Impact of macrophagic activity on tissue structure in patients suffering from clinically isolated syndrome suggestive of multiple sclerosis: a multicentric USPIO enhancement study at 3T

Adil Maarouf[1,2], Jean Christophe Ferré[3,4], Wafaa Zaaraoui[1], Elise Bannier[5], Christian Barillot[4], Isabelle Berry[6], Gilles Edan[7], Damien Galanaud[8], Jean Pelletier[1,9], Christophe Portefaix[10], Ayman Tourbah[11], Jean-Phillipe Ranjava[1], and Bertrand Audoin[1,9]

[1]CRMBM, CNRS UMR 7339, Marseille, France, [2]Dept. of Neurology, CHU REIMS, Reims, France, [3]Dept. of Neuroradiology, CHU Rennes, RENNES cedex 9, France, [4]Visages U746, INSERM INRIA IRISA, Rennes, France, [5]Neurinfo, INSERM INRIA IRISA, Rennes, France, [6]Dept. of Biophysics, CHU Toulouse, Toulouse, France, [7]Dept. of Neurology, CHU Rennes, Rennes, France, [8]Dept of Neuroradiology, Pitié Salpêtrière Hospital, Paris, France, [9]Dept. of Neurology, CHU Marseille, Marseille, France, [10]Dept. Radiology, CHU Reims, Reims, France, [11]Dept. of Neurology, CHU Reims, Reims, France

· **Purpose** - Macrophage infiltration is an important pattern in inflammatory processes associated to multiple sclerosis (MS). Several studies have supported a close link between macrophage infiltration and axonal loss [1 - 5] that is a major substrate for permanent neurological disability in patients [6 7]. Recently, new contrast agents based on ultra-small super paramagnetic particles of iron oxide (USPIO) have been developed. USPIO modify locally the longitudinal and transversal relaxation times. Phagocytosis of USPIO by monocytes/macrophages cells allows in vivo and noninvasive labelling of regions with macrophage infiltration. Four previous studies have validated the feasibility of using USPIO in vivo in patients suffering from multiple sclerosis [8 - 11]. All these studies focused on radiological patterns description and spatial distributions of USPIO enhancements in MS. The studies evidenced that USPIO provides distinct and complementary information to gadolinium-enhanced MRI. Nevertheless, the macrophage activity assessed in vivo by USPIO enhancement had never been studied at the earliest stage of MS (Clinically Isolated Syndrome or CIS), neither the impact of macrophagic infiltration on the tissue structure.

The aim of this longitudinal and multicentric study was to determine the prevalence of USPIO enhancement in patients with a CIS and the impact of macrophage activity at early and medium term on tissue integrity assessed by magnetization transfer ratio (MTR).

· **Methods** - *Patients and Study Design:* Five french university hospitals included 35 patients within 3 months after a first demyelinated clinical episode. Inclusion was based on the following criteria: (i) age between 18 and 45 yo; (ii) occurrence of the first presumed inflammatory demyelinating event in the central nervous system; (iii) no previous history of neurological symptoms suggestive of demyelination; (iv) no possible alternative diagnoses; (v) dissemination in space according to McDonald criteria, 2005; (vi) EDSS (Expanded Disability Status Scale) between 0 and 5 at baseline; (vii) first injection of USPIO within 3 months after the first clinical episode; (viii) no corticoids in the month before USPIO injection and no previous administration of immunomodulatory or immunosuppressive drug; (ix) no previous history of asthma, allergy, injection of iron oxide particles within 5 months; (x) no pregnancy. The local ethics committees approved the protocol and all subjects gave informed consent.

Image acquisition: Patients were imaged with a 3T MRI system at baseline and 12-month (Verio; Siemens or Achieva; Philips). Conventional and quantitative brain MRI (qMRI) have been performed before and after (24h) USPIO injection including in the first day transverse fast spin-echo proton density-weighted and T2-w sequences (Verio: TR/TE1/TE2 = 6530/8.8/88ms and Achieva: TR/TE1/TE2 = 2269/8.2/90ms; all other parameters were the same: 44 contiguous sections, 3-mm section thickness, 256-mm FOV, matrix 256×256), transverse spin echo T1-w sequence (Verio: TR/TE = 500/8.4 ms and Achieva: TR/TE = 600/9.3 ms; all other parameters were the same: 44 contiguous sections, 3-mm section thickness, 256-mm FOV, matrix 256×256) before and 5 minutes after intravenous administration of 0.1 mmol/kg of gadolinium chelate (Gd; Dotarem; Guerbet) and transverse proton density-weighted spoiled gradient-echo sequence (Verio: TR/TE=750/4.5ms and Achieva: TR/TE=65.8/5.1ms, all other parameters were the same: 44 contiguous sections, 3-mm section thickness, 256-mm FOV, matrix 256×256) performed without (M0) and with (Ms) magnetization transfer saturation pulse (Gaussian shape, 1.5-kHz off-water resonance, 1000°). After the first examination, 40 micromol of iron/kg body weight of USPIO (SHU-555C; Bayer Schering Pharma) was injected. The second day (24h) transverse spin echo T1-w sequence post-USPIO was performed.

Image analysis: All exams were blindly analyzed by three experts. The visual analysis consisted in post Gd and post USPIO T1-w enhanced lesions count and pattern analysis: ring like enhancement, focal enhancement and return to iso-intensity of a pre-contrast hypo-intense lesion[9]. The overlap of USPIO and gadolinium enhancement was assessed using a 3-point scale: <20%, 20-80% and >80% of the enhancement volume. MTR maps were calculated on a voxel-by-voxel basis according to the following equation: MTR (%) = ((M0 - Ms)/M0) x100, where M0 and Ms were the images obtained without and with the MT saturation pulse, respectively. T2 lesions were delineated onto the T2-w images by means of a semi-automated method.

In order to investigate the rates of MTR in neighbouring normal-appearing white matter (NAWM) regions by going away in a progressive and concentric way from the lesions, we labelled each lesion automatically in related components by means of the floodill algorithm (Brainvisa). Then for each lesion, we defined its crowns of interest using set theory coupled with classical mathematical morphology operators[12]. Thus, we created three crowns after three dilations of the original lesion. Finally, these obtained masks have been applied to the MTR maps with two constraints: taking into account only the white matter and exclusion of the lesions and the nearby dilations from each crown.

The optimized post-processing pipeline included (1) Coregistration of magnetization transfer (Ms) images on the T2-w images using the normalized mutual information procedure (SPM5); (2) Subtraction of the T2 lesions mask from the coregistered Ms to obtain the lesion-free Ms image; (3) Segmentation of grey and white matter (GM and WM) with VBM 5; (4) Segmentation of MTR maps using the T2-lesions, the three regions of dilatation, the GM and the WM masks obtained previously.

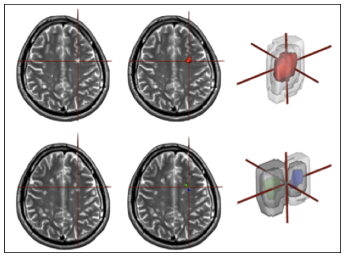

Fig. 1 : 3D representation of lesions dilatation

Statistical analysis: Wilcoxon test for the comparison of patient's features ($p<0.05$). ANOVA with Wilcoxon test for the comparison between the mean MTR values.

Safety: Patients were monitored clinically after each USPIO injection and every three months. All data were collected in case report forms.

· **Results** - *Radiological analysis:* All MR exams were blindly analyzed by 3 experts. USPIO enhancement was hyper intense on T1-w images. At baseline,16 patients out of 35 had a contrast enhancement, and among them, 82 gadolinium-positive lesions and 17 USPIO-positive lesions were seen; only one of USPIO-positive lesion was gadolinium-negative. The overlap of enhanced lesion areas between gadolinium and USPIO was always partial (<20% or 20-80%), suggesting that the presence of USPIO in the cerebral parenchyma appears to be due to a different mechanism than passive passage though leaked blood brain barrier. The 16 patients were divided into two groups: 9 patients with at least one USPIO-positive lesion and 7 patients only with gadolinium-positive lesions. The two groups did not differ in term of age, sex, mean disease duration and EDSS (a clinical score of disability).

Lesional and perilesional structure according to enhancement: In the 16 USPIO and gadolinium-positive lesions, the mean MTR value was significantly lower than in the 66 only gadolinium-positive lesions ($p=0.01$), reflecting a more important tissue destructuration. In the dilatations, this decrease was not significant. At 12-month, the mean MTR values in the lesions that were at baseline USPIO and gadolinium-positive were still significantly lower compared to the lesions that were only gadolinium-positive at baseline ($p=0.03$). None of these lesions were still enhanced at 12-month. Furthermore, the mean MTR value was significantly lower in the first and third level of dilatation in the normal-appearing white matter (NAWM) ($p=0.04$).

T2-w lesion load according to enhancement: At baseline, total T2-w lesion load was non-significantly higher in the group of patients with at least one USPIO-positive lesion (12.9 cm3 versus 5.4 cm3, $p=0.68$). However, the total T2-w lesion was significantly higher in the group of patient that had at baseline at least one USPIO-positive lesion (11.4 cm3 versus 3.9 cm3, $p=0.02$).

Structure of the normal-appearing white matter and grey matter according to enhancement: All patients were classified into 3 groups: (i) a group of 9 patients having at least one enhanced lesions after injection of USPIO at baseline; (ii) a group of 7 patients having at least one lesion enhanced after gadolinium injection and no lesion after USPIO injection at baseline; and (iii) a group of 16 patients without any enhancement at baseline. Three patients did not show up at the 12-month follow up. There was no significant difference between the mean values of MTR in the normal-appearing white matter or the grey matter between the 3 groups at baseline and 12-month.

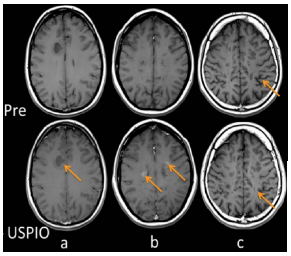

Fig. 2: Cross-sectional patterns of lesion enhancement. Pre and post USPIO injection T1-w SE :
(a) Ring like enhancement pattern
(b) Focal enhancement pattern
(c) Return to isodensity of a previously T1-w hypodense lesion pattern

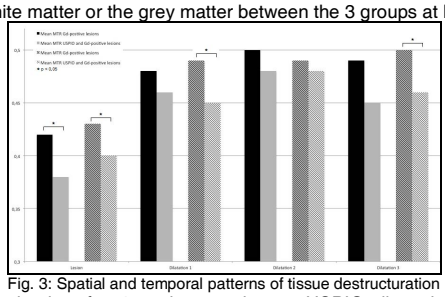

Fig. 3: Spatial and temporal patterns of tissue destructuration

· **Conclusion** - From the earliest stage of multiple sclerosis, we highlighted the presence *in vivo* of activated macrophages. USPIO allowed to approach the mechanisms involved in the initial and specific inflammation, and to confirm the presence of tissue destructuration that was higher and persistent in lesions with significant macrophages burden, but also to their neighbours. We demonstrated that macrophages play a role in the local tissue destructuration in MS, although the underlying mechanisms remain unknown. Taking into account the existence of this most important and persistent tissue destructuration of USPIO-positive lesions and their perilesional NAWM and the predictive value of the lesion load at 12-month, USPIO seems to be a predictive biomarker of the disease. Complementary explorations are necessary to apply these procedures at the individual level.

· **References** – (1) Trapp et al, 1998; (2) Bitch et al, 2000; (3) Aboul Enein et al, 2006; (4) Smith et al, 2001; (5) Redford et al, 1997; (6) Kornek et al, 1999; (7) Trapp et al, 1999; (8) Dousset et al, 2006; (9) Vellinga et al, 2008; (10) Vellinga et al, 2009; (11) Tourdias et al, 2012; (12) Serra, 1982.