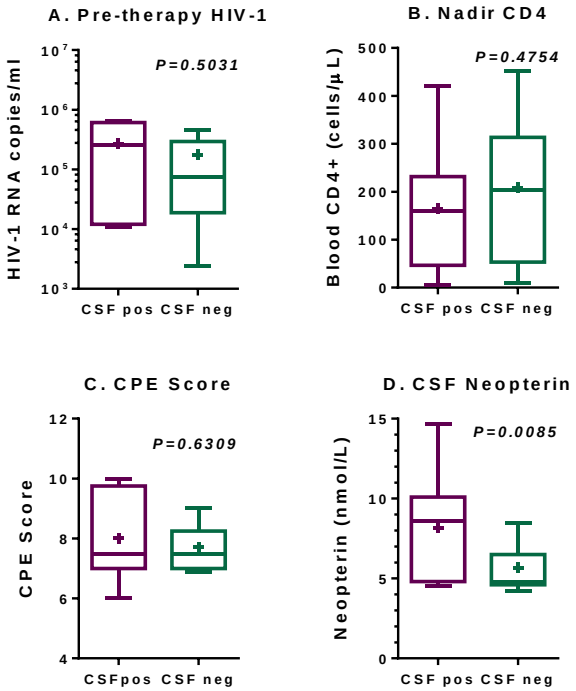


Yilmaz A et al. *J AIDS* 2008; 47: 168

Low levels of HIV RNA in CSF after up to 10 years of suppressive therapy - associated with local immune activation

Table 2. SCA HIV-1 RNA measurements.

	CSF	Plasma	P-value
Proportion of positive sampels	12/70	39/68	P<0.0001
HIV-1 RNA concentrations during suppressive therapy (mean HIV-1 RNA copies/mL, range)	0.3 (0.2-3.9)	1.1 (0.2-15)	P<0.0001
Time of suppressive therapy to time point with detectable HIV-1 RNA (median years, range)	3 (2-10)	2 (0.4-11)	P=0.0917

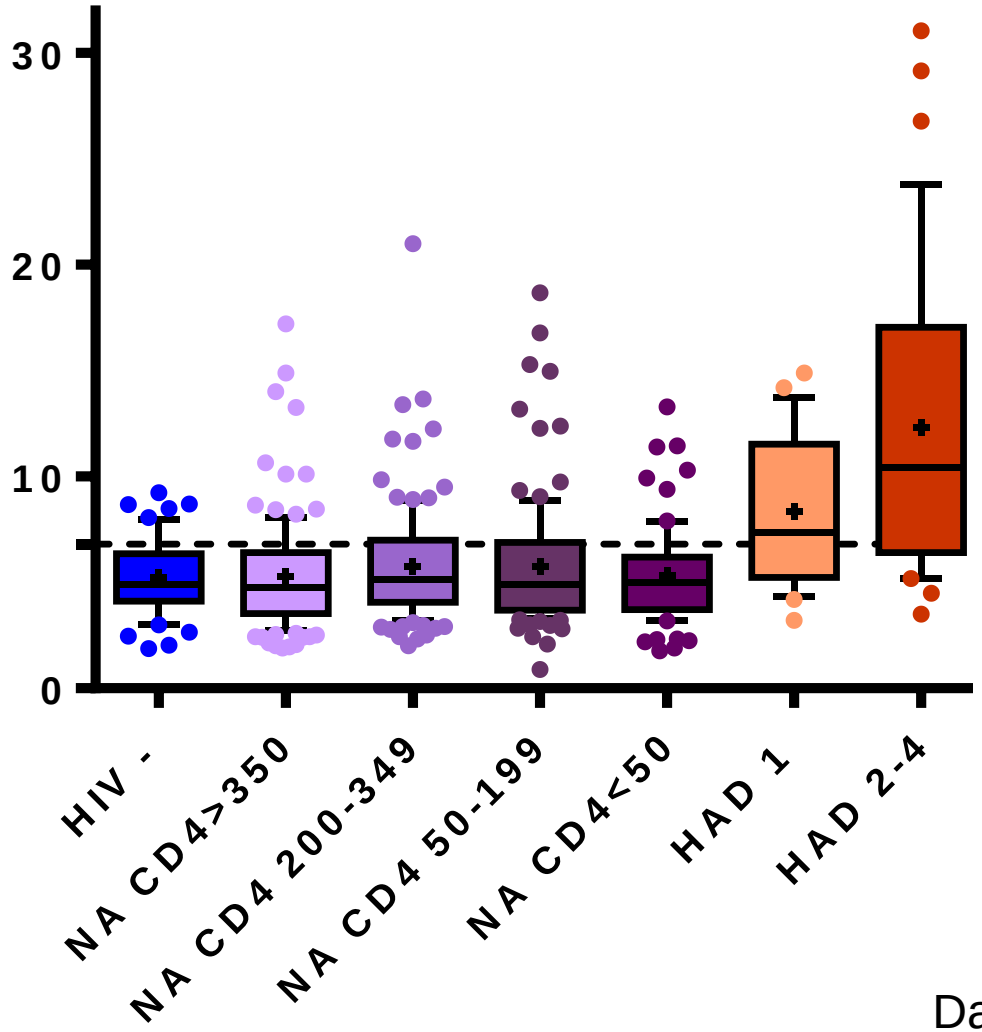


Dahl V et al. Submitted

CSF Biomarkers

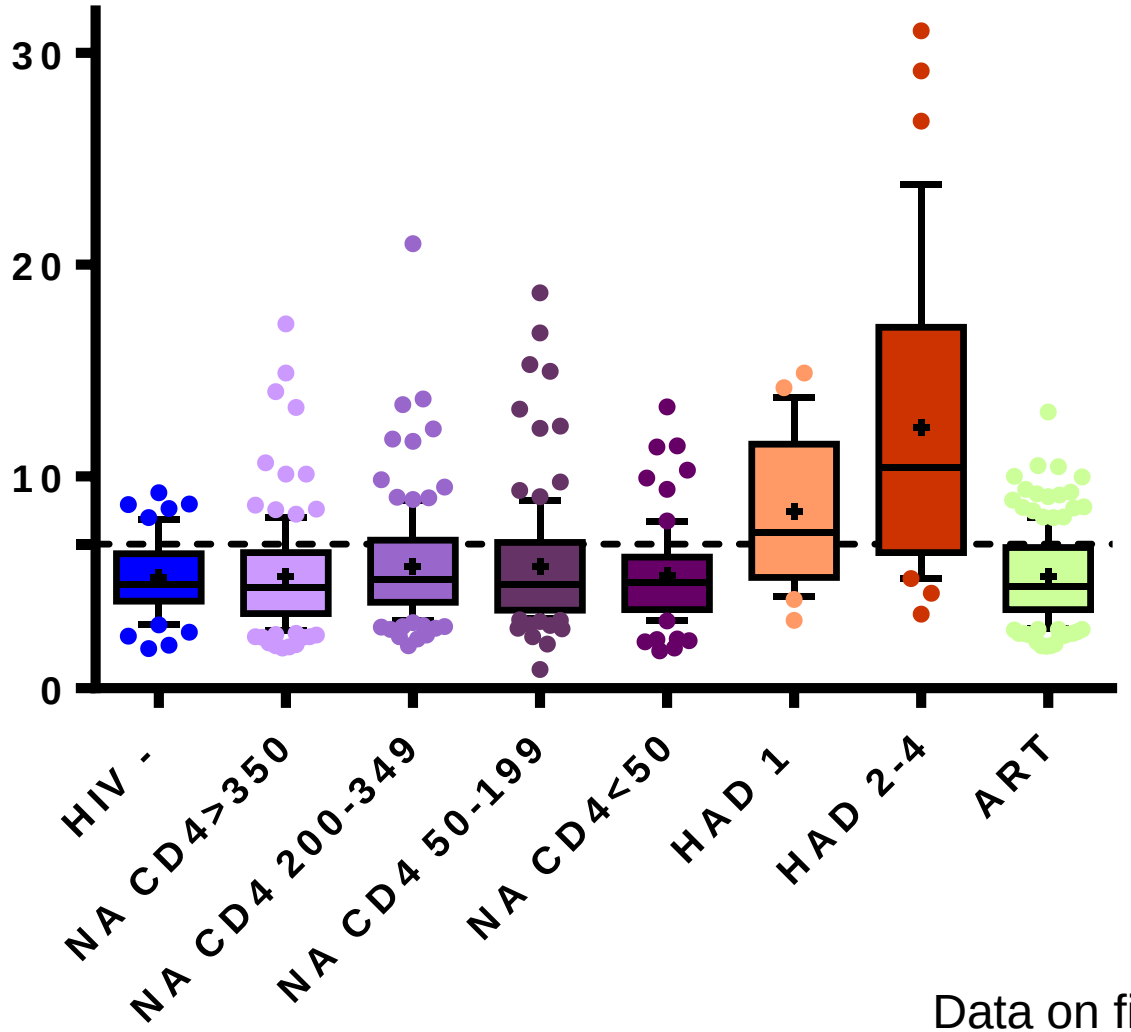
- Viral
- Immunoactivation
- **Blood-brain barrier integrity**
- Neuronal injury

Alb Ratio



Data on file (n = 710)

Alb Ratio



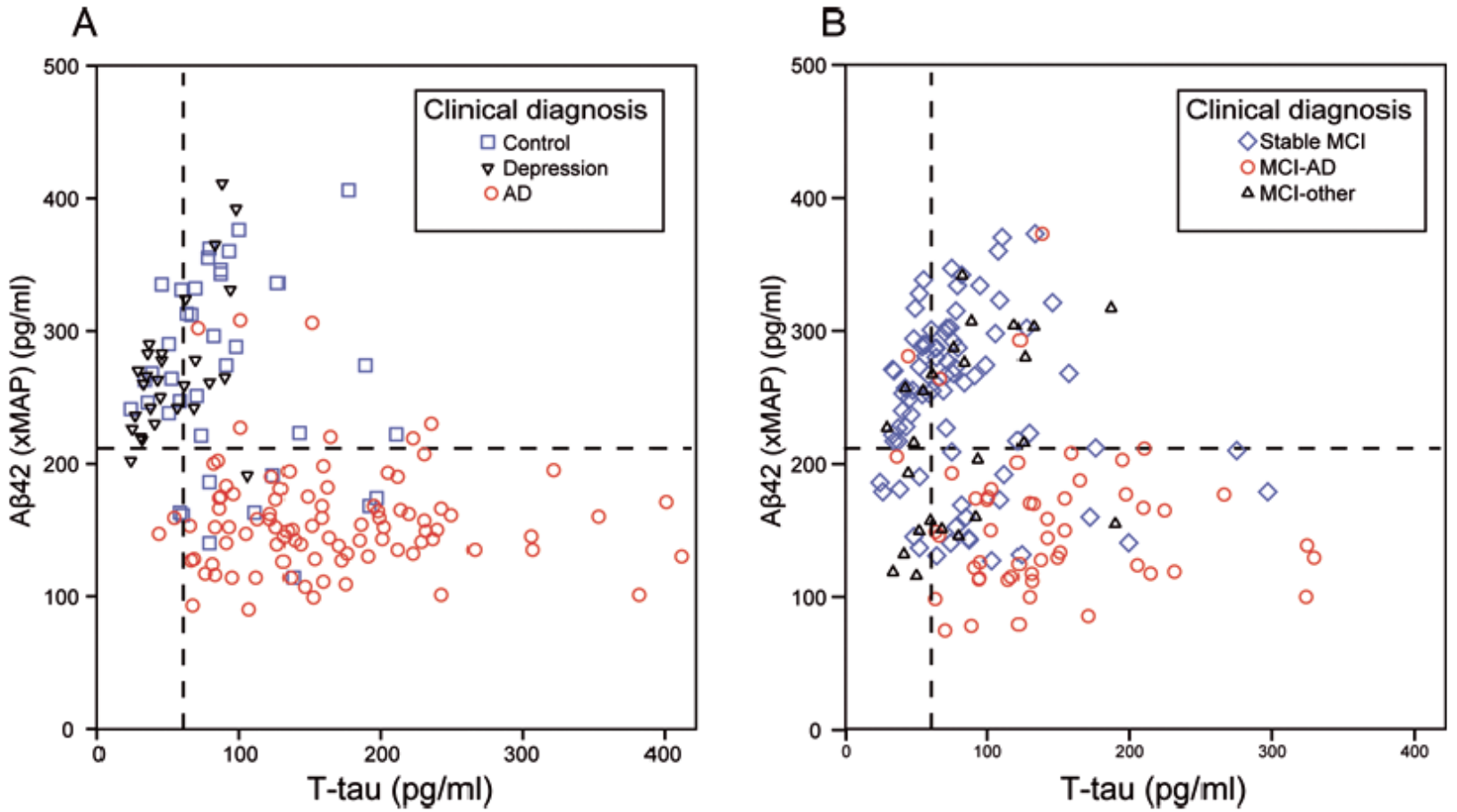
Data on file (n = 710)

CSF Biomarkers

- Viral
- Immunoactivation
- Blood-brain barrier integrity
- **Neuronal injury**

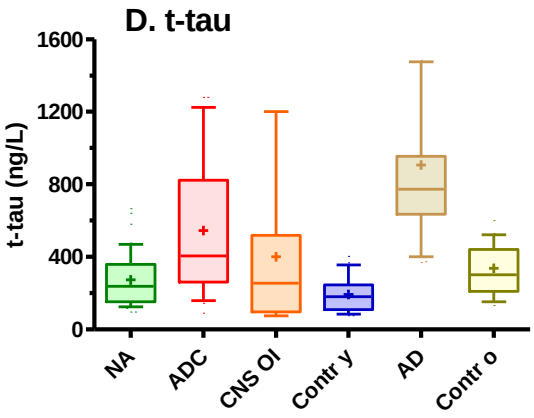
CSF Biomarkers
t-tau
p-tau
Amyloid beta
soluble amyloid precursore proteins (sAPP)
Neurofilament Light Protein (NFL)
Neurofilament Heavy Protein (NFH)
Tubulin
Actin
Neuron-specific enolase (NSE)
14-3-3
N-acetyl aspartic acid (NAA)
Tissue transglutaminase (tTG)
N-lysine isopeptide
Ceramide
Sulphatide
Gangliosides
Glial fibrillary acidic protein (GFAP)
S100b
Etc

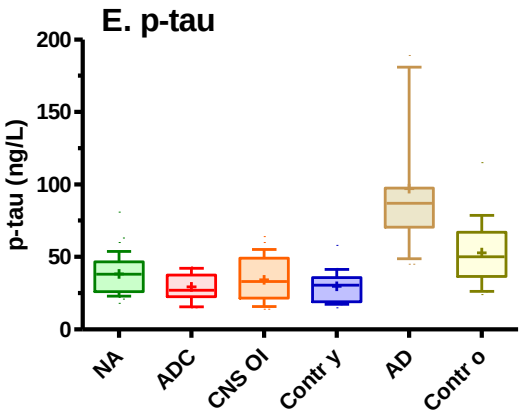
Prediction of progression to Alzheimer's Disease

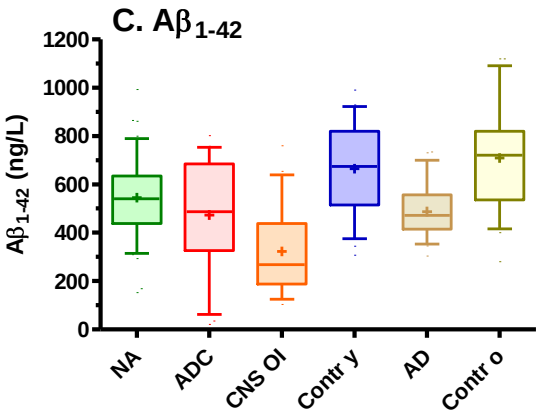


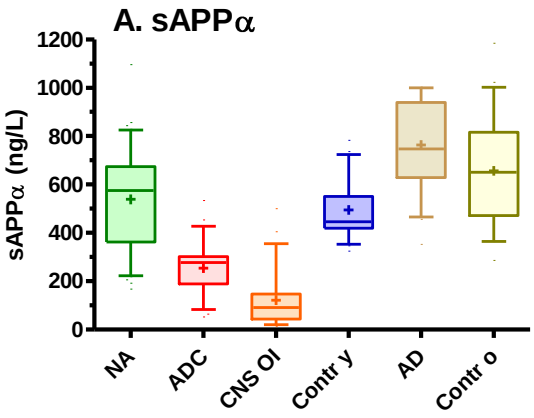
Hertze J et al. *JAD* 2010; 21 :1119

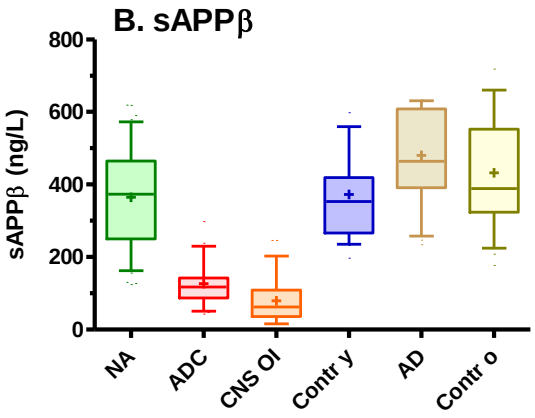
Amyloid and tau cerebrospinal fluid biomarkers HIV infection











n = 150

NA	**n = 40**
ADC	**n = 21**
CNS OI	**n = 25**
Contr	**n = 43**
AD	**n = 21**

All HIV+ without ART

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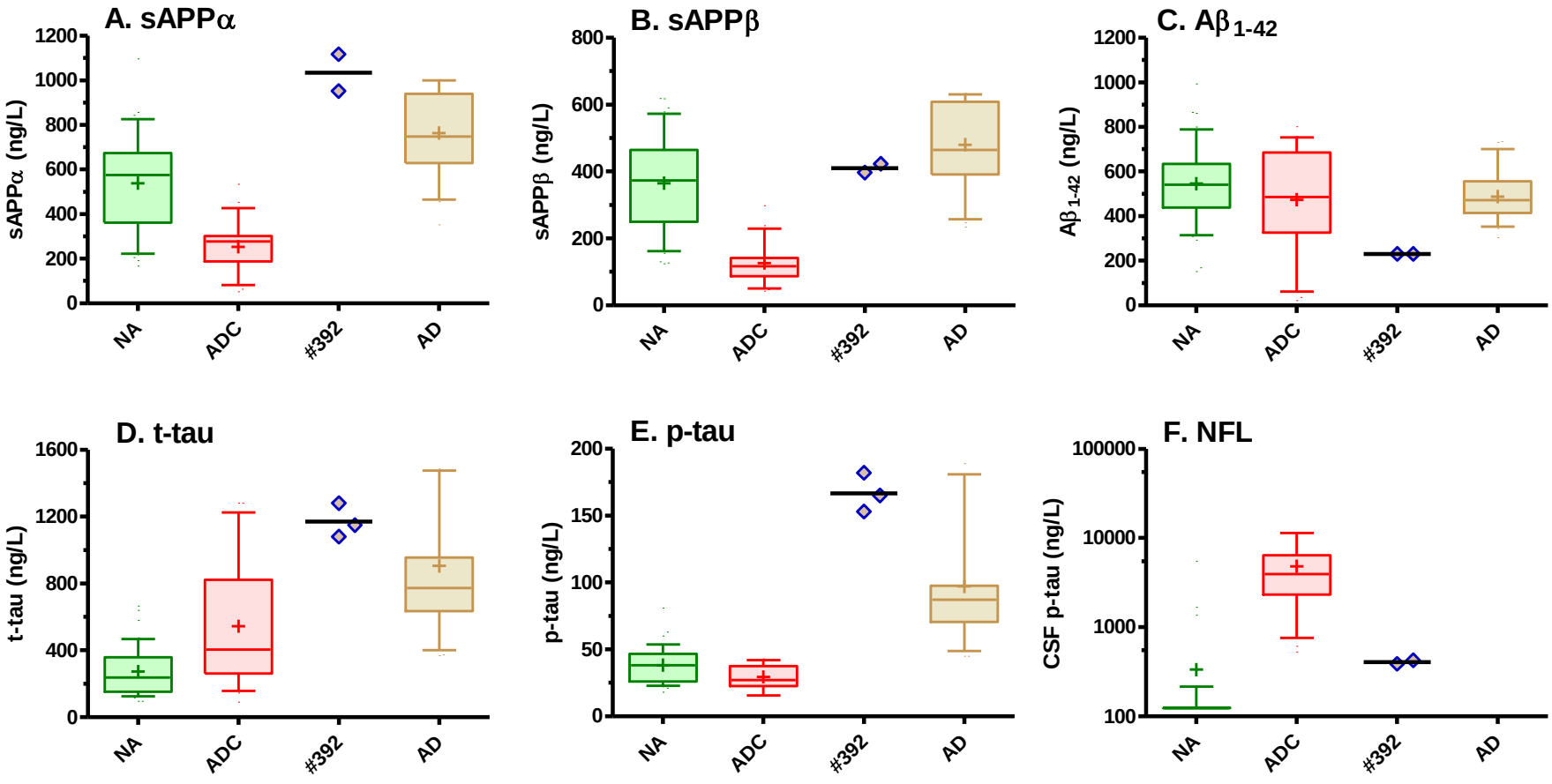
Gisslén M et al. *BMC Neurology* 2009; 9:63

Alzheimers disease in an HIV-infected subject



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In manuscript

HIV-associated dementia vs Alzheimer's disease

	HAD	AD
t-tau	++	+++
p-tau	=	++
AB42	-	-
sAPPα	---	=
sAPPβ	---	=
NFL	+++	+

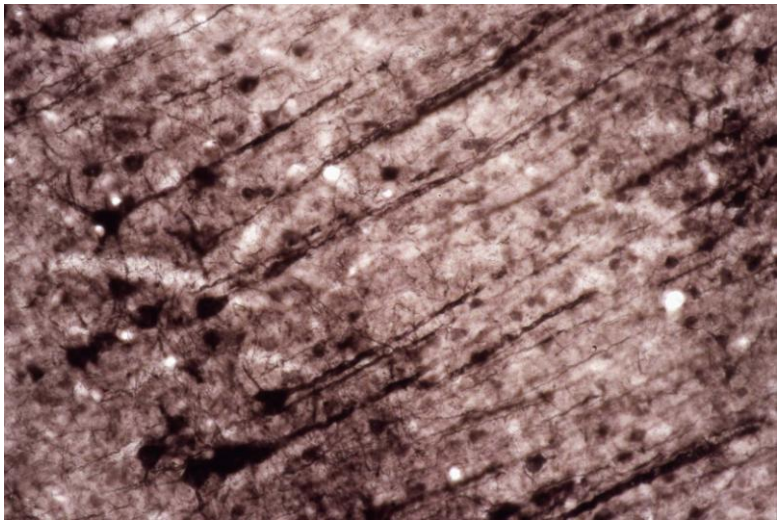
Gisslén M et al. *BMC Neurology* 2009; 9:63

CSF ”Alzheimer biomarkers”

- Clinical usage
  - Differential diagnostics
    - HIV-associated dementia
    - Alzheimer’s disease
    - Vascular dementia

CSF biomarkers of neuronal injury

- Neurofilament light protein (NFL)



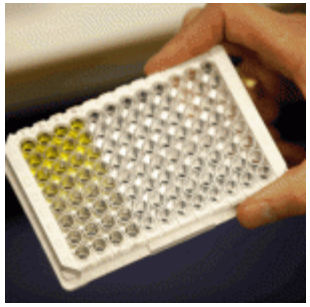
- A major structural element of neurons
- Mainly found in large myelinated neurons
- A triplet protein, the light subunit (NFL) is the essential component of the neurofilament core
- Main function: to maintain the axonal calibre and thereby having a crucial role for morphological integrity and conduction velocity of nerve impulses

Hoffman PN et al. *PNAS* 1987; 84(10): 3472-6

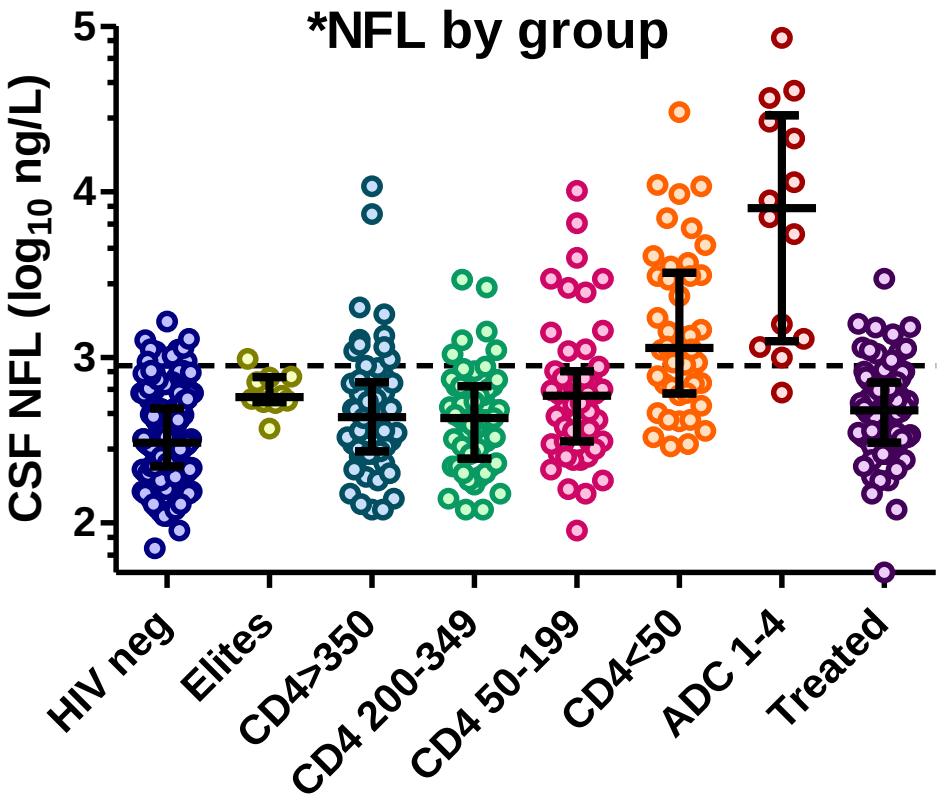
CSF NFL methods

- Commercial assay
- UmanDiagnostics (www.umandiagnostics.com)

- NF-Light® Neurofilament ELISA RUO
  - enzymatic two site immunoassay for quantitative determinations of NF-light in human body fluids

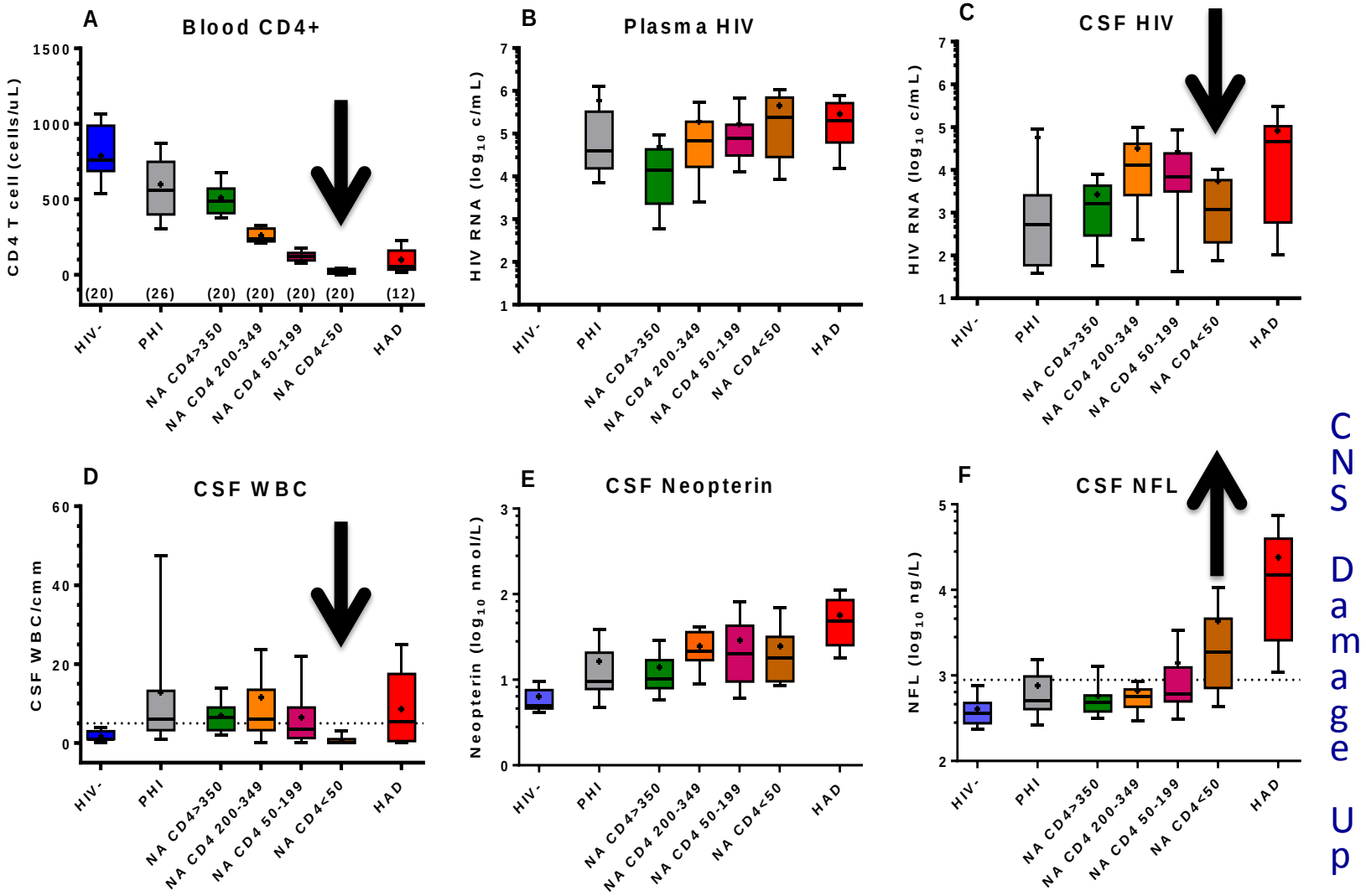


CSF NFL



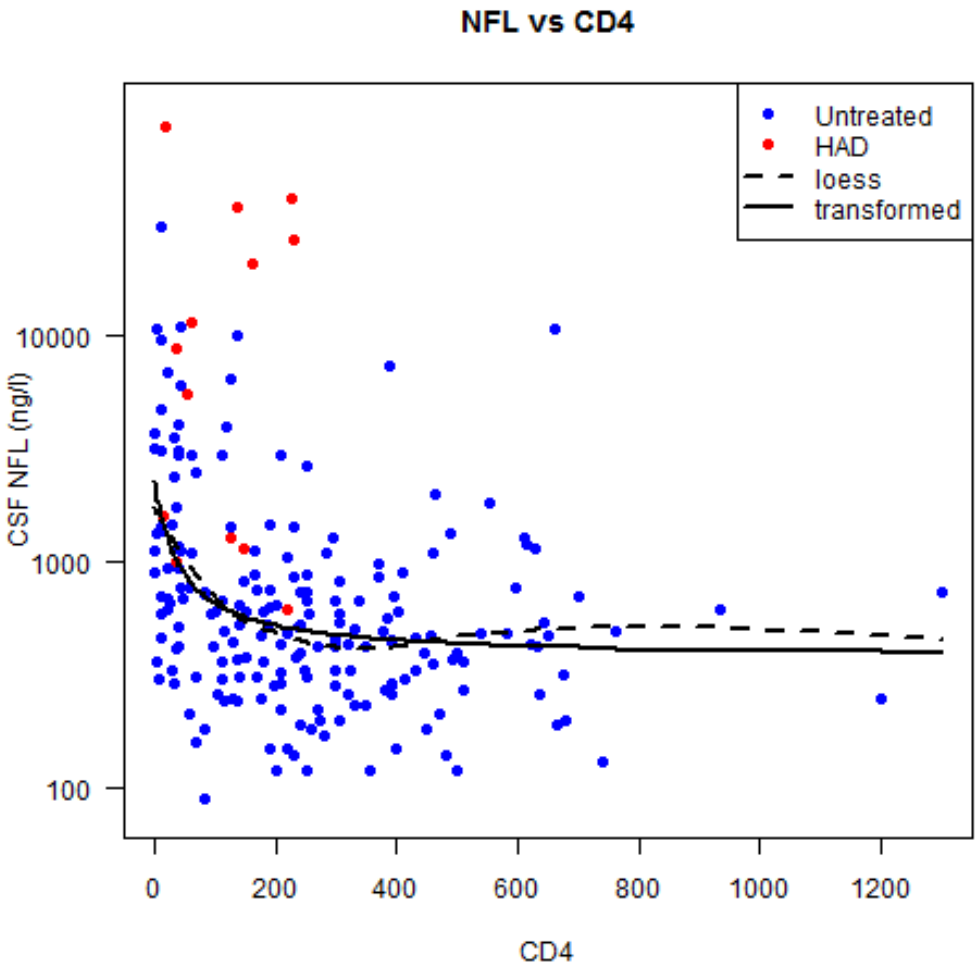
Data on file (n = 518)

Biomarkers Over the Entire Range of Infection: CD4 Low, Pleocytosis Drops, CSF VL Drops, CSF NFL Up



R. Price, UCSF

In asymptomatic HIV; signs of neuronal destruction mainly found in patients with low CD4 cell counts.



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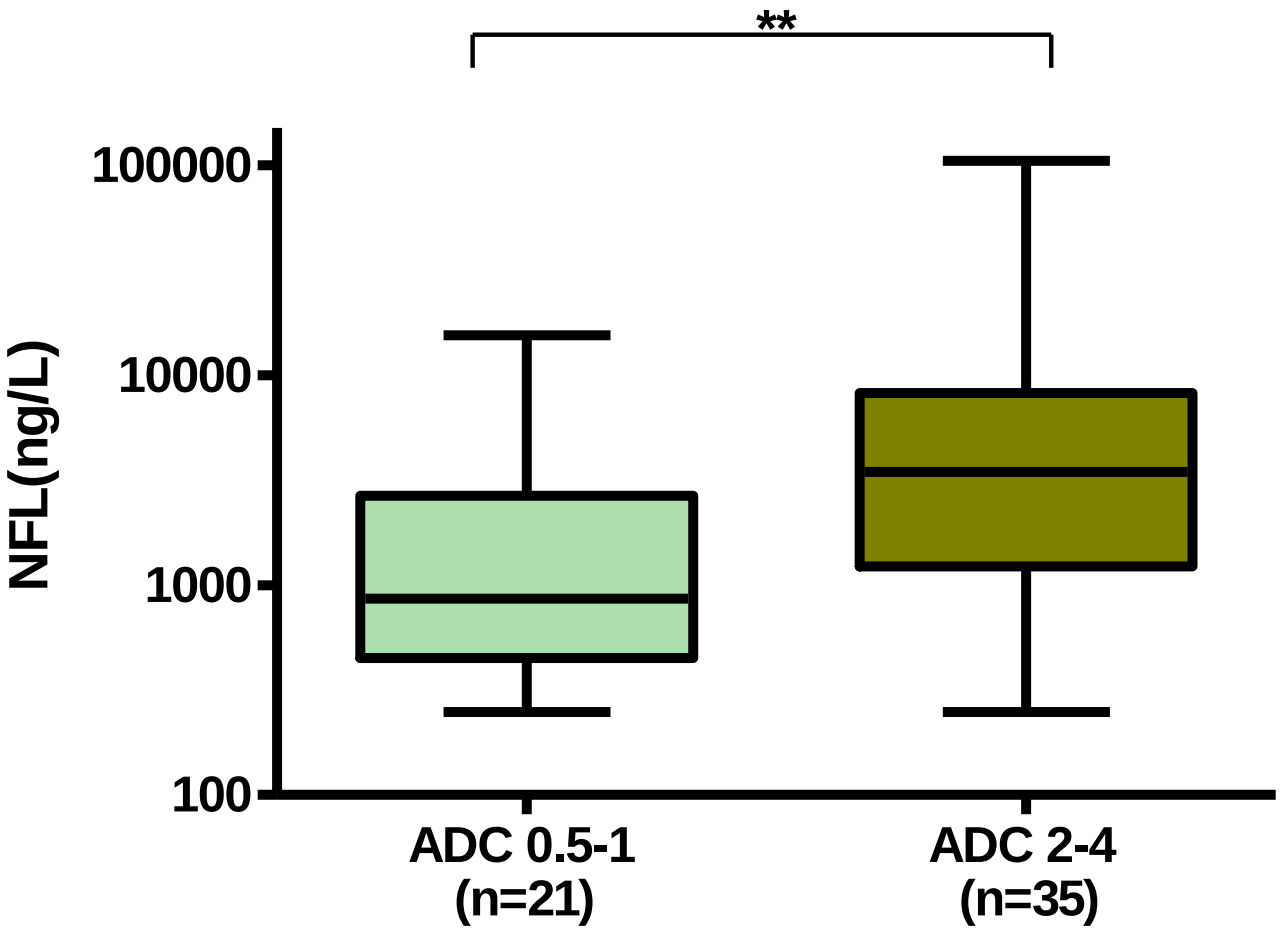
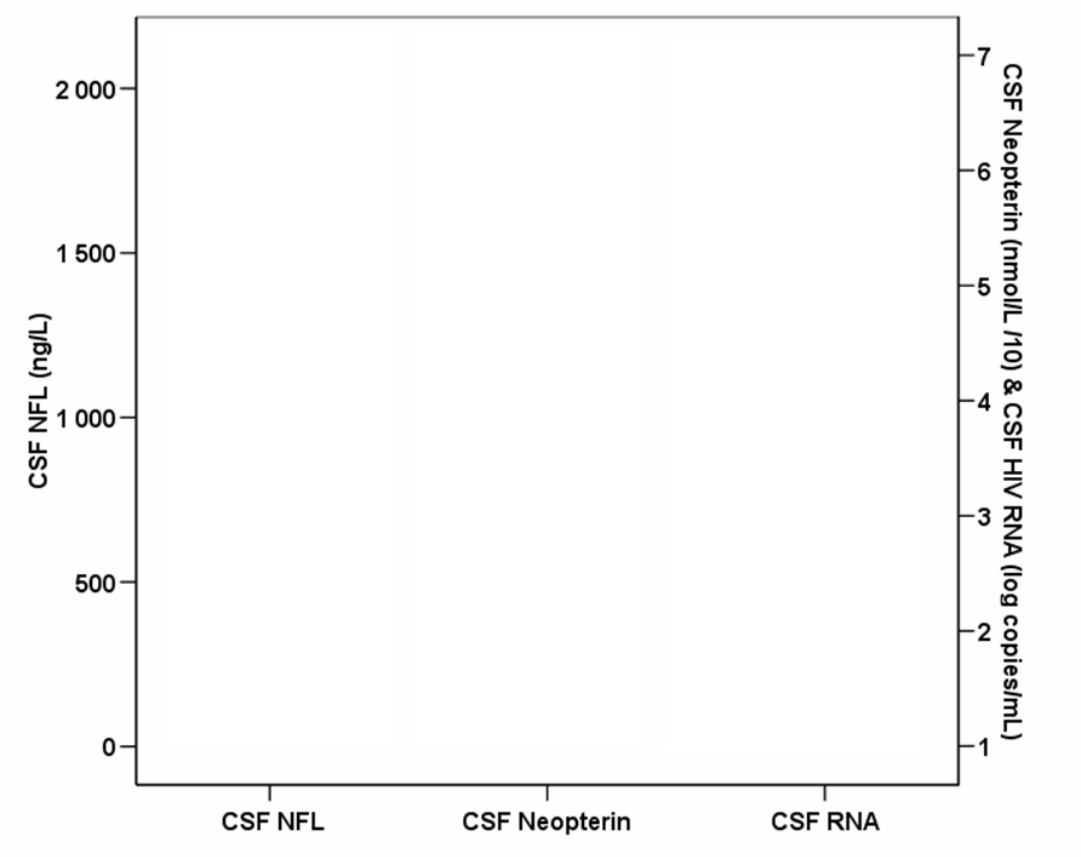
Jessen Krut J, Mellberg T et al. *PLoS One* 2014 Feb 11;9(2):e88591

CSF NFL levels related to severity of dementia



Abdulle S et al. *J Neurol* 2007; 254: 1026

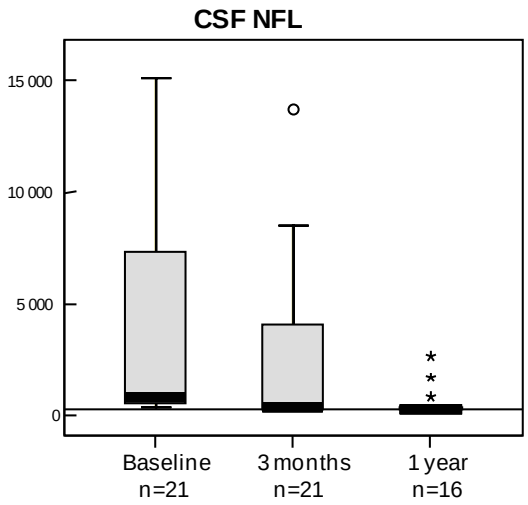
CSF biomarkers 1-2 years before development of dementia (pre-HAART era)

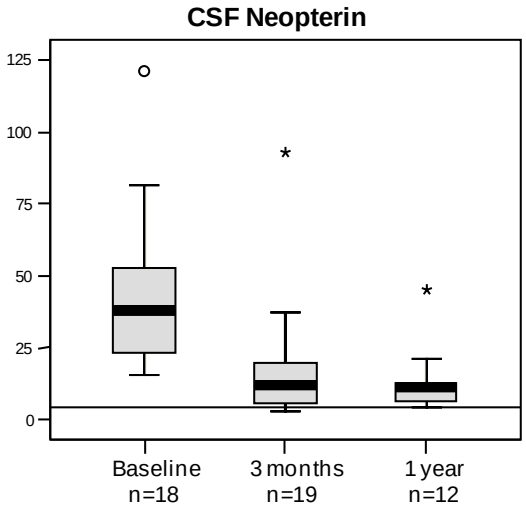


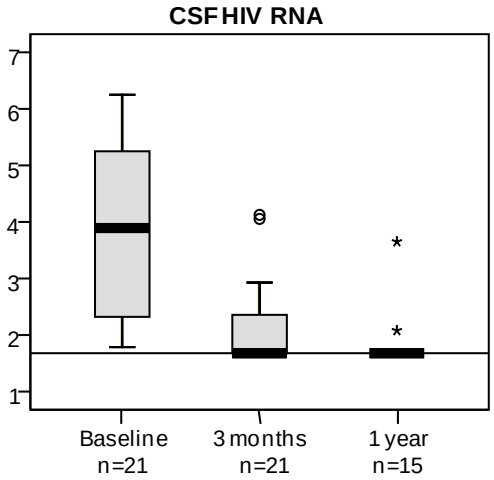
Cases developed HAD 1-2 years after examination
Controls did not (CD4 matched)

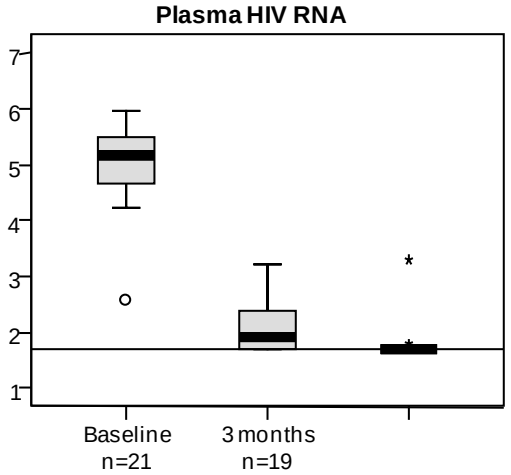
Gisslén M et al. *J Infect Dis* 2007; 195:1774

CSF NFL decrease with ART









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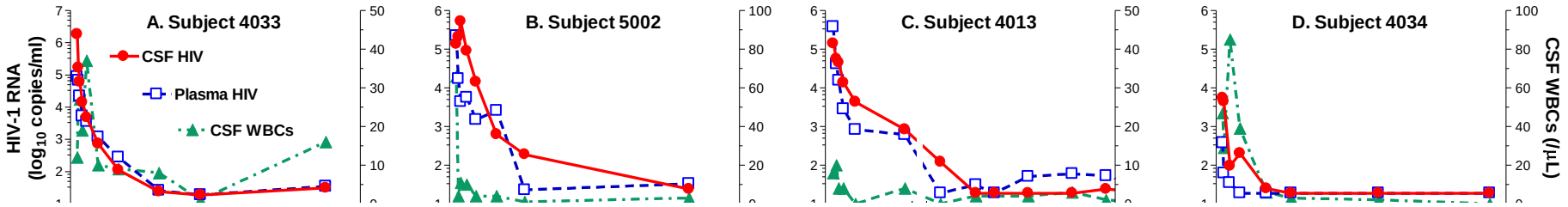
Mellgren Å et al. *Neurology* 2007; 69 : 1536

CSF NFL decrease with ART

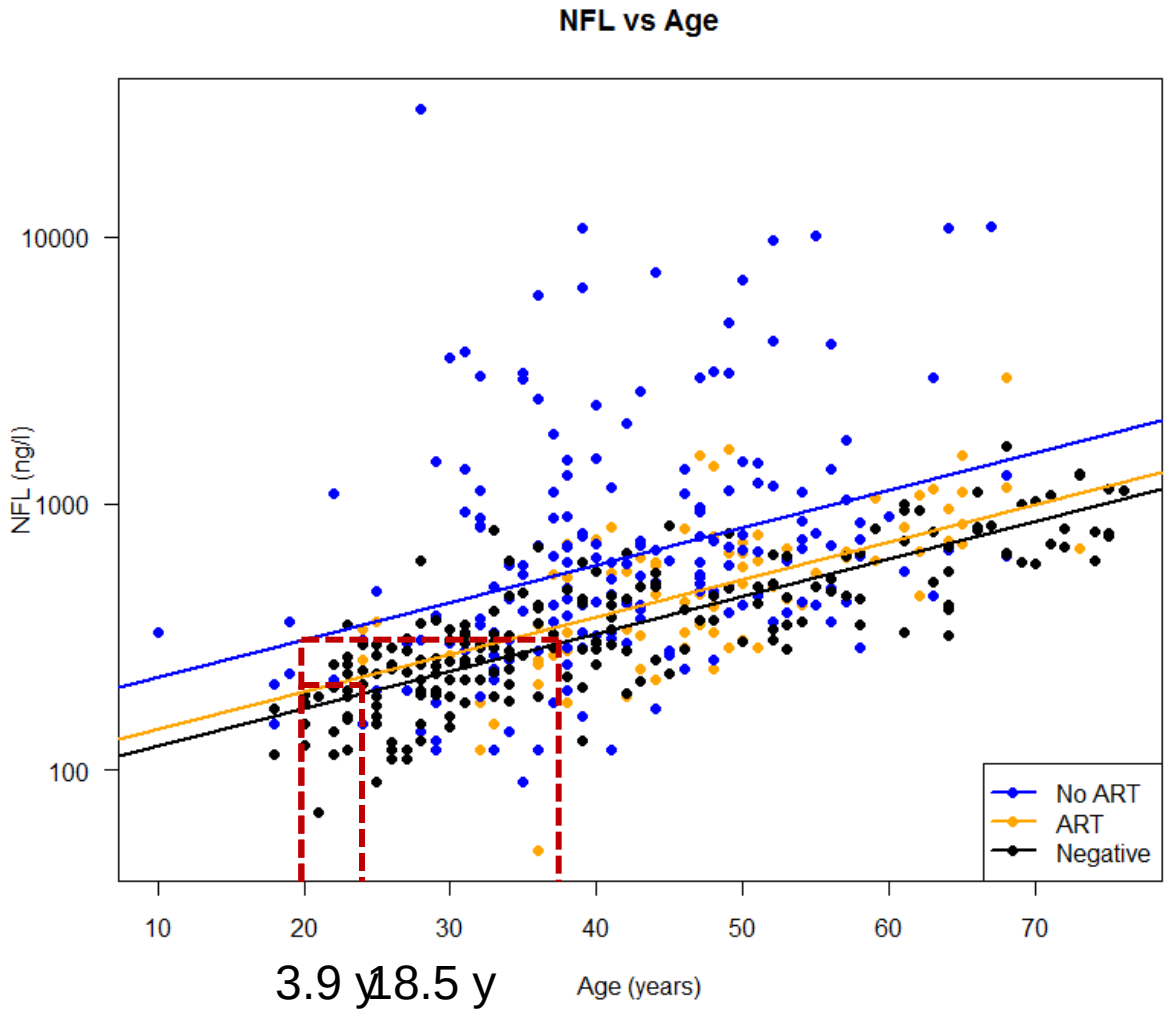


Abdulle S et al. *J Neurol* 2007; 254: 1026

CSF NFL

- Very sensitive marker of axonal injury
- Potential clinical usage
  - Pathological correlate to HAND
  - Confirming HAD diagnosis
  - Objective evaluation of milder forms of HAND (ANI/MND)?
  - Evaluation of neuronal injury by CSF viral breakthrough during treatment?

HIV and aging independently affect axonal disruption as measured by CSF NFL



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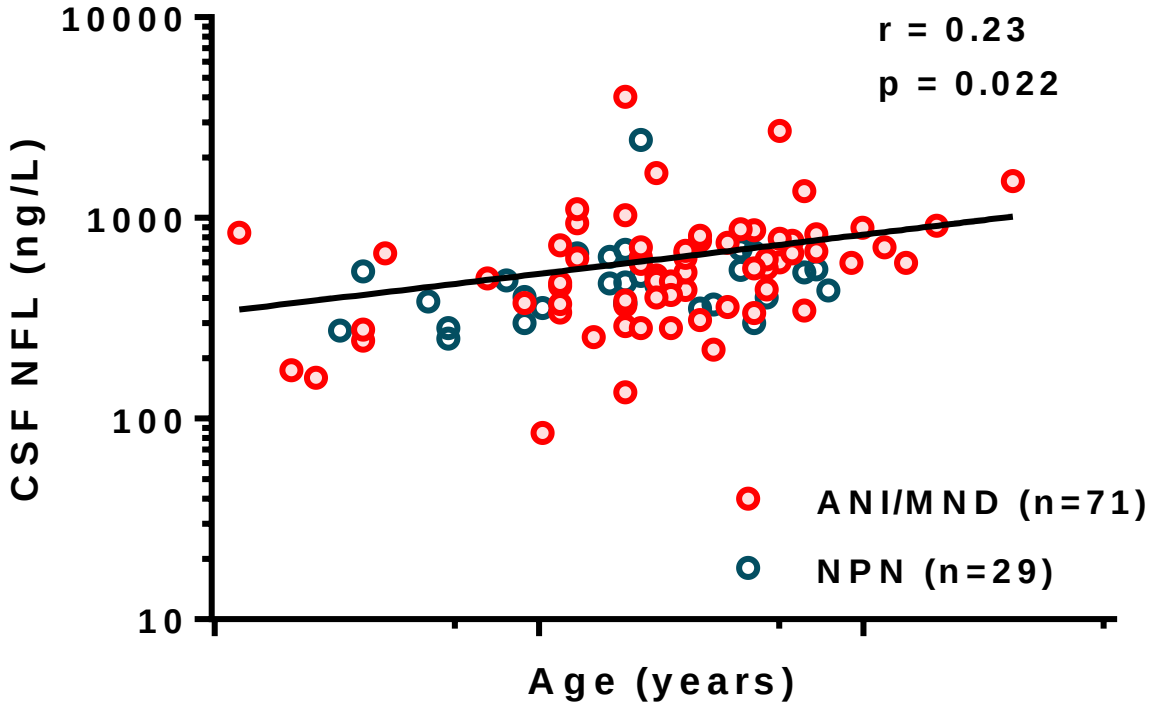
Jessen Krut J, Mellberg T et al. *PLoS One* 2014 Feb 11;9(2):e88591

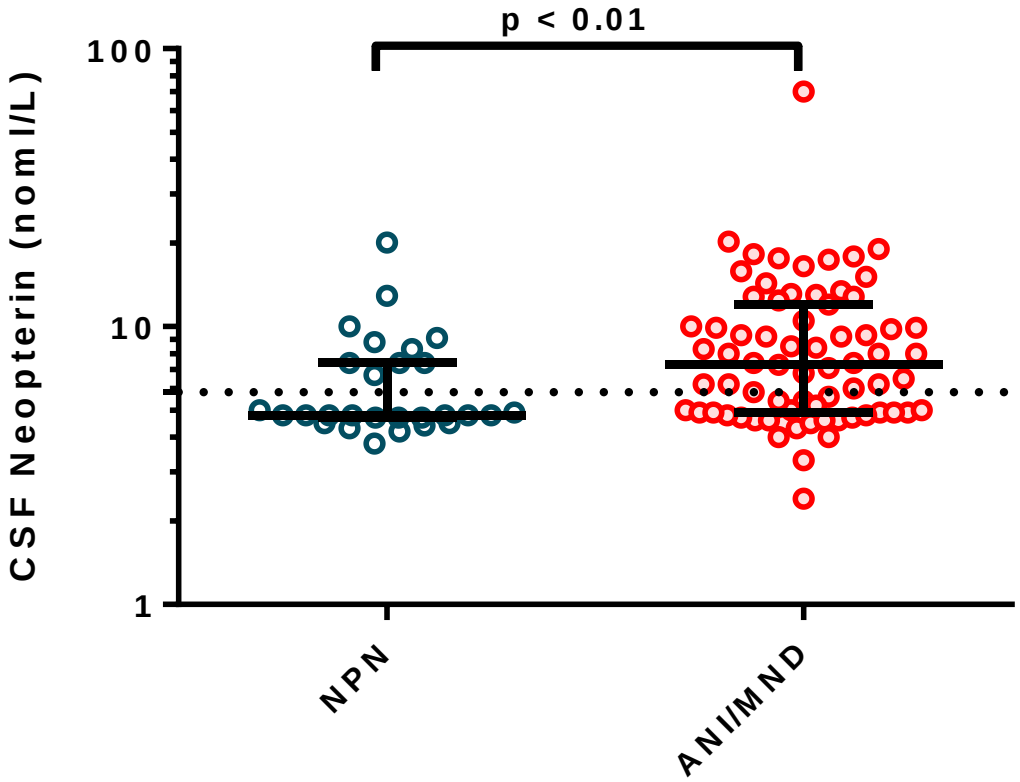
CSF NFL in treated subjects (P-RNA <50) with mild neurocognitive disease (CHARTER)



No sign difference between asympt and ANI/MND

Edén A et al. *CROI* 2014 Abstract #490

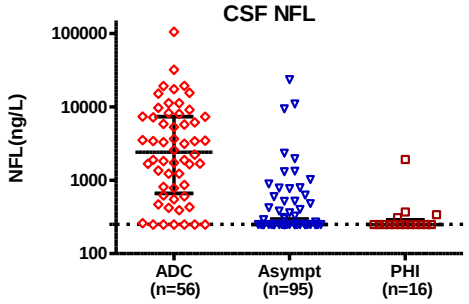
CSF Neopterin in treated subjects (P-RNA <50) with mild neurocognitive disease (CHARTER)

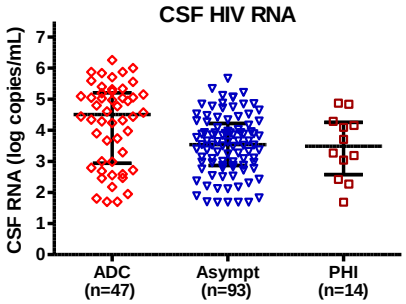


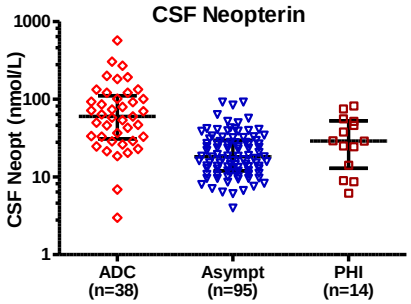
Edén A et al. *CROI* 2014 Abstract #490

CSF biomarkers in HAND Conclusions

- There is a need for an approach that focuses on objective markers of ongoing CNS-injury, as a complement to NP testing.
- Markers should be based on current pathogenetic concepts of HIV-related injury.
- A combination of CSF biomarkers (viral, immunological and damage) is a promising alternative.
- Tau- and amyloid CSF biomarkers promising for differential diagnosis of dementia







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Matti Ristola, Finland
Anders Sönnerborg, Sweden
Magnus Gisslén, Sweden

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Lena Lindborg, Stockholm
Veronica Svedhem Johansson, Stockholm

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Margaret Johnson, UK
David Margolis, USA
Graeme Moyle, UK
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www.hivnordic.se

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