FERRARA, 27 maggio 2014

Il Sistema Venoso Cerebrale Extracranico: un potenziale contributore alla neurodegenerazione?

RELATORE

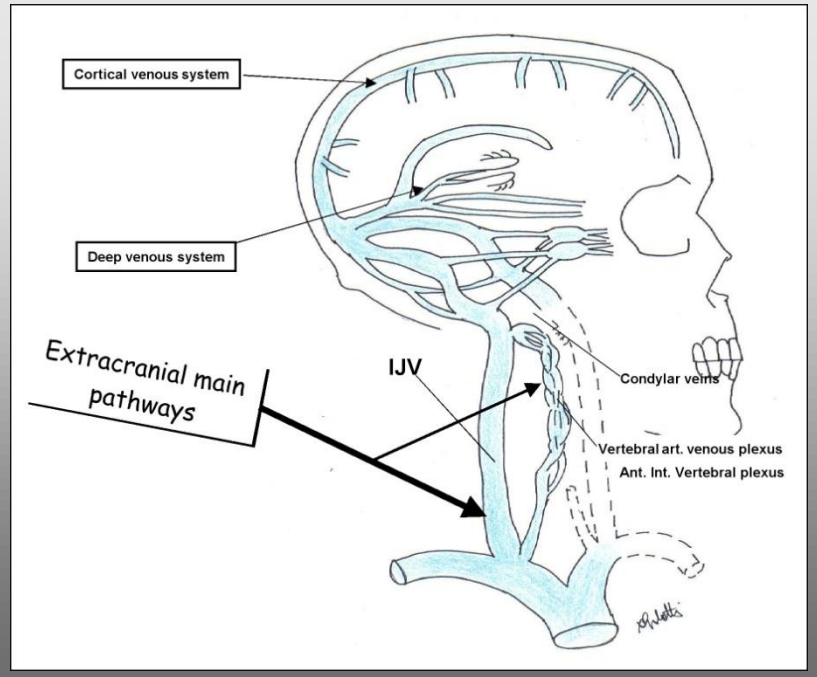
Prof Paolo Zamboni

Sezione di Medicina e Chirurgia Traslazionale, Dipartimento di Morfologia e Chirurgia e Medicina Sperimentale

DISCUSSANT

Prof Enrico Granieri

Sezione di Scienze neurologiche, psichiatriche e psicologiche - Clinica Neurologica, Dipartimento di Scienze Biomediche e Chirurgico Specialistiche



PRIMARY

VENOUS OBSTRUCTION

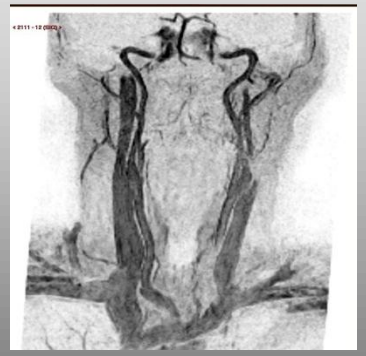
GUIDELINES

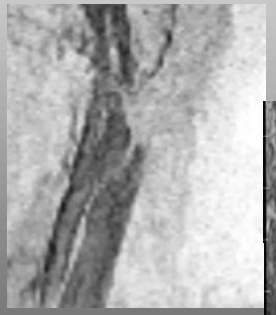
Diagnosis and Treatment of Venous Malformations Consensus Document of the International Union of Phlebology (IUP): updated 2013

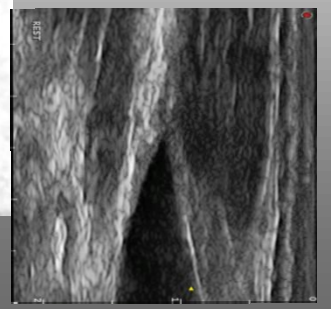
B. B. LEE, I. BAUMGARTNER, P. BERLIEN, G. BIANCHINI, P. BURROWS, P. GLOVICZKI, Y. HUANG
J. LAREDO, D. A. LOOSE, J. MARKOVIC, R. MATTASSI, K. PARSI, E. RABE, M. ROSENBLATT,
C. SHORTELL, F. STILLO, M. VAGHI, L. VILLAVICENCIO, P. ZAMBONI

Chronic Cerebrospinal Venous Insufficiency by Truncular VM

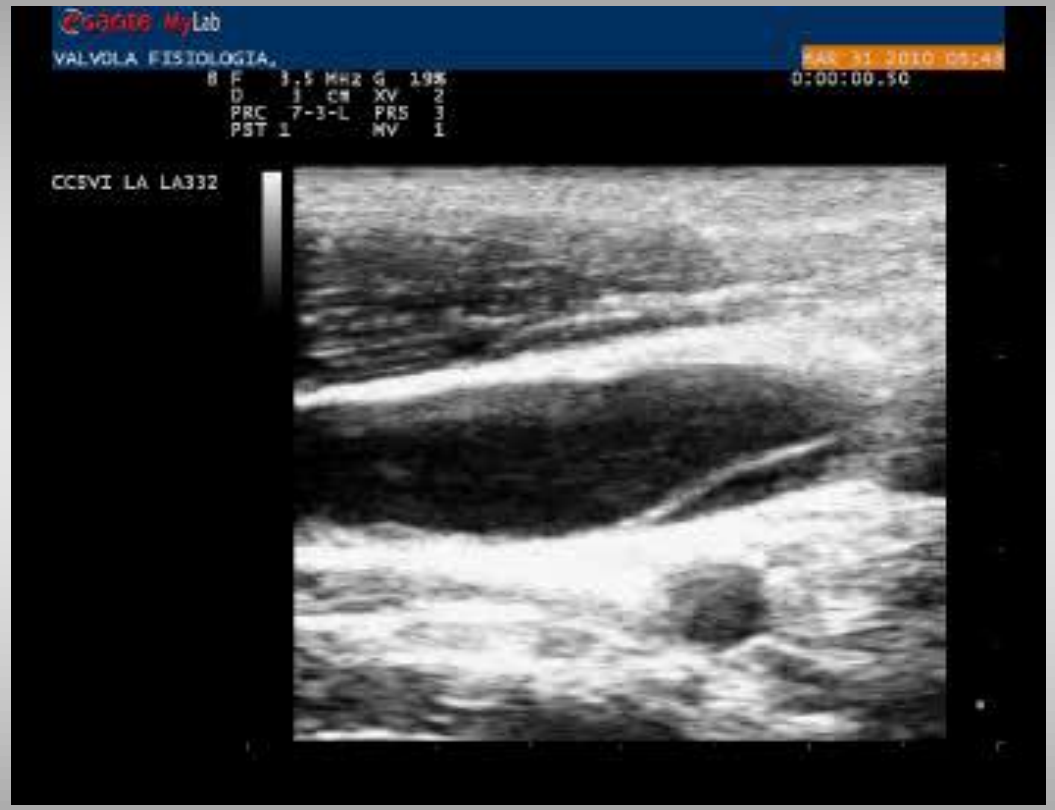
Truncular VMs are the result of the developmental defects of vascular trunk formation during the later stage of embryogenesis, Truncular VMs are subdivided into obstruction (intraluminal defects, segmental aplasia or hypoplasia), and dilation (aneurysms). [1, 30, 31] Obstructive lesions can be respectively subdivided in intraluminal obstacles (septa. webs, membranes, fixed and rudimental valves) and in wall stenosis (hypoplasia, agenesis). [1, 30, 31]

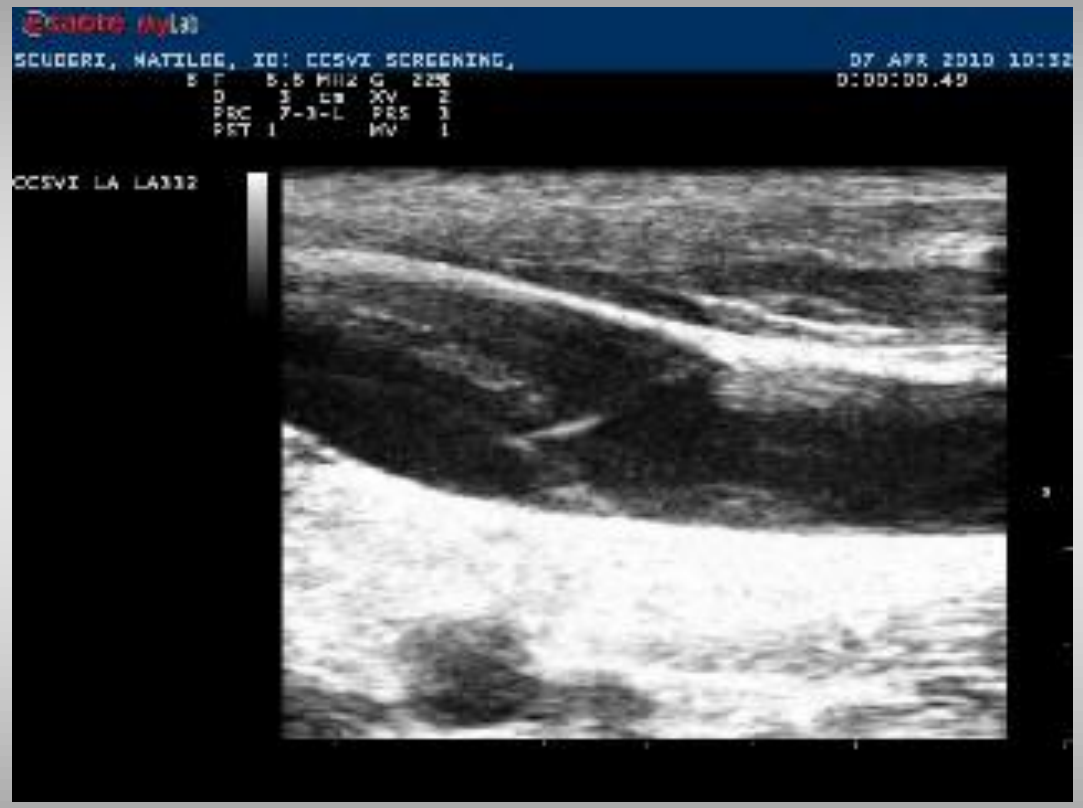






PRIMARY VENOUS OSTRUCTION
INTRALUMINAL OBSTACLES

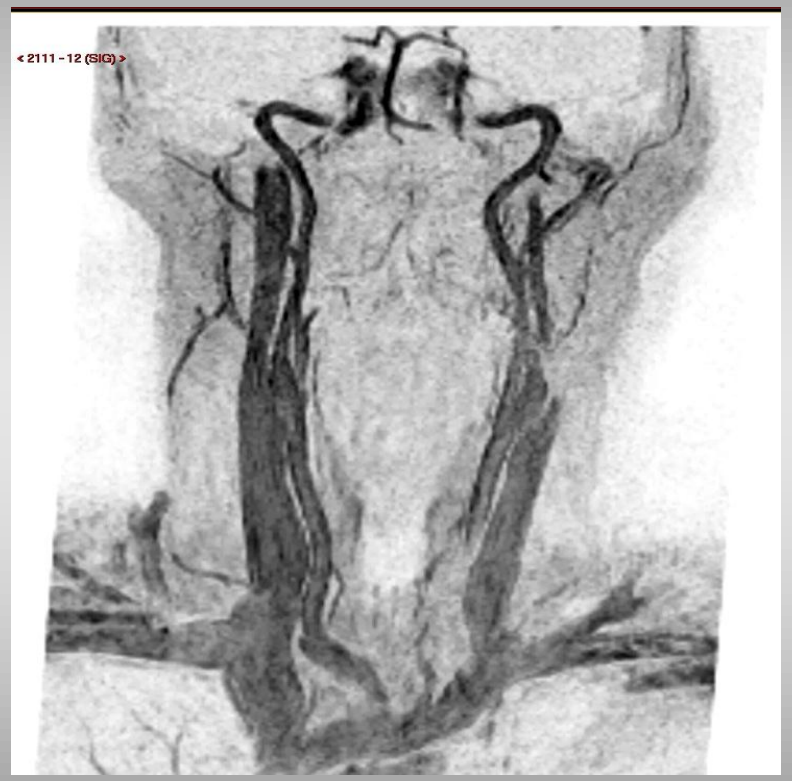


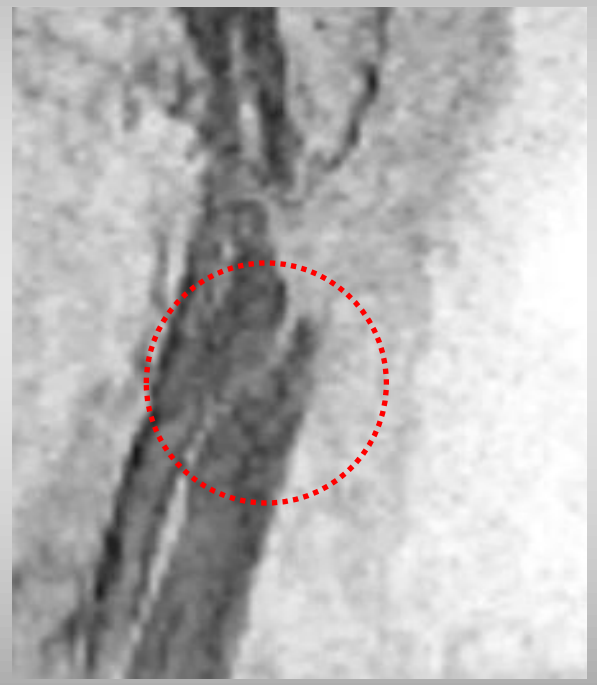


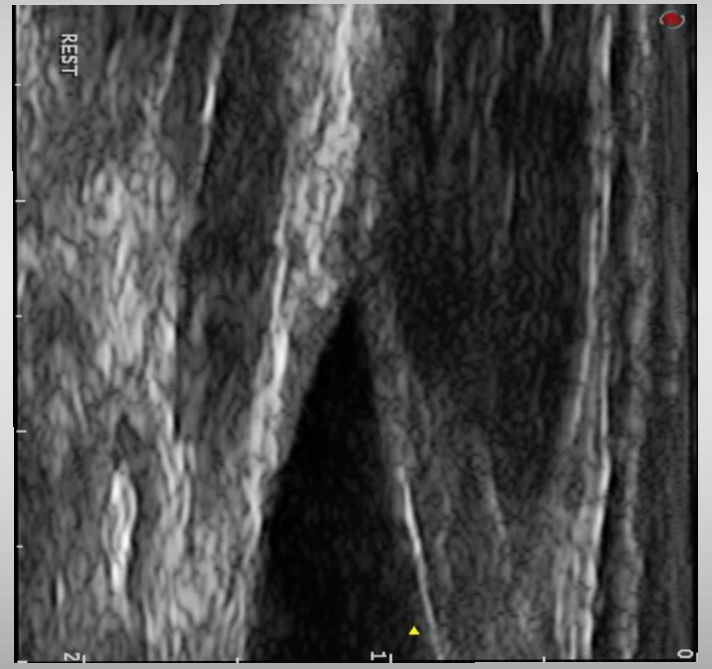
PRIMARY

VENOUS OBSTRUCTION

Muscular entrapment-external compression





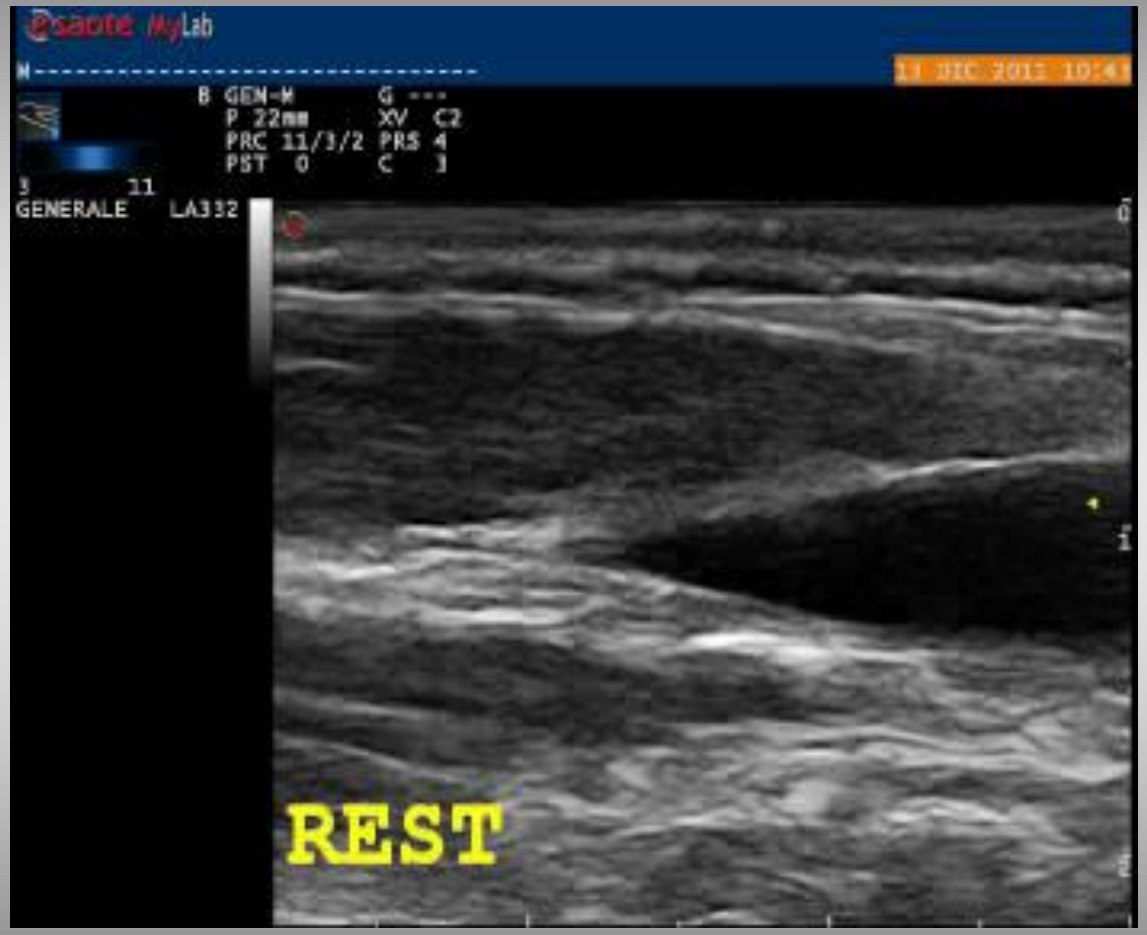


Multimodality imaging techniques comparison

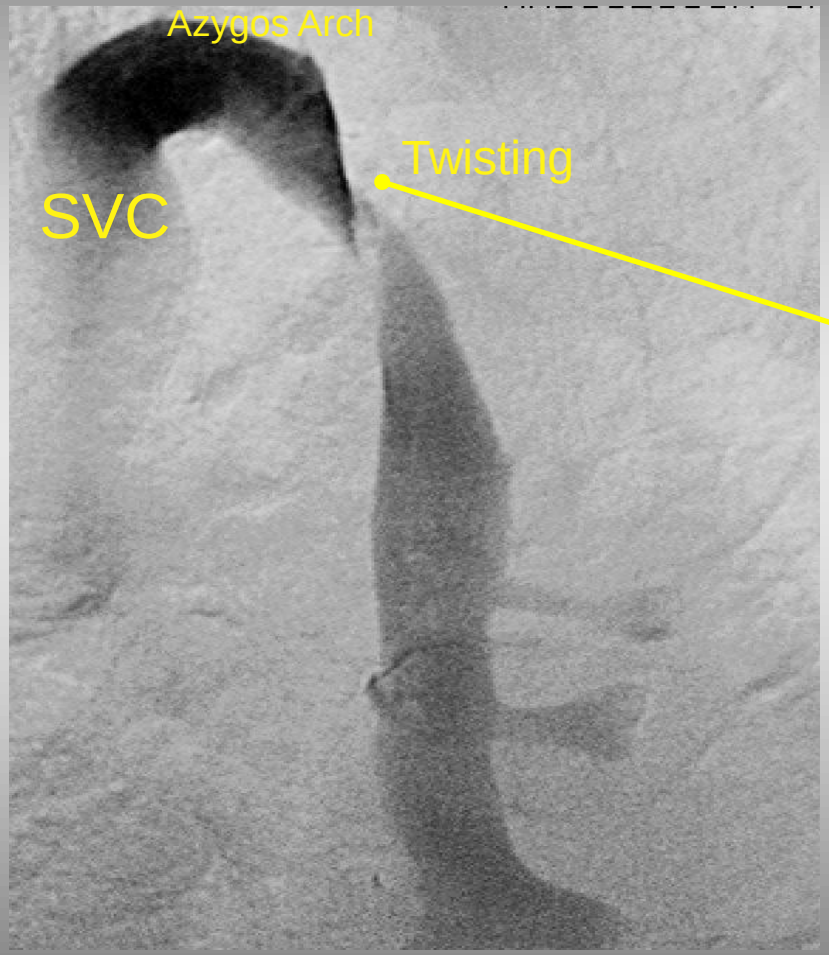
Vascular Disease Center
University of Ferrara

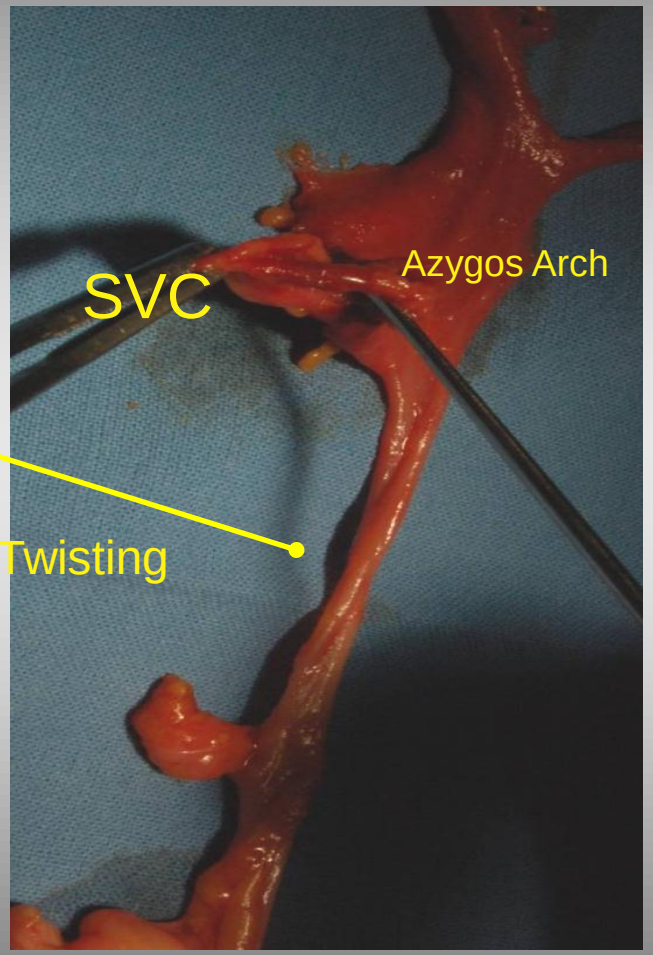
Chief: Prof. *Paolo Zamboni*
email: zmp@unife.it

REAL TIME ULTRASOUND AT 1 REST 2 BITE 3 YAWN



WALL STENOSIS TWISTING





Cosa è stato misurato?

- Ritardato svuotamento attraverso gli assi principali venosi
- Aumento del flusso attraverso le vene collaterali
- Outflow cerebrale con incrementata resistenza idraulica
- Ridotto flusso in uscita dal cranio in favore di gravità e stasi nei vasi del collo

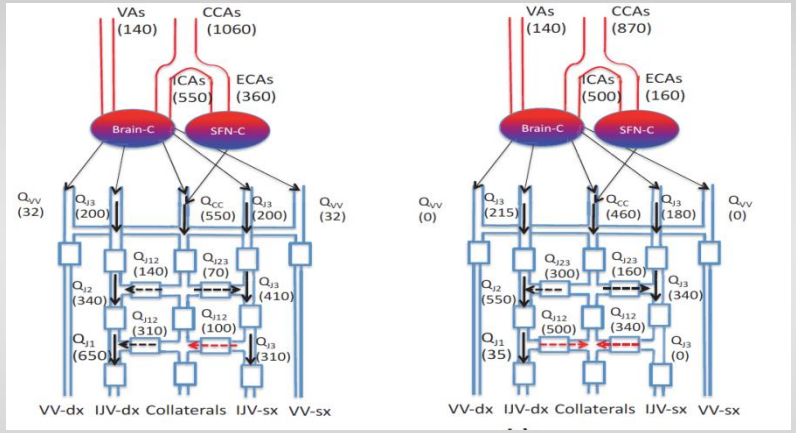
Veroux *JVIR 2013*
Monti *Am J Neurorad 2014*
Mancini *PLOS one 2014*

Feng *Neurol Res 2012*
Zamboni *BMC Neurology 2013*

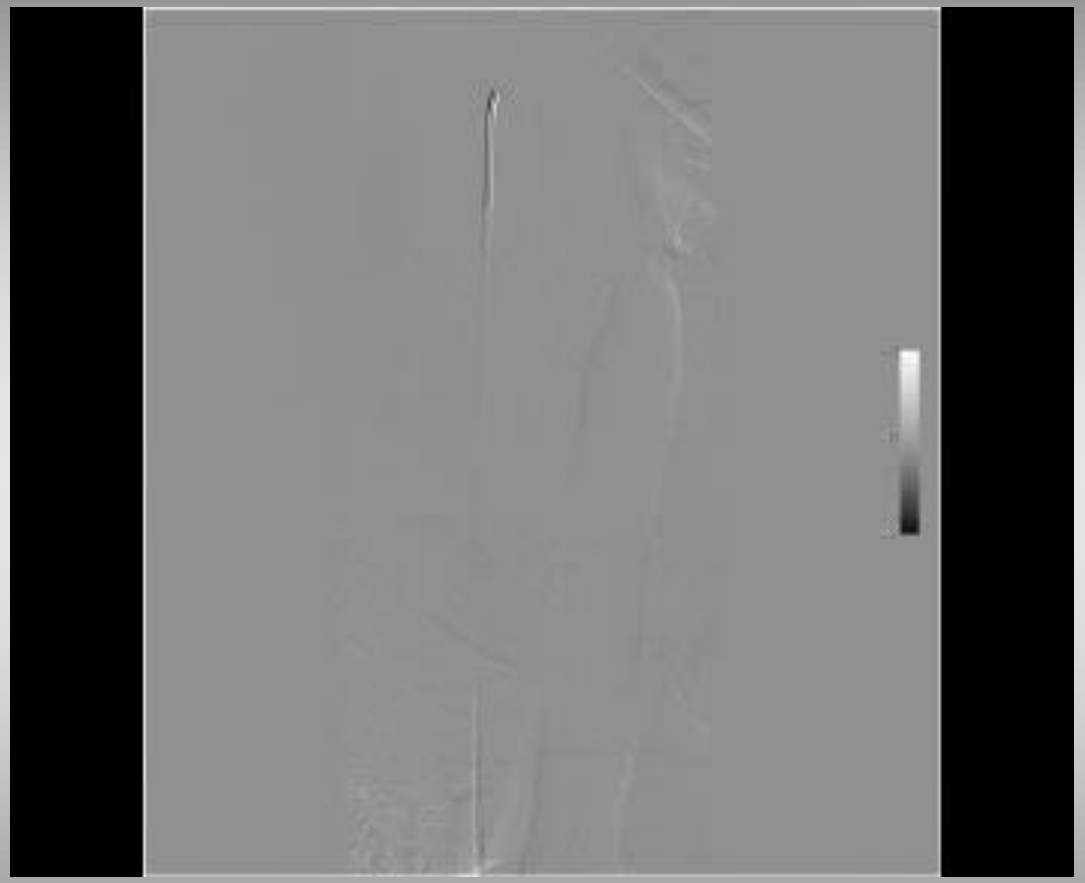
Beggs *Phlebology 2013*

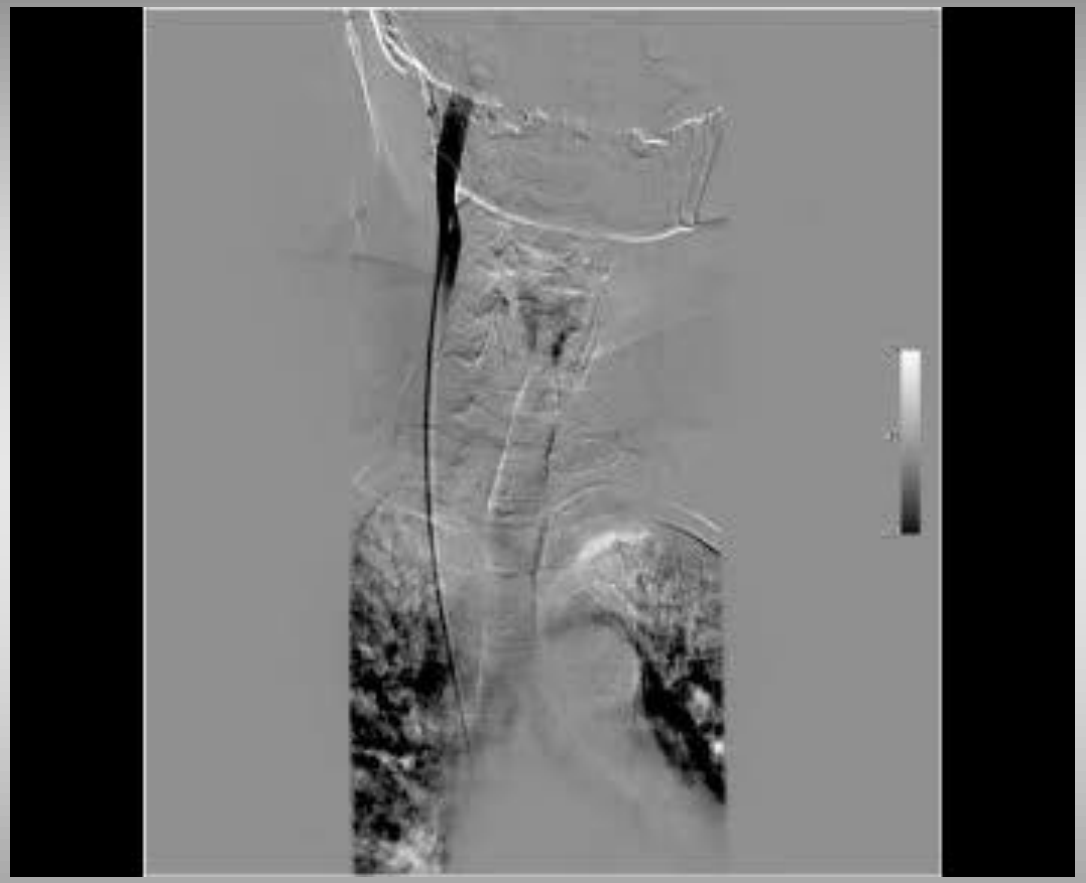
Zamboni *JVS 2012*

HEALTHY CONTROL **CCSVI**



> 85% Total Inflow **p<0.001** <50% Total Inflow





Patologie venose extracraniche e neurodegenerazione

- AD and brain aging
- Parkinson
- Meniere
- Normotensive hydrocephalus
- SM

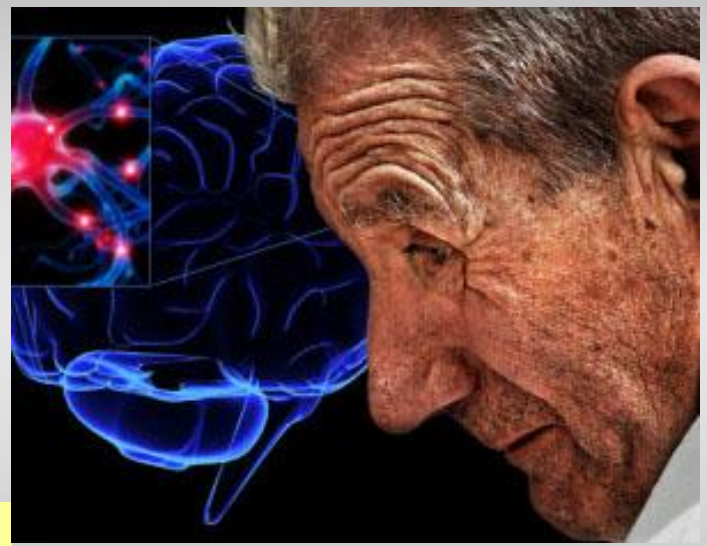
Chung et al *J Alzheimers Dis. 2014*
Lanzillo et al *BMC Neurol. 2013*

Liu et al *J Vasc Surg 2014*

Filipo et al *Eur Arch Otorhinolaryngol. 2013*
Di Berardino et al *Phlebology. 2014*

Beggs et al *BMC Med. 2013*

Zamboni *et al J Neur Neurosurg Psichiatry 2009*
Zivadinov et al *Neurology 2010*



Sono note conseguenze fisiopatologiche?

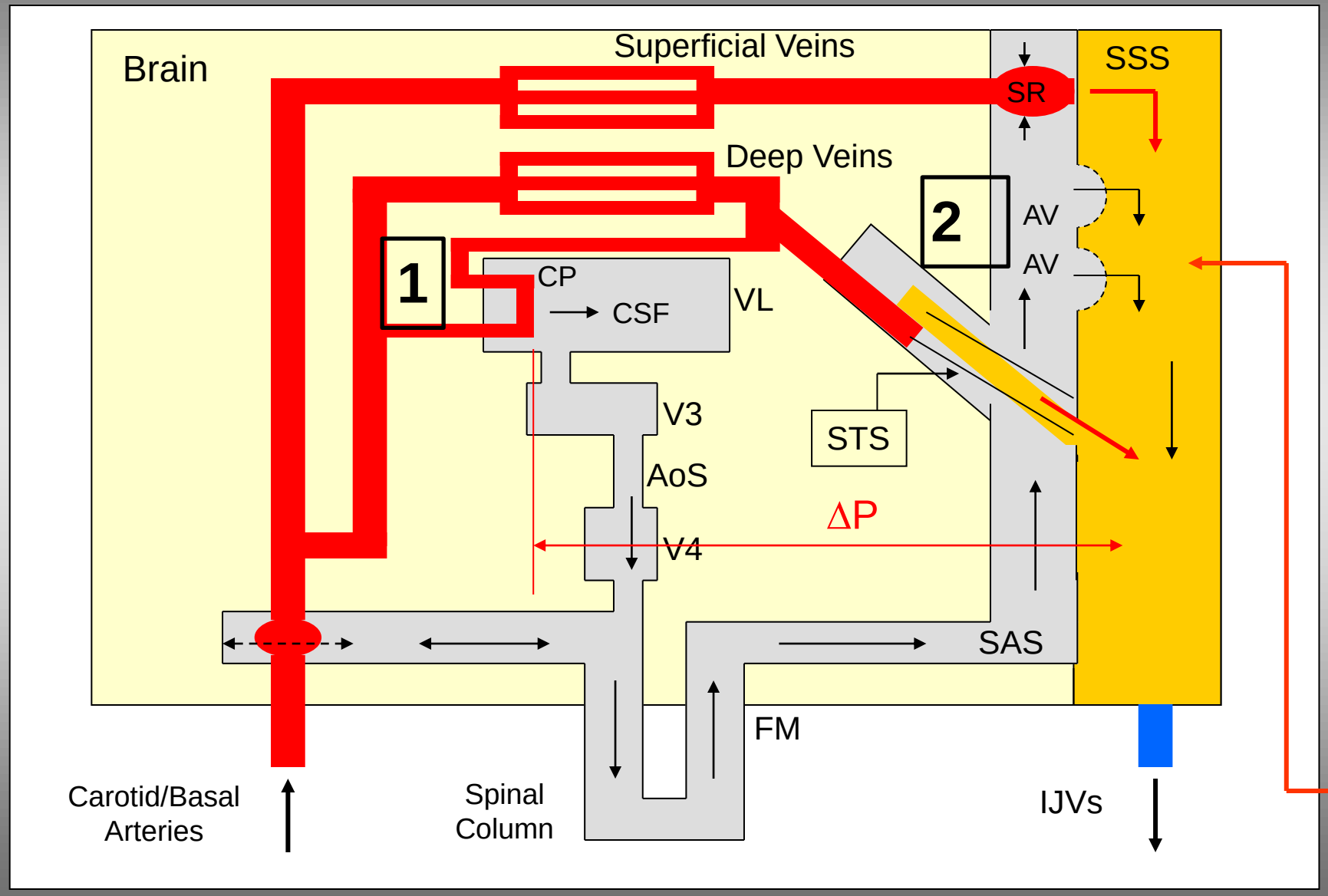
- **Riduzione della portata e della velocità del flusso liquorale**

Zamboni *Funct Neur 2009*
Beggs *J Magn Reson Imaging 2013*
Beggs *BMC Neur 2013*
Zivadinov *JVIR 2013*
Magnano *J Magn Reson Imaging 2012*

- **Ridotta perfusione cerebrale**

Zamboni *BMC Medicine 2011*
D'haeseleer *Lancet Neurol 2011*
Utriainen *Neurol Res 2012*
Garaci *Radiology 2012*
Guttmann *J Neuroimaging 2012*
Zivadinov *BMC Neurology 2011*

CSF Bulk Flow

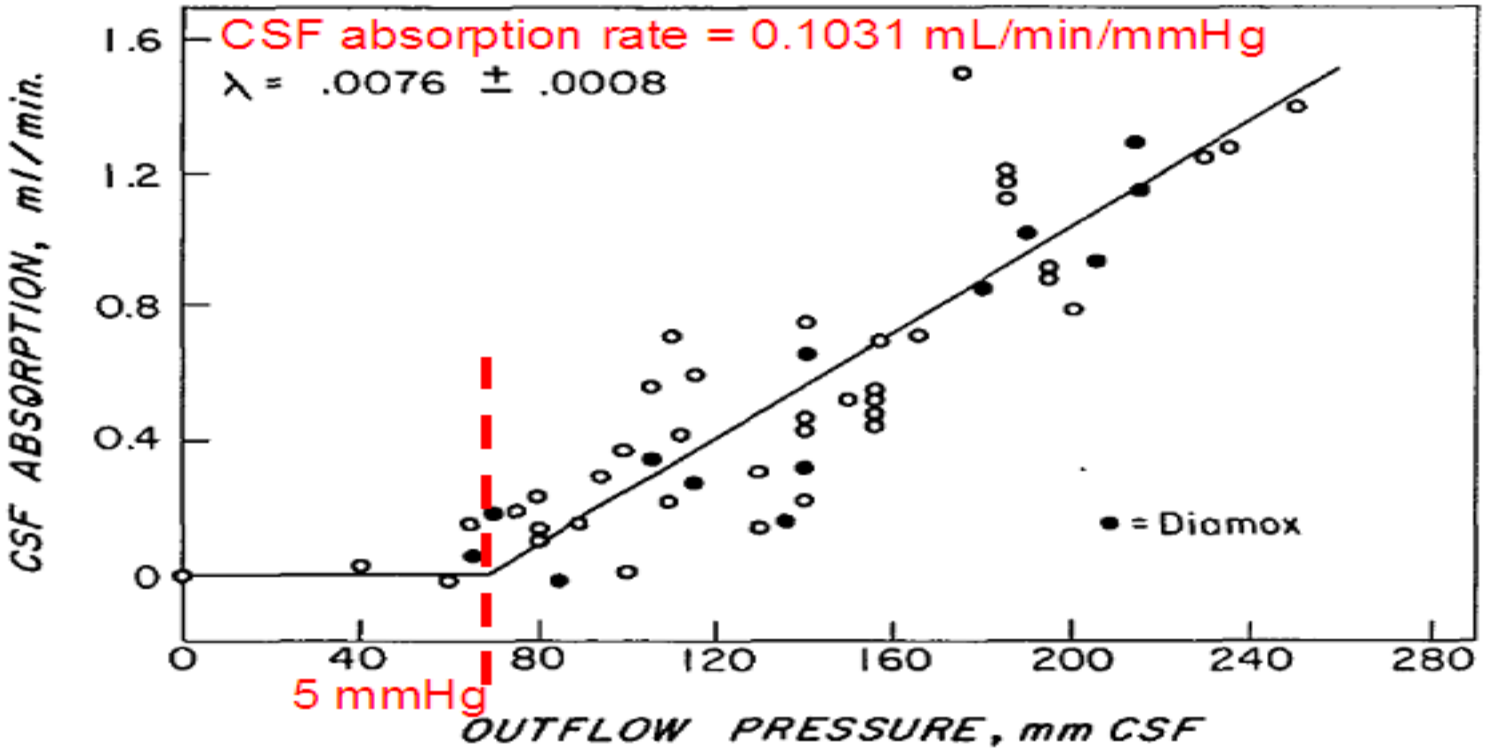


Legend

AV Arachnoid villi
AoS Aqueduct of Sylvius
CP Choroid plexus
CSF Cerebrospinal fluid
FM Foramen magnum
IJV Internal jugular vein
SAS Sub-arachnoid space
SR Starling resistor
SSS Superior sagittal sinus
STS Straight sinus
VL Lateral ventricle
V3 Third ventricle
V4 Fourth ventricle

Venous hypertension here will tend to inhibit absorption of CSF by the SSS.

CSF Absorption into SSS

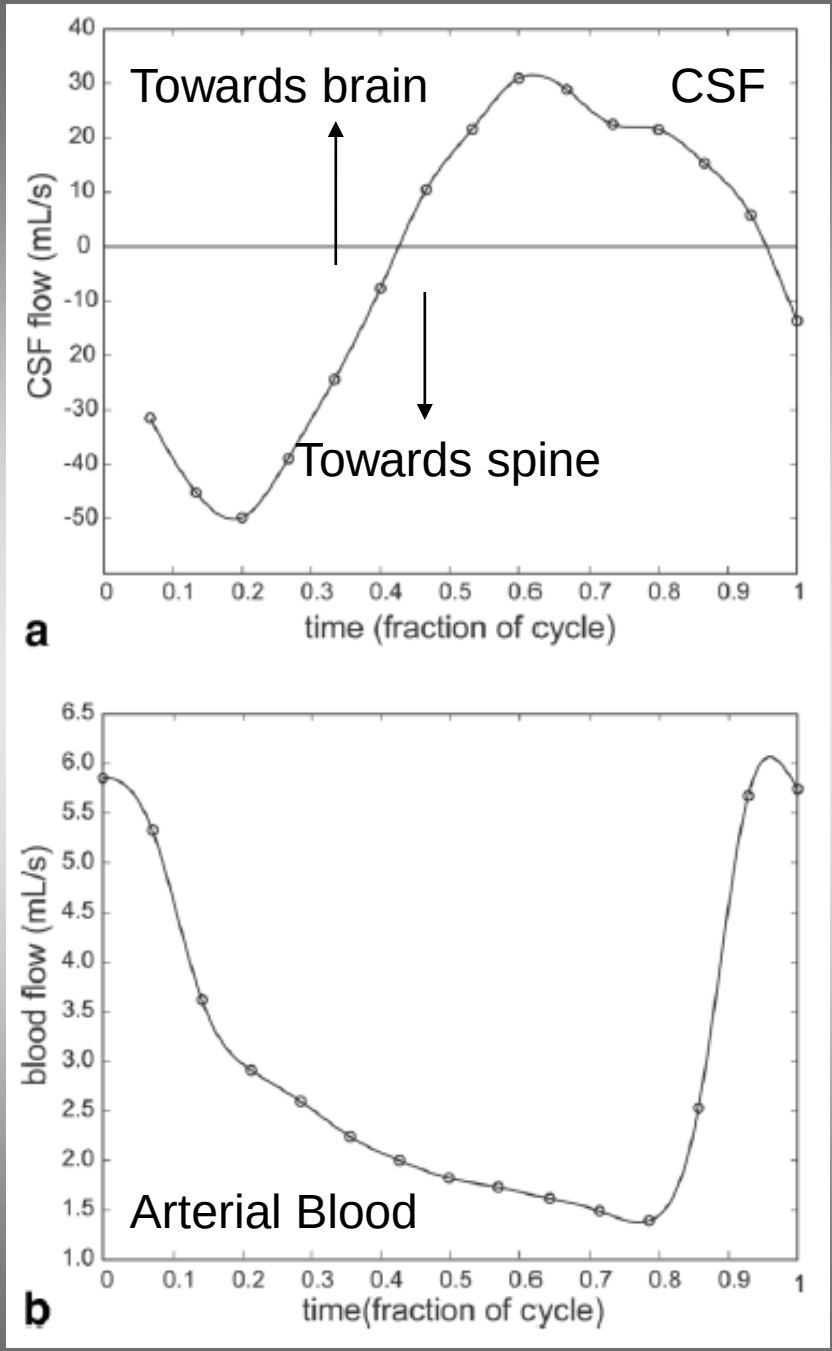


CSF absorption vs CSF pressure

- CSF absorption is driven by pressure difference between SAS and SSS at a rate of approximately 0.1031 mL/min/mmHg.
- Minimum of 5 mmHg CSF pressure required to permit CSF absorption through the arachnoid villi into the superior sagittal sinus.

Source: Cutler RWP, et al. *Brain* 1968, **91**(4):707-720

CSF Flow in Aqueduct of Sylvius calculation by advanced MRI in normal and CCSVI conditions



In CCSVI associated to MS during diastole, the retrograde flow of CSF back towards the third ventricle was approximately twice (i.e. 37.13 mm^3/ beat) that of healthy controls (i.e. 19.30 mm^3/ beat) .
By comparison, during systole the displacement in the opposite direction was about the same for both cohorts
(i.e. approx. 32 mm^3/ beat)

Source: Zamboni P, et al. Funct N.eur 2009; 24; 107-112
Naish JH, et al. Magn Reson Med. 2006; 56; 509-516.

Physical correlation between CSF flow and SSS venous pressure

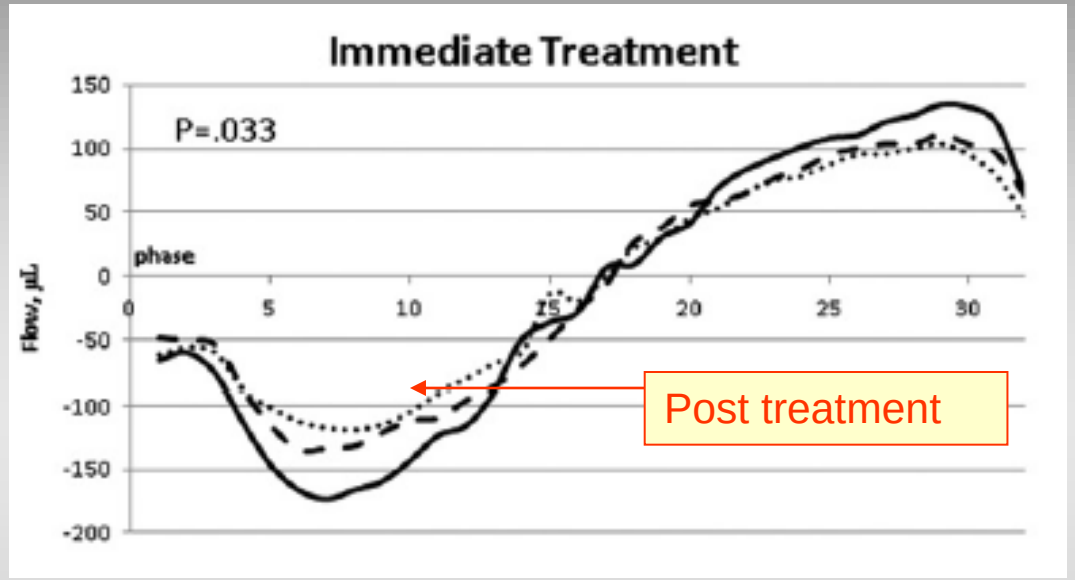
Cerebrospinal Fluid Flow and Velocity Differences Between the Study Groups

CSF parameter	HC (*n*=35)	MS (*n*=67)	*P* value
Net flow (μL/beat)	−7.1±5.5	−3.7±9.4	0.005
Net negative flow (μL/beat)	−30.5±19.2	−37.8±22.8	0.11
Net positive flow (μL/beat)	23.5±16.5	34.1±20.0	0.004
Peak negative velocity (cm/s)	−8.5±3.5	−9.4±3.8	0.22
Peak positive velocity (cm/s)	6.6±3.6	7.3±2.6	0.04
Average short axis length (mm)	1.6±0.3	1.7±0.3	0.06

- Magnano et al found CSF absorption to be reduced in CCSVI and MS patients by approx. 3.4 mm^3/beat.
- This equates to a mean reduction in the SAS-SSS pressure difference of about 2.3 mmHg.
- Magnano et al found aqueductal CSF net positive flow (i.e. towards the brain) to be 45.1% greater in CCSVI and MS patients compared with controls (p = 0.004).

Source: Magnano C, et al. *Magnetic Resonance Imaging*. 36; pp. 825-834; (2012)

Venous Angioplasty (temporary improvement of the CCSVI condition) and CSF Pulsatility

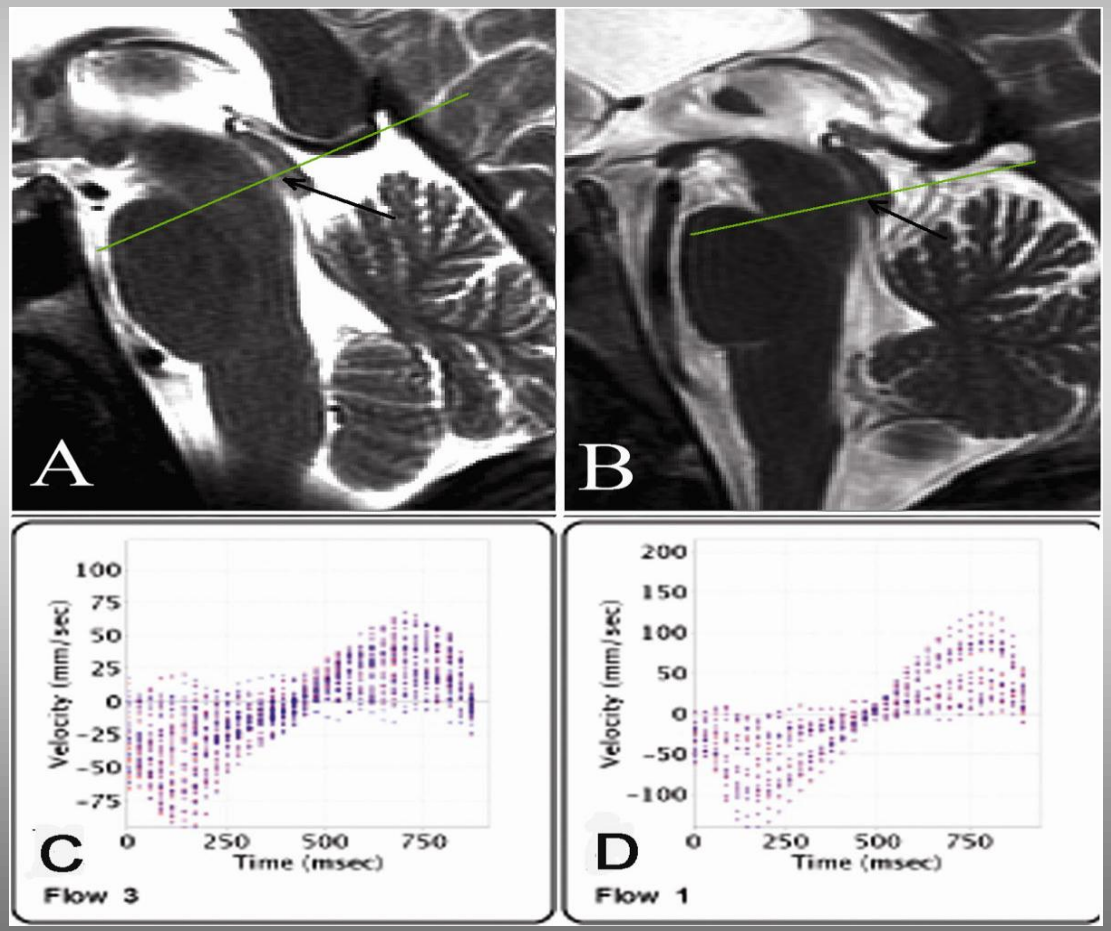


Prior to treatment

Zivadinov et al showed that the intervention of venous angioplasty in CCSVI positive MS patients, had a normalizing effect on CSF pulsatility. In control cases over 6 months no CSF flow variation were assessed.
This reinforces the opinion that MS is characterised by mild venous hypertension.

Zivadinov R, et al. JVIR. 2013; 24; 107-112

CASE CONTROL WITH BLINDED OUTCOME MEASURE



MR-T2 LESION VOLUME : Fup 6m reduced MR T2 lesion volume in treated vs non treated p<.08 ***(EJEVS 2012)***

CSF FLOW DYNAMICS: At month 6, significant improvement in CSF flow (p<0.001) and velocity (p=0.013) was detected in the treated group vs not treated group ***(JVIR 2013)***

RESTRICTED VENOUS OUTFLOW AND REDUCED REABSORPTION OF CEREBRO SPINAL FLUID: A PIECE OF THE PUZZLE?

J Magn Reson Imaging. 2012 Oct;36(4):825-34. doi: 10.1002/jmri.23730. Epub 2012 Jun 25.

Cine cerebrospinal fluid imaging in multiple sclerosis.

- **Altered CSF flow and velocity measures were associated with more severe T1 and T2 lesion volumes, ($P < 0.01$ for all).**

- **In CIS patients, conversion to clinically definite MS in the following year was related to decreased CSF net flow ($P = 0.007$).**

- **Slow CSF flow is also linked with neurodegeneration in Alzheimer disease and in normotensive hydrocephalus**

1: Daouk J, et al Relationship between cerebrospinal fluid flow, ventricles morphology, and DTI properties in internal capsules: differences between Alzheimer's disease and normal-pressure hydrocephalus. Acta Radiol. 2013 Oct 17. [Epub ahead of print]

2: Hosseinzadeh S, et al. Elevated CSF and plasma microparticles in a rat model of streptozotocin-induced cognitive impairment. Behav Brain Res. 2013 1;256:503-11.

3: Erickson MA, et al. Lipopolysaccharide impairs amyloid Œ≤ efflux from brain: altered vascular sequestration, cerebrospinal fluid reabsorption, peripheral clearance and transporter function at the blood-brain barrier. J Neuroinflammation. 2012,29;9:150.

4: Stomrud E, et al. CSF biomarkers correlate with cerebral blood flow on SPECT in healthy elderly. Dement Geriatr Cogn Disord. 2012;33(2-3):156-63.

5: Santos AN, et al. Amyloid-Œ≤ oligomers in cerebrospinal fluid are associated with cognitive decline in patients with Alzheimer's disease. J Alzheimers Dis. 2012;29(1):171-6.

6: Banks WA, et al. Impairments in brain-to-blood transport of amyloid-Œ≤ and reabsorption of cerebrospinal fluid in an animal model of Alzheimer's disease are reversed by antisense directed against amyloid-Œ≤ protein precursor. J Alzheimers Dis. 2011;23(4):599-605.

Journal of Alzheimer's Disease 39 (2014) 601–609
DOI 10.3233/JAD-131112
IOS Press

Jugular Venous Reflux and White Matter Abnormalities in Alzheimer's Disease: A Pilot Study

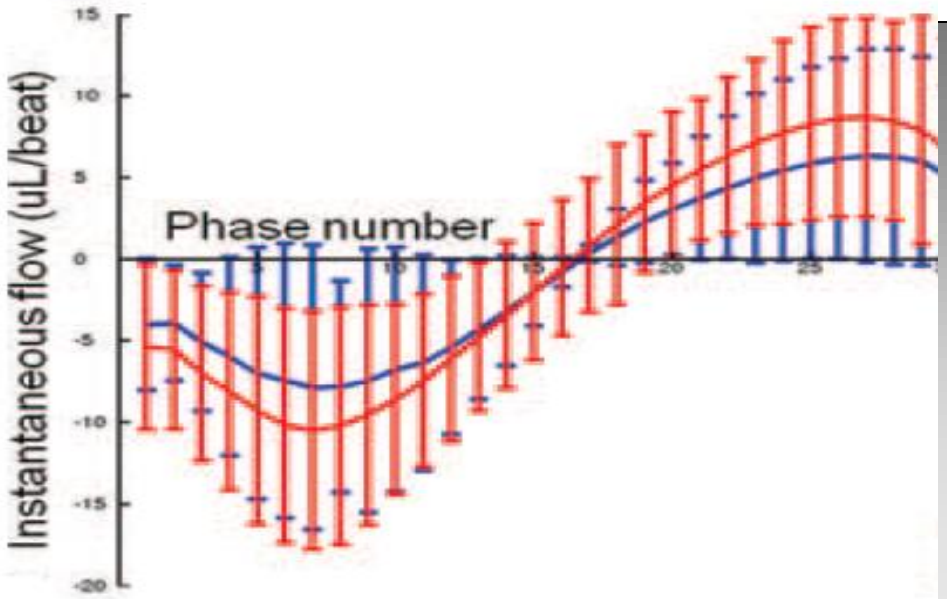
Chih-Ping Chung[a,b], Clive Beggs[c], Pei-Ning Wang[a,b,1], Niels Bergsland[d], Simon Shepherd[c], Chun-Yu Cheng[a,b,e], Deepa P. Ramasamy[d], Michael G. Dwyer[d], Han-Hwa Hu[a,b] and Robert Zivadinov[d,*,1]

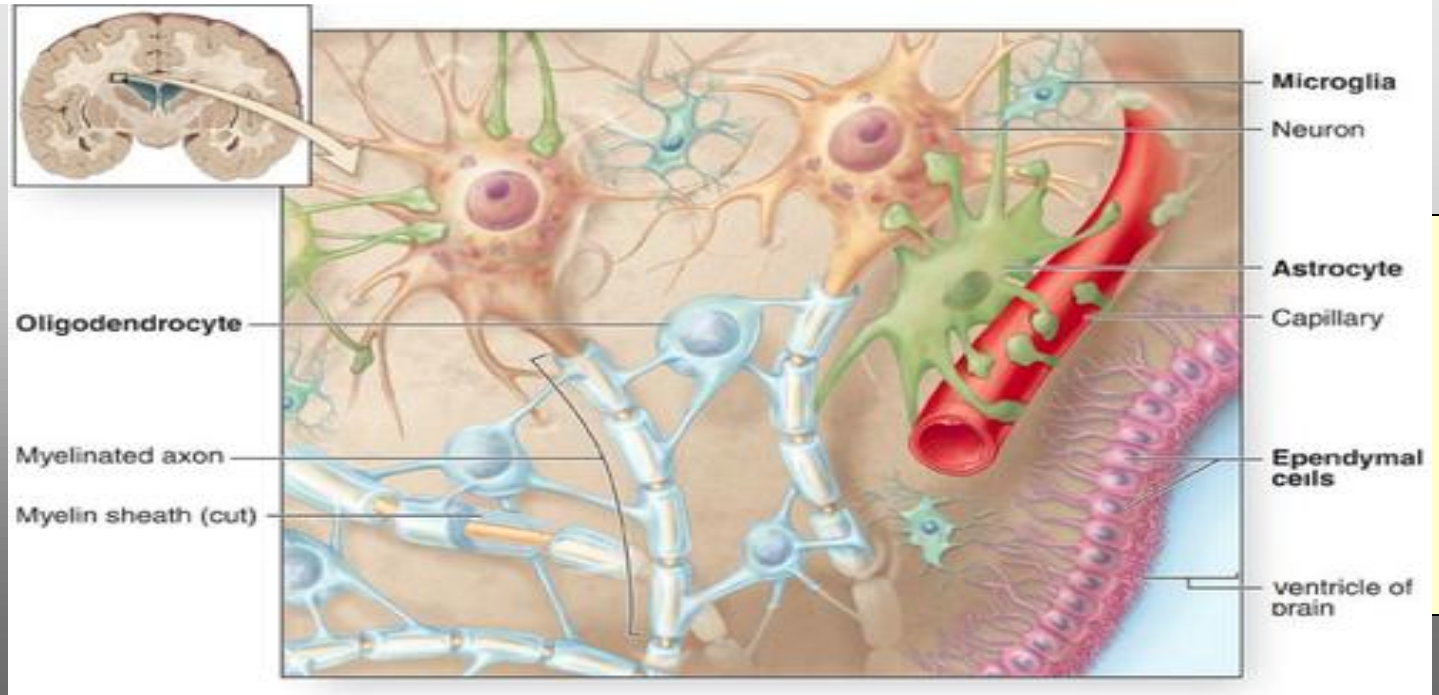
Jugular venous reflux

reduced CSF re-absorption

more MRI lesion in AD

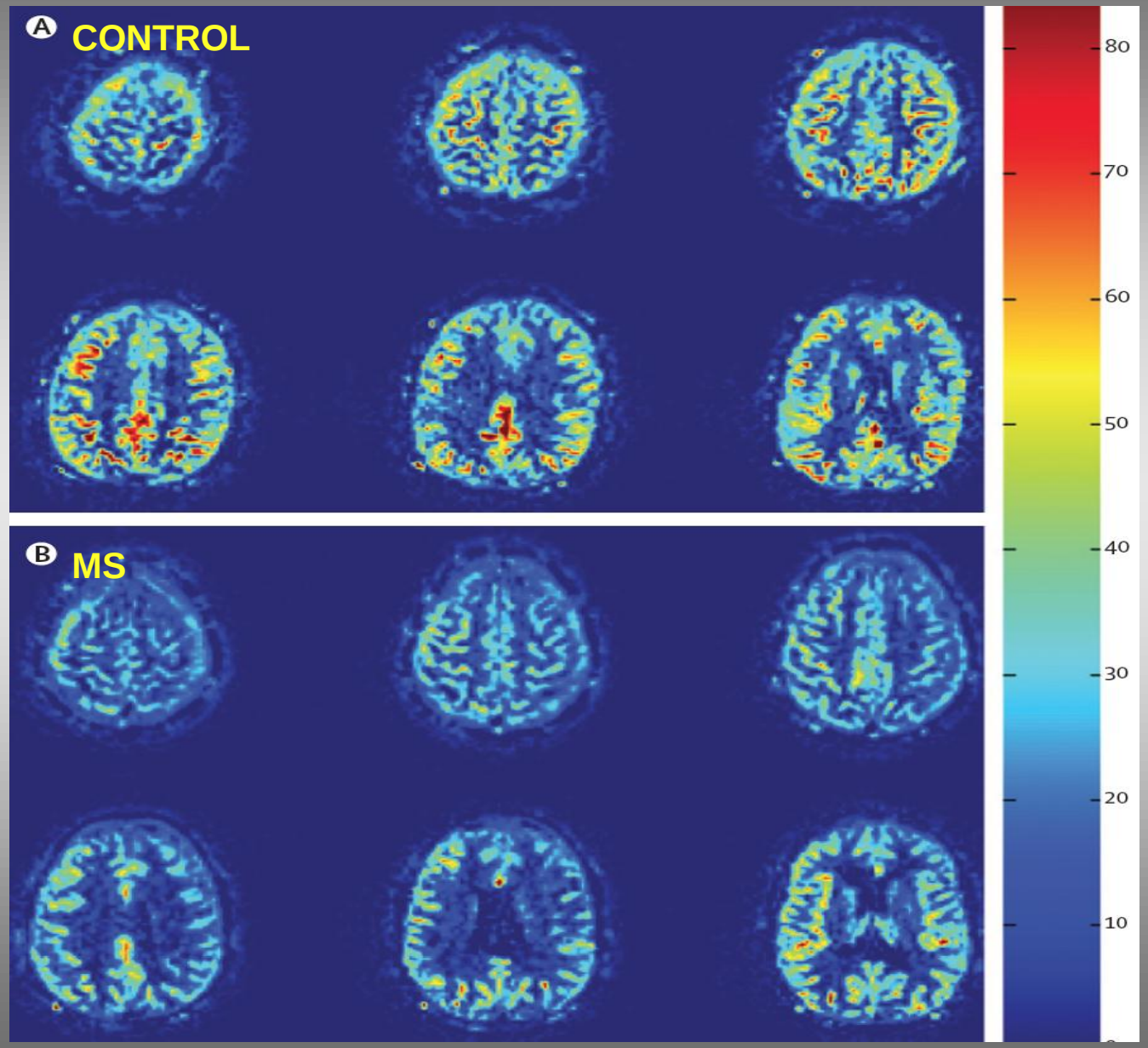
Work to progress: the impact of Ventricular Reflux





The impact of ventricular reflux is not well understood. White matter edema is likely to alter the chemical composition of the interstitial fluid in the periventricular region.

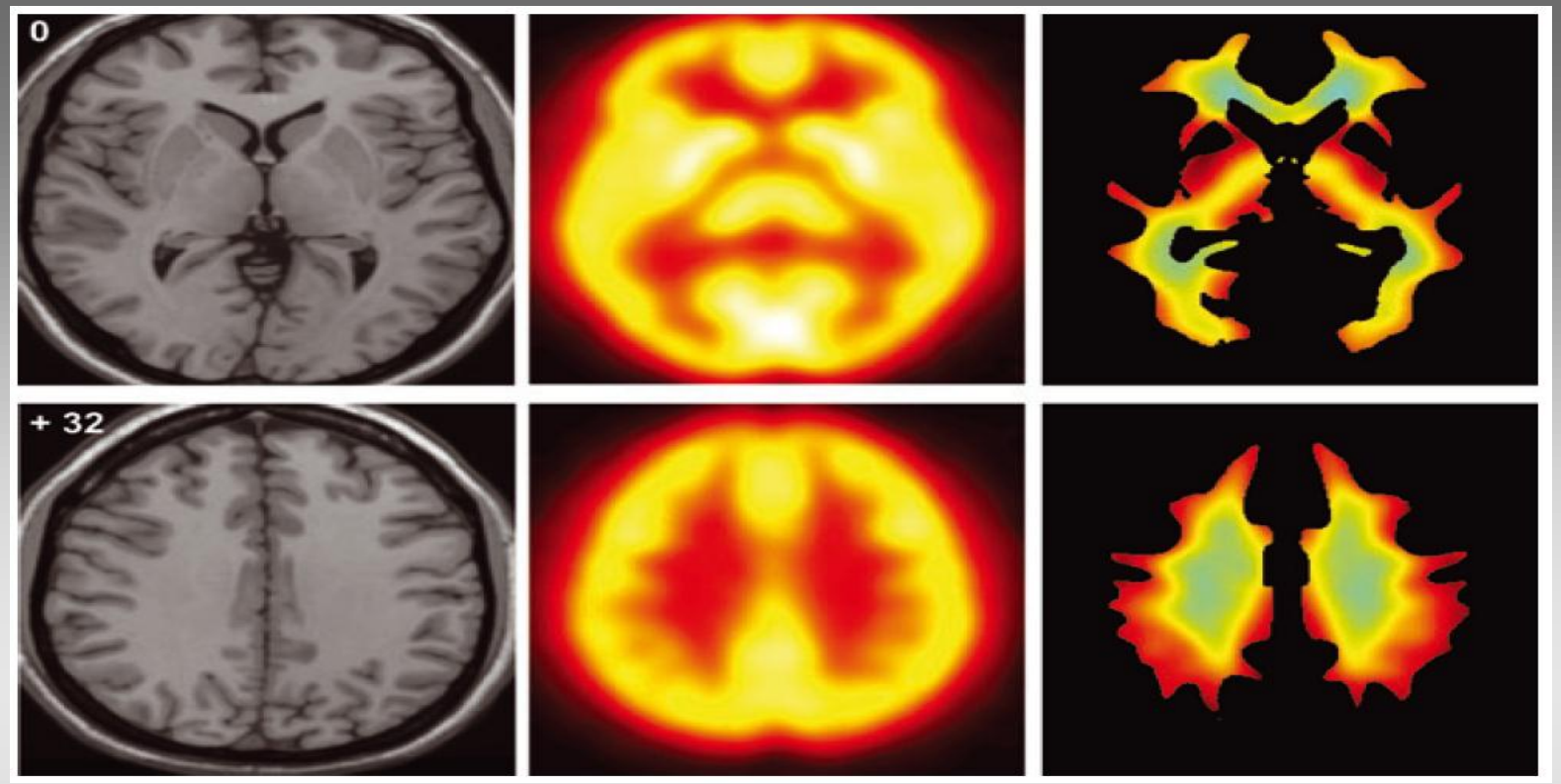
MR GLOBAL HYPOPERFUSION IN MS



Diffuse hypoperfusion in MS is a FACT. It cannot be explained with autoimmunity but CCSVI is a valuable hypothesis

Lancet Neurology 2011

- 1294 SPECT-MRI in MS cases, early and late RR, SP respectively.
- Comparison with normal cerebral perfusion patterns provided by SPECT atlas of normal healthy individuals



International Consortium for Brain Mapping.

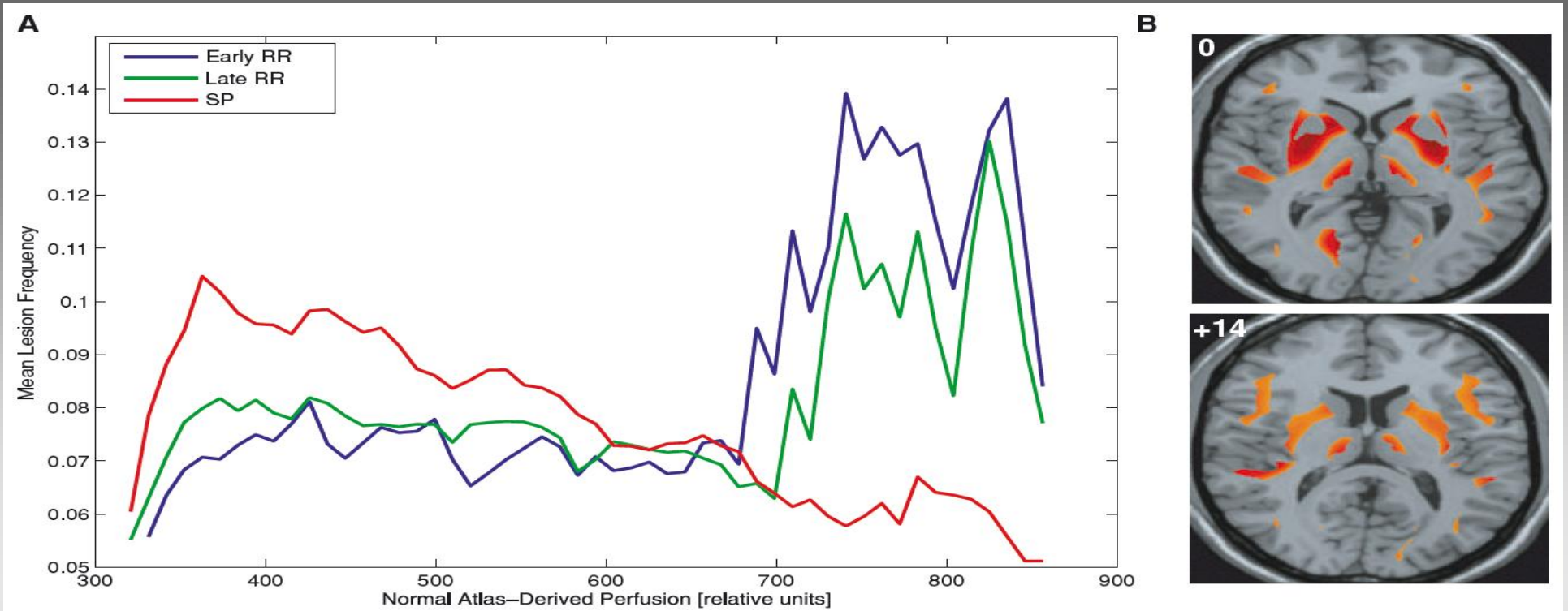
ICBM Atlas of normality

SPECT Atlas of normality generated from 47 healthy subjects.

Harvard Medical School

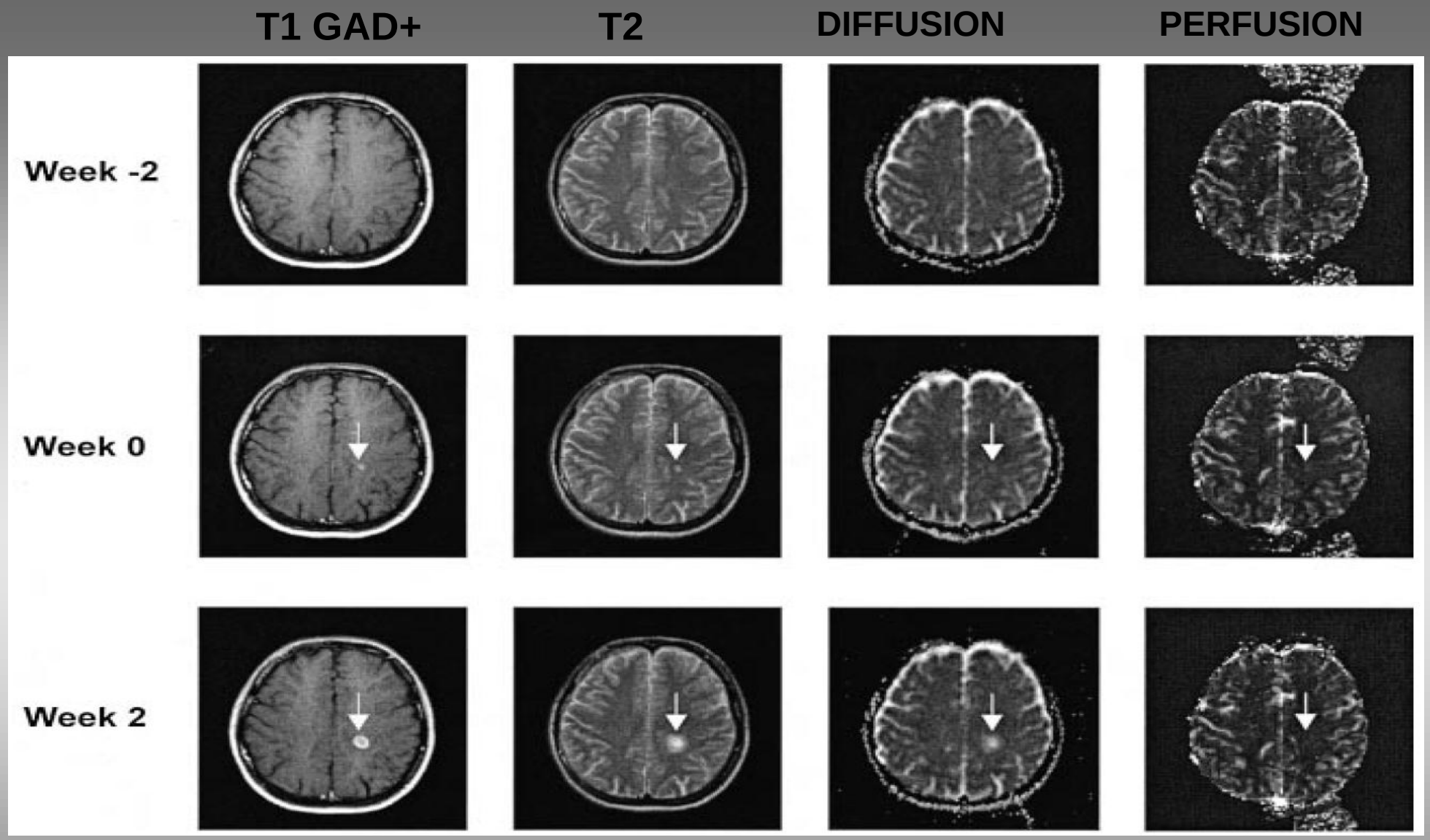
Variability of perfusion of the white matter in normal cases.

Guttmann, J Neuroimaging 2012



Chronic plaques were more prevalent in WM regions with lower relative perfusion. Lesions in more highly perfused regions were more commonly observed in early RR MS and therefore, may be more likely to successfully remyelinate and resolve.

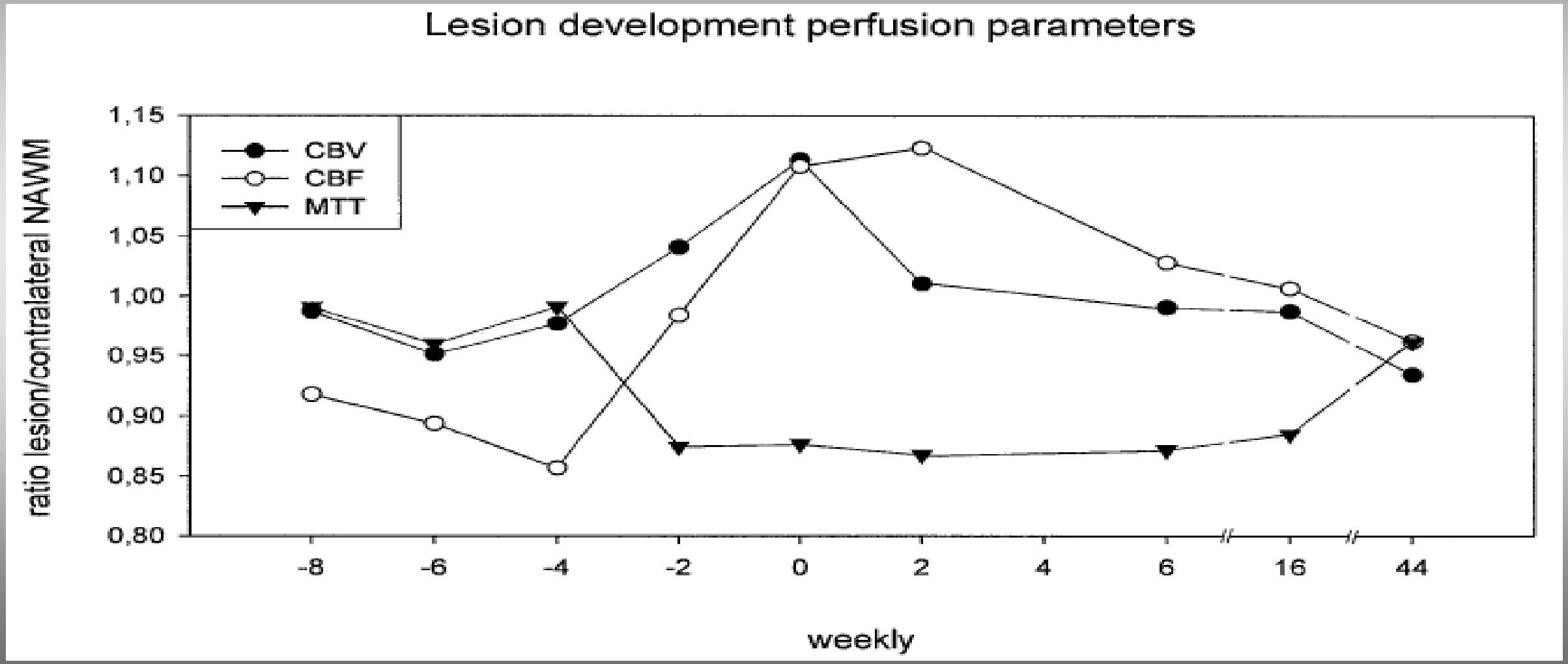
Guttmann, J Neuroimaging 2012



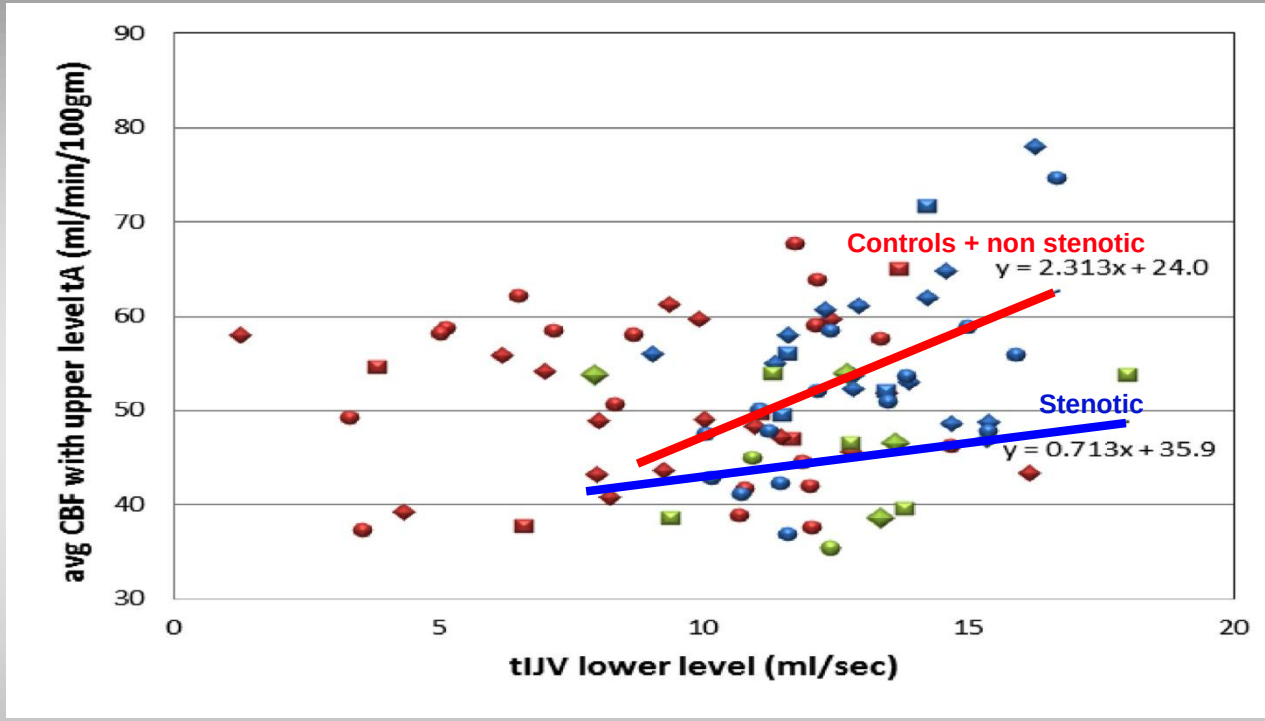
HYPOPERFUSION PRECEDES PLAQUE FORMATION

Brain 2004

Diffuse brain hypoperfusion precedes plaque formation (Brain 127,111-119, 2004)



RELATIONSHIP BETWEEN BRAIN PERFUSION AND JUGULAR FLOW: THE SIGNIFICANCE OF STENOSIS

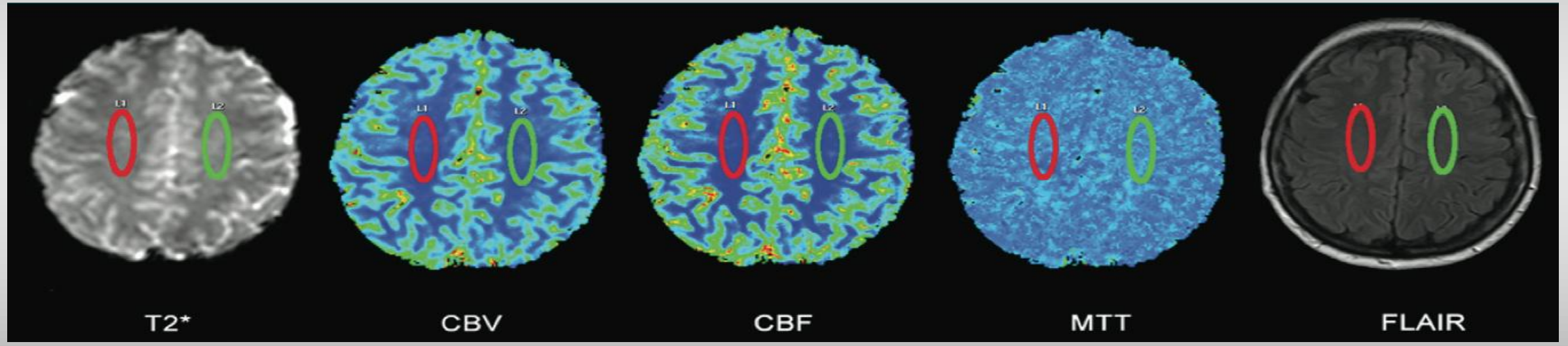


- Evidence already exists for reduced perfusion in patients with MS but there has been no attempt to correlate this with obstructed venous outflow.

- 2D magnetic resonance imaging (MRI) flow techniques demonstrate that flow in the internal jugular veins in humans is linearly related to global brain perfusion

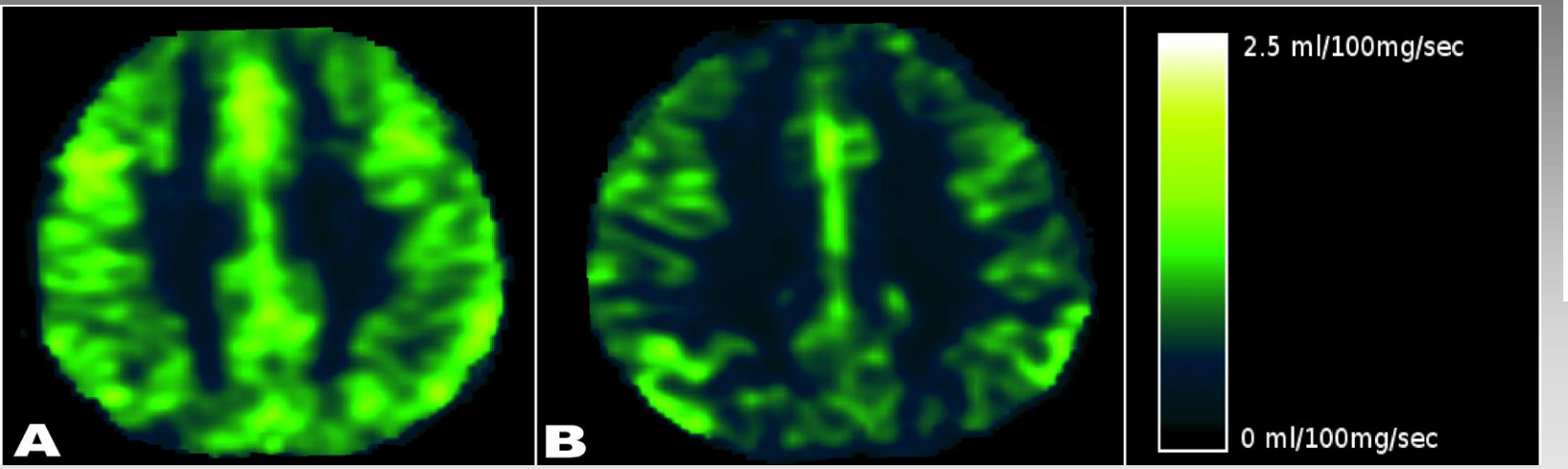
Neurol Res. 2012 Oct;34(8):780-92

The data support a role of CCSVI in cerebral hemody- namic changes, such as a decrease of CBV and CBF, re- gardless of the presence of MS.



Garaci et al, Radiology: Volume 265: October 2012

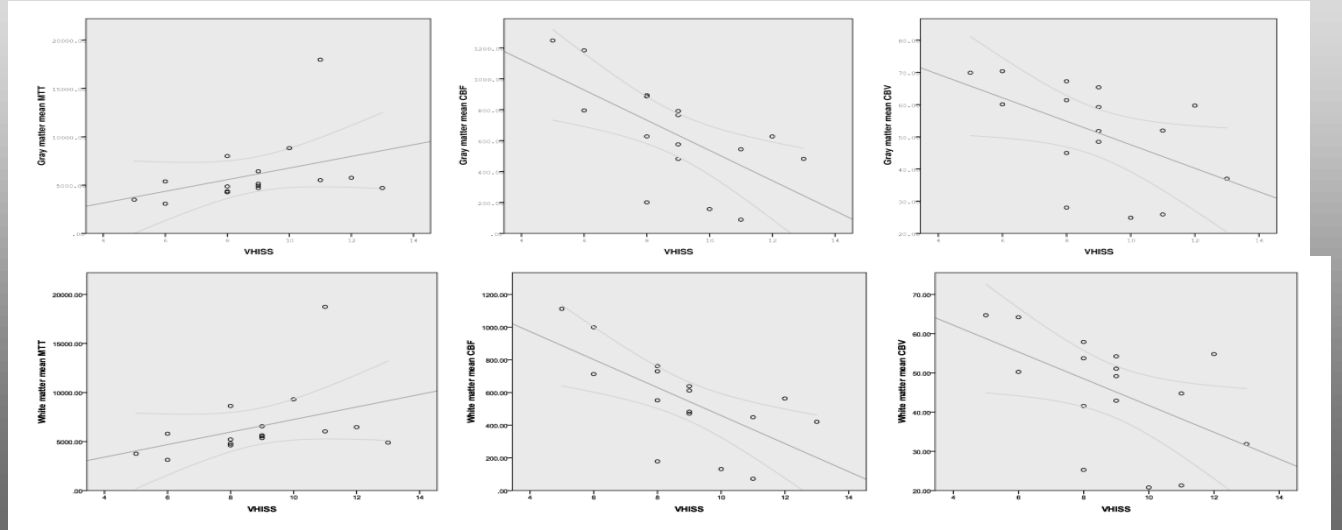
CCSVI is related to brain hypoperfusion



A: CBF in a 33 yo, relapsing remitting, CCSVI-MS patient with a VHISS 5.
B: CBF in a 38 yo, relapsing remitting, CCSVI-MS patient with a VHISS 12.
The dark areas indicate lower CBF in the patient with higher VHISS.

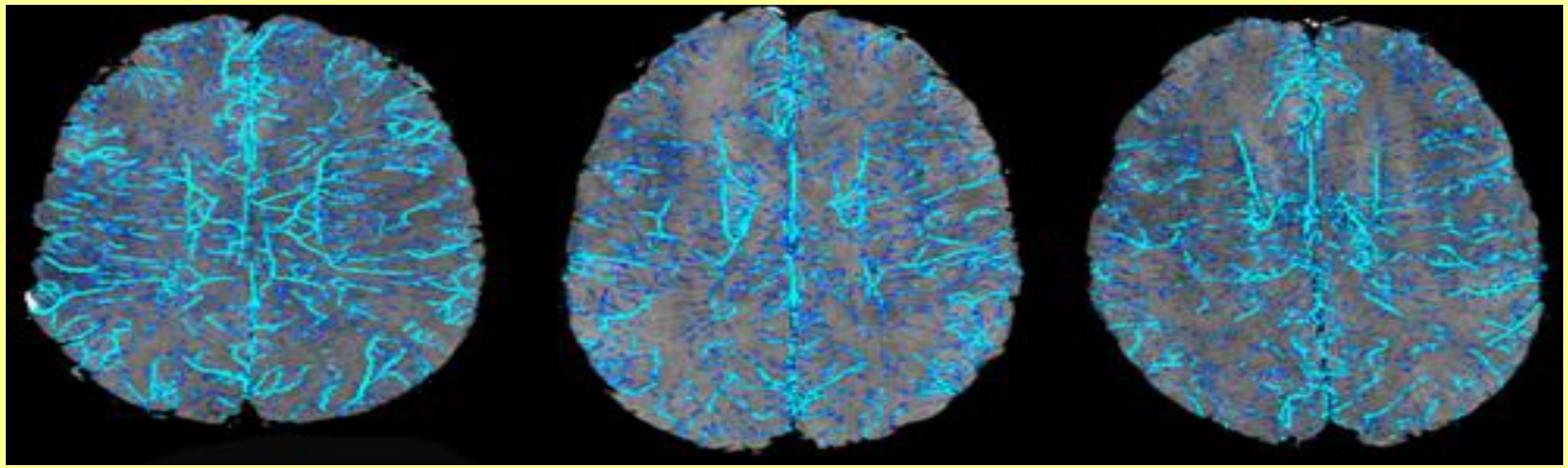
Cerebral blood flow (CBF) at MRI perfusional study

Robust correlation between VHISS and MR perfusional parameters (r= -0.70 to -0.71, p<0.002).



BMC Medicine 2011

MS & Loss of Cerebral Veins



HC RR SP

- MS is associated with a marked deduction in cerebral vein volume (VV).
- VV (for all vein diameters): HC = 82.9 mL; MS = 66.9 mL;

  Reduction = 19.3%; p<0.0001
- VV (for veins <0.3 mm): HC = 53.8 mL; MS = 45.0 mL;

  Reduction = 16.4%; p<0.0001 [Strongly correlated with CCSVI (p<0.003)]

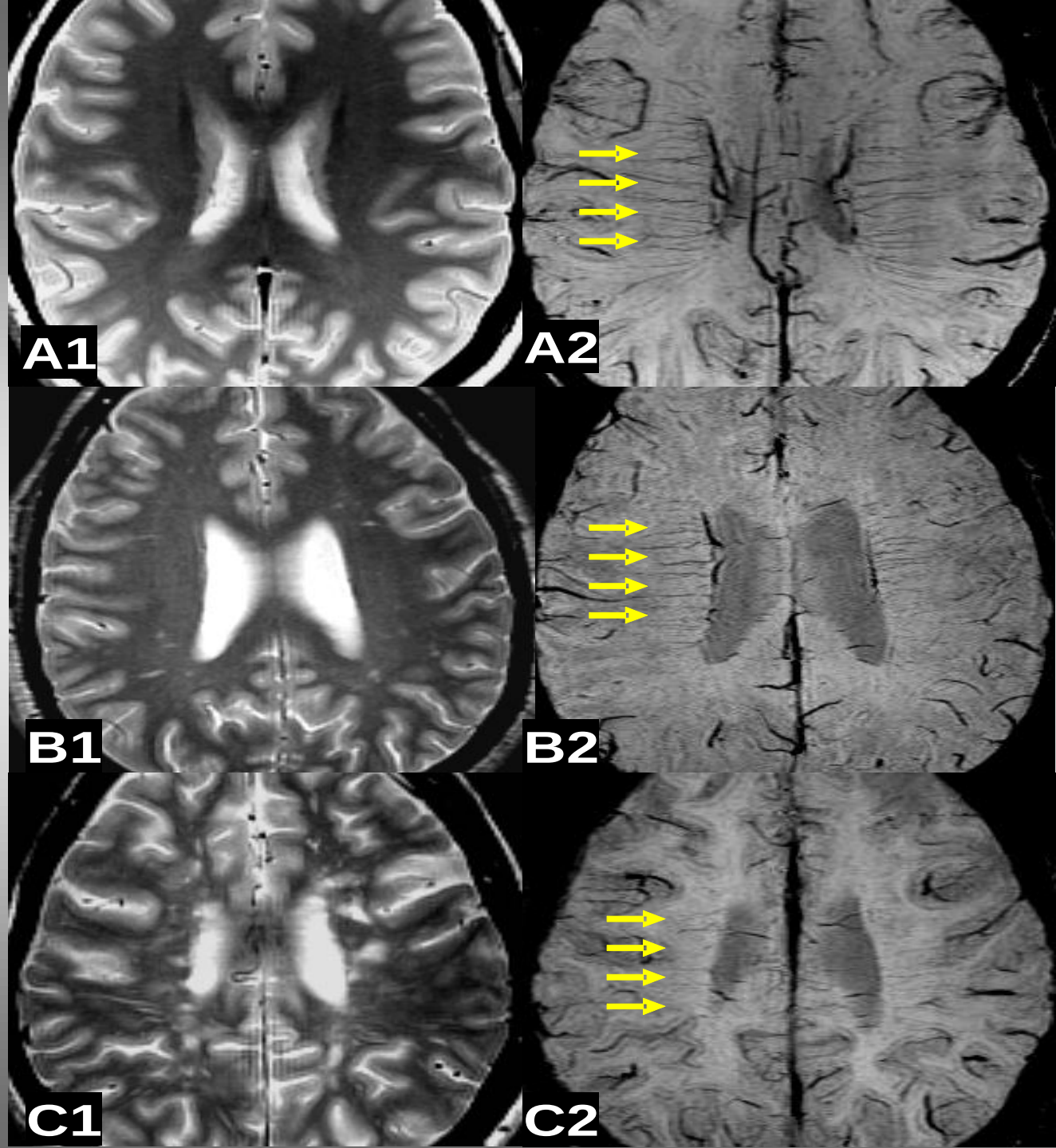
Source: Zivadinov R, et al. BMC Neurology 2011, 11:128

SWI Venography

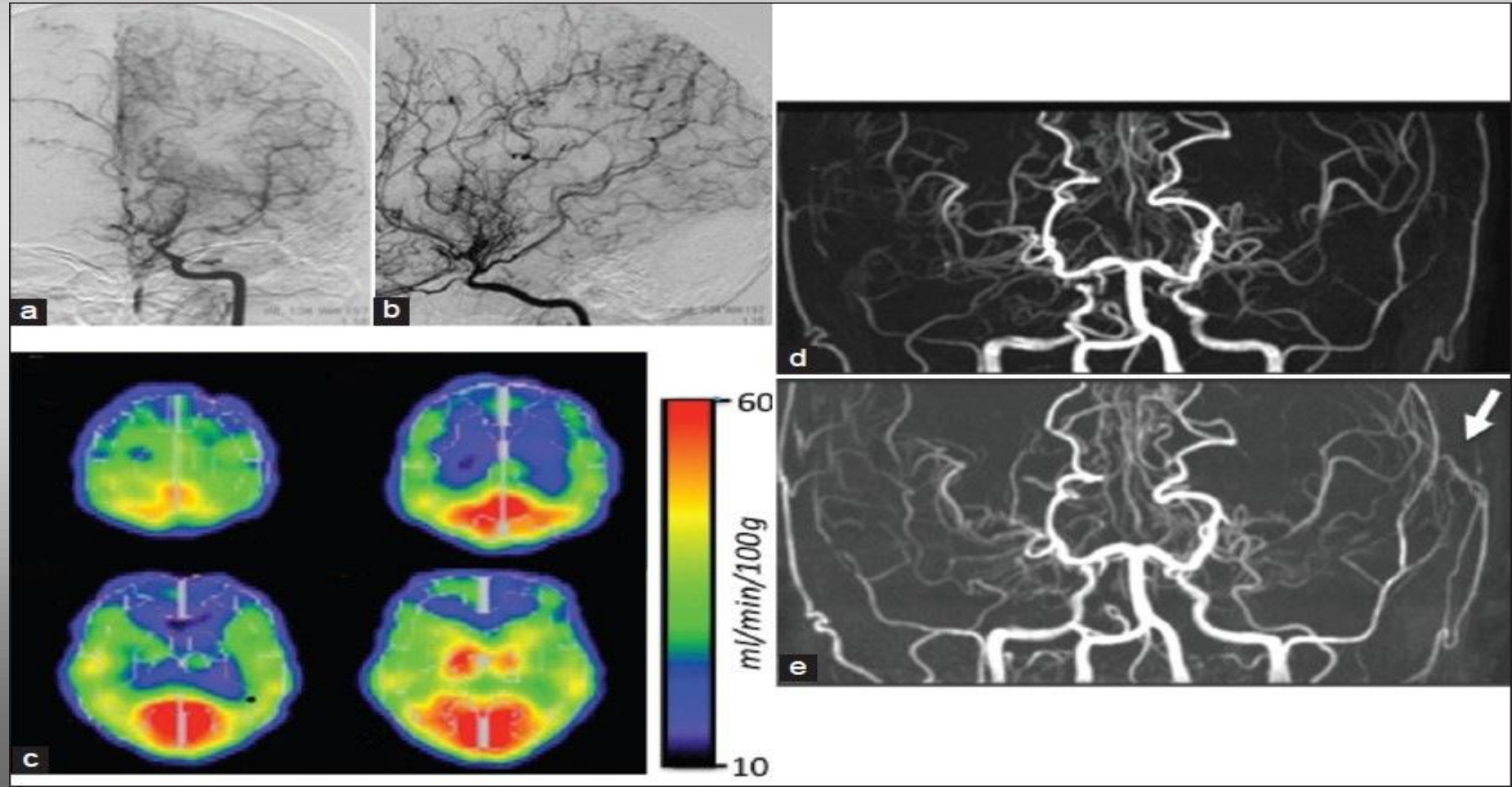
normal control (A) and two MS patients (B, C) demonstrate a significantly reduced number of veins in perviventricular NAWM in patients compared to controls. MS patient with more lesions (C) has less venous structures depicted on SWI mIP image than MS patient with fewer lesions (B).

Do vessels degrade because flow is shunted away from them?

Slide courtesy of Yulin Ge, NYU



Work to progress: increased venous outflow resistance in relation to arteriolar shunting and reduced perfusion

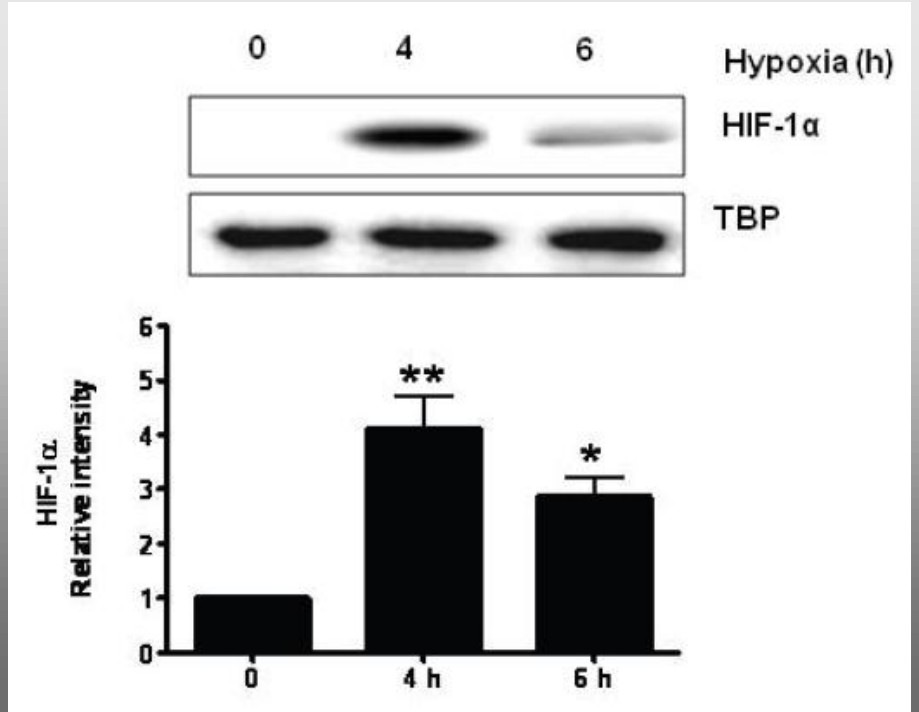


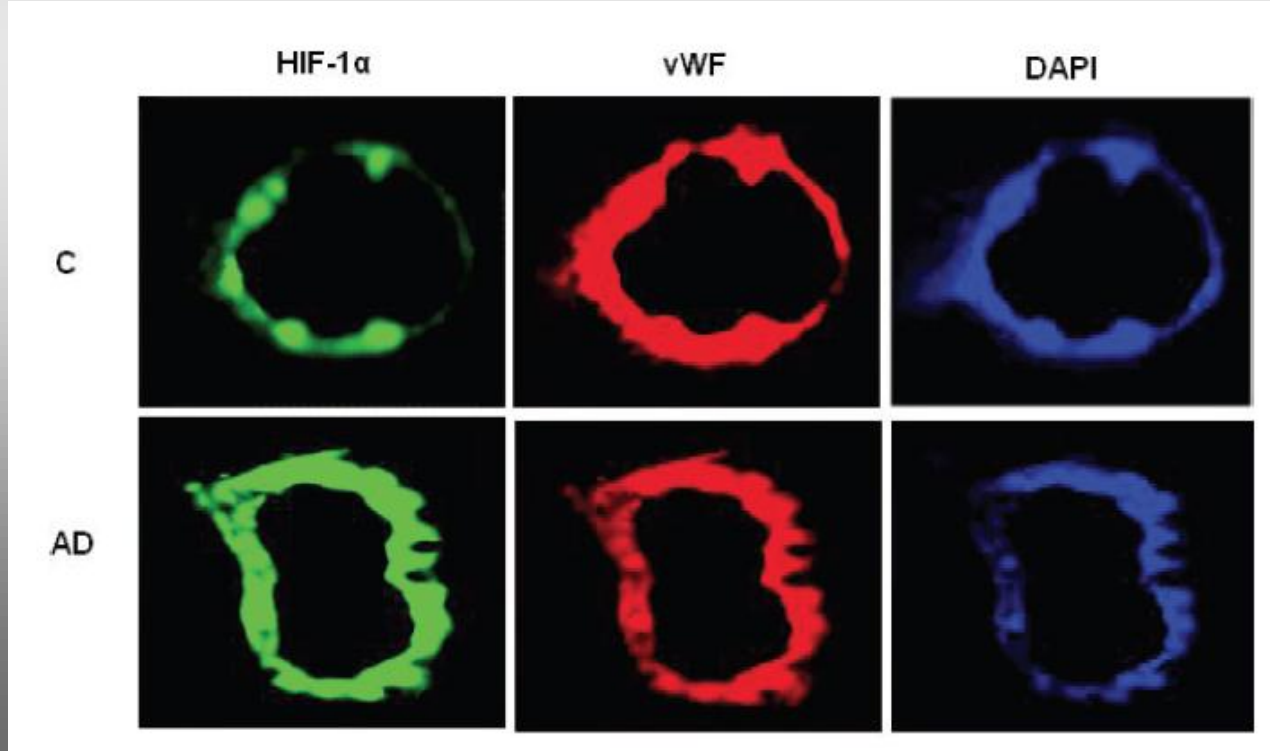
Int J Clin Exp Pathol 2011;4(6):616-627
www.ijcep.com /IJCEP1105004

Original Article

Brain microvasculature and hypoxia-related proteins in Alzheimer's disease

Paula Grammas, Debjani Tripathy, Alma Sanchez, Xiangling Yin, Jinhua Luo



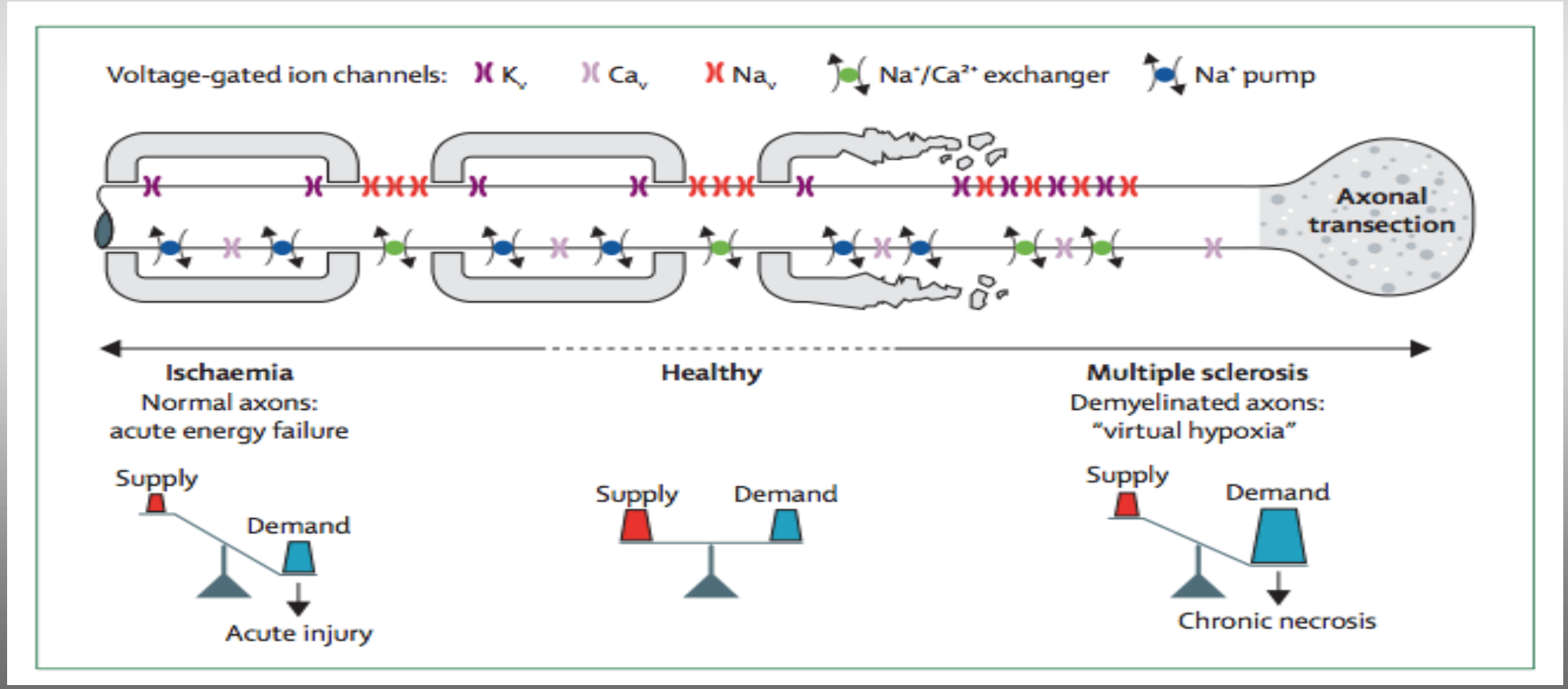


CONSEQUENCE OF HYPOPERFUSION IN MS

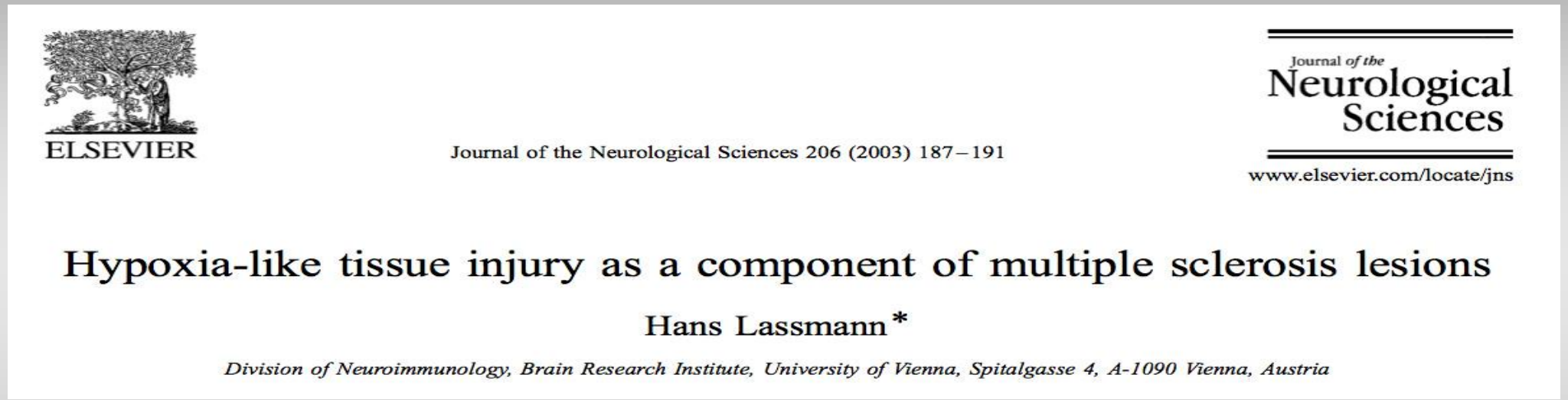
Lancet Neurology 2009

Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis

Bruce D Trapp, Peter K Stys



Oligodendrocyte susceptibility to hypoperfusion



ELSEVIER

Journal of the Neurological Sciences 206 (2003) 187–191

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Hypoxia-like tissue injury as a component of multiple sclerosis lesions

Hans Lassmann*

Division of Neuroimmunology, Brain Research Institute, University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria

- ROS (increased i-NOS in macrophages and microglia)
- Mitochondria impairment (defective phosphorilation)
- Hypoxia associated molecules (expression HIF 1 alfa)

ORIGINAL ARTICLES

Multiple Sclerosis

Distribution of Inflammatory Cells in Newly Forming Lesions

Andrew P. D. Henderson, MBBS,[1] Michael H. Barnett, MBBS, PhD,[1,2] John D. E. Parratt, MBBS, PhD,[1] and John W. Prineas, MBBS[1]

Interpretation: Early loss of oligodendrocytes is a prominent feature in tissue bordering rapidly expanding MS lesions. Macrophage activity is largely an innate scavenging response to the presence of degenerate and dead myelin. Adaptive immune activity involving T and B cells is conspicuous chiefly in recently demyelinated tissue, which may show signs of oligodendrocyte regeneration. The findings suggest that plaque formation has some basis other than destructive cell-mediated immunity directed against a myelin or oligodendrocyte antigen.

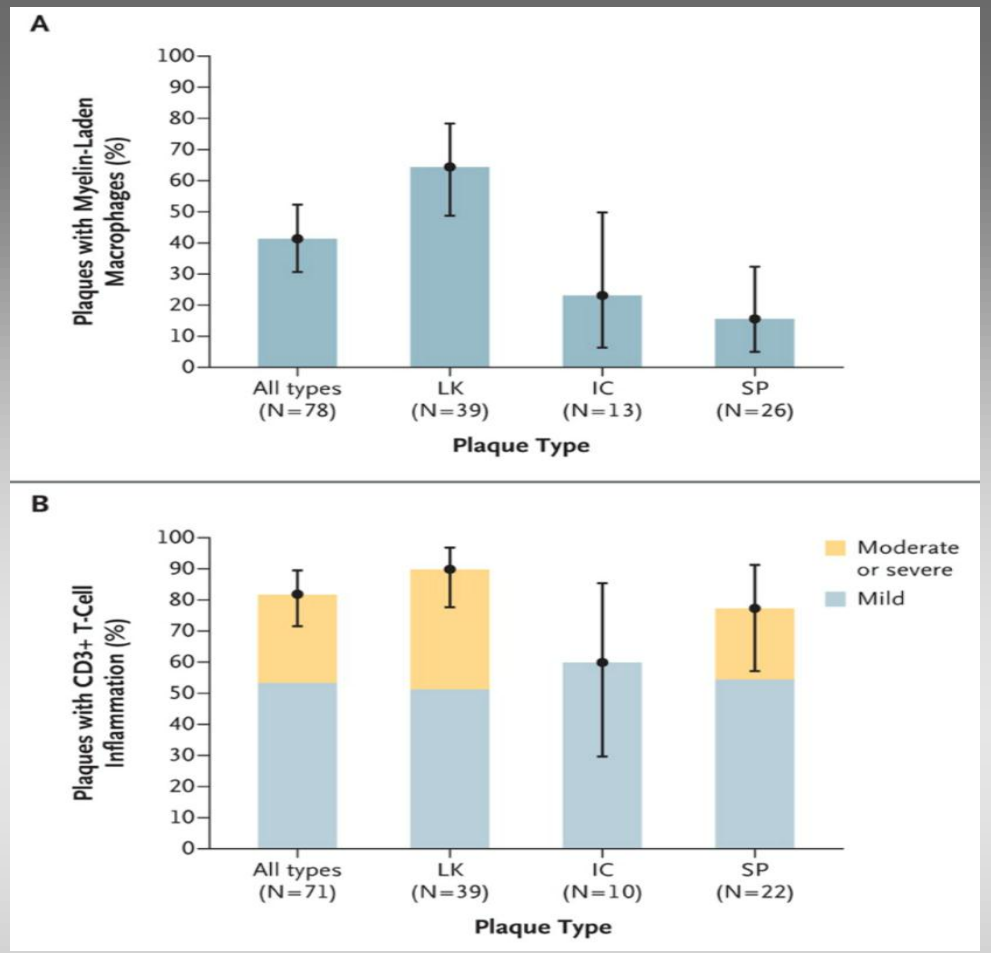
Ann Neurol 2009;66:739–753

NEW MS LESIONS

1. Loss of oligodendrocytes
2. Dead myelin
3. Myelin-laden macrophages
4. Only subsequently T and B cells infiltration

• Damage to axon can occur without the presence of inflammation (Int MS J. 2009 Jun;16(2):57-6; Ann Neurol. 2009 Dec;66(6):739-53

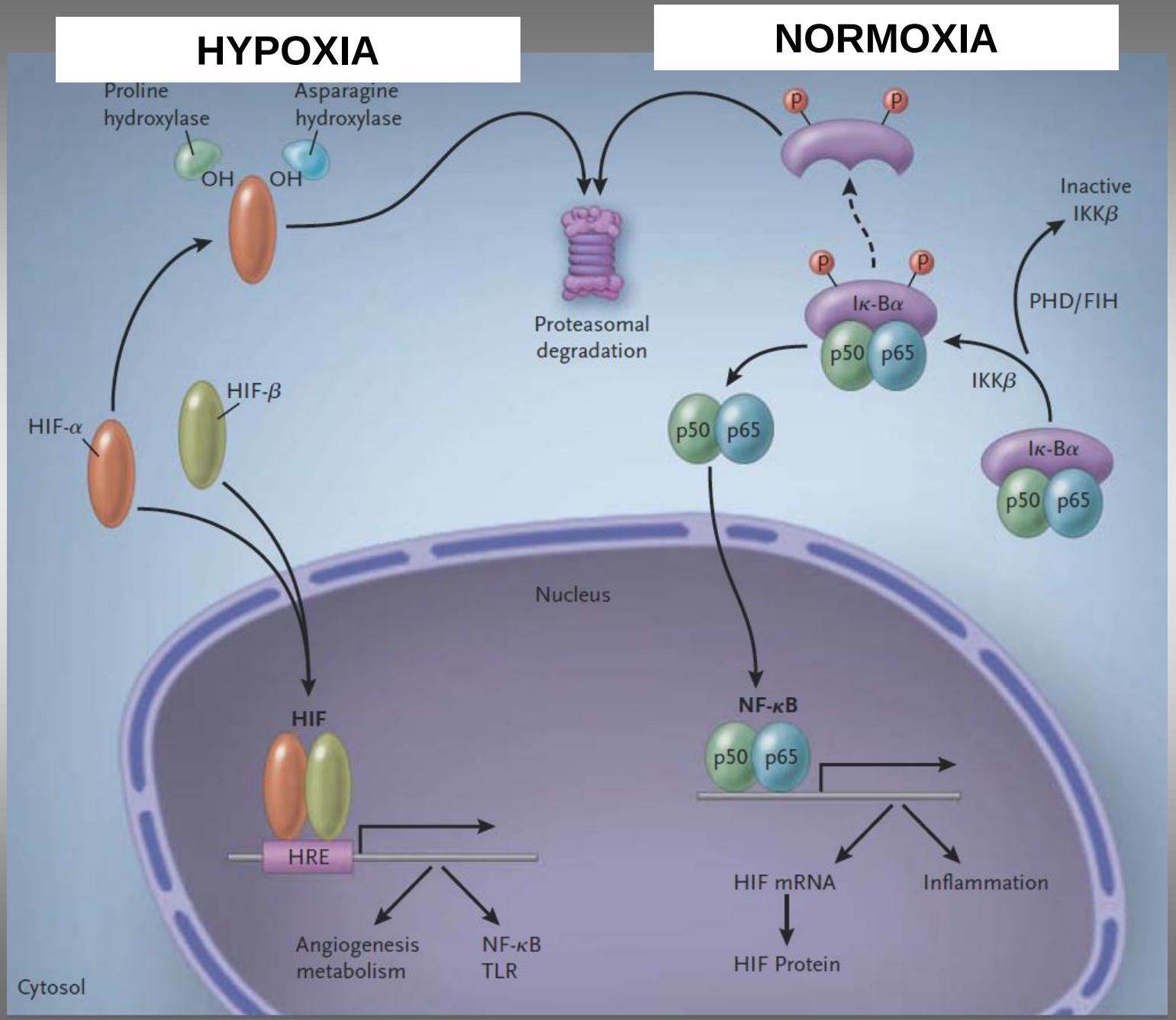
• Axon demyelination was seen in early lesion without inflammatory cells (NEJM 2011).



DEMYELINATION AND AXONAL DAMAGE PRECEDE T-CELL - INFLAMMATORY CELL INFILTRATION

ANN NEUR 2009; NEJM 2011

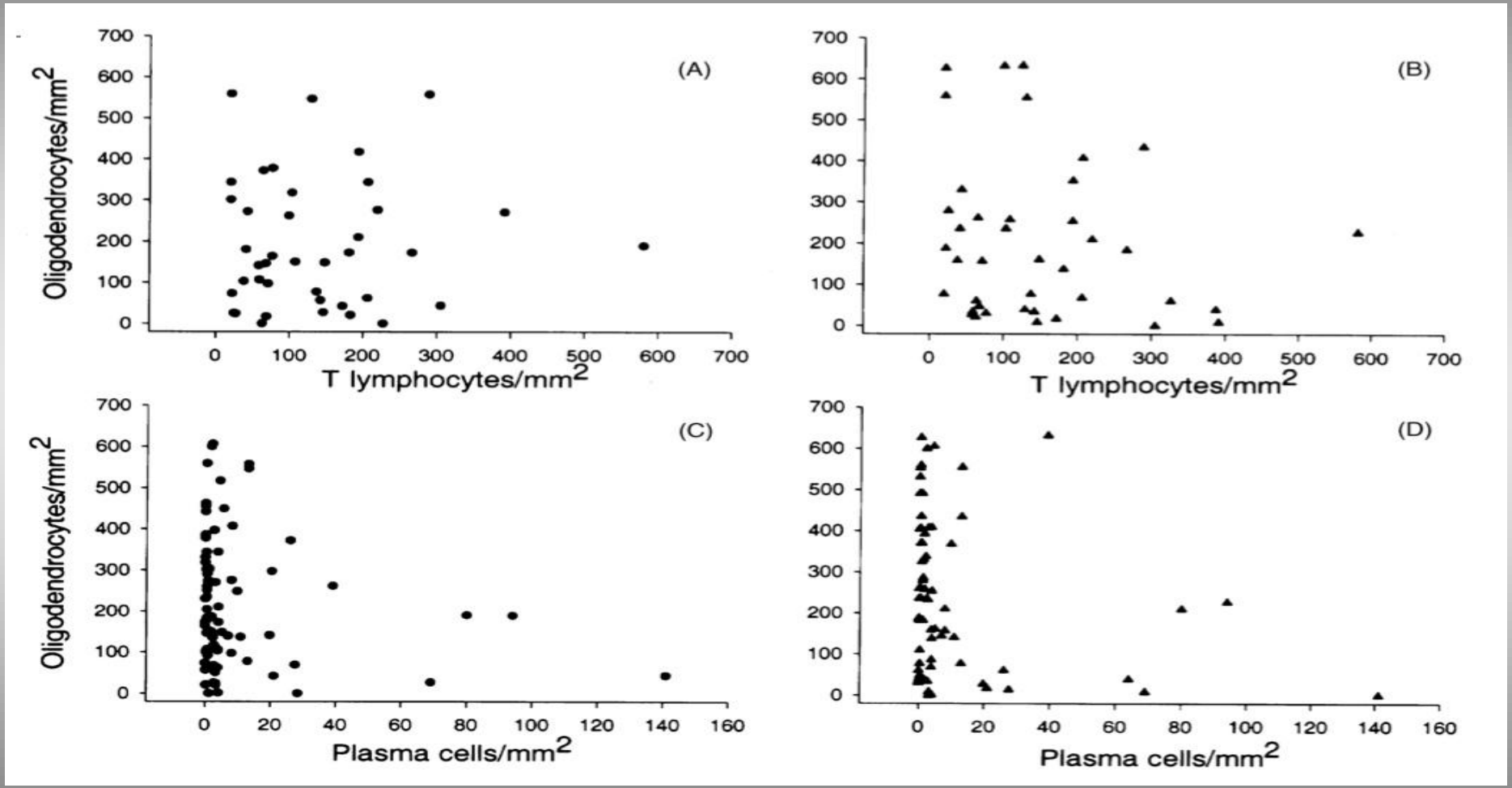
The loop between hypoxia and NF-kB



1. **Hypoxia induces HIF alfa and beta translocating to the nucleus**
2. **Binding with hypoxia response promoter HRE and gene interaction**
3. **Genes of the NF-kB**
4. **In addition, in hypoxia the p50 and p65 subunits of NF-kB are no more inhibited, translocate to nucleous, and in turn activate inflammatory genes**

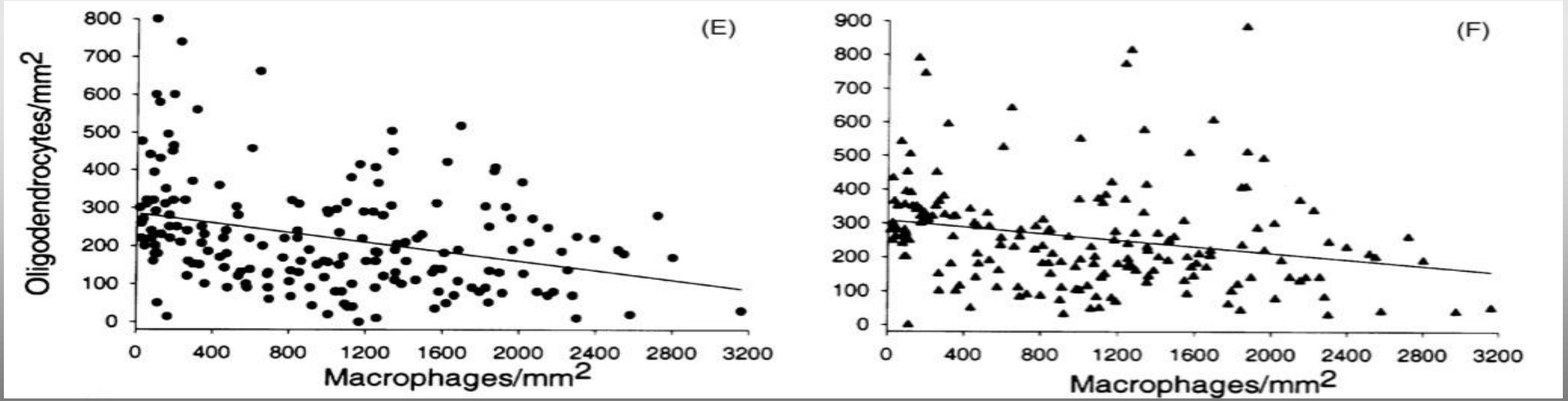
Source: N Engl J Med 2012

NO CORRELATION BETWEEN LOSS OF OLIGODENDROCYTES AND T AND PLASMA CELLS INFILTRATION… BUT



Lucchinetti, Brain 1999

….INVERTED CORRELATION WITH INFILTRATION OF MACROPHAGES TAKEN UP MYELIN DEBRIS



Lucchinetti, Brain 1999

PATHOLOGIC PATTERNS OF MS

I. Massive tissue distruction mediated by CD8+T cells infiltrates and macrophages.

II. Massive deposition of immunoglobulins and component of activated complement

Are chronologically late events?

III. Oligodendrocytes apoptosis, “dying back” oligodendrogliopathy

IV. Neurodegeneration and oligodendrocytes death also in the periplaque WM

Are effects of the hypoperfusion?

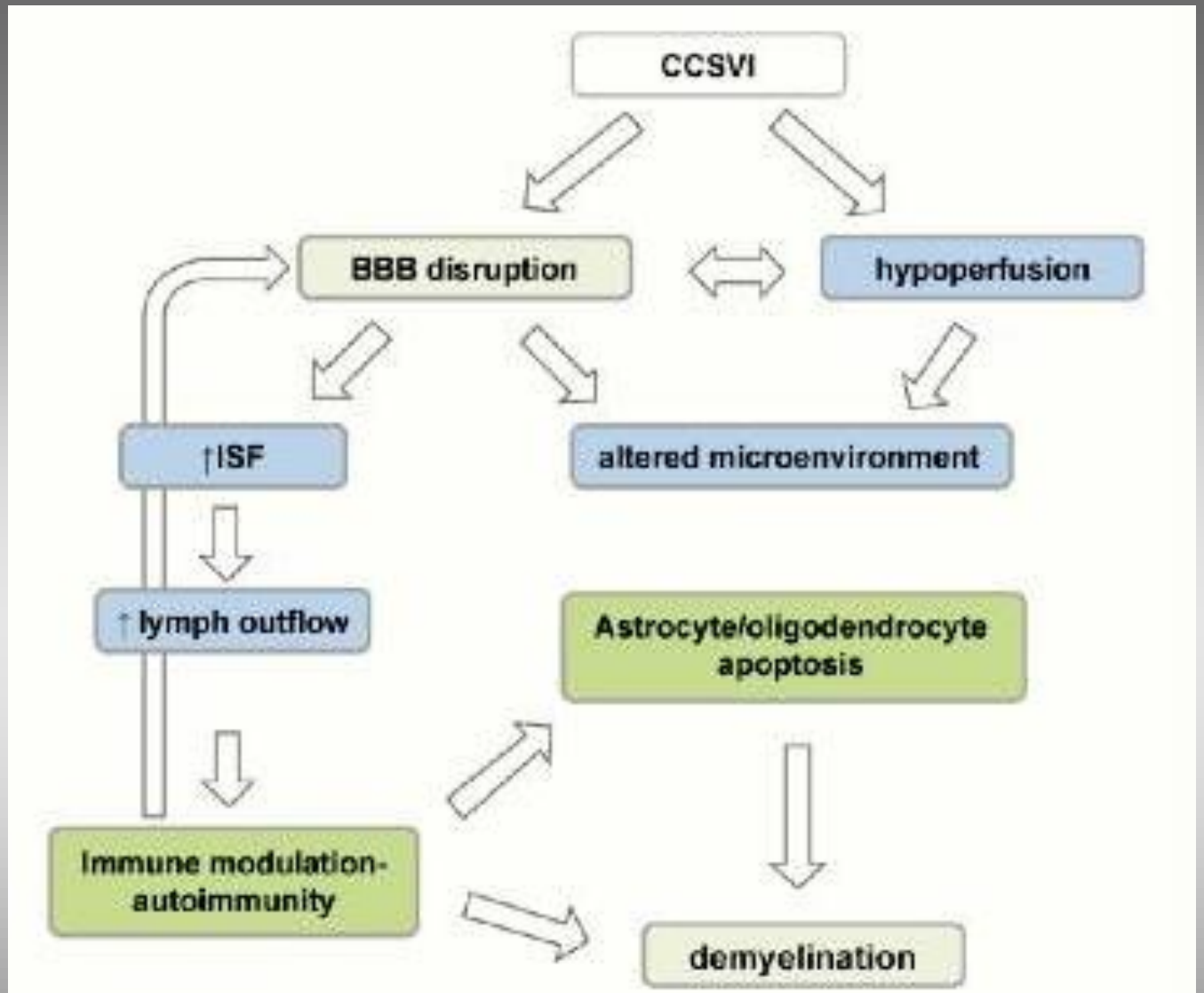


Figure 1: Interrelations of factors in the combined hydrostatic-immune paradigm of CCSVI. In blue: hydrostatic factors, in green: immune factors, in grey: mixed hydrostatic and immune factors.

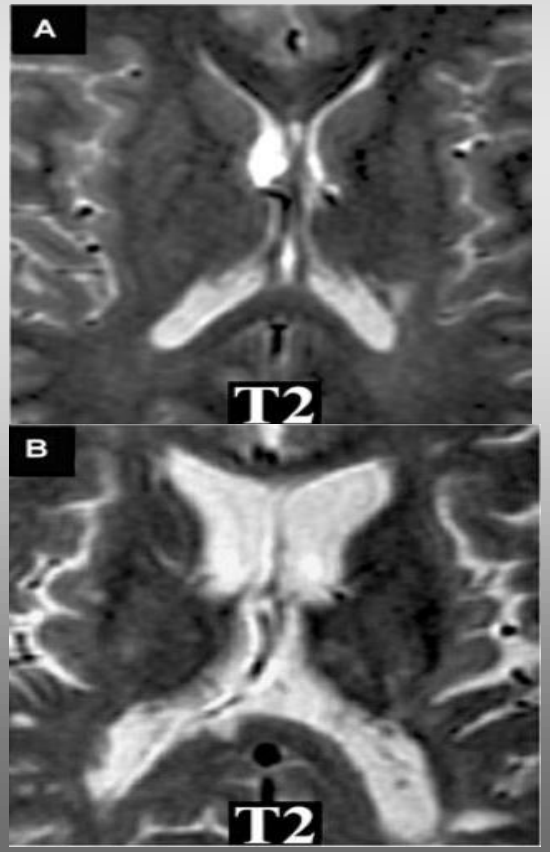
Tsamopoulos et al. J Mult Scler 2014

Late event

Deep Gray Matter Involvement on Brain MRI Scans Is Associated with Clinical Progression in Multiple Sclerosis

Mohit Neema, MD, Ashish Arora, MD, Brian C. Healy, PhD, Zachary D. Guss, BA, Steven D. Brass, MD, MPH, Yang Duan, MD, Guy J. Buckle, MD, Bonnie I. Glanz, PhD, Lynn Stazzone, NP, Samia J. Khoury, MD, Howard L. Weiner, MD, Charles R.G. Guttmann, MD, and Rohit Bakshi, MD

J Neuroimaging. 2009



- **Iron deposition** associated with leakage of the blood-brain barrier may exacerbate the inflammatory process.
- Leads to **further damage** to oligodendrocytes and myelin.
- It is an **end stage biomarker** of tissue damage
- **It correlates with scores on the Expanded Disability Status Scale (EDSS)**

Journal of Neurology, Neurosurgery, and Psychiatry 1988;**51**:260–265

Perivascular iron deposition and other vascular damage in multiple sclerosis

C W M ADAMS

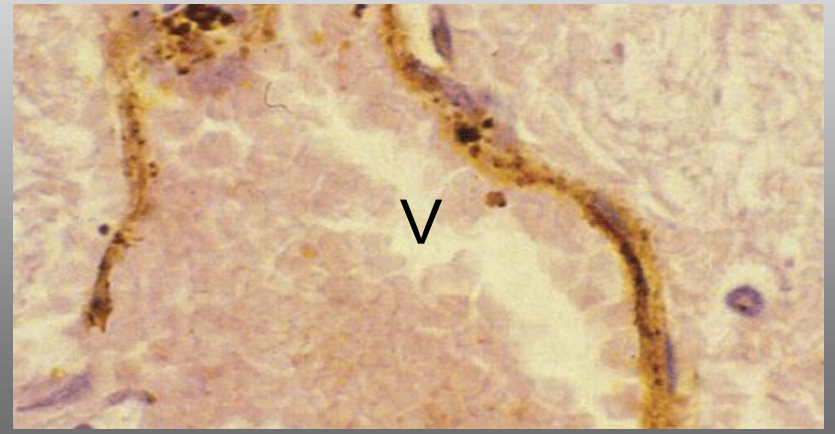
From the Division of Histopathology, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, University of London, UK

Fibrin cuff 26%

Microbleeding 47%

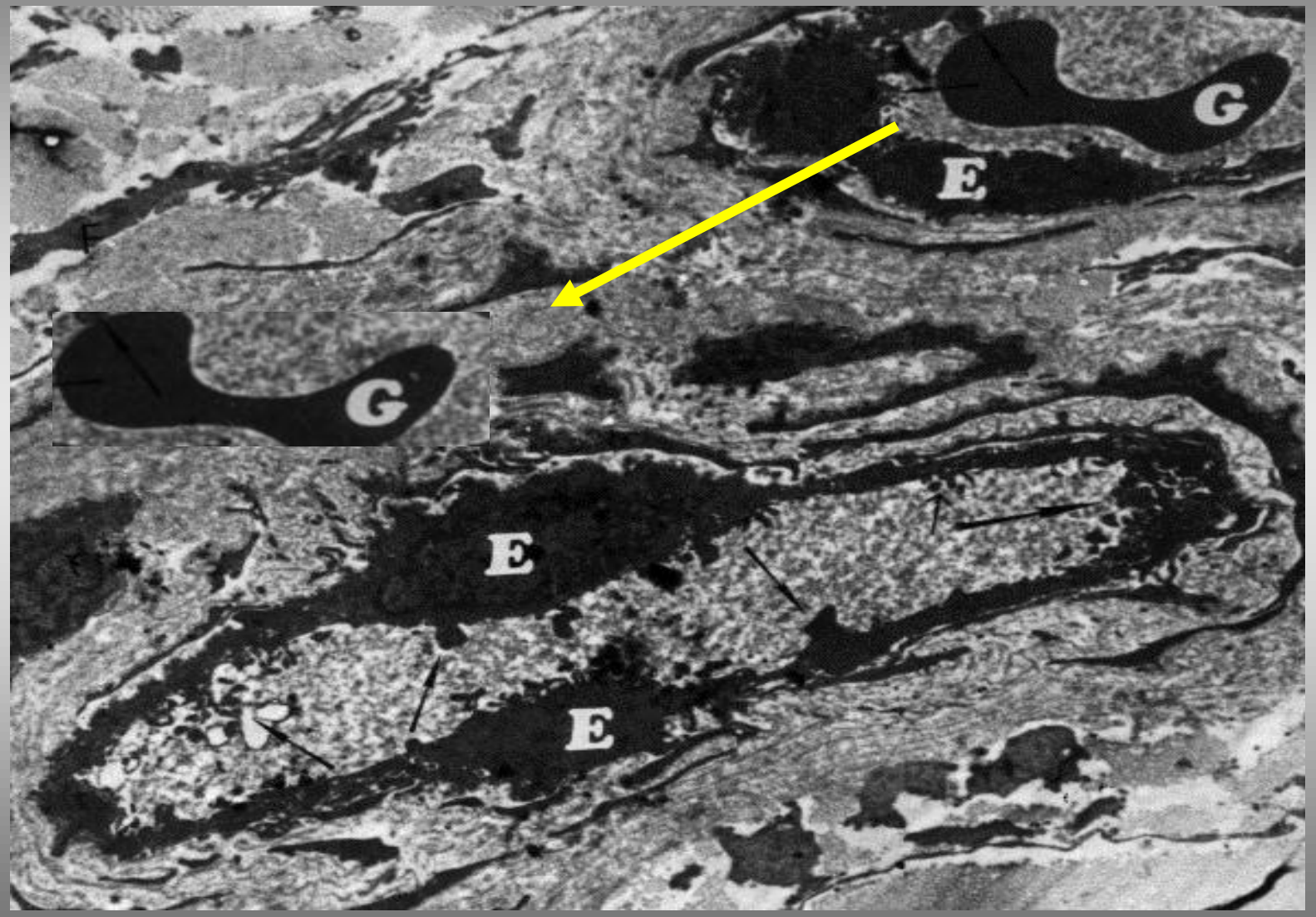
SUMMARY Evidence of damage to cerebral vein walls was sought in 70 cases of multiple sclerosis. Seventy control cases were also examined. The multiple sclerosis cases showed venous intramural fibrinoid deposition (7%), recent haemorrhages (17%), old haemorrhages revealed by haemosiderin deposition (30%), thrombosis (6%) and thickened veins (19%). In all, 41% of all multiple sclerosis cases showed some evidence of vein damage. Occasional control cases showed haemosiderin deposition in the brain but, unlike the multiple sclerosis cases, these were diffuse and almost entirely related to coexistent cardiovascular or cerebrovascular disease. Haemosiderin deposition was common in the substantia nigra and other pigmented nuclei in all cases. It is concluded that the cerebral vein wall in multiple sclerosis is subject to chronic inflammatory damage, which promotes haemorrhage and increased permeability, and constitutes a form of vasculitis.

- Venous wall and perivenous tissue both show typical histology of chronic venous stasis
- Major evidence in the subcortical gray matter
- Neglected part of MS pathology

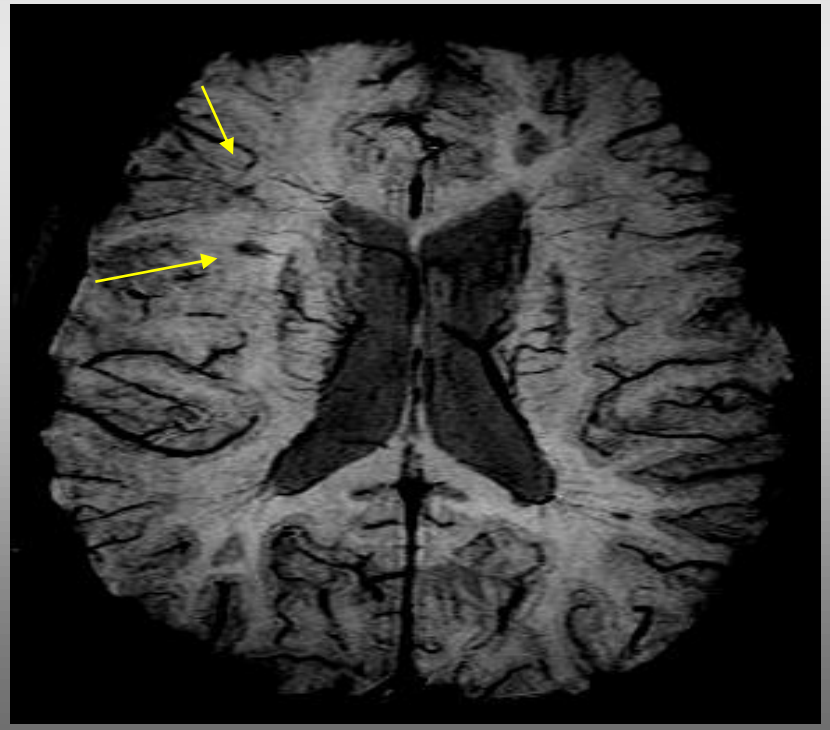


Perivenous iron deposition. Is Heme iron the iron in MS?

RBC EXTRAVASATION IN CHRONIC VENOUS INSUFFICIENCY.



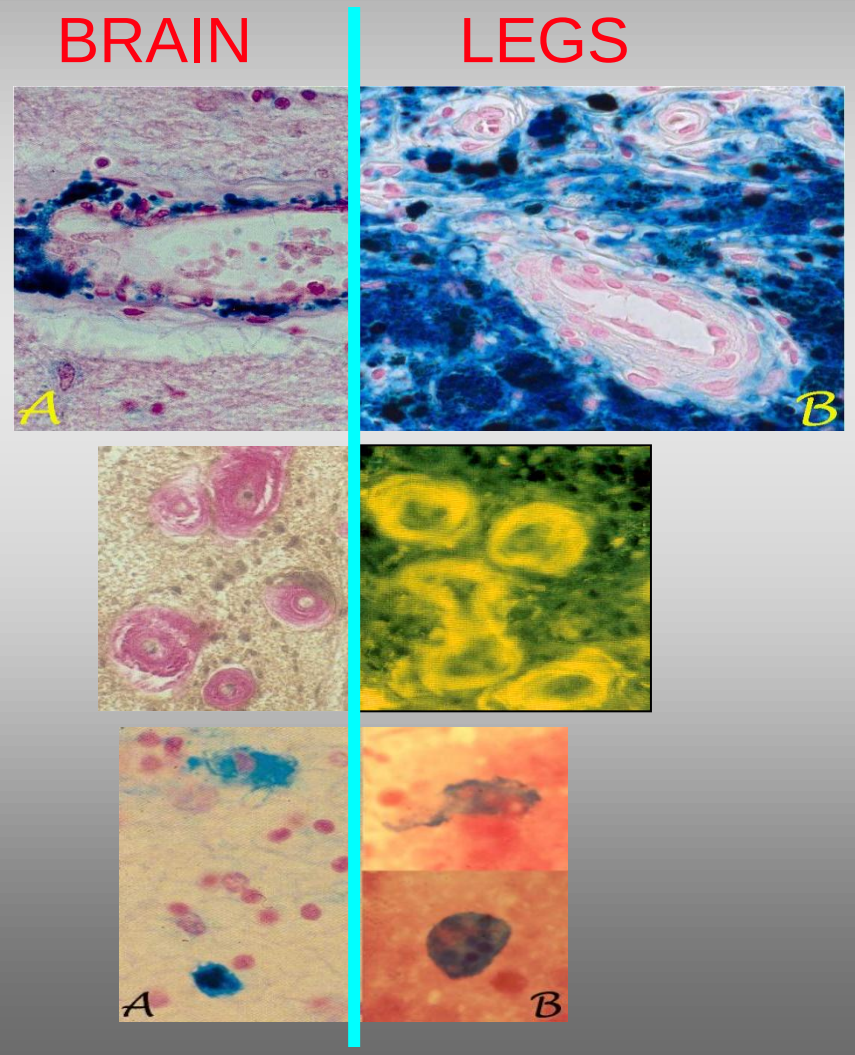
PARALLEL CVI in brain and legs



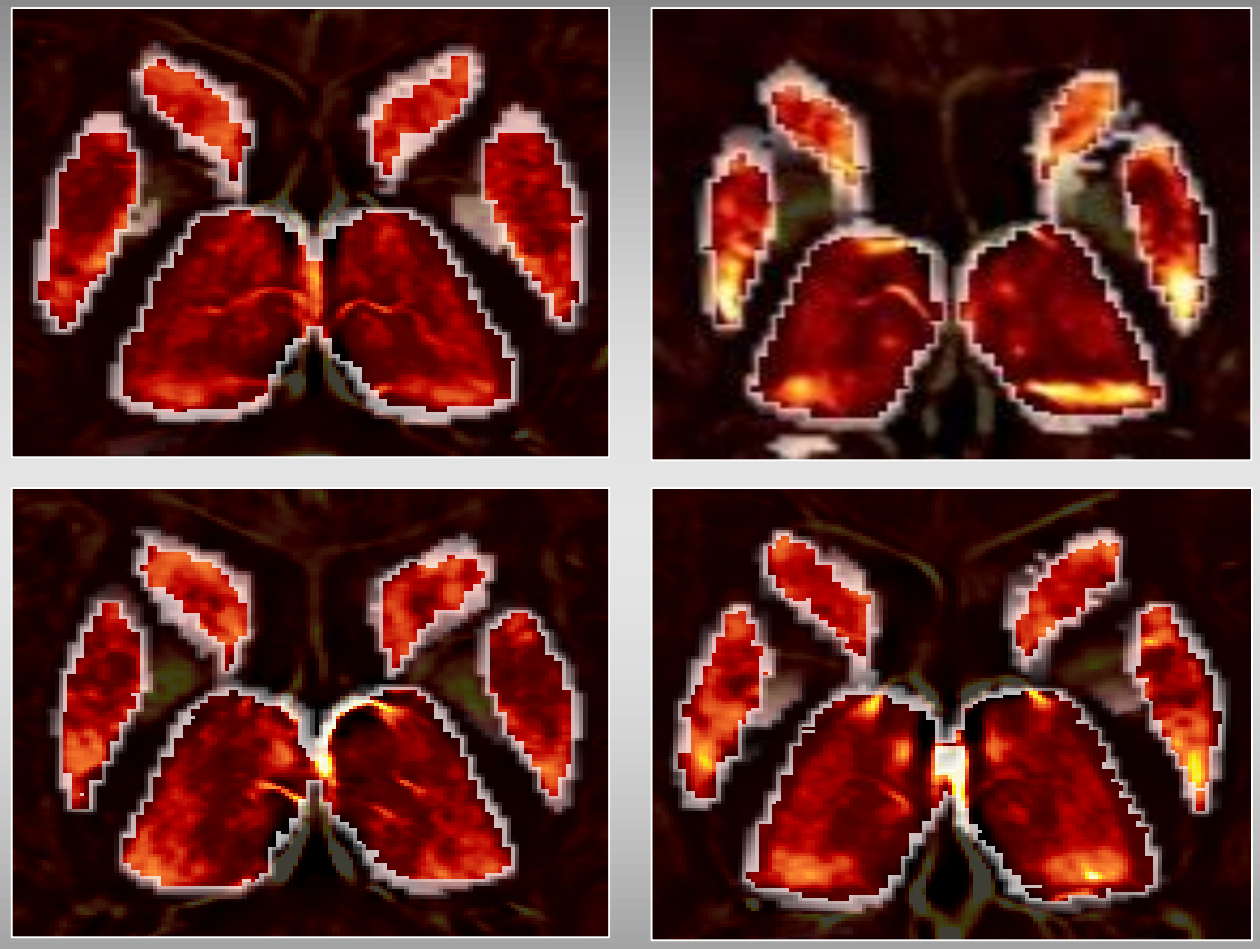
Peri-venular iron deposition

Fibrin cuffs

Iron laiden macrophages



CCSVI and IRON DEPOSITS on SWI



Normal Control

Multiple Sclerosis

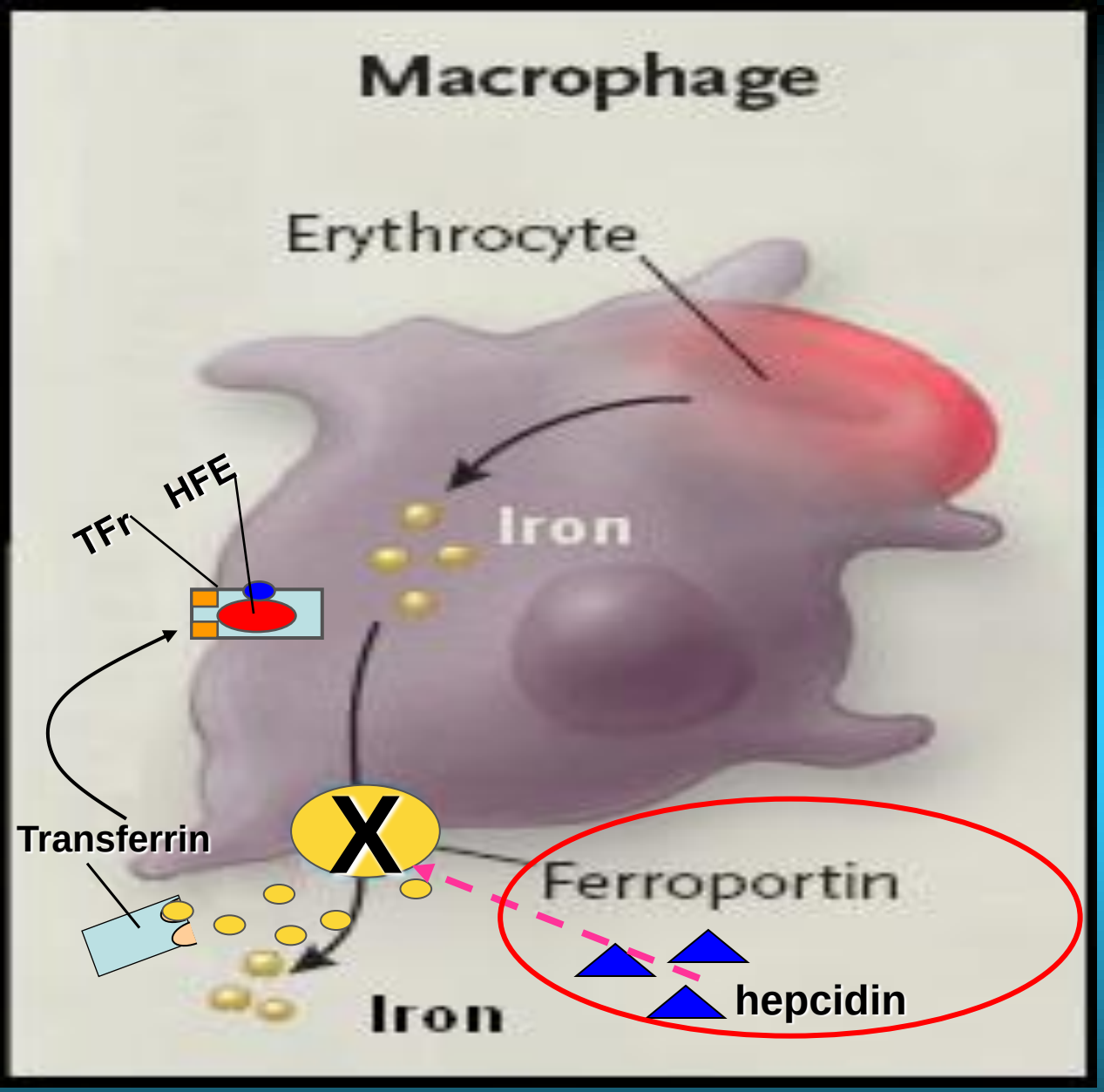
- **Significant correlations between extracranial blockages and iron loading in the pulvinar nucleus of thalamus, thalamus, globus pallidus, and hippocampus and in T2-LV, T1-LV**

Zivadinov et al. 2010, Haackee M 2010

- **Increased iron stores correlates with the disability (EDSS)**

Bakshi 2008

Iron trafficking genes



(modified from Fleming 2005 N Eng J Med)

FPN1 is the main iron cellular expoter,

HEPC FPN modulator

IRON TRAFFICKING GENES AND DISEASES PROGRESSION

- HEPC-582GG; 414 MS (RR 273; SP 103; PP 38), 414 HC.

Over represented in PP/SP 4.4 (1.8-10).

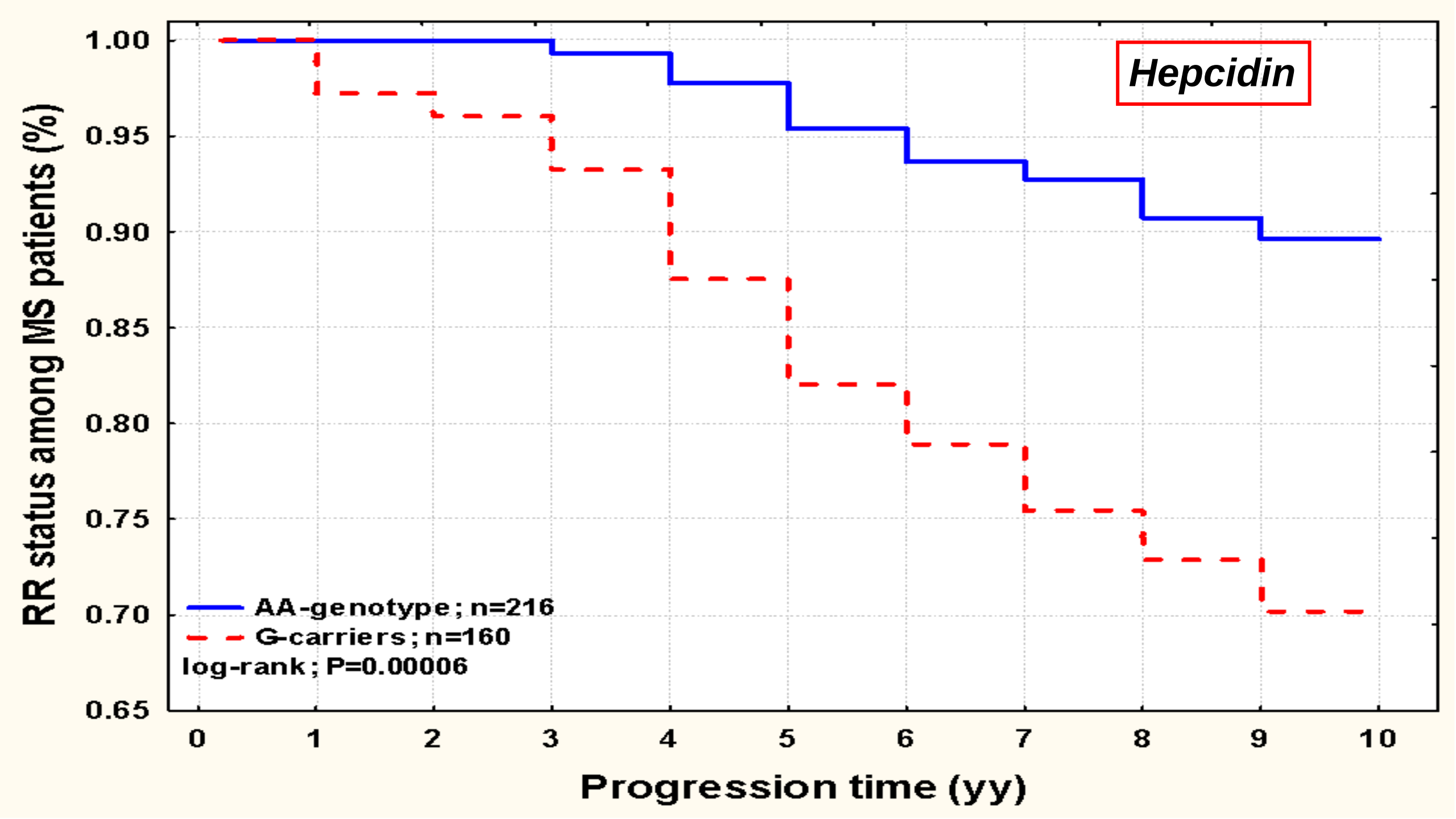
In homozygotes significantly increases EDSS; PI; MS SS;

The chance to switch into progression is increased; HR 3.6 (1.8-6.8) log rank p = 0.00006

- FPN1-8GG over represented in the whole MS population

4.4 (1.9-10) $p < 0.0001$

Source: Gemmati et al BMC Med Genet. 2012 Aug 10;13:70


Hepcidin
RR status among MS patients (%)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
AA-genotype; n=216
G-carriers; n=160
log-rank; P=0.00006
0
1
2
3
4
5
6
7
8
9
10
Progression time (yy)

Università degli Studi di Ferrara

Dottorato in Scienze Biomediche e Biotecnologiche

Curricula in Fisiopatologia del Sistema Nervoso Endocrino e Vascolare

Dottorato in Fisica