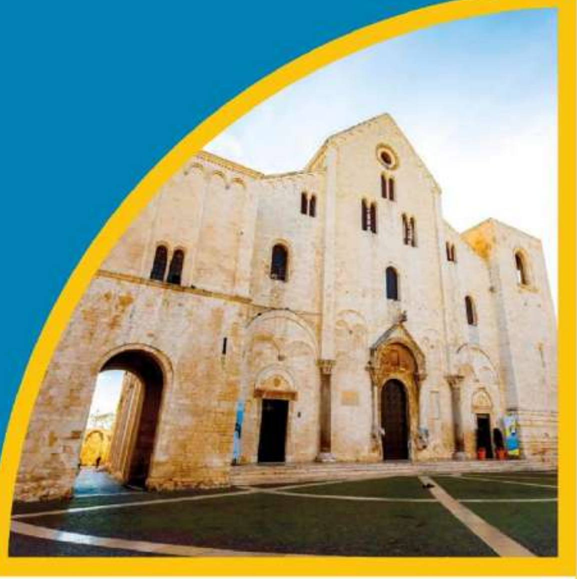


RIUNIONE ANNUALE SIN APPULO-LUCANA

3-4 Novembre 2022

Nicolaus Hotel Bari

CON IL PATROCINIO DI

Sin
SOCIETÀ ITALIANA DI NEUROLOGIA

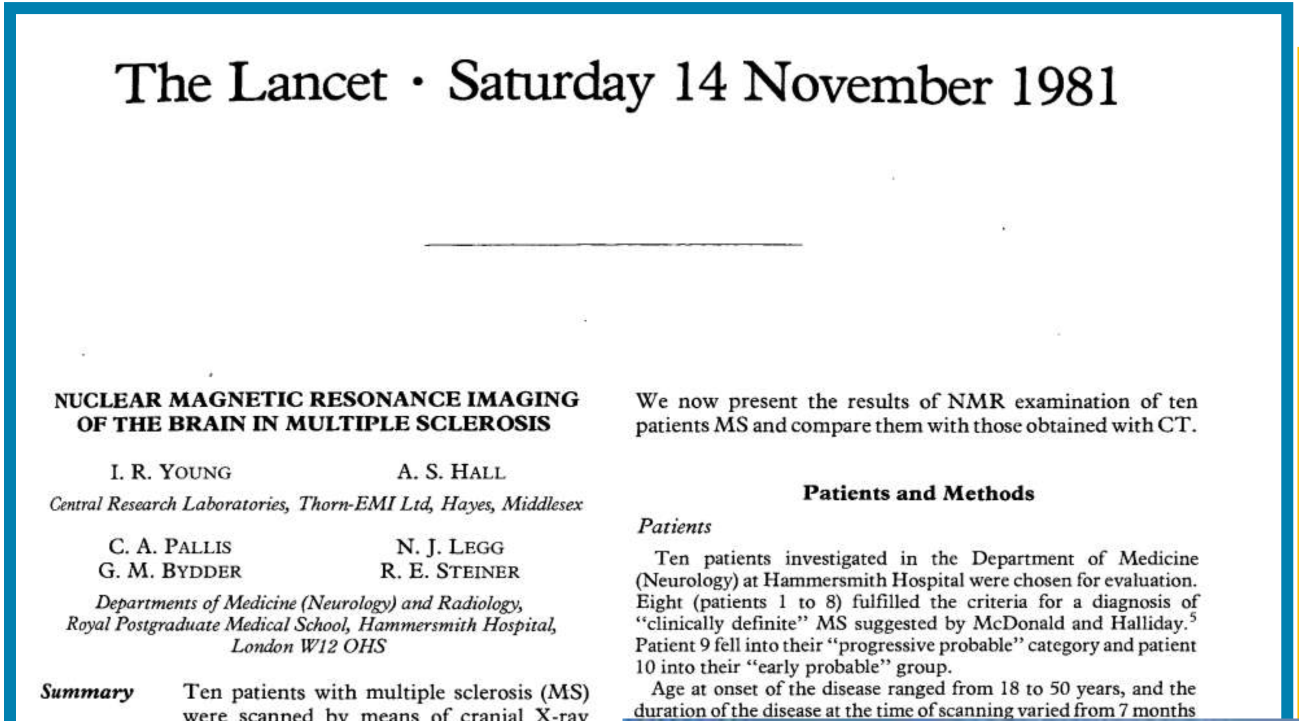
RESPONSABILI SCIENTIFICI

Prof.ssa Maria Trojano
Prof. Damiano Paolicelli

Biomarker di malattia con tecniche di Neurimaging strutturale e funzionale non invasiva

Dott.ssa Alessia Manni

Dipartimento di
Biomedicina Traslazionale Neuroscienze “DiBraiN”
Università degli Studi di Bari «Aldo Moro»



The Lancet · Saturday 14 November 1981

NUCLEAR MAGNETIC RESONANCE IMAGING OF THE BRAIN IN MULTIPLE SCLEROSIS

I. R. Young A. S. Hall
Central Research Laboratories, Thorn-EMI Ltd, Hayes, Middlesex

C. A. Pallis N. J. Legg
G. M. Bydder R. E. Steiner
Departments of Medicine (Neurology) and Radiology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS

Summary Ten patients with multiple sclerosis (MS) were scanned by means of cranial X-ray computed tomography (CT) with and without intravenous contrast enhancement, and by nuclear magnetic resonance (NMR) using an inversion-recovery sequence. Altogether 19 lesions varying in size between about 7 mm × 5 mm and 13

We now present the results of NMR examination of ten patients MS and compare them with those obtained with CT.

Patients and Methods

Patients

Ten patients investigated in the Department of Medicine (Neurology) at Hammersmith Hospital were chosen for evaluation. Eight (patients 1 to 8) fulfilled the criteria for a diagnosis of "clinically definite" MS suggested by McDonald and Halliday.[5] Patient 9 fell into their "progressive probable" category and patient 10 into their "early probable" group.

Age at onset of the disease ranged from 18 to 50 years, and the duration of the disease at the time of scanning varied from 7 months

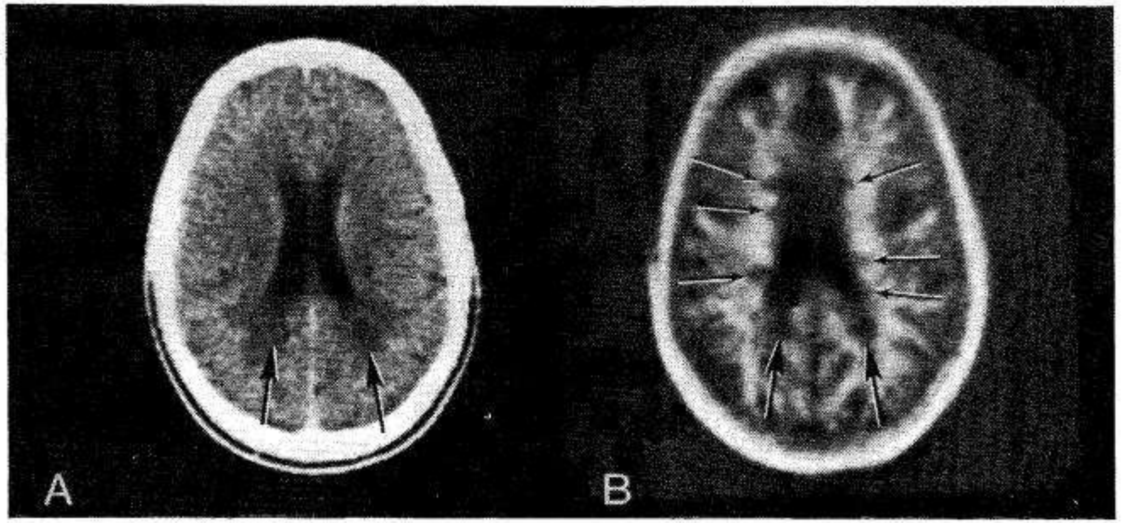
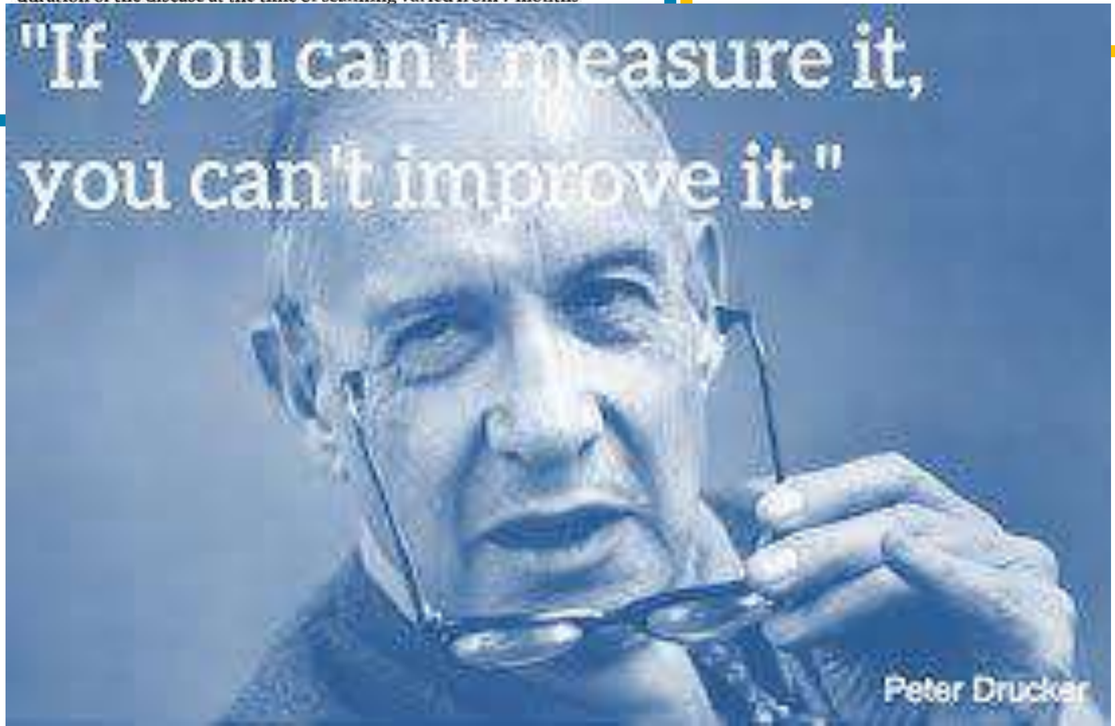
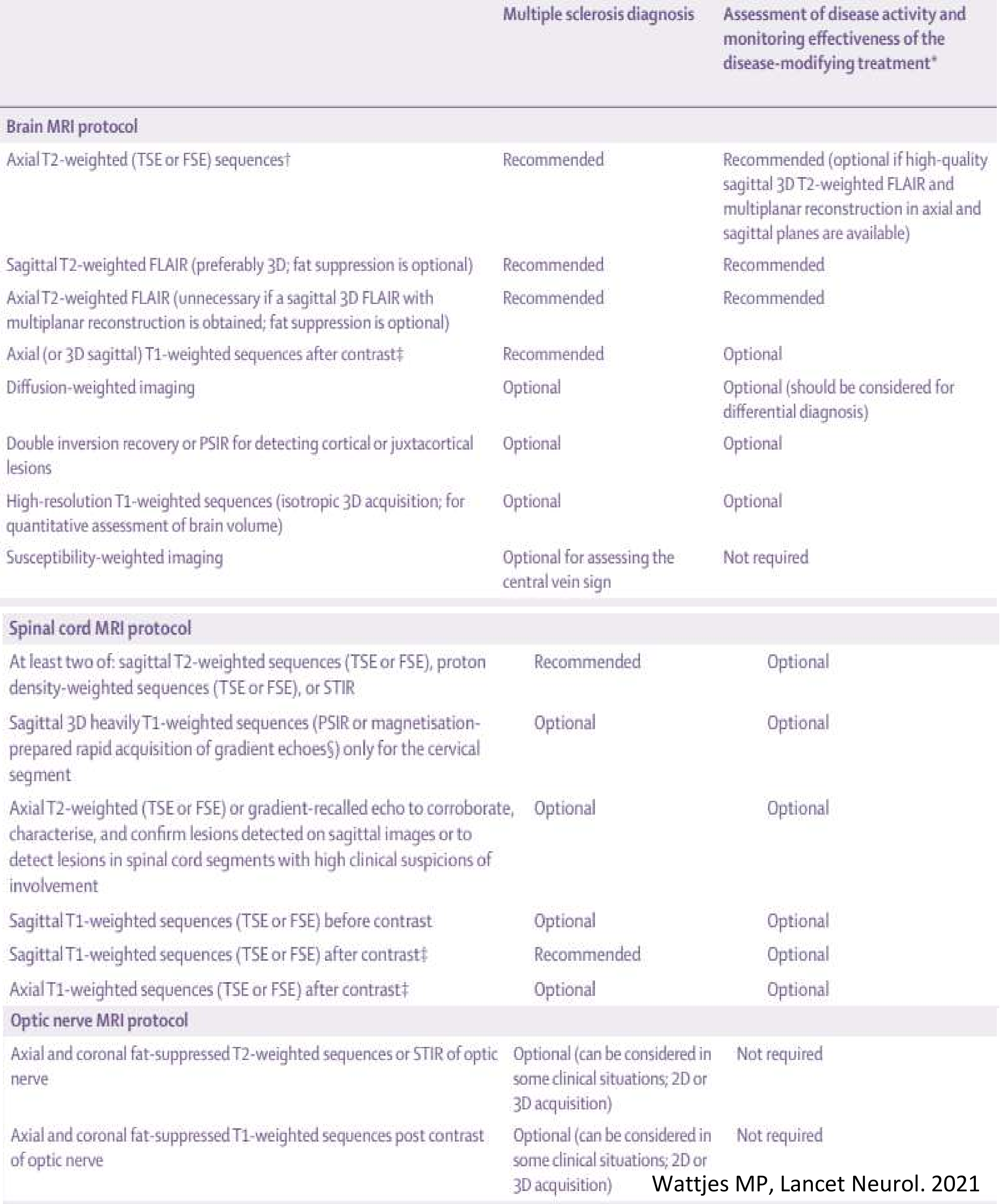


Fig. 1—Comparable CT (A) and NMR (B) scans in patient 10 at mid-ventricular level.

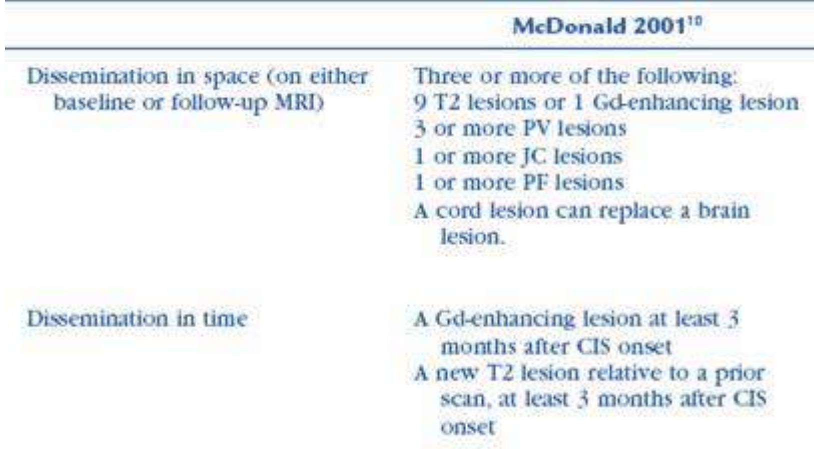
The two posterior periventricular lesions seen on the CT scan are also seen on the NMR scan (large arrows). In addition 6 smaller lesions are seen on the NMR scan at the lateral margin of the lateral ventricles (small arrows). The sharply defined area on the medial margin of the left posterior horn is a circular artefact.





	Multiple sclerosis diagnosis	Assessment of disease activity and monitoring effectiveness of the disease-modifying treatment*
Brain MRI protocol		
Axial T2-weighted (TSE or FSE) sequences†	Recommended	Recommended (optional if high-quality sagittal 3D T2-weighted FLAIR and multiplanar reconstruction in axial and sagittal planes are available)
Sagittal T2-weighted FLAIR (preferably 3D; fat suppression is optional)	Recommended	Recommended
Axial T2-weighted FLAIR (unnecessary if a sagittal 3D FLAIR with multiplanar reconstruction is obtained; fat suppression is optional)	Recommended	Recommended
Axial (or 3D sagittal) T1-weighted sequences after contrast‡	Recommended	Optional
Diffusion-weighted imaging	Optional	Optional (should be considered for differential diagnosis)
Double inversion recovery or PSIR for detecting cortical or juxtacortical lesions	Optional	Optional
High-resolution T1-weighted sequences (isotropic 3D acquisition; for quantitative assessment of brain volume)	Optional	Optional
Susceptibility-weighted imaging	Optional for assessing the central vein sign	Not required
Spinal cord MRI protocol		
At least two of: sagittal T2-weighted sequences (TSE or FSE), proton density-weighted sequences (TSE or FSE), or STIR	Recommended	Optional
Sagittal 3D heavily T1-weighted sequences (PSIR or magnetisation-prepared rapid acquisition of gradient echoes§) only for the cervical segment	Optional	Optional
Axial T2-weighted (TSE or FSE) or gradient-recalled echo to corroborate, characterise, and confirm lesions detected on sagittal images or to detect lesions in spinal cord segments with high clinical suspicions of involvement	Optional	Optional
Sagittal T1-weighted sequences (TSE or FSE) before contrast	Optional	Optional
Sagittal T1-weighted sequences (TSE or FSE) after contrast‡	Recommended	Optional
Axial T1-weighted sequences (TSE or FSE) after contrast‡	Optional	Optional
Optic nerve MRI protocol		
Axial and coronal fat-suppressed T2-weighted sequences or STIR of optic nerve	Optional (can be considered in some clinical situations; 2D or 3D acquisition)	Not required
Axial and coronal fat-suppressed T1-weighted sequences post contrast of optic nerve	Optional (can be considered in some clinical situations; 2D or 3D acquisition)	Not required

Wattjes MP, Lancet Neurol. 2021



	McDonald 2001[10]
Dissemination in space (on either baseline or follow-up MRI)	Three or more of the following: 9 T2 lesions or 1 Gd-enhancing lesion 3 or more PV lesions 1 or more JC lesions 1 or more PF lesions A cord lesion can replace a brain lesion.
Dissemination in time	A Gd-enhancing lesion at least 3 months after CIS onset A new T2 lesion relative to a prior scan, at least 3 months after CIS onset

Includere la RMN nei criteri diagnostici

Definizione di precisi protocolli RMN

RMN come Biomarker: definizione e ruolo

«Caratteristica» che può essere misurata in maniera obiettiva e può essere considerata come indicatore di:

un normale processo biologico
un processo patologico
una risposta farmacologica ad una terapia

Biomarkers Definitions Working Group. Clin Pharmacol Ther. 2001

La RMN come biomarker ideale:

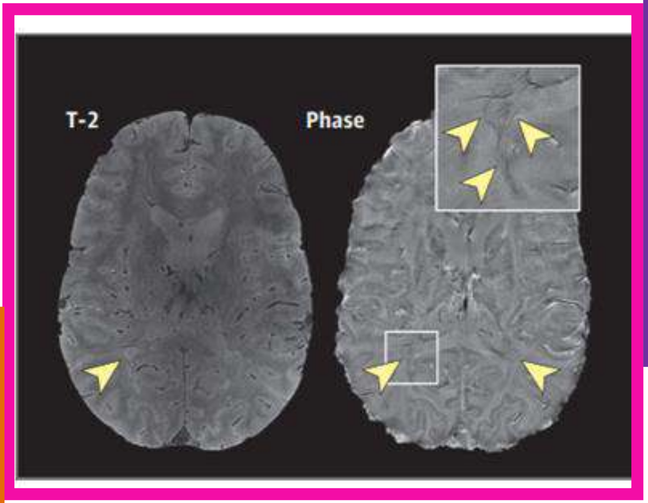
- correla bene con l'accumulo di disabilità nella storia naturale della malattia
- cattura tutti gli effetti (positivi e negativi) del trattamento
- Ha elevata sensibilità

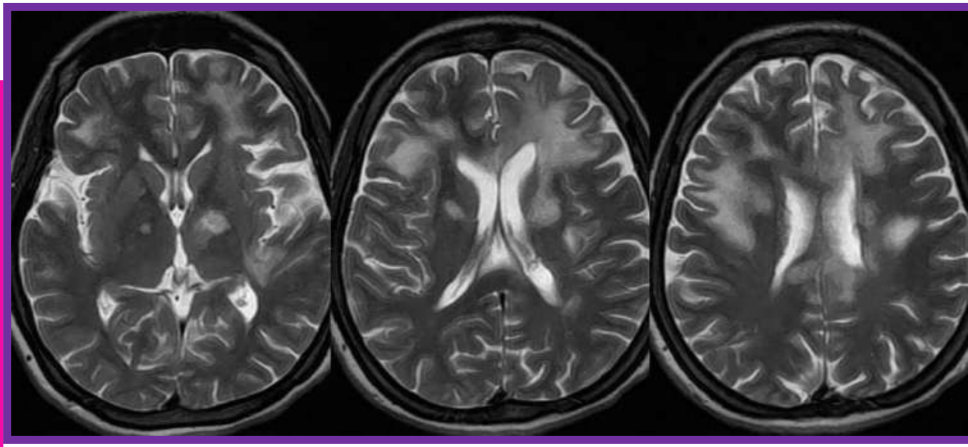
- Definito quindi ***"surrogato" di un end-point clinico:***

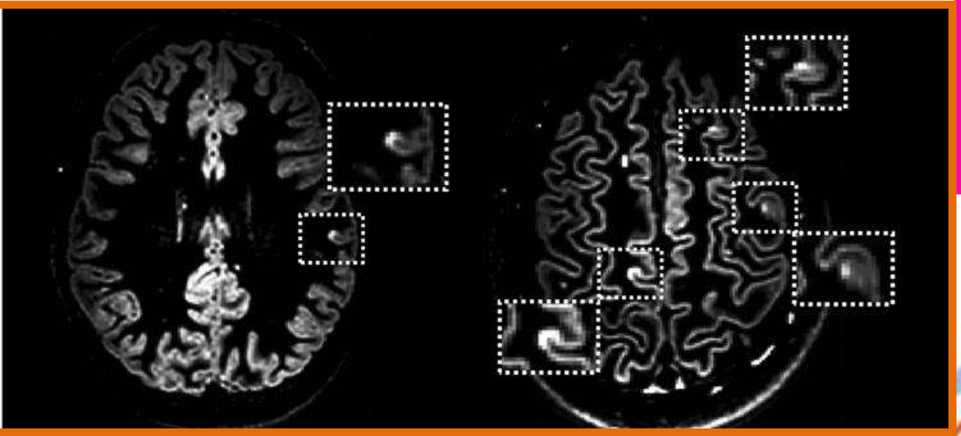
  può sostituirlo e fornire informazioni circa diagnosi, prognosi o efficacia terapeutica, **in un tempo più breve** rispetto a quello necessario all' end-point

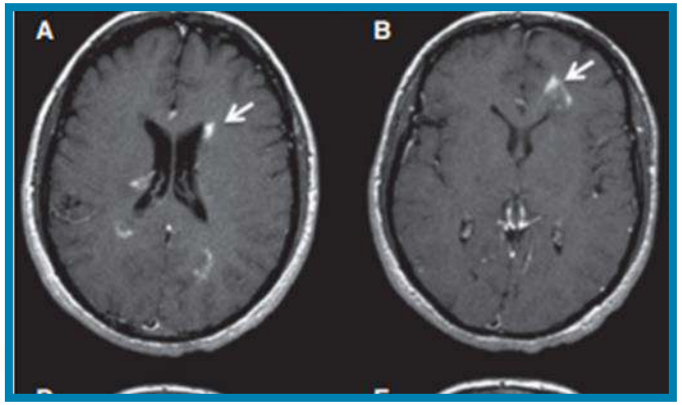
Comabella et al, 2014

RMN come Biomarker





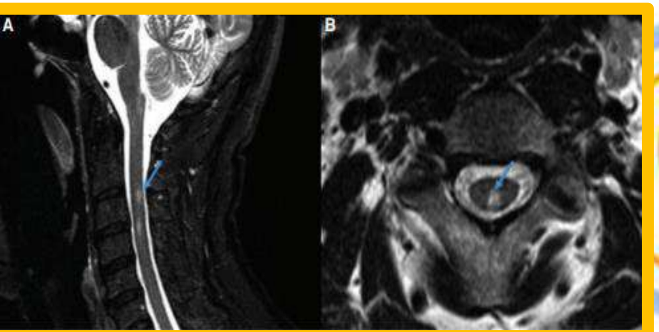




DIAGNOSTICO

ATTIVITA' DI MALATTIA

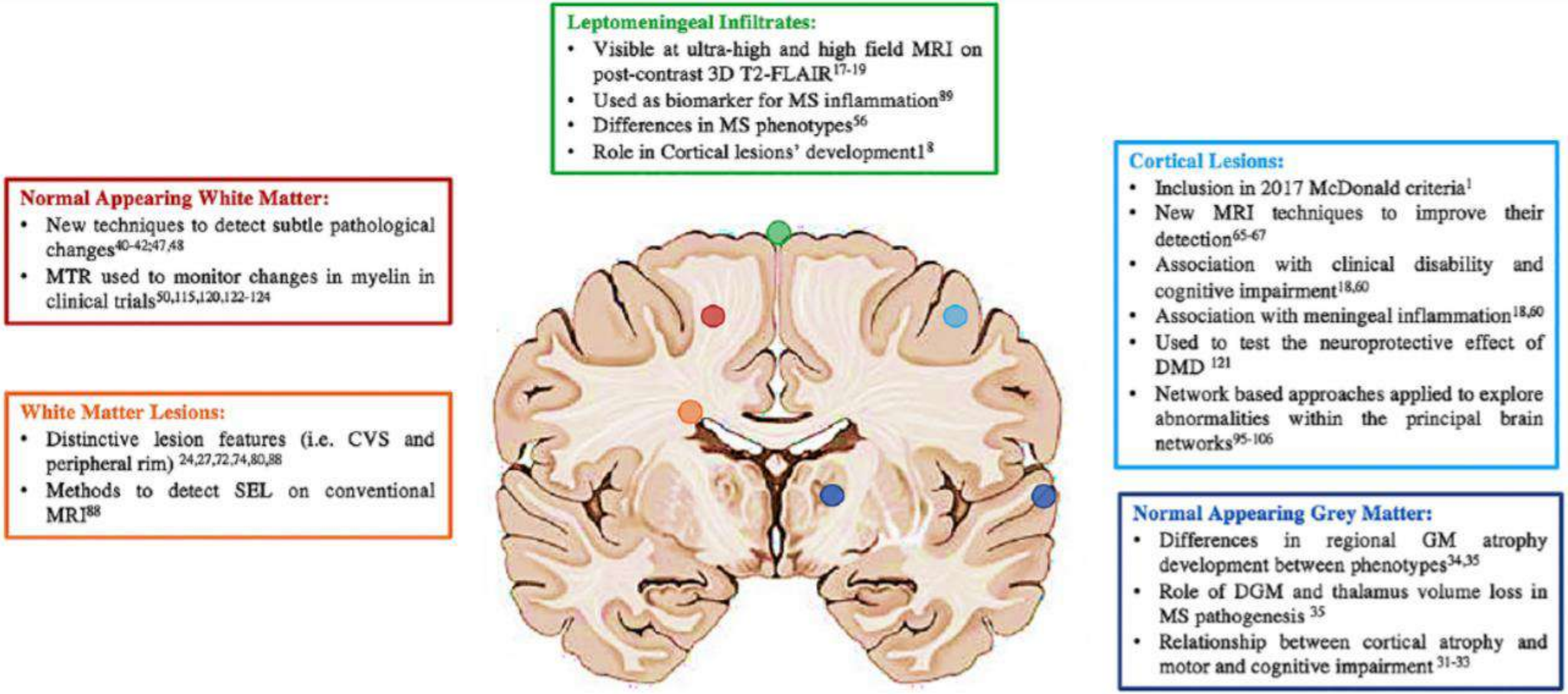
RISPOSTA AL TRATTAMENTO



PROGNOSTICO

DRUG SAFETY

Nuovi Biomarker di RMN: overview



Leptomeningeal Infiltrates:
- Visible at ultra-high and high field MRI on post-contrast 3D T2-FLAIR[17-19]
- Used as biomarker for MS inflammation[89]
- Differences in MS phenotypes[56]
- Role in Cortical lesions' development1[8]

Normal Appearing White Matter:
- New techniques to detect subtle pathological changes[40-42;47,48]
- MTR used to monitor changes in myelin in clinical trials[50,115,120,122-124]

White Matter Lesions:
- Distinctive lesion features (i.e. CVS and peripheral rim) [24,27,72,74,80,88]
- Methods to detect SEL on conventional MRI[88]

Cortical Lesions:
- Inclusion in 2017 McDonald criteria[1]
- New MRI techniques to improve their detection[65-67]
- Association with clinical disability and cognitive impairment[18,60]
- Association with meningeal inflammation[18,60]
- Used to test the neuroprotective effect of DMD [121]
- Network based approaches applied to explore abnormalities within the principal brain networks[95-106]

Normal Appearing Grey Matter:
- Differences in regional GM atrophy development between phenotypes[34,35]
- Role of DGM and thalamus volume loss in MS pathogenesis [35]
- Relationship between cortical atrophy and motor and cognitive impairment [31-33]

Figure 1. Advances in brain imaging in multiple sclerosis in different brain locations.
CVS, central vein sign; DGM, deep grey matter; DMD, disease-modifying drug; ihMT, inhomogeneous magnetization transfer; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MWF, myelin water fraction; NODDI, neurite orientation dispersion and density imaging; PET, positron emission tomography; qMT, quantitative magnetization transfer; SEL, slowly expanding lesion; TSC, total sodium concentration.

Cortese et al, Ther Adv Neurol Disord. 2019

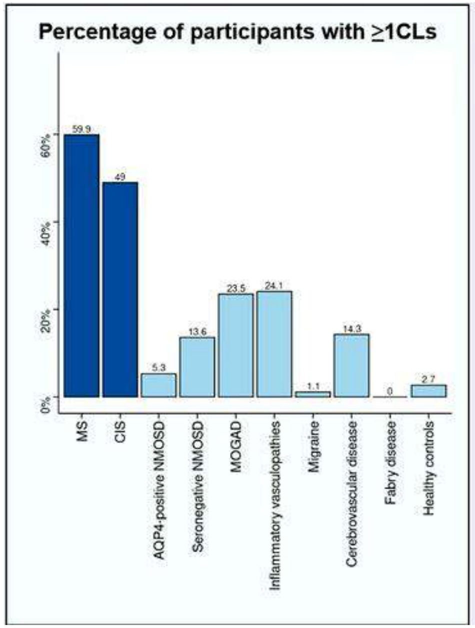
RMN strutturale: ruolo delle Lesioni Corticali

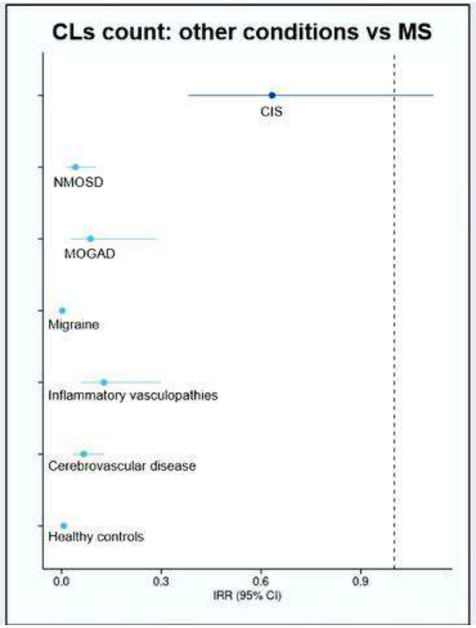
- ➢ **Gli attuali criteri diagnostici (Thompson *et al.* 2018) e le linee guida (Filippi *et al.* 2016) includono lesioni intracorticali, leucocorticali e iuxtacorticali**

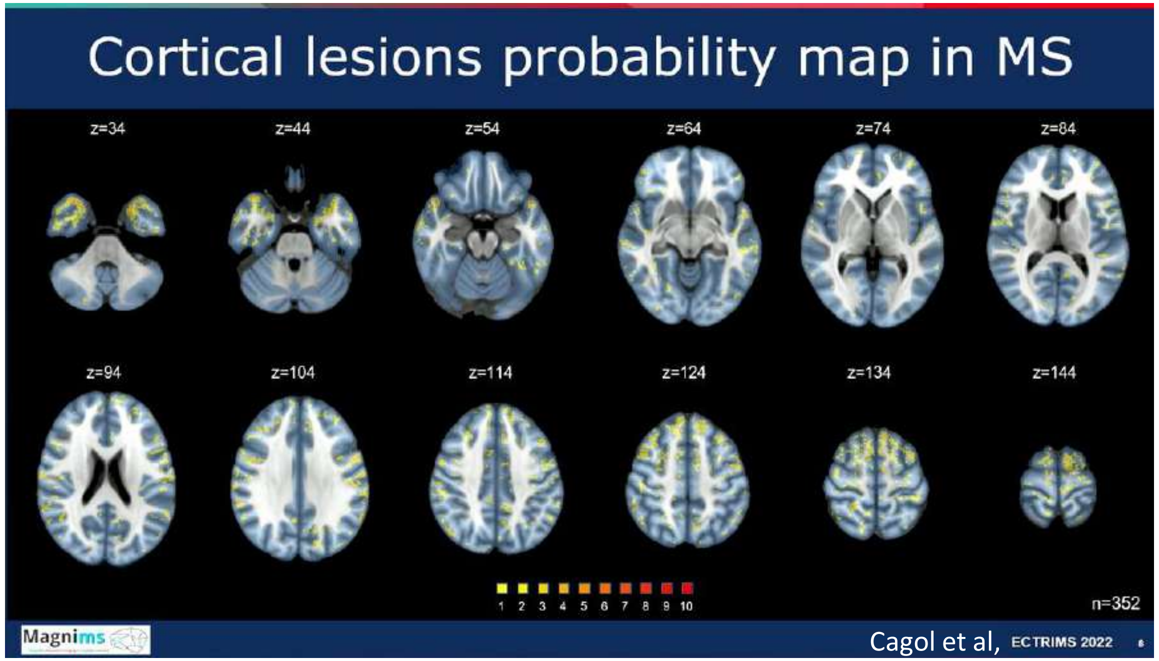
✓ La diagnosi di SM può essere difficile nella pratica clinica per SM-Mimicks

✗ Le sequenze MRI avanzate raccomandate per la loro identificazione non sono ampiamente applicate in ambito clinico e possono essere di difficile interpretazione (Filippi *et al.* , 2019)

❑ 1051 soggetti da 14 centri


Percentage of participants with ≥1CLs


CLs count: other conditions vs MS


Cortical lesions probability map in MS

Cagol et al, ECTRIMS 2022

❑ 60% delle SM e 50% delle CIS con LC

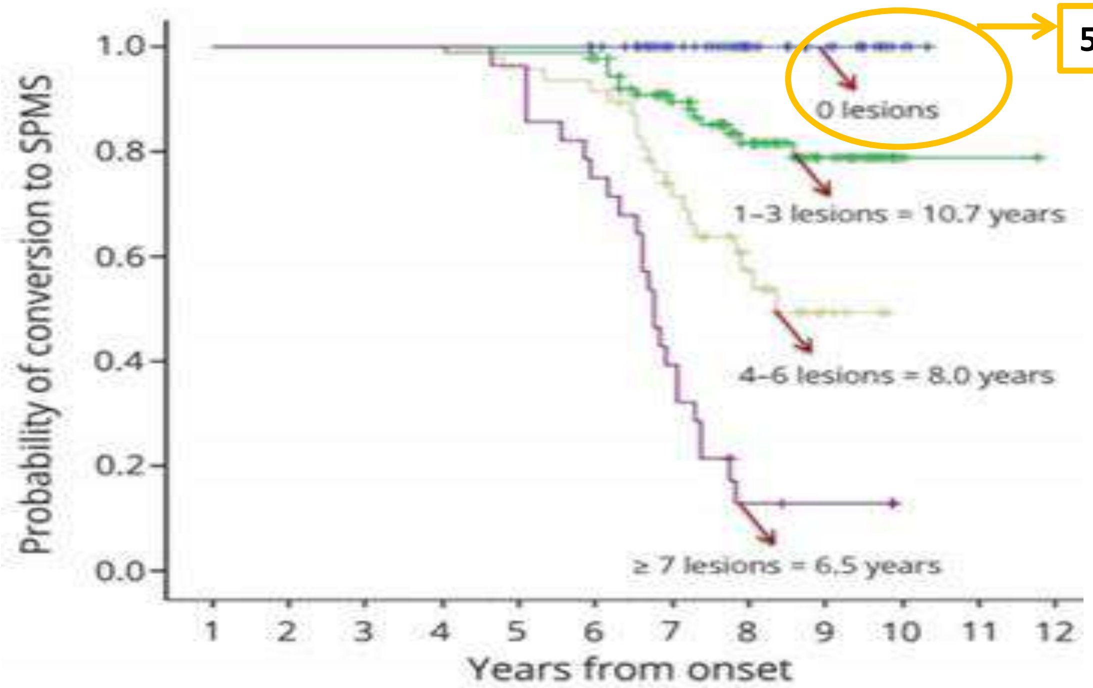
❑ Nessuna differenza significativa nelle LC fra SM/CIS, ma le SM hanno ↑ LC di ogni altra patologia

❑ Lobi temporali

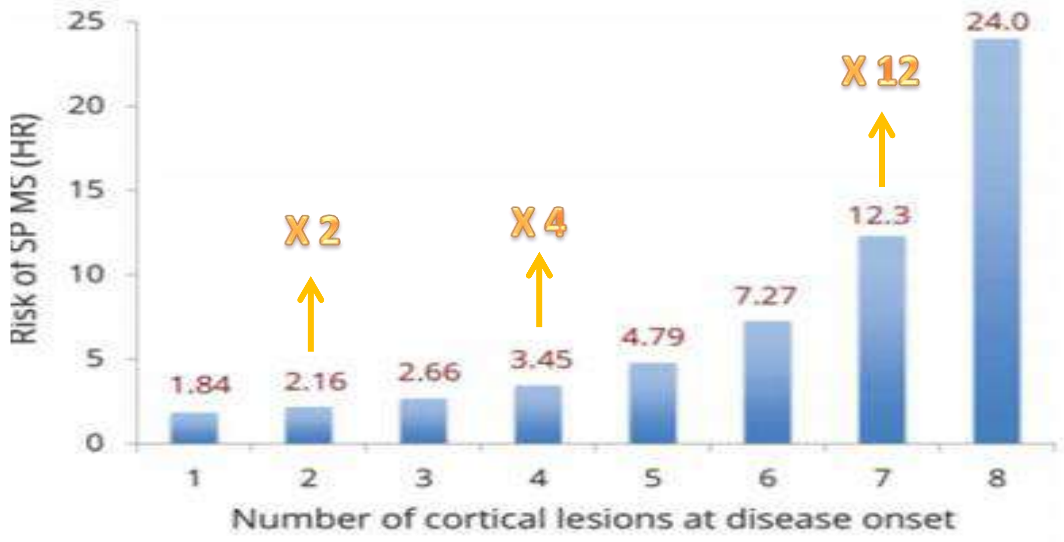
❑ Regioni superiori degli emisferi, specie nei lobi frontali

RMN strutturale: ruolo delle Lesioni Corticali

Ruolo nel predire la conversione clinica: da RMS a PMS



53 pazienti senza CLs= nessuno nella fase SP



- Valutato il numero delle Lesioni Corticali (CLs) e della sostanza bianca (WM), e il tasso di assottigliamento corticale, in 219 RRMS con 1 (n=116), 2 (n=53) e ≥3 (n = 50) ricadute nei primi 2 anni
- Follow-up= 7.9 anni
- ***Danno corticale più severo all'esordio → più alto rischio di conversione ad SP***

Scalfari et al, Neurology 2018

RMN strutturale: CVS (+LC)

- Il **Central Vein Sign, CVS** differenzia la SM da altre malattie con caratteristiche radiologiche analoghe

↓

Sistema automatico, **CVSnet,** che si basa su ***pre-selezione manuale*** (time-consuming) delle lesioni CVS- e CVS+, ed esclude alcune lesioni (***CVSe***: *lesioni confluenti, eccentriche rispetto alla vena, legate a più vene*)

⬇

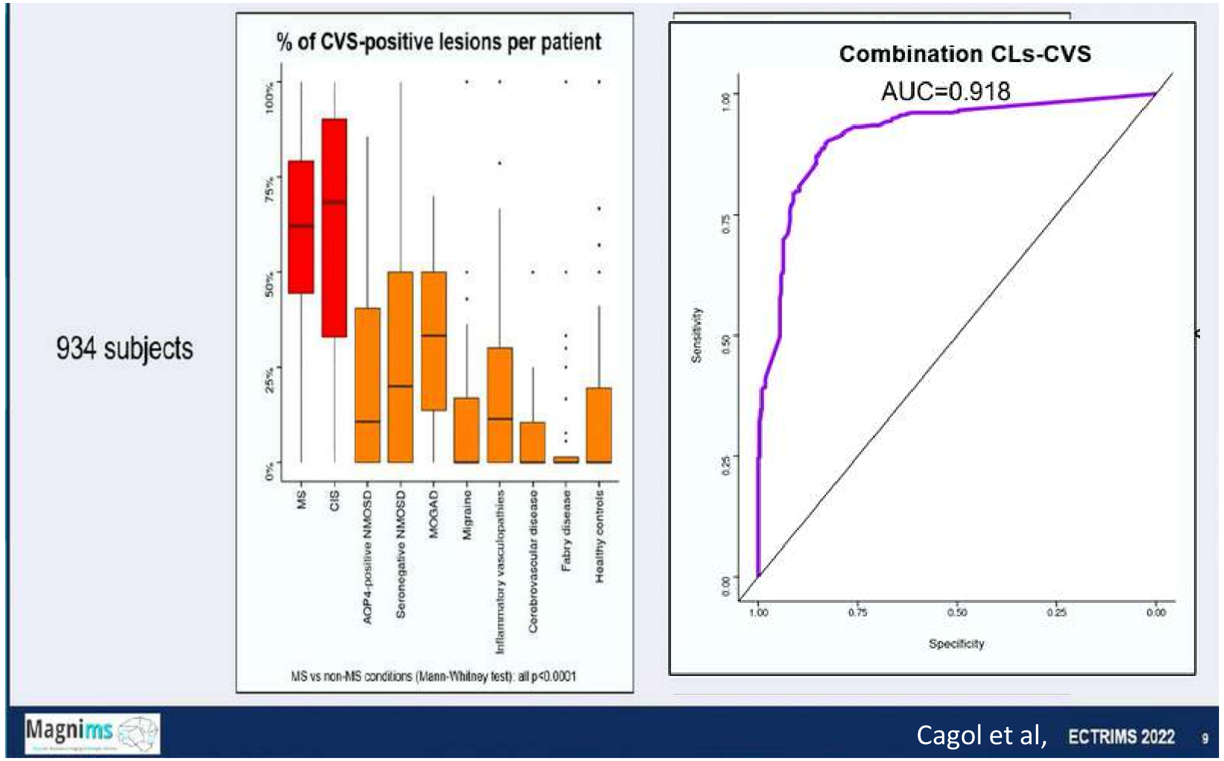
Prototipo **CVSNet modificato** in grado di analizzare la presenza di CVS per tutti i tipi di lesioni cerebrali, con **valutazione completamente automatizzata**.

⬇

✓ ***Accelerare la valutazione del CVS come biomarcatore diagnostico importante***

✓ ***Aumentarne sensibilità, specificità ed accuratezza per lesioni CVS+, CVS- e CVSe***

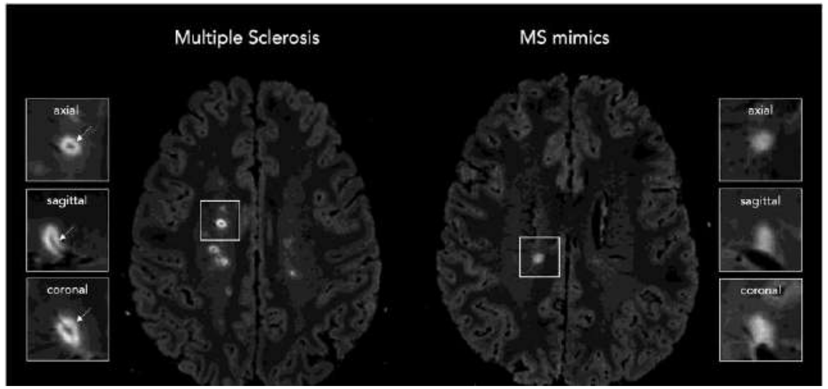
Maggi P, et al, *NMR Biomed* (2020)


934 subjects

MS vs non-MS conditions (Mann-Whitney test): all p<0.0001

Cagol et al, ECTRIMS 2022 9

❑ 62% delle SM e 68% delle CIS con CVS

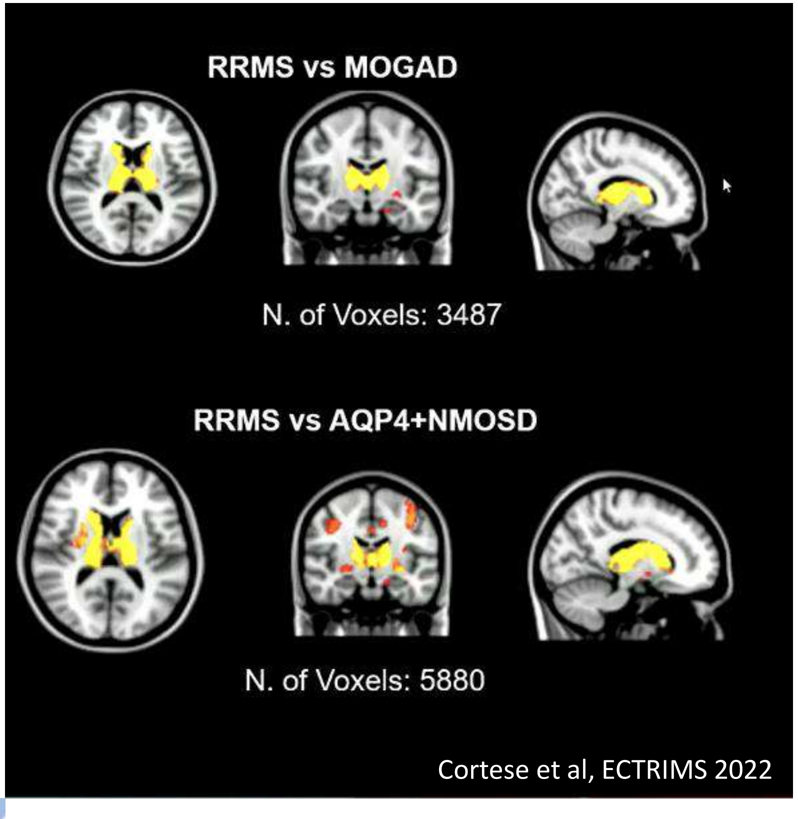


RMN strutturale: ruolo dell'atrofia della grigia

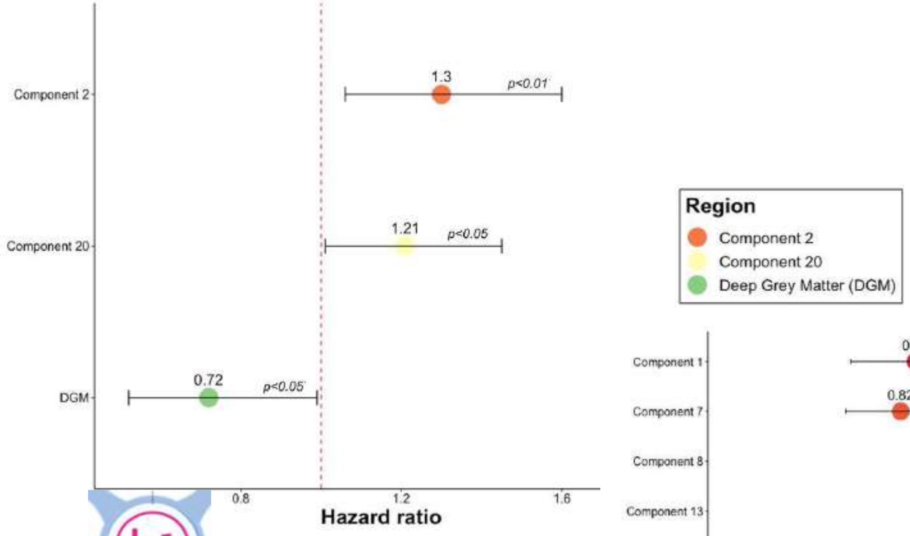
- ✕ Per anni la SM è stata ritenuta una patologia esclusivamente della SB
- ✓ Recenti studi hanno dimostrato come sia una patologia che interessa ab initio anche la sostanza grigia

- La perdita di volume della grigia (GM) contribuisce all'atrofia cerebrale
- La progressione della disabilità è stata prevista meglio da alcuni dei modelli covarianti delle regioni GM, che da singole misure regionali o dell'intero cervello.

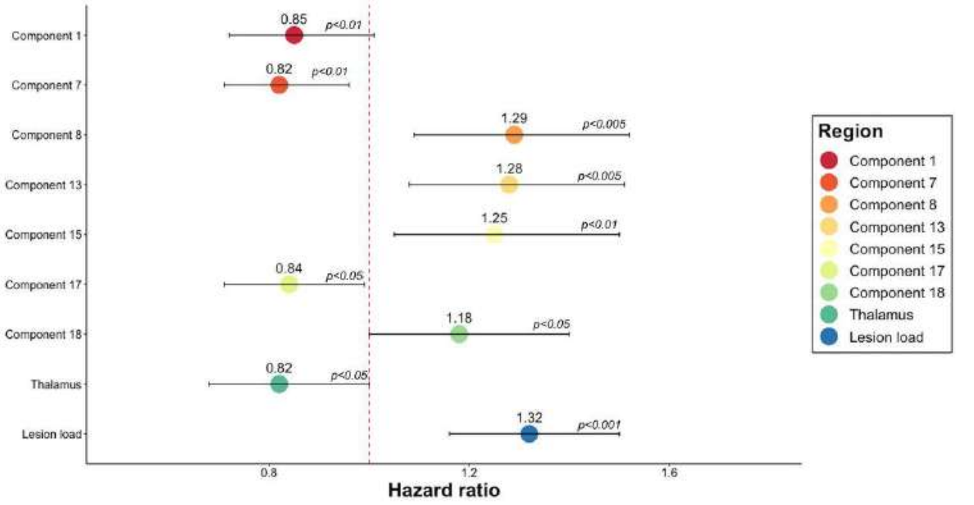
- ☐ 665 RMN da 16 centri
- ☐ Nella SM prevalentemente coinvolta la SG profonda



Cortese et al, ECTRIMS 2022



- ☐ Dati da RCT ASCEND (163 siti in 17 paesi)
- ☐ 1003 pazienti con SMSP



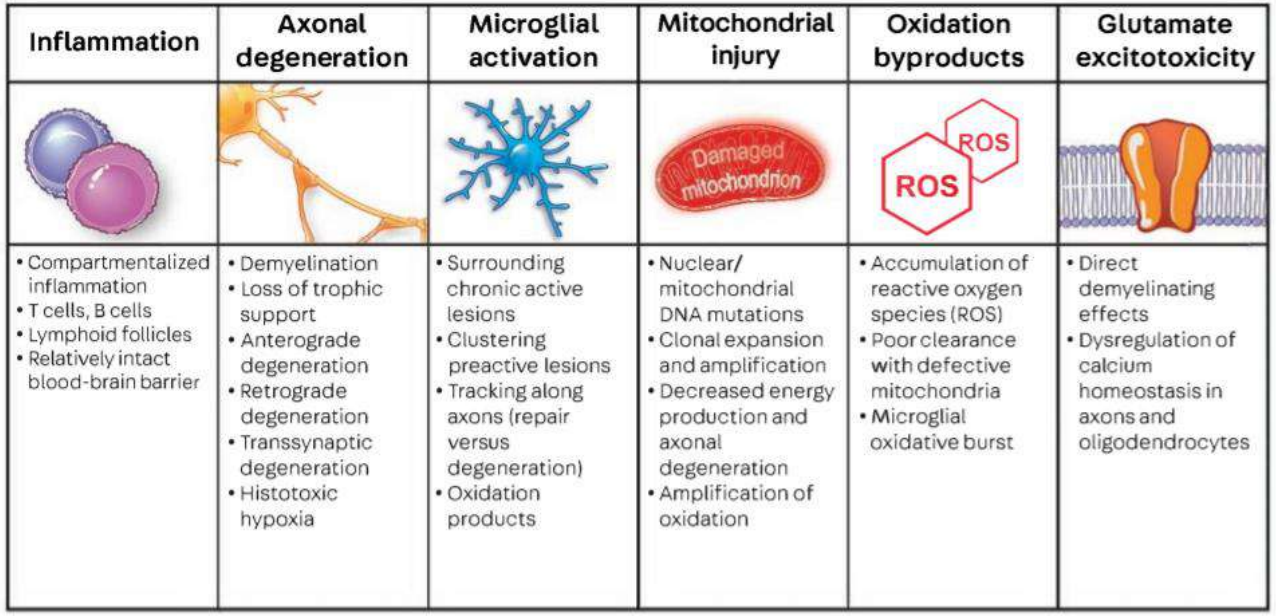
Elisa Colato et al. J Neurol Neurosurg Psychiatry 2021;92:995-1006

Biomarcatori: associazione con i diversi processi patologici

➢***Biomarker = utilizzato per rilevare o confermare la presenza di una malattia o condizione di interesse o per identificare individui con un sottotipo della malattia.***

2016 FDA-NIH Biomarker Working Group

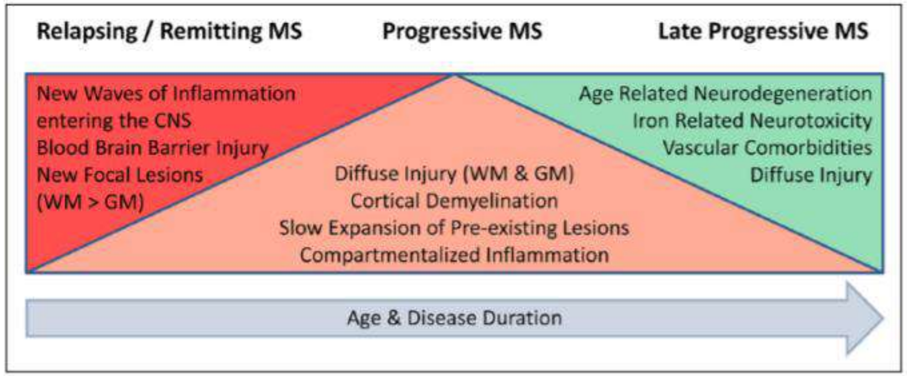
Nel corso degli ultimi anni→ focus sulla neurodegenerazione e sulle forme progressive di malattia



Damaged mitochondrion
ROS
ROS

Inflammation	Axonal degeneration	Microglial activation	Mitochondrial injury	Oxidation byproducts	Glutamate excitotoxicity
• Compartmentalized inflammation • T cells, B cells • Lymphoid follicles • Relatively intact blood-brain barrier	• Demyelination • Loss of trophic support • Anterograde degeneration • Retrograde degeneration • Transsynaptic degeneration • Histotoxic hypoxia	• Surrounding chronic active lesions • Clustering preactive lesions • Tracking along axons (repair versus degeneration) • Oxidation products	• Nuclear/ mitochondrial DNA mutations • Clonal expansion and amplification • Decreased energy production and axonal degeneration • Amplification of oxidation	• Accumulation of reactive oxygen species (ROS) • Poor clearance with defective mitochondria • Microglial oxidative burst	• Direct demyelinating effects • Dysregulation of calcium homeostasis in axons and oligodendrocytes

D. Ontaneda Continuum (Minneap Minn) 2019 Jun;25(3):736-752



Relapsing / Remitting MS **Progressive MS** **Late Progressive MS**

New Waves of Inflammation entering the CNS
Blood Brain Barrier Injury
New Focal Lesions (WM > GM)

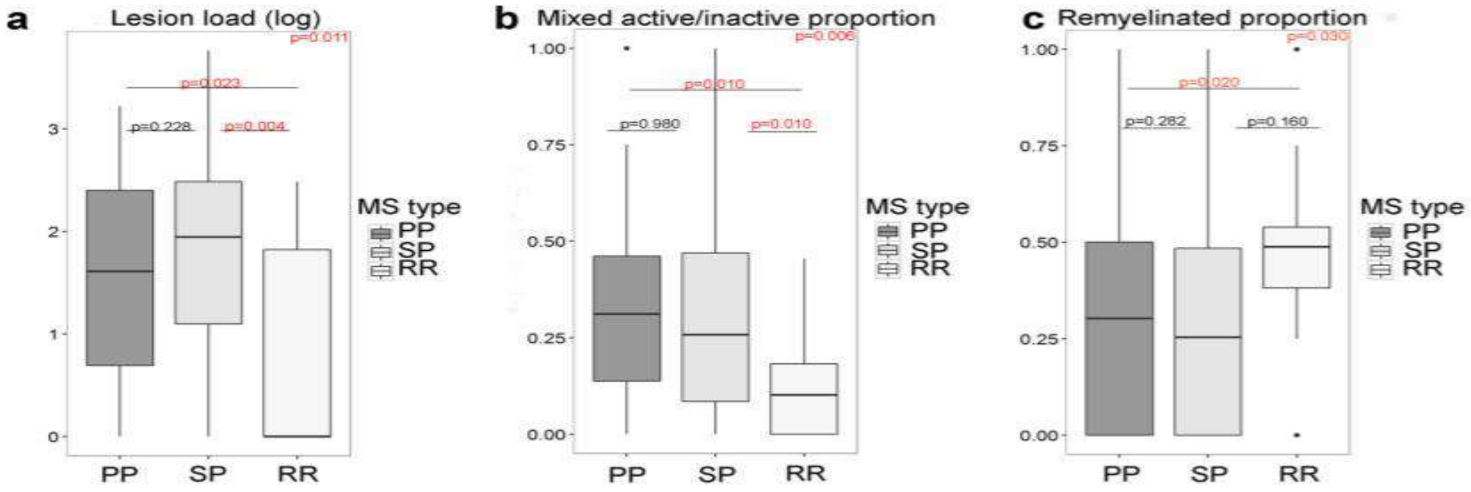
Age Related Neurodegeneration
Iron Related Neurotoxicity
Vascular Comorbidities
Diffuse Injury

Diffuse Injury (WM & GM)
Cortical Demyelination
Slow Expansion of Pre-existing Lesions
Compartmentalized Inflammation

Age & Disease Duration

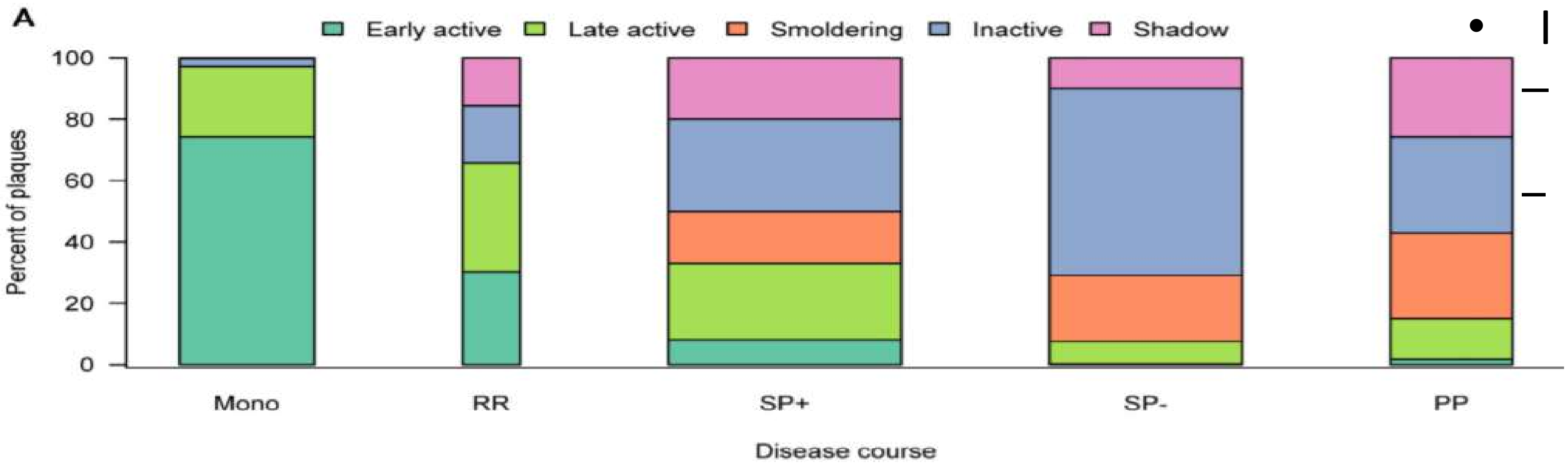
H Lassmann Multiple Sclerosis Journal 2017, Vol. 23(12) 1593–1599

… dalle lesioni anatomo-patologiche….



Lucchetti S et al, Acta Neuropathologica (2018) 135:511–528

- 182 casi autoptici SM (Paesi Bassi)
- I pazienti progresivi hanno
  a. carico lesionale ↑
  b. ↑lesioni attive/inattive (croniche attive)
  c. ↓ lesioni rimielinizzate rispetto a RR MS

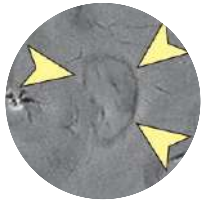


JM Frischer et al. Ann Neurol. 2015

- 120 casi autoptici(USA/Austria)
- I pazienti progressivi hanno
  - ↑Smoldering , associate con attivazione della microglia e neurodegenerazione
  - ↑“Shadow” sono aree rimielinizzate ma più suscettibili ad eventuale secondo attacco infiammatorio

… alla RMN strutturale (nuove metodiche): ruolo delle Slowly expanding lesions

- SEL possono essere identificate su RM 7T e 3T in vivo come lesioni croniche non-Gd+ con bordo paramagnetico



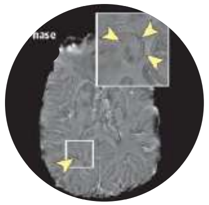
Rim lesion volume

&

Disability on EDSS

Correlation:
r=0.270;
p=0.0003

*Spearman correlation analysis



Rim lesion number

&

Disability on EDSS

Correlation:
r=0.248;
p=0.0005

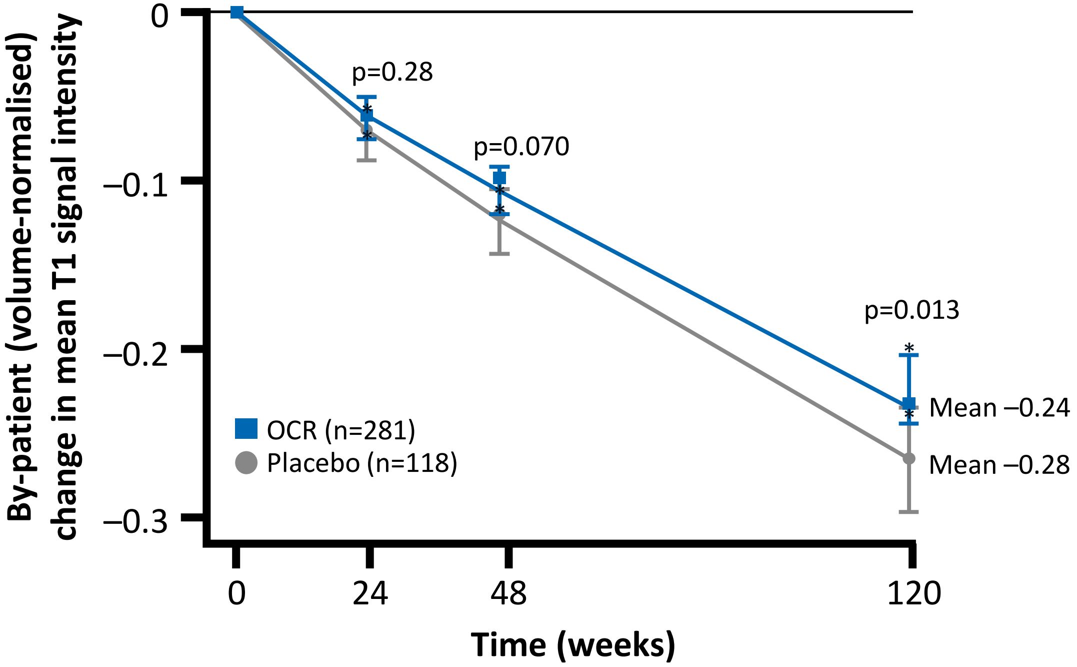
*Spearman correlation analysis

- 192 pazienti (f.u. 7 aa):
  - Persone con ≥4 SEL raggiungevano disabilità precocemente
  - Le lesioni «rimless» si riducevano volumetricamente nel corso del tempo, (-3,6%/anno), le SEL si espandevano (2,2%/anno; P <0,001)

Absinta M, *et al. JAMA Neurology* 2019

❑ Paz trial ORATORIO

❑ Terapie ad ↑ efficacia hanno maggiore azione sulle SEL



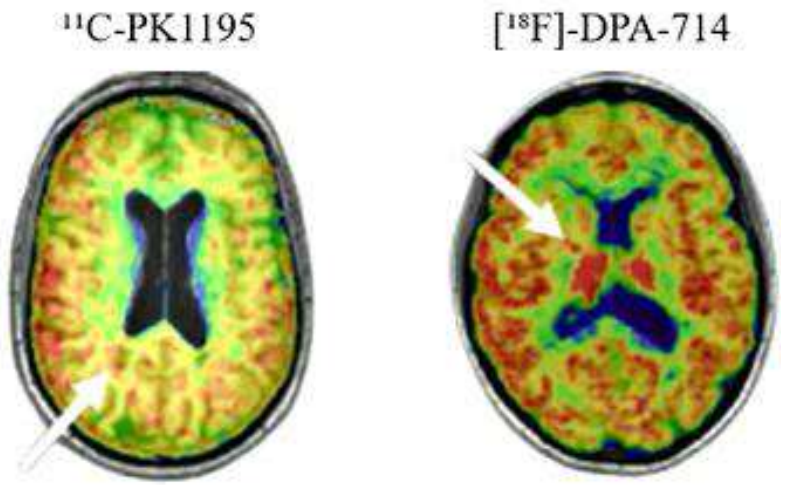
Elliot C, *et al. Brain* 2019;142:2787–99.

PET e Slowly expanding lesions (I)

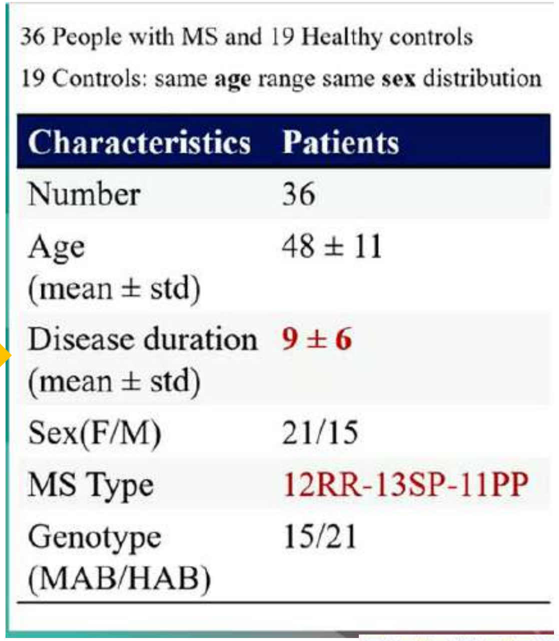
Biological specificity

Positron Emission Tomography

Target: **TSPO**


^{11}C-PK1195
[^{18}F]-DPA-714

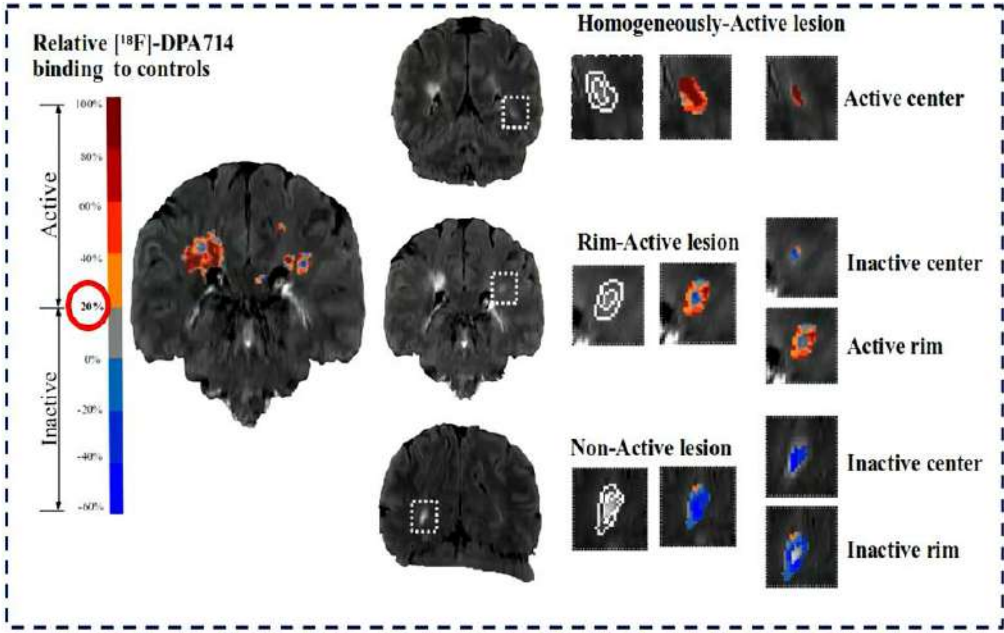
Bodini , *et al. 2021*

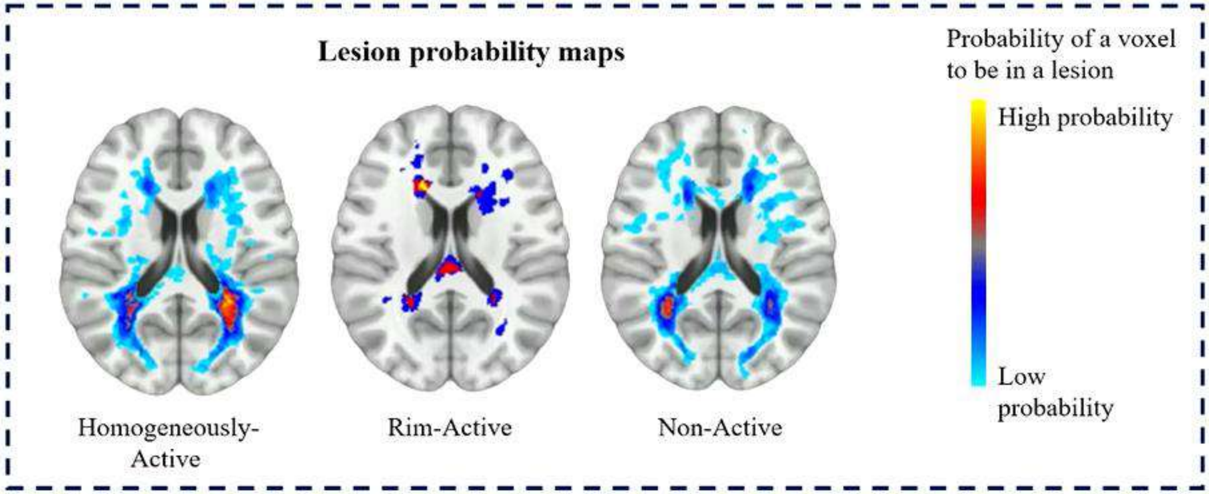


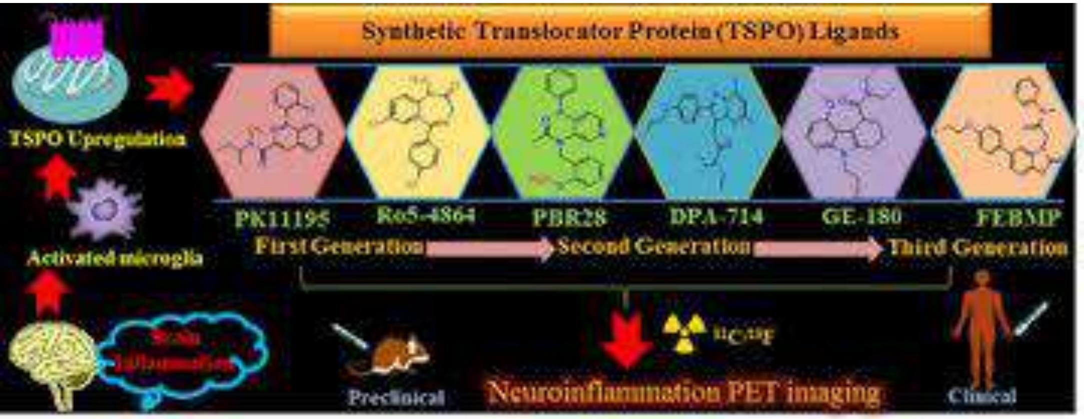
36 People with MS and 19 Healthy controls

19 Controls: same **age** range same **sex** distribution

Characteristics	Patients
Number	36
Age (mean ± std)	48 ± 11
Disease duration (mean ± std)	**9 ± 6**
Sex(F/M)	21/15
MS Type	12RR-13SP-11PP
Genotype (MAB/HAB)	15/21


Relative [^{18}F]-DPA714 binding to controls
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
Active
Inactive
Homogeneously-Active lesion
Active center
Rim-Active lesion
Inactive center
Active rim
Non-Active lesion
Inactive center
Inactive rim


Lesion probability maps
Probability of a voxel to be in a lesion
High probability
Low probability
Homogeneously-Active
Rim-Active
Non-Active

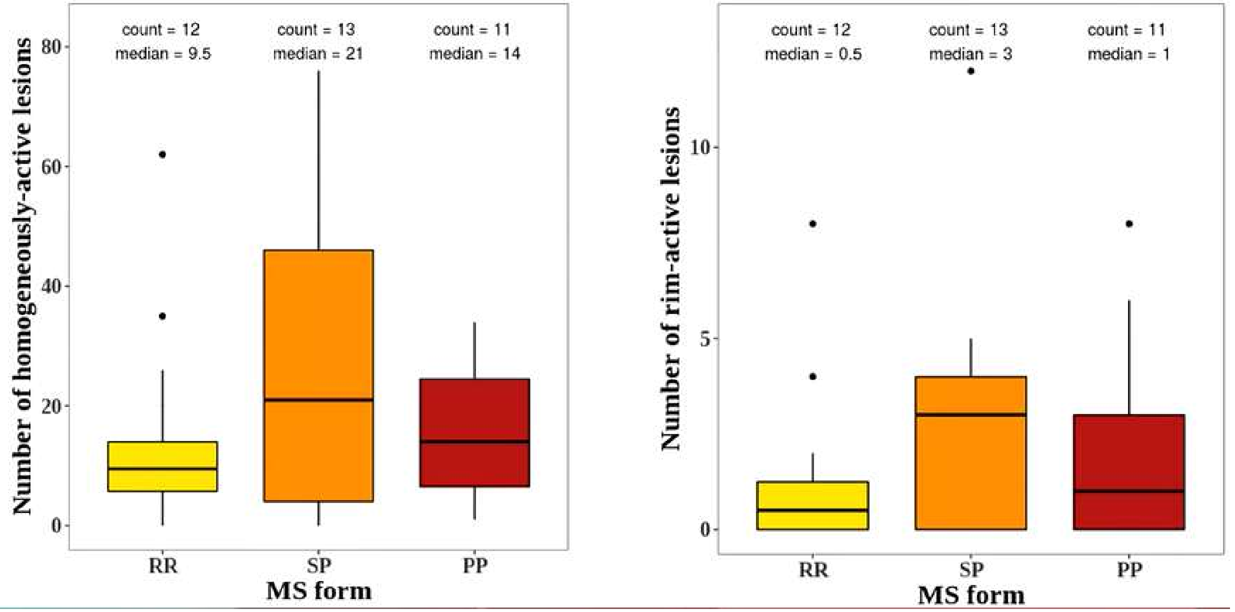

Synthetic Translocator Protein (TSPO) Ligands
TSPO Upregulation
Activated microglia
Brain Inflammation
PK11195
Ro5-4864
PBR28
DPA-714
GE-180
FEBMP
First Generation
Second Generation
Third Generation
Preclinical
11C/18F
Neuroinflammation PET imaging
Clinical

Singh , *et al. 2022*

Hamzoui et al, ECTRIMS 2022

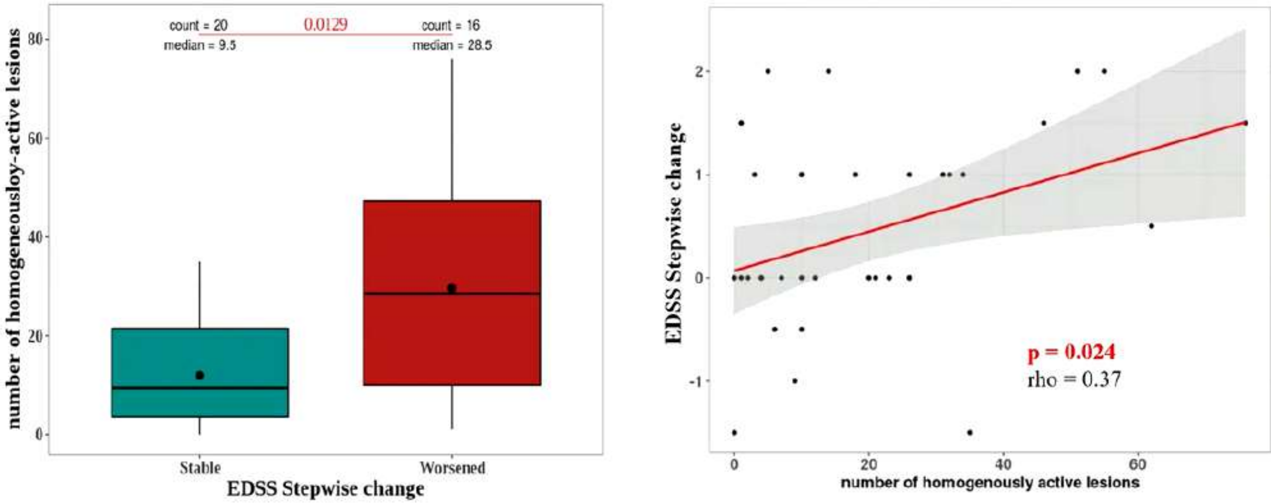
PET e Slowly expanding lesions (II)

Maggiore prevalenza delle SEL nelle forme progressive



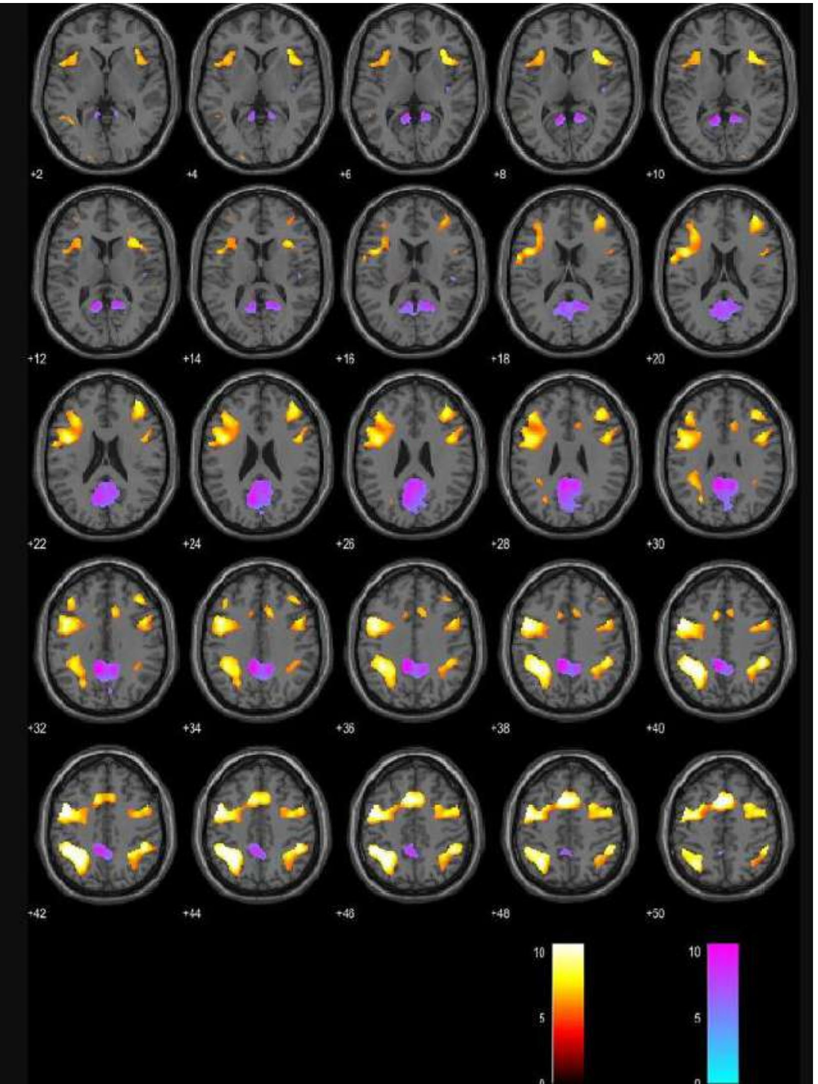
Correlazione delle SEL con peggioramento dell’EDSS

- **2-years change:** EDSS stepwise change



Hamzoui et al, ECTRIMS 2022

RMN funzionale e fatica


+2
+4
+6
+8
+10
+12
+14
+16
+18
+20
+22
+24
+26
+28
+30
+32
+34
+36
+38
+40
+42
+44
+46
+48
+50
10
5
0
10
5
0

- ➢ **La fatica o *fatigue* è uno dei sintomi più comuni della SM: è riportata dal 75 -95% dei pazienti; è considerata dal 55-75% dei malati uno dei sintomi più invalidanti (Amato MP 2012)**

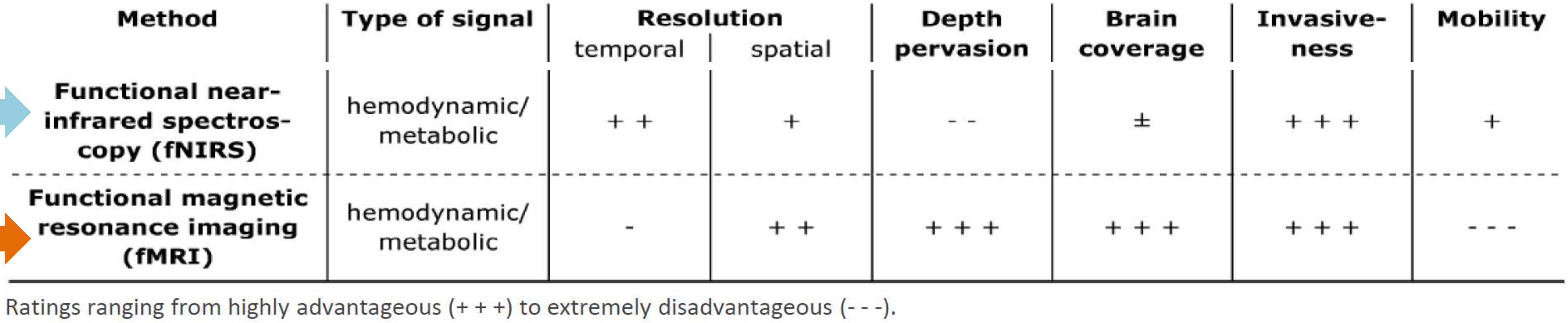
- 42 SMRR
- SDMT /PASAT
- BDI II/ MFIS29
- ➢ ↑attivazione nella BA 40 sinistra, nella BA 40 destra (giro sopramarginale) e nella BA 6 sinistra (corteccia premotoria) nei pazienti con un punteggio più alto su MFIS

Iancheva et al, 2019

Metodiche di Neuroimaging Funzionale:

Vantaggi e Svantaggi

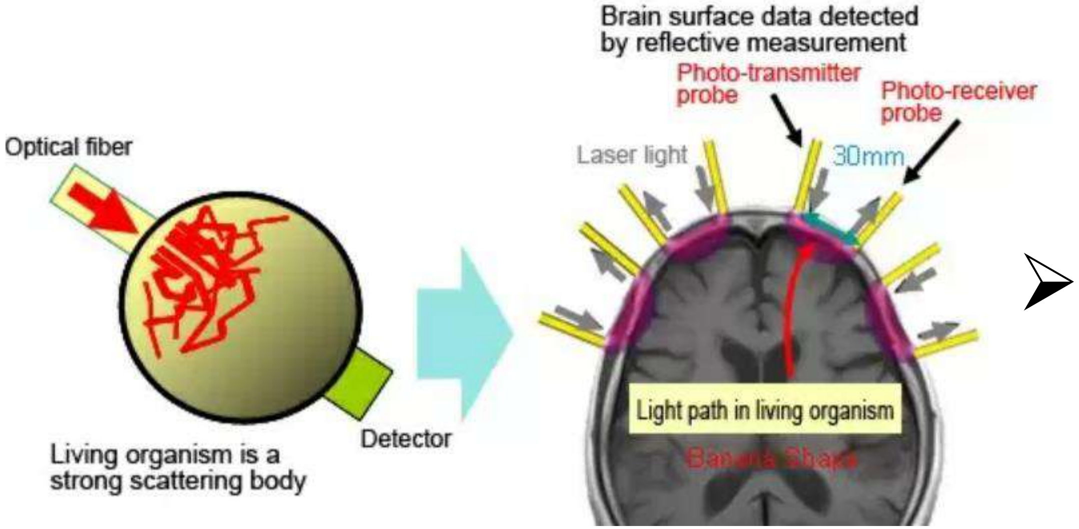
Le diverse metodiche di Neuroimaging Funzionale differiscono soprattutto per la loro capacità di **risoluzione spaziale** e **temporale** delle funzioni cerebrali.



Method	Type of signal	Resolution		Depth pervasion	Brain coverage	Invasiveness	Mobility
		temporal	spatial				
Functional near-infrared spectroscopy (fNIRS)	hemodynamic/ metabolic	+ +	+	- -	±	+ + +	+
Functional magnetic resonance imaging (fMRI)	hemodynamic/ metabolic	-	+ +	+ + +	+ + +	+ + +	- - -

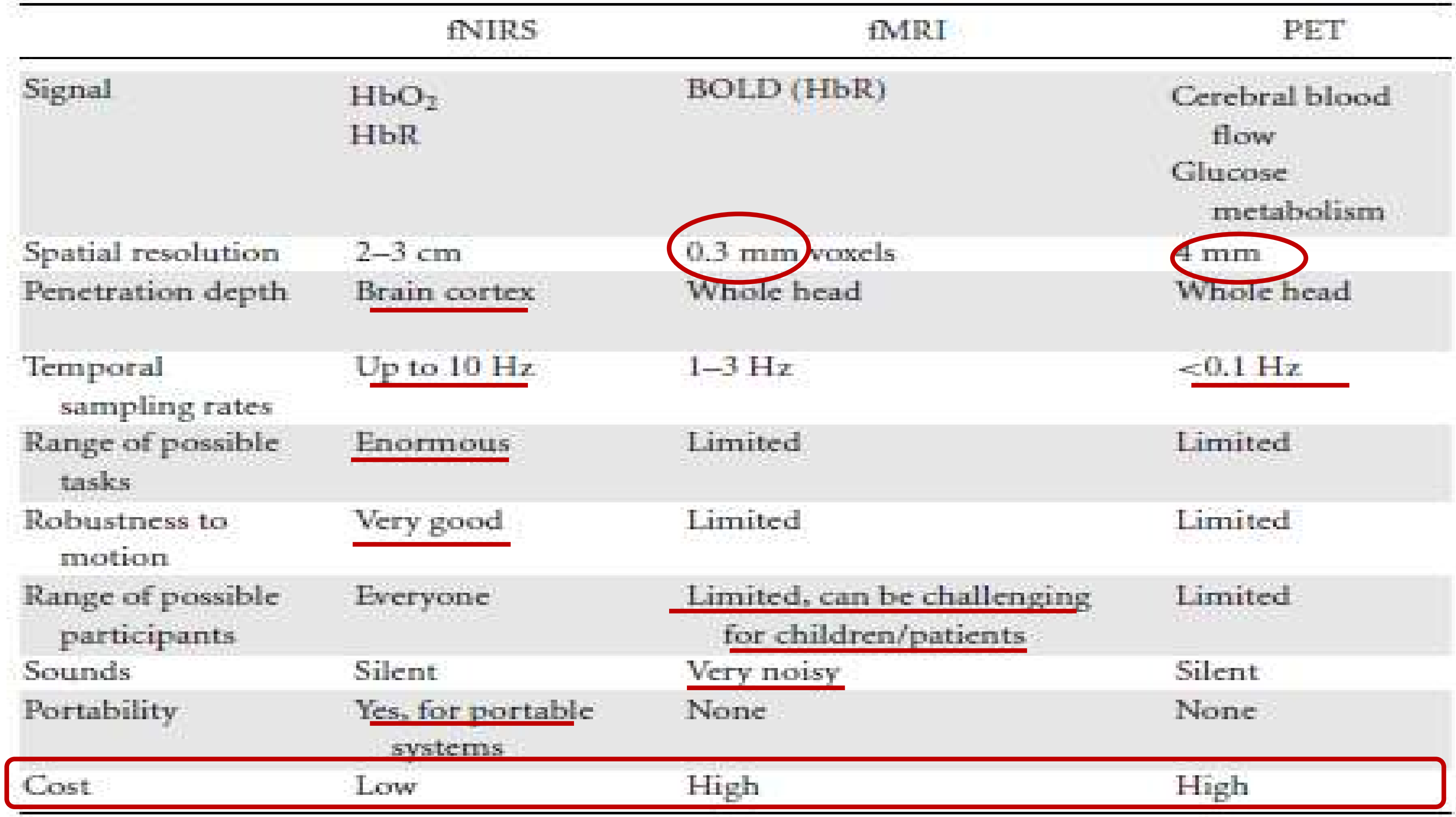
Ratings ranging from highly advantageous (+ + +) to extremely disadvantageous (- - -).

fNIRS: come funziona?


Optical fiber
Living organism is a strong scattering body
Detector
Brain surface data detected by reflective measurement
Photo-transmitter probe
Photo-receiver probe
Laser light
30mm
Light path in living organism
Banana Shape

- La NIRS utilizza ***la radiazione ottica***, precisamente la ***banda spettrale nel vicino infrarosso (NIR, 700-950 nm)*** per studiare i tessuti biologici
- La strumentazione permette di ottenere dati che riproducono il reale stato dell’ossigenazione del tessuto cerebrale, mediante il dosaggio “assoluto” dell’HbO2 e Hb e quindi della StO2.

Metodiche di Neuroimaging Funzionale a confronto

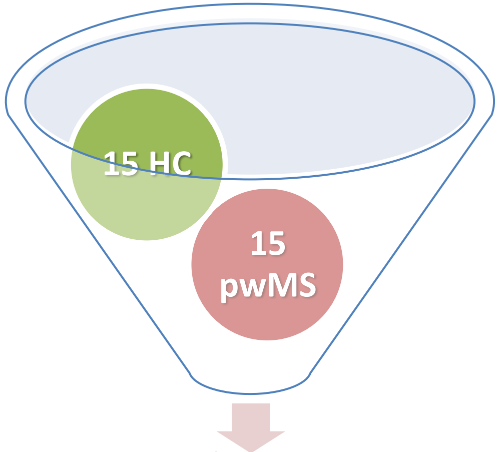


	fNIRS	fMRI	PET
Signal	HbO_2 HbR	BOLD (HbR)	Cerebral blood flow Glucose metabolism
Spatial resolution	2–3 cm	0.3 mm voxels	4 mm
Penetration depth	Brain cortex	Whole head	Whole head
Temporal sampling rates	Up to 10 Hz	1–3 Hz	<0.1 Hz
Range of possible tasks	Enormous	Limited	Limited
Robustness to motion	Very good	Limited	Limited
Range of possible participants	Everyone	Limited, can be challenging for children/patients	Limited
Sounds	Silent	Very noisy	Silent
Portability	Yes, for portable systems	None	None
Cost	Low	High	High

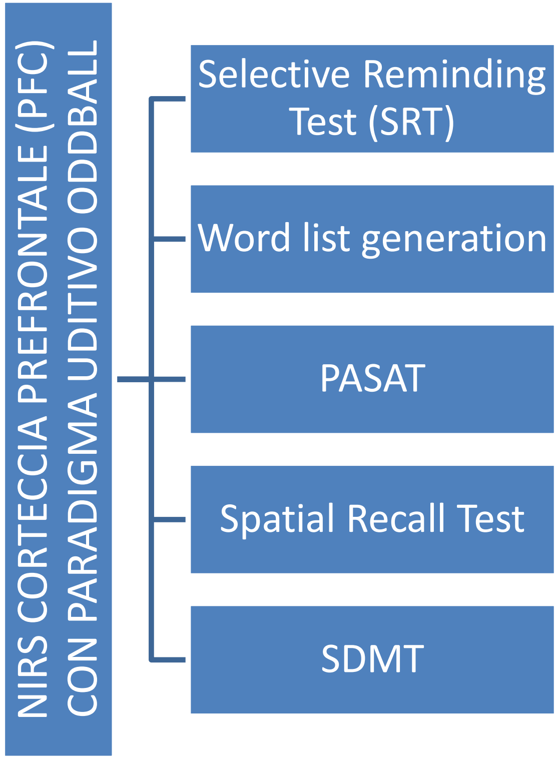
Table adapted from Pinti P, et al. Ann N Y Acad Sci. 2018

NIRS e fatica cognitiva: il nostro studio

Studio sulla Fatica Cognitiva nella Sclerosi Multipla: ricerca di Biomarcatori Neurofisiologici di malattia e valutazione dell'efficacia del Trattamento Riabilitativo Multimodale



Match per età, sesso, livello di istruzione



NIRS CORTECCIA PREFRONTALE (PFC) CON PARADIGMA UDITIVO ODDBALL

- Selective Reminding Test (SRT)
- Word list generation
- PASAT
- Spatial Recall Test
- SDMT

- PwMS (70% F , età 38.1±6.1)
- SRT long-term retrieval :

  0.87±1.41 in HCs

  vs p=0.03

  -0.42±1.09 in pwMS

- SRT long-term storage :

  0.97±1.21 in HC

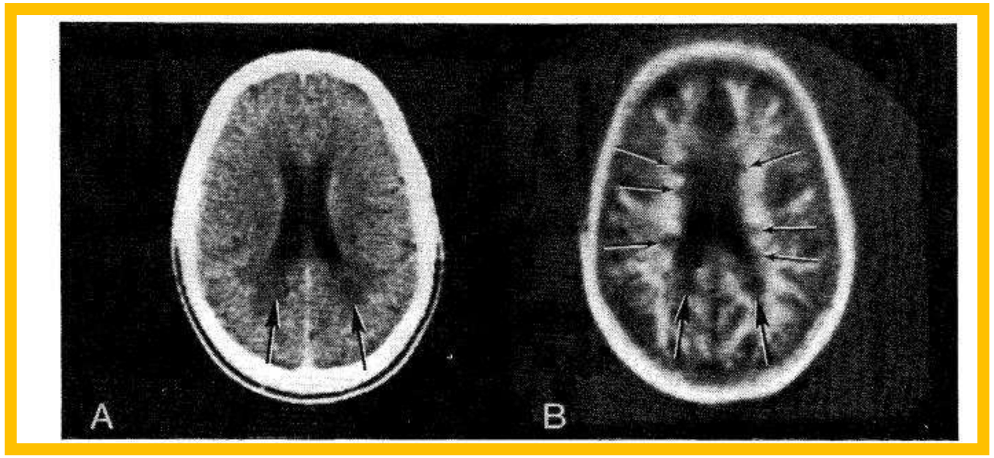
  vs p=0.023

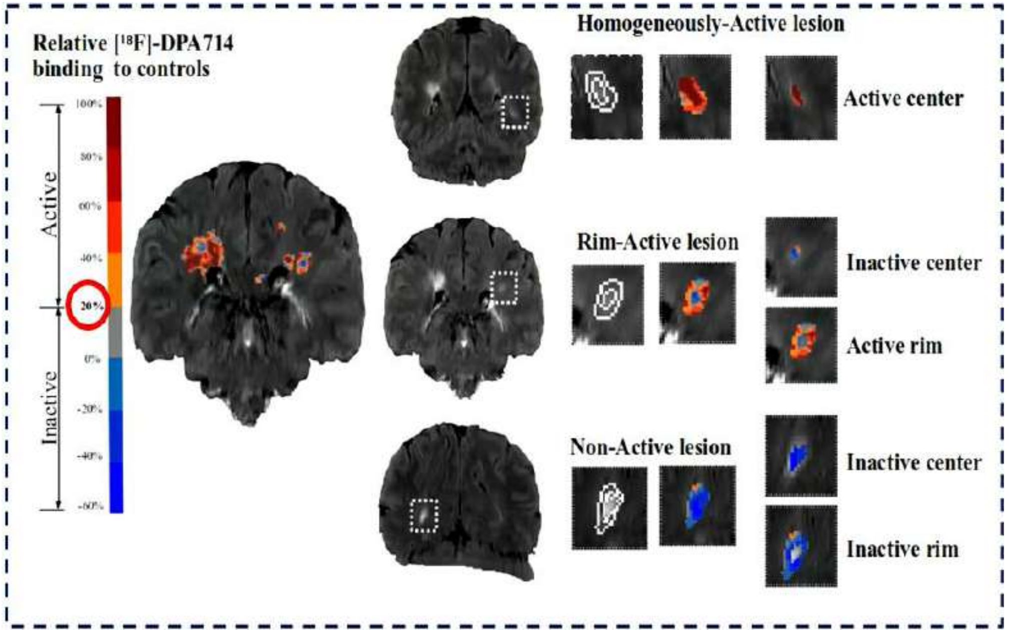
  -0.47±0.6 in pwMS

- ↑ attivazione emodinamica PFC (p<0.05) in SM nei primi 4 min del task

- **pwMS, hanno necessità di maggiore attivazione corticale per compiere lo stesso task**

Take home messages




Relative [18F]-DPA714 binding to controls
Active
Inactive
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
Homogeneously-Active lesion
Active center
Rim-Active lesion
Inactive center
Active rim
Non-Active lesion
Inactive center
Inactive rim

- I clinici che si occupano di SM hanno necessità sempre crescenti di poter condividere ed analizzare dati che devono includere una **fenotipizzazione clinica, radiologica e biologica** , da una popolazione eterogenea, ben rappresentativa della pratica clinica quotidiana

- **Le persone con SM mostrano crescente necessità di aver accesso a strumenti clinici che possano aiutarli a monitorare e comprendere la malattia**

L'integrazione di dati acquisiti con nuove tecnologie e migliori metodiche di pre-esistenti strumenti (MRI) permette di ottenere informazioni complete da utilizzare nella cura del paziente e nella ricerca clinica

↓

Obiettivo finale: sviluppare nuovi approcci alla medicina personalizzata nella SM

Grazie per l’attenzione

«Anche se la finestra è la stessa, non tutti quelli che si affacciano vedono le stesse cose: la veduta dipende dallo sguardo»

A.Merini