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Università Campus Bio-Medico di Roma

Corso di dottorato di ricerca in

Scienze della Plasticita' d'organo e della rigenerazione tissutale
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Double Inversion Recovery MR in cerebrovascular disease

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DOUBLE INVERSION RECOVERY MR in CEREBROVASCULAR DISEASE

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All my awesome friends and colleagues...

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Abbreviations

DIR	Double Inversion Recovery
DIRlns	Double Inversion Recovery intracortical lesions
MR	Magnetic Resonance
DWI	Diffusion Weighted Imaging
VCI	Vascular Cognitive Impairment
MCA	Middle Cerebral Artery
ACA	Anterior Cerebral Artery
PCA	Posterior Cerebral Artery
MCIs	Cortical Microinfarcts

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INTRODUCTION

Double Inversion Recovery MRI

Investigation of cerebral cortex is of paramount importance in Neuroscience. In the last twenty years, development of new MR tools able to image grey matter *in vivo* has driven significant advance in understanding the cortical damage in many neurological disease, i.e. Multiple Sclerosis or Epilepsy.

To implement MR segmentation of brain tissues (grey matter, white matter, cerebrospinal fluid), in 1994 Redpath and Smith designed a new MRI sequence named Double Inversion Recovery[1].

Inversion Recovery sequence works through nulling the signal of a single tissue by applying an inversion pulse with an appropriate inversion time (TI). In Double Inversion Recovery an additional inversion pulse induces the suppression of the signals of two tissues (i.e. cerebrospinal fluid and white matter signals) with different relaxation time (TR) simultaneously. This allows grey matter segmentation from white matter and CSF without the need for image MR post-processing.

DIR was later implemented by Bedell BJ & Narayana PA [2] in order to make sequence acquisition time shorter and DIR suitable for clinical usage.

DIR has been increasingly used in Multiple Sclerosis to detect and score intracortical lesions since the publications of seminal papers by the Amsterdam Neuroimaging Group demonstrating the greater sensitivity of DIR compared to conventional imaging for intracortical lesions visualization and its high pathology specificity in agreement with post-mortem histopathology [3–5].

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Cerebrovascular risk factors and brain damage

Cerebrovascular risks factors including hypertension, hyperlipidemia, diabetes mellitus, obesity, alcohol abuse, cigarette smoking are very common in aged individuals. Beyond overt stroke, cerebrovascular risk factors and atherosclerosis may cause multiple asymptomatic brain microinfarcts accrual over time[6,7]. Moreover evidence have been accumulating that they are associated with white matter lesions and gray matter atrophy[8].

Cerebrovascular risk factors and subclinical early carotid artery disease are associated with later vascular cognitive impairment (VCI) also in individuals without clinical stroke, according to data collected in large prospective cohorts as the Baltimore Longitudinal Study of Aging or The Framingham Study cohort [9–11].

From the pathological perspective, there is dispute about the role of various types of vascular lesions (large cortical infarcts, lacunar infarcts, subcortical white matter disease, strategically placed subcortical infarcts, or a combination of these) that contribute to cognitive impairment and vascular lesions can also lower the threshold for the clinical manifestation of Alzheimer disease.

Several clinico-pathological studies show that the likelihood of late dementia grows with increasing burden of macro and microscopic infarcts[12]. Among them, cortical microinfarcts seem to be the strongest determinant for cognitive decline according to post-mortem studies in elderly demented subjects.

So far data about the association in vivo between intracortical lesions and VCI are not available, firstly because CMI are very difficult to visualize *in vivo* by the currently available neuroimaging techniques[13]. Furthermore the relation between cerebrovascular risk profile and cortical microischemic damage has not been clarified yet.

Small cortical infarcts are a common collateral findings in patients with symptomatic stroke[14]. A recent paper found that, in patients with symptomatic brain infarct, carotid disease was significantly associated with small cortical infarcts (SCI) ipsilateral to the side of carotid disease, independently from other cerebrovascular risk factors[15] .

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Moreover studies of microembolic signals (MES) detected by transcranial Doppler have suggested an association between small cortical infarcts, spotty lesions in the cortex and emboli from carotid plaques [16–20]. These results lend support to the hypothesis that the predominant mechanism of SCI is mainly microembolism from carotid disease.

Cortical infarcts may be localized in the 'distal field' regions where blood perfusion is low[21]. Ten % of all brain infarcts are in fact watershed infarcts, which by definition involve the junction of the distal fields of 2 nonanastomosing arterial system. These infarcts have been traditionally attributed to heamodynamic impairment (i.e. distal hypoperfusion)[22].

In contrast with this widely prevalent interpretation, several pathologic investigations have emphasized an association between border zone infarction and microemboli[23]. Preferential propagation of emboli in the border zone regions also has been found in experimental studies[24].

Currently border zone infarction seem to be better explained by a synergistic combination of hypoperfusion and embolization: carotid artery disease would be the source of microemboli which are as many as much severe and ulcerate the plaque is and hypoperfusion, which is most likely to be impaired in border zone regions, would reduce the washout of emboli inducing distal microvessel occlusion[25].

Since vascular disease is involved in the etiology of brain changes on MRI and development of dementia, patients with cerebrovascular disease can be considered a high-risk group, either for disability and mortality but also for cognitive decline.

Identifying novel MR markers of brain ischemic damage potentially affecting cognitive functioning in otherwise asymptomatic individuals with cerebrovascular risk factors is fundamental in order to adopt adequate preventive treatment.

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AIM of the thesis

The impact of cerebrovascular risk factors on brain injury appears to begin in middle life and additively increases the likelihood of later life VCI. Post-mortem studies show that cerebral microinfarcts (CMIs) burden and especially cortical microischemic damage are correlated with VCI. In vivo CMIs go largely undetected in clinical–radiological correlation studies that rely on conventional structural MR[13]. DIR is a convenient MR sequence able to selectively image grey matter, widely used to score intracortical demyelinating lesions in Multiple Sclerosis. DIR is considered high pathologically specific after the demonstration of the good agreement between change in the tissue MR signal *ex vivo* and demyelinating cortical lesions found at autopsy[5]. Few reports are available in patients with mixed dementia, including VAD and CADASIL of cortical microinfarcts detected by DIR.[26]. However the ischemic origin of those lesions has not been proved, as studies comparing post-mortem and DIR findings in cerebrovascular disease are lacking. Though the concomitance with cerebral microbleeds in the same MR seems supportive of this hypothesis.

On this backgroud, aims of the work are:

1. To provide the proof of concept that DIR is able to detect in vivo intracortical ischemic lesions and to compare the power of DIR in detecting micro-ischemic cortical lesions early tracked by DWI occurring during CAS with conventional imaging
2. To examine the prevalence of DIR intracortical lesions in vivo in patients with cerebrovascular risk factors with and without carotid artery stenosis and to explore the power of cerebrovascular risk factors and carotid artery stenosis severity in explaining microischemic lesions accrual.

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EXPERIMENTAL SETS

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Double Inversion Recovery Is Feasible to Detect Small Intracortical Ischemic Brain Lesions

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AIM 1

Backgroud

Diffusion-weighted MRI (DWI) is the most sensitive (80-90%) available technique measuring tissue damage in acute cerebral ischemia and it is commonly used in clinical and experimental settings. DWI signal contrast is based on differences of random motion of water molecules (diffusion capacity). If the diffusion capacity is restricted, signal intensity increases. Diffusion restriction can be visible within minutes after the onset of cerebral ischemia. It is thought to reflect intracellular (cytotoxic) edema, resulting from failure of energy-dependent transmembrane Na/K pumps[27,28].

Patients submitted to carotid endoarterectomy and carotid artery stenting (CAS) are at high risk of periprocedural cerebral micro-embolism. [29–33]

Compared to surgery, CAS is associated with higher rate (37-50% VS 10-17%) of microembolic ischemic events recordable either by transcranial Doppler sonography or by applying diffusion-weighted imaging (DWI) which detects new embolic brain lesions after the intervention[30,34,35].

This finding is largely related to the manipulation of catheters, guidewires, and sheaths in the supra-aortic vasculature, but it may also be the consequence of a diagnostic angiography, which is usually performed before CAS. Moreover new ischemic lesions occurring after CAS are mostly located in the cerebral hemispheric cortex, especially in the cortical border zone between the major arteries. Lesions size is usually very small (<5mm).

The meaning and reliability of these early detected areas are still a matter of debate. Several studies investigating the radiological evolution of post-procedural brain DWI lesions in fact failed in tracking those lesions on follow up MR imaging [32]. Although evidence have demonstrated that DWI restriction of neural tissue is potentially reversible when reperfusion is restored quickly after ischemia[36], studies in rat models of cerebral ischemia showed that permanent resolution of DWI lesions does not necessarily indicate complete salvage of brain tissue from ischemic injury [37]. The reversibility of DWI lesion might be

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explained by the transient nature of the ischemic injury, but powerlessness of the current applied MR sequence in catching areas of damaged tissue should be considered.

Therefore, we chose to study patients undergoing CAS as they are a convenient experimental model to evaluate the natural history of ischemic lesions from the time of induction to the chronic phase. DWI restricted lesions occurring after CAS may be used as 'markers' to be followed up upon time through conventional and non conventional imaging.

Materials and Methods

Twelve patients affected by unilateral severe carotid artery stenosis, diagnosed according to standardized criteria[38,39] and candidate to carotid angioplasty with stent placement were enrolled in the study. Plaque characteristics and degree of stenosis were always confirmed by neck vessels computed-tomography.

Each patient underwent careful neurological and cardiological examination at admission. No patient had recent (<3 months) stroke or transient ischemic attack or possible or probable embolizing cardiopathy.

Intracranial vessels were also examined both with transcranial color-coded sonography and angio-MR: no patient had significant stenosis of large intracranial arteries.

Therapy with 100 mg aspirin and 75 mg clopidogrel once a day was initiated at least 5 days before CAS in all patients.

Neurological assessment with administration of NIH stroke scale and brain MR were performed the day before CAS (E0) and the day after the procedure (E1). At E1, a neck vessels duplex sonography was repeated to confirm CAS good outcome.

Clopidogrel was continued for 6 months after CAS and aspirin was administered indefinitely. Each patient received the best medical therapy to control vascular risk factors. Patients were followed-up by telephone interview every 3 months and clinically re-evaluated every 6 months: no patient

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presented symptoms suggestive of stroke during the entire follow-up. Patients underwent follow-up MR (E2) within 24 months from CAS.

Stent placement

Every CAS in the present study was performed in local anaesthesia in a dedicated operating room equipped for endovascular procedures by the same two experienced radiologists. A femoral access was used to introduce a guidewire up to the origin of the internal carotid artery. Carotid and cerebral angiogram was then performed to confirm the level and the degree of arterial narrowing. The guidewire was then manipulated across the stenosis and a balloon was passed over the device to lie across the stenosis, where it was inflated manually. Thereafter, a deployment of the stent into area of narrowing was done. At the end of the procedure an angiogram was performed to assess the result. Distal cerebral protection devices were always used.

MR imaging

MR was performed with an Achieva 1.5 T and a 8 channels head Phase-Array coil with parallel imaging capabilities (SENSE) (Philips Medical Systems, Best, The Netherlands). The following brain MR sequences were collected at E0, E1 and E2 scanning in axial plane: T1 weighted imaging, 2D FLAIR, DWI with apparent diffusion coefficient maps. At E2 axial DIR sequence was added (see **table 2** for sequence pulse parameters). MR imaging analysis was conducted by consensus of two experienced independent investigators blinded with respect to clinical patients' data.

New DWI restricted areas arising after the procedure were checked at E1. For each DWI restricted area found at E1 the evolution on the corresponding FLAIR and DIR E2-scans was assessed (see **Study Timeline**).

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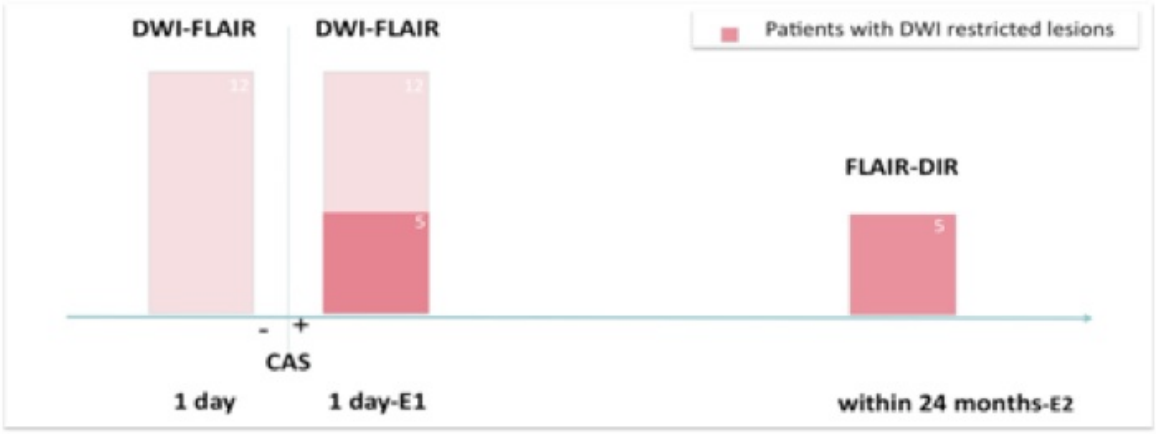

Study Timeline

Results

Baseline characteristics of the 12 patients (3 female, mean age 73±6) are fully described on **Table 1.** Eight presented a right stenosis. Mean degree of stenosis in our group was 79±9%. Six patients had a mild contralateral internal carotid stenosis (ranging from 30 to 50%). None had a contralateral severe stenosis. All patients had a complete recanalization after the procedure, confirmed by means both of post-procedural angiography and then of duplex sonography. Major complications were observed neither during the intervention nor during the follow-up period. Nine patients out of 10 were asymptomatic at E0; only one patient had a NIH stroke scale =1 at the basal evaluation as he presented a mild stroke 6 months before the procedure. One patient reported a transient (< 2 hours) left upper limb weakness early after CAS. Neurological examination and NIH stroke scale did not changed from E0, E1 and E2 for all patients.

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Table 1. Baseline characteristics of the study population

Hypertension	11 (91%)
Diabetes mellitus	4 (33%)
Smoking habit	6 (50%)
Hypercholesterolemia	10 (83%)
Coronary artery disease	4 (33%)
Previous stroke (≥ 3 months)	1 (9%)
Carotid artery stenosis side	Right 8 (67%)
Degree of stenosis, mean %	77 (9%)
Contralateral internal artery stenosis < 30% 30-50%	 4 (33%) 8 (67%)

Table 2. Pulse Sequence Parameters (Achieva 1.5 T, Philips Medical Systems, Best, The Netherlands)

	DWI	FLAIR	DIR
Field of view (FOV), mm	240	240	240
Acquisition matrix , mm	192x113	260x231	240x177
Repetition time (TR), ms	3220	8000	13109
Echo time (TE), ms	88	125	25
Inversion time (TI), ms	-	2500	3400
b-value, s/mm^2	1000	-	-
Slice Thickness, mm	5	3	3
Interslice gap, mm	1	0	0
Number of slices	23	40	40
Acquisition time, mints	01:07	03:12	04:48
Number of signals averages (NSA)	1	2	2

Neuroradiological findings

No patient presented DWI restricted regions at pre-procedural scan (E0). After CAS (E1), 5 out of 12 patients developed DWI restricted areas with respect to E0 MR scan. All of them were ipsilateral to the treated artery. A total of 23 small (<6 mm) intracortical DWI restricted lesions were found. All of them lied in cortical borderzones, but one. One lesion was symptomatic. For detailed description of DWIrls radiological features see **Figure 1**.

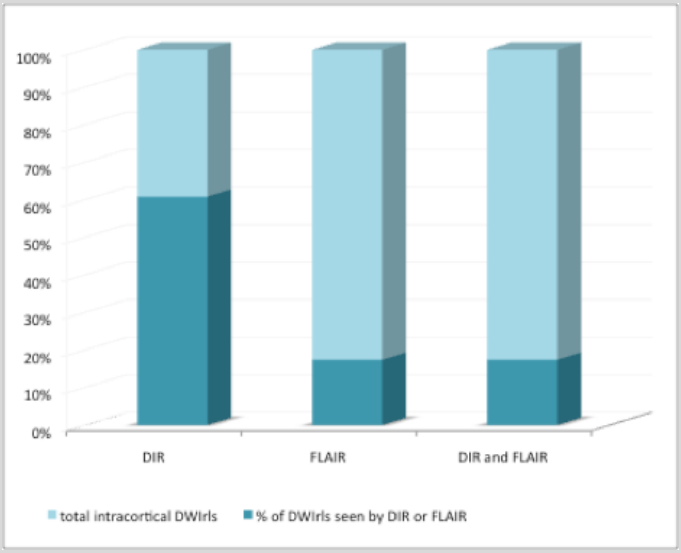

Figure 1
Three different MR evolution patterns of DWIrls have been observed at E2:
a) hyperintense areas detectable both by DIR and FLAIR
b) hyperintense areas detectable by DIR but not by FLAIR
c) no lesions detectable either by DIR or by FLAIR

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We observed three different MR evolution paradigms of DWI restricted areas:

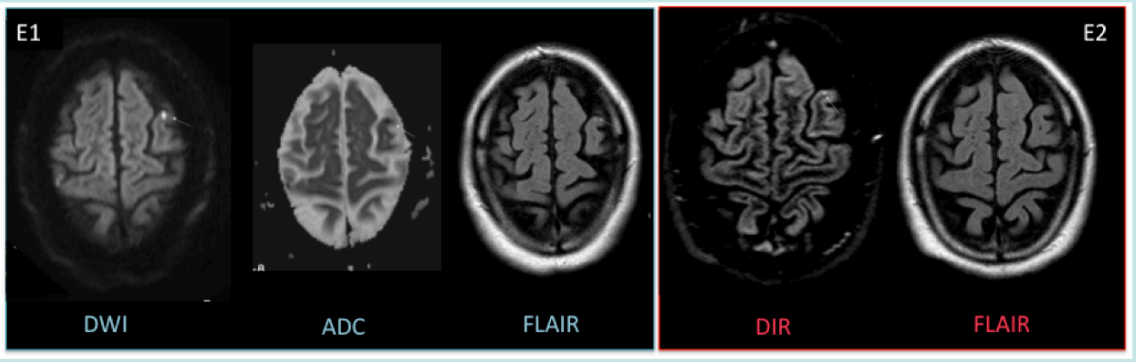
a) DWIrls (n=4) detectable by both DIR and 2DFLAIR E2-scans as intracortical hyperintense areas, but better outlined by DIR than 2D FLAIR against the surrounding grey matter;

b) DWIrls (n=14) detectable by DIR and not by 2DFLAIR E2-scans as intracortical hyperintense areas (see **Figure 2**);

c) DWIrls (n=9) detectable neither by E2 DIR nor by E2 2DFLAIR.

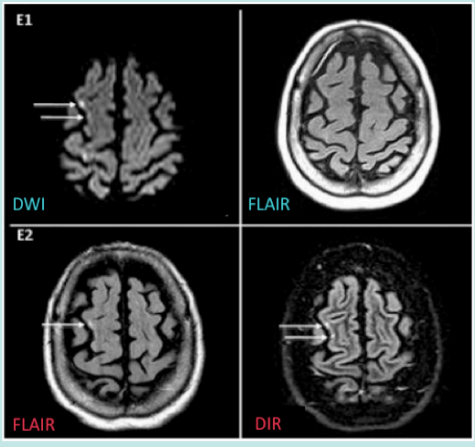
According to Pearson's chi-squared test, DIR more likely unveiled gliotic lesions originating from DWIrls compared to concurrent FLAIR (p=0,078).

Figure 2. MR evolution of intracortical ischemic lesions (DWIrls) occurring after CAS



A. At E1 DWI shows in the middle frontal gyrus an area of diffusion restriction which is hypointense on ADC map. At E2 DIR detects an hyperintense lesion corresponding to the early DWIrl. The same lesion is not visible on the concurrent FLAIR. Conversely FLAIR_E1 unveils the lesion as a slight hyperintensity. Discrepancy between early and late FLAIR might be explained by initial perilesional vasogenic oedema that dissolves over time.

B. At E1 DWI shows two DWIrls in the right lateral superior frontal gyrus. On follow up scan (E2), DIR is able to track both lesions, suggesting that early DWI restricted areas, even if small sized, turn into permanent gliotic damage. Follow up FLAIR detects only the largest lesion and misses the smallest one.



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Discussion

This is the first study showing that double-inversion recovery sequence is feasible to visualize cortical ischemic lesions occurring after carotid artery stenting long time after their onset with good spatial resolution. According to our observations, DIR seems to provide a greater sensitivity in lesions detection compared to conventional imaging as it may unveil small intracortical lesions that are barely or even not detectable by 2D FLAIR. So far DIR has been widely used to identify intracortical demyelinating lesions in Multiple Sclerosis[5]. However, in our sample the ischemic origin of such intracortical lesions detected by DIR is guaranteed by the fact that we chose to follow up and analyze only those lesions corresponding to areas with DWI restriction, which is considered an optimal surrogate marker of ischemia[40]. CAS is a procedure at high risk for cerebral microembolic events[29]. The number of patients with DWI restricted lesions found in our sample (5 out of 12) is in line with the incidence observed in previous DWI studies (from 20 to 50%)[41]. Whether or not DWIrls evolve into chronic ischemic damage is still matter of debate[42], as many studies failed in detecting chronic ischemic scars corresponding to DWI restricted areas on follow-up MR scan. A recent substudy of the ICSS showed that 50% of patients undergoing CAS and 17% of patient undergoing carotid endarterectomy had at least one new DWI lesion detected on post-treatment scans performed a median of 1 day after treatment[32]. At 1 month, only 17% of DWI lesions detected at post-treatment scans in the stenting group and 53% DWI lesions in the endarterectomy group had a corresponding hyperintense FLAIR signal. Authors propose a full recovery of tissue injury after transient local ischemia for those early DWI lesions not associated with visible MR damage at 1 month. However rats model studies [37] proved that reversibility of DWIrls does not necessarily mean normal histological outcome and that even short lasting hypoperfusion is associated with chronic microischemic tissue damage. Differences in lesional volumes might explain the higher proportion of permanent tissue damage on FLAIR corresponding to DWI findings after endarterectomy with respect to stenting

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procedure. In MS comparison between post-mortem histopathology and conventional MR imaging showed that MR visibility of cortical lesions is determined only by lesion size and not by any distinctive underlying pathology[43].

In a prospective study of DWIrls outcome occurring after CAS, Palombo et al.[44] reported a reversibility rate of 60% of acute ischemic lesions on delayed MR imaging, depending on lesion size and location. Namely cortical and smaller (<5 mm) DWIrls were not disclosed by follow-up FLAIR. In our study DWIrls were mainly located in the cortical borderzones, in agreement with previous reports showing that ischemic lesions occurring during CAS usually involve distal territories of cerebral arteries.[45]. Interventional angiography-related microembolisms have been demonstrated to mostly lodge in the cortical borderzones, as well[46].

Our results suggest that DIR may increase the detectability of both small (<6 mm) and intracortical ischemic lesions occurring after CAS which are below the detection power of 2D FLAIR currently used by clinical studies. This may allow a more complete assessment of gray matter damage in patients at high risk of distal microembolisms.

However, in our sample two DWIrls were not detectable by both E2 DIR and FLAIR, suggesting either a transient ischemic event or a poor resolution also of DIR for microscopic brain damage.

DWIrls occurring after CAS are not often related to clinical deficits, as in 22 out of 23 lesions of our report. The long-term clinical impact of DWIrls occurring early after CAS is still unclear. Several studies suggest a possible relationship between the number of DWI lesions and worsening of cognitive performance after CAS[47,48]. Probably the underestimation of the brain vascular permanent damage by currently used brain imaging in comparison to post-mortem studies[6] might explain these conflicting results.

This study has some limitations, in fact the small number of patients included and of DWIrls observed doesn't allow to provide conclusive data about superiority of DIR compared to conventional imaging. A larger sample of ischemic cortical lesions is needed to confirm these observations.

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Conclusion

In conclusion, according to our data we suggest that DIR is feasible to assess the cortical ischemic lesion load. In addition to 2D FLAIR, it can improve the intracortical micro-ischemic lesions detection for a more selective capability of imaging brain cortex and for providing a higher contrast ratio between lesions and grey matter[49], as it has been already demonstrated for intracortical MS lesions. The application of DIR sequence in the field of cerebrovascular disease could be helpful to evaluate in vivo the cortical ischemic burden that has been already proved by histopathological studies to impact on cognitive decline significantly more than white matter lesion load [50] DIR may also provide new clinical cues in order to better define the risk of patients suffering from severe carotid stenosis not only in terms of major vascular events, but also of accumulating cortical micro-damage.

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Intracortical Ischemic Lesion Burden Measured by DIR Is Related to Carotid Artery Disease Severity

Tesi di dottorato in Scienze della plasticità d'organo e della rigenerazione tissutale per il recupero funzionale, di Doriana Landi, discussa presso l'Università Campus Bio-Medico di Roma in data 23/04/2013.
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AIM 2

Background

Cortical microinfarcts (CMIs) represent a very common finding at brain autopsy. CMIs have long been considered as benign consequences of brain aging. However, increasing evidence is in favour of an association between CMIs and both vascular cognitive impairment (VCI) and Alzheimer Disease, representing a potential bridge between these two conditions[51].

Conventional imaging usually fails to detect those lesions in vivo, given the low power on grey matter damage estimate.

Epidemiological studies suggest that cerebrovascular risk factors and carotid artery disease are correlated either with brain vascular damage and cognitive impairment in vivo.

Preliminary results by our group (see above) demonstrated that Double Inversion Recovery MR at 1.5 Tesla is able to detect small cortical ischaemic lesions, offering a great opportunity to estimate the grey matter focal damage in vivo in a general clinical setting.

Thus, with this second work we intended to benefit from this convenient neuroimaging approach to investigate the impact of cerebrovascular risk factors and increasing degree of carotid artery stenosis on DIR lesion burden in vivo.

Materials and methods

62 subjects (31 Males, 31 Females) with common cerebrovascular risk factors were consecutively recruited through the Cerebrovascular Disease outpatients clinic of the Fatebenefratelli, Isola Tiberina Hospital from July 2012 to December 2012.

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Concurrently to the screening visit, all patients underwent Echocolordoppler of the neck vessels (Sequoia, Acuson Siemens, USA) and transcranial doppler (Multidop DWL, Germany) in order to exclude intracranial stenosis.

Carotid stenosis degree and plaque characteristics were estimated according to standardized criteria[39]. According to individual ultrasound (US) findings, each subject was assigned to a class of carotid disease severity: absence, mild (30-50%) and moderate-severe stenosis (51-80%).

Intima-media thickness (IMT) was measured at the far wall of the distal common carotid artery, 10 mm proximal to the bifurcation, with the mean value calculated on a 10-mm segment of the artery through a semiautomatic software[52].

Subjects with contraindications to MR scanning (claustrophobia, metal objects such as cardiac pace-maker or implantable devices), other source of microembolism included atrial fibrillation, history of major stroke, arteritis, small vessels disease (amyloid angiopathy, CADASIL), hematological disorder have been excluded.

MR imaging

Brain MR scan was performed with an Achieva 1.5 T and 8 channels head Phase-Array coil with parallel imaging capabilities (SENSE) (Philips Medical Systems, Best, The Netherlands). The MR scanning protocol included the following sequences with full brain coverage and bi-commissural line as reference: Diffusion Weighted Imaging, 2D-FLAIR sequence (TR/TE/FA/TI = 8000/125/90/2500, acquisition matrix 260x231mm, slices 40, slice thickness 3 mm, interslice gap 0 mm), Double Inversion Recovery (TR/TE/FA/TI = 13109/25/90°/3400, acquisition matrix 240x177 mm , NSA 2, slices 40, slice thickness 3 mm, interslice gap 0 mm).

MR metrics

DIR have been analyzed by two experienced investigators (DL&DL), blind to clinical and US data (presence/absence of carotid stenosis, carotid stenosis

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side). DIR lesions (DIRlns) have been scored according to the *Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI* [53].

Total intracortical lesions number and localization have been computed on DIR sequence in each subject and for each hemisphere (left and right). DIRlns have been classified by lobe and vascular territory (anterior, middle and posterior cerebral artery, external borderzones).

Only those areas for which a consensus between the two observers was reached were considered as lesions and entered the final count. Namely lesions with very low contrast compared the surrounding grey matter and not clearly intracortical were excluded. Cerebellar and deep grey matter lesions were not scored.

Moreover, as DIR can give rise to many artifacts (intracortical and meningeal vessels artifacts, bone artifacts), suspected lesions were compared with the correspondent signal on 2D Fluid Attenuated Inversion Recovery and eventually not scored if not confirmed by FLAIR.

Statistical Analysis

Data analysis has been performed by using SPSS Version 19.

As indicated in epidemiological literature for multivariate analysis the sample size has not been defined on the basis of a power analysis but on the Cases to Variable ratio. Subjects enrolled were subdivided in three groups according to carotid artery stenosis severity (0, 1, 2). DIRlns have been reported as total number and number per hemisphere and vascular territories.

An univariate analysis was conducted to explore cerebrovascular risk factors frequency distribution within groups (0, 1, 2). Then a Linear Regression Analysis was applied to evaluate the power of those factors resulting differently distributed within groups and of age in explaining DIRlns. Finally, Analysis of the Variance (ANOVA) was performed considering DIRlns as dependent variable and group as independent variable. Age entered the ANOVA as covariate. DIRlns value was normalized by logarithmic transformation.

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Results

1. Study population

Baseline demographic and lifestyle characteristics of the 62 subjects included in the study and atherosclerosis burden are described in **Table 1 and Figure 1.**

Demography and lifestyle	
Age, years, (SD)	71.4 (11.5)
Antiplatet therapy, mono	45%
Antiplatet therapy, double	10%
Hypertension	62%
Diabetes	9%
Hypercholesterolemia	48%
Smoking habit	39%
Carotid Transient Ischaemic attack	20%
Coronary artery disease	14%
Ultrasound features	
Side carotid artery stenosis, left	10%
Side carotid artery stenosis, right	13%
Bilateral carotid artery stenosis	24%
No carotid artery stenosis	53%
Degree of stenosis, %	
< 30%	53%
30-50%	26%
51-80 %	21%
IMT mean	33%
0	32%
1	32%
2	

Table 1

Group 0 (no carotid artery stenosis) included 33 subjects (53%), Group 1 (mild carotid artery stenosis) 16 subjects (26%), Group 2 (moderate-severe carotid artery stenosis) 13 subjects (21%).

Group 2 and 3 showed a higher prevalence of hypertension (0, 50%, 1, 66.7%, 2, 85 %), hypercholesterolemia (0, 36.7%, 1, 46.7%, 2, 76.9%) and antiplatelet therapy assumption (0, 36.7%, 1, 60.0%, 2, 92.3%). Given the few cases of diabetes m ellitus, coronary artery disease, Transient Ischemic attack and hypertrigliceridemia in the population under study, it was not possible to perform within group comparisons for these factors. No difference was found about smoking habits within groups.

2. Neuroradiological findings

A total number of 247 intracortical DIRlns (median 3/patient, range 0-20) were scored in 17 out of 62 patients. One hundred and nineteen DIRlns out of 247

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were located in the left hemisphere and 128 DIRIns in the right hemisphere. One hundred and twenty one lied in the MCA, 51 in the ACA, 20 in the PCA and 54 in the external border zones. The majority of lesions was localized in the conjunction between ACA/MCA (39) VS MCA/PCA (14) and ACA/PCA (1). One hundred and thirty five were found in the frontal lobe, 52 in the parietal lobe, 18 in the temporal lobe, 16 in the occipital lobe and 26 in the insula (**Table 2**).

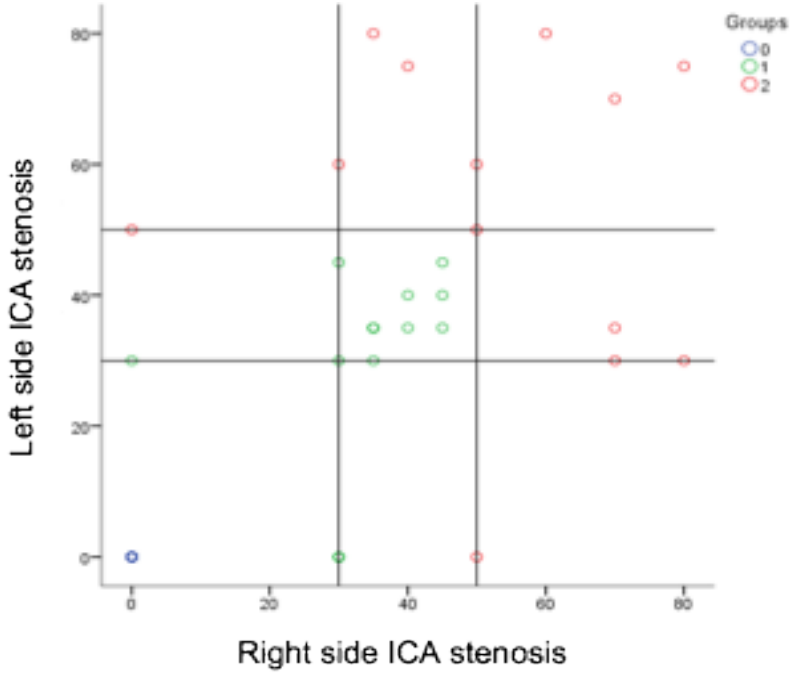

Figure 1 Schematic representation of carotid artery stenosis by side and severity

The mean number of DIRIns was higher in the group 2 and 3 (see **Figure 2**).

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	n
DIRIns	247
DIRIns left	119
DIRIns right	128
Vascular territories	
CMA	121
ACA	51
PCAP	20
Borderzones	55
ACA/MCA	39
MCA/PCA	14
PCA/ACA	1
Brain lobes	
Frontal	135
Parietal	52
Temporal	18
Occipital	16
Insula	26

Table 2 Schematic representation of radiological results

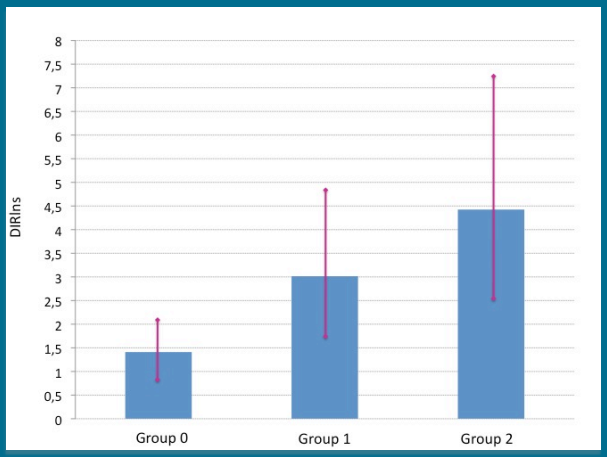

Figure 2 shows DIRIns distribution among groups.

3. DIRIns and carotid artery disease

Among cerebrovascular risks and demographic variables explored (hypertension, hypercholesterolemia, smoking, IMT and antiplatelet therapy) Linear regression analysis showed a significant association only between age and DIRIns (Pearson's=.496, $p<0.01$). After correcting for age, DIRIns load resulted significantly dependent on carotid artery stenosis severity ($F=5.56$, $p<0.01$) as reported in **Figure 3**.

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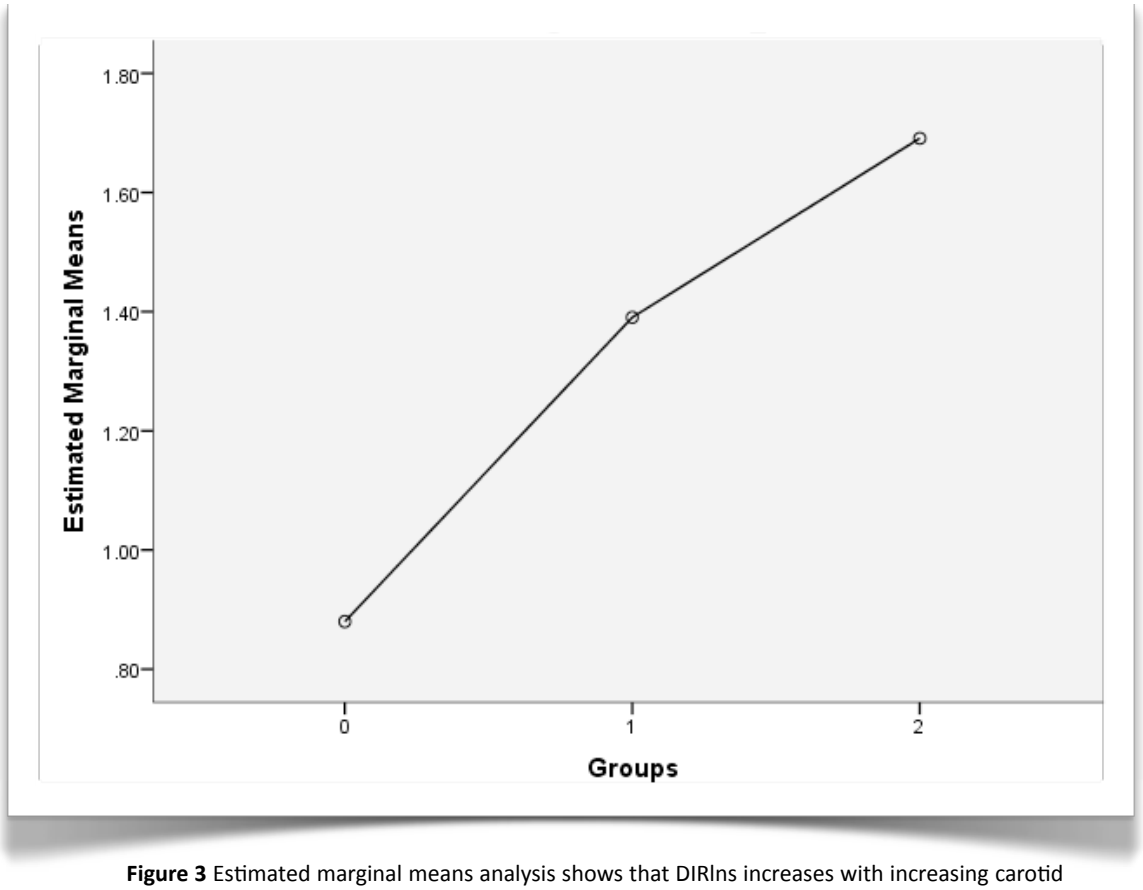

Figure 3 Estimated marginal means analysis shows that DIRIns increases with increasing carotid artery stenosis. DIRIns are reported as lognDIRIns.

Discussion

This is the first study exploring intracortical ischemic lesion load in patients with cerebrovascular risk factors by applying Double Inversion Recovery *in vivo*.

Our results show that among all cerebrovascular risk predictors the main contributor to intra-cortical lesion accrual is the severity of carotid artery stenosis after correcting for age.

Epidemiological studies have extensively shown that increasing grades of carotid stenosis are associated with greater likelihood of developing symptomatic brain ischemic events[54,55].

Moreover, previous studies in patients with symptomatic stroke have reported that the risk of developing small DWI restricted lesions in the cortical areas near the main ischemic region depends mainly on the presence of severe carotid stenosis[15].

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Microembolism originating from unstable atherosclerotic plaques seems to be the main responsible for distal multiple infarcts [56–59]

The proof that microembolization subtends distal ischemic damage comes from studies in experimental models of primates in which microbeads repeatedly injected in the internal carotid artery migrated preferentially to the cortex and especially to cortical borderzones determining acute small infarcts[60].

According to our results we argue that chronic cortical ischemic damage might share the same pathogenetic mechanisms of acute stroke in patients with carotid artery stenosis.

Although the primum movens of both symptomatic and asymptomatic ischemic lesions seems similar, the histopathological consequence of the occlusion of small and large vessels may be different.

Wang et al. (2012), in fact, demonstrated that microinfarct natural history follows a distinct course compared to larger regional infarcts such as those induced by middle cerebral artery occlusion. Within the classical ischemia model, ischemic cell death in fact is considered irreversible within 3 hours[61] and complete within 24 h after injury[62], while small lesions are characterized by long-lasting gliosis, delayed neuronal loss (28 days), and demyelination[63]. We might speculate that this very last feature may participate into the enhancement of DIR signal *in vivo*.

Moreover, when multiple penetrating vessels in the same cortical region are experimentally occluded, microinfarcts tend to coalesce leading to a larger ischemic area due to clots propagation beyond the initial core of the microinfarct. Thus, also in humans it cannot be excluded that areas of intracortical infarcts may grow from a small set of initially isolated microinfarcts[64].

In our cohort, in the subgroup of patients with severe carotid stenosis, we observed (results not reported) areas with high DIR signal inhomogeneity, surrounding clearly outstanding intracortical lesions ('dirty cortex'). Although artifacts due to technical issues or intracortical small vessels cannot be excluded, we can also argue that visible cortical lesions are 'the tip of the

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iceberg' of a wide regional cortical ischemic damage originating from widespread local microembolism.

The number of cortical infarcts that we found in our patients ranges from 0-20. A recent revision of post-mortem studies shows that the average number of microinfarcts detected by histopathology varies from 1 to 37 (size from microscopic to < 5 mm). However this number seems reliably underestimated. Westover et al. have recently estimated in post-mortem specimens that 1 or 2 microinfarcts implies a maximum-likelihood estimate of 552 or 1,104 microinfarcts throughout the brain[65].

In agreement with experimental observations these authors also comment that microinfarcts are not uniformly distributed in the brain but tend to localize preferentially in the cerebral cortex.

A very recent paper by van Veluw et al. (2013) tried to identify cortical microinfarcts in vivo by 7T MR on the FLAIR, T2, and T1 weighted scans. In the 22 elderly cognitively preserved subjects under study they found 15 cortical lesions that were likely to be microinfarcts in 6 subjects[66].

Despite their small size and number, evidence is accumulating that microinfarcts are associated with dementia even after controlling for other brain vascular damage including macroscopic infarcts and white matter hyperintensities[67,68]. In rodents the occlusion of even only one single penetrating vessel in the somatosensory cortex led to cognitive dysfunction, suggesting that every cortical penetrating vessel can be critical for normal cognitive status[64]. Thus even very small infarcts are not clinically benign.

Subclinical carotid atherosclerosis has been proved to be independently associated with lower cognitive function and dementia in cross-sectional studies[11]. A prospective study in patients from the Baltimore Longitudinal Study of Aging recently assessed the prospective risk of dementia after 14 years of follow-up in those subjects with early subclinical atherosclerosis (bilateral plaques and IMT increase)[10] confirming previous observation coming from the large cohort of the Framingham Study[9].

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Cortical microischemic damage may represent the missing link between small-vessel disease and cognitive impairment in vivo.

In agreement with pathological studies, in our cohort we found that age correlated with DIRlns. A longer exposition to carotid microembolization and cerebrovascular risk factors might explain the greater accumulation of lesions over time, although these results need to be confirmed in larger groups.

Further studies are needed to explore the correlation between cognition and grey matter focal damage in vivo in cerebrovascular disease.

Conclusions and Perspectives

With these sets of experiments we were able to prove that DIR is a feasible and sensitive tool to investigate focal microischemic grey matter damage. Moreover DIR lesion load increases with age and carotid atherosclerosis.
Future development of this work will explore:

1. the role of cerebral heamodynamics in DIRlns accrual having in mind the synergistic effect of carotid artery stenosis and vasomotor reactivity in cerebral blood supply [69]
2. the role of microischemic grey matter damage on cognition *in vivo*, as it has been already proved by pathology
3. the relationship between intracortical lesion load and other radiological markers of brain damage (white matter hyperintensities, grey matter volume)

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Appendix

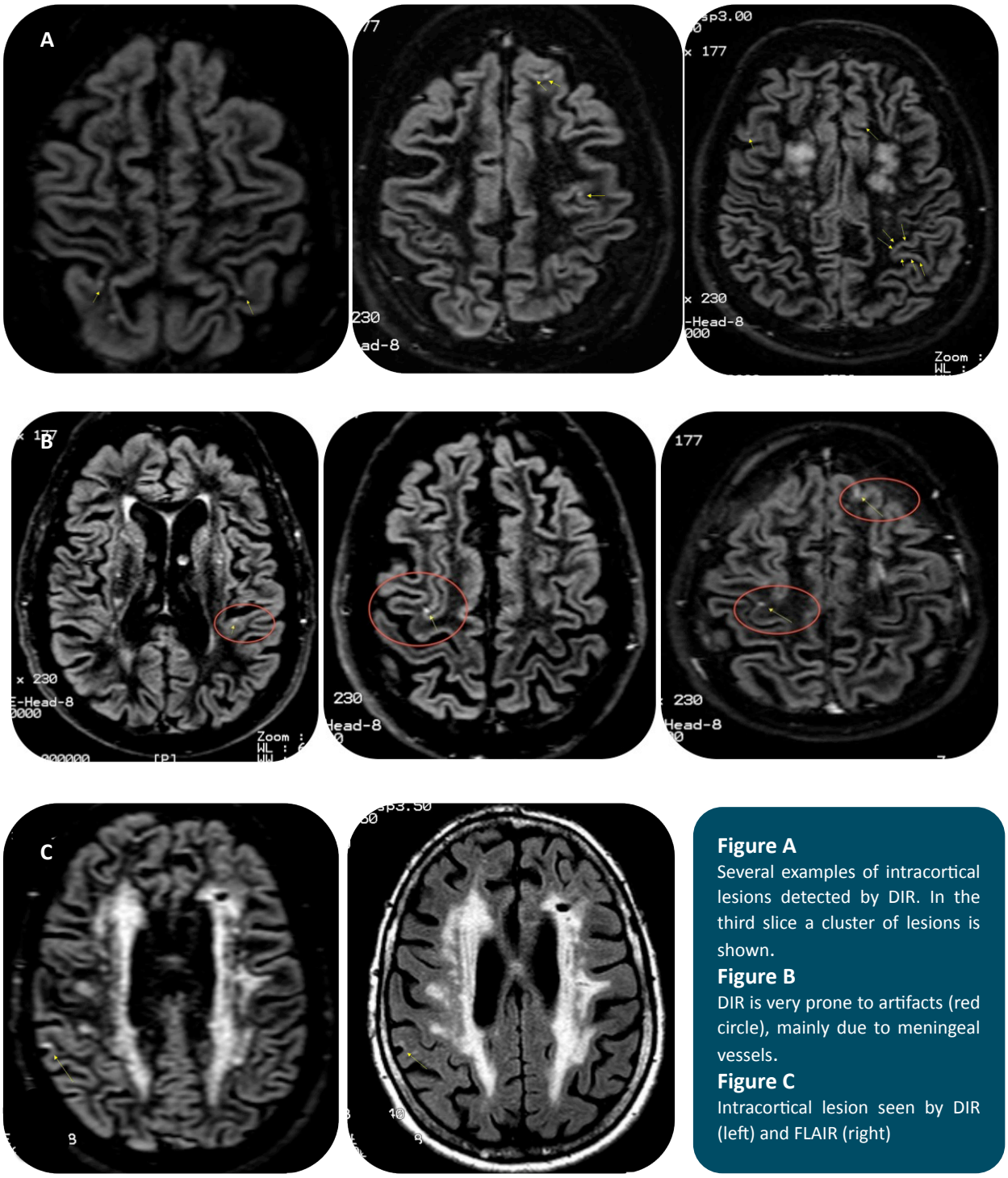

Figure A
Several examples of intracortical lesions detected by DIR. In the third slice a cluster of lesions is shown.

Figure B
DIR is very prone to artifacts (red circle), mainly due to meningeal vessels.

Figure C
Intracortical lesion seen by DIR (left) and FLAIR (right)

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