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Original article

Clinical performance of a collagen-based hydroxyapatite bone graft substitute in procedures of spinal arthrodesis

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SUMMARY: Aims. *Spinal fusion is a common procedure used for surgical treatment of spinal deformity. In recent years, many bone graft substitutes have been developed to provide good arthrodesis when the available autologous bone harvested from the patient is not enough. Besides, bioactive synthetics have undergone changes to stimulate a beneficial response within the bone, which are based on chemical reactions allowing cellular turnover and the enhancement of new bone formation. In this context, a collagen-based hydroxyapatite bone graft substitute enriched in magnesium has shown promising results in achieving fusion for the treatment of adult scoliosis. Aim of the present clinical study was to evaluate the performance and safety of a collagen-based hydroxyapatite bone graft substitute enriched in Magnesium, to stimulate bony fusion in patients undergoing posterolateral spinal fusion.*
Materials and Methods. *Twenty patients were consecutively enrolled and prospectively evaluated. Patients underwent instrumented posterolateral spinal fusion using a biomimetic hydroxyapatite composite scaffold. Radiological evaluations were performed at 12 months follow-up to evaluate fusion.*
Results. *The percentage of bony fusion recorded was of 95% at twelve months follow-up. No intra-operative or post-operative adverse events were recorded.*
Conclusions. *The present study provides clinical evidences of the fusion properties of a collagen-based HA scaffold, enriched in magnesium, for posterolateral spinal fusion. The safety profile and the osteointegrative properties makes the device a valid alternative to local autograft bone.*

KEY WORDS: *Bioinorganic ions, Bone graft substitutes, Mg-enriched hydroxyapatite, Posterolateral fusion.*

INTRODUCTION

Vertebral arthrodesis is one of the most common surgical procedures for the treatment of deformities, trauma and degenerative pathologies affecting the spinal column[17]. Thanks to the use of bone grafts and instruments (i.e. metal rods and screws), the procedure allows to create fusion between two or more adjacent vertebrae and to stabilize the vertebral column. The biological processes involved in bone regeneration require three critical elements: an osteogenic potential that is capable of directly providing cells to the newly forming bone, osteoinductive factors able to cause osteoblastic differentiation of osteoprogenitor stem cells, and the presence of an osteoconductive scaffold that facilitates neo-

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LIST OF ACRONYMS AND ABBREVIATIONS: **BMPs** = Bone Morphogenetic Proteins; **DBM** = Demineralized Bone Matrix; **HA** = HydroxyApatite; **Mg-HA** = Magnesium doped-HydroxyApatite; **TCP** = TriCalcium Phosphate.

vascularization and supports the bone ingrowth[15]. An ideal bone graft should possess all these features, together with excellent biological compatibility and biological safety[4].

To date, autologous bone, either local or harvested from the iliac crest, is still considered the "gold standard", thanks to its osteoinductive, osteoconductive and osteogenic properties. However, contraindications and drawbacks have been frequently reported, such as limited quantity and donor site pain[17]. In order to overcome these limitations, many alternatives have been developed during decades and are currently available on the market, among which growth factors (BMPs), allogeneic or heterologous bone, DBM and synthetic ceramic materials[1,10]. However, although being extensively investigated[1,2,10,17], clinical data concerning these alternative materials are still scarce in terms of quantity and quality, type of studies, evaluations performed and conclusions reached[10,17]. As such, an understanding of the precise biological mechanism of each bone substitute is necessary for achieving successful results.

Synthetic ceramic-based bone graft substitutes, such as beta-TCP and HA, have been developed as osteoconductive scaffolds able to fill defects, support bone remodelling and regeneration, thanks to their chemico-physical features and properties which are very similar (up to 70%) to the human mineral bone component[2,10].

In the last years, moreover, the incorporation of bioinorganic ions such as silicon, magnesium, strontium, zinc and copper, has been extensively investigated[20], being actively involved in ion channel processes and cellular signalling recruiting, specifically, osteoblasts and osteoclasts for regenerative processes of new bone formation[20]. The therapeutic use of bioinorganic ions, such as magnesium, doped into various materials, including hydroxyapatite, tricalcium phosphate and collagen, showed osteoblastic cellular attachment, proliferation and newly bone formation in in vivo experiments[13] and clinical applications[7]. Additionally, the incorporation of these ions in synthetic bone grafts like hydroxyapatite confers low cost, longer shelf life and lower risk as compared to other bone graft alternatives[20].

☐ AIMS

In the present clinical study, we investigated the use of a biomimetic HA scaffold, enriched in magnesium and type I collagen of equine origin. Hydroxyapatite represents the majority of the human mineral bone component (65% weight) while type I collagen is a fibrous protein representing the most important structural component of the extracellular matrix of many human tissues. The presence of type I collagen allows the scaffold chemico-physical biomimetic properties, ensuring great stability on site. The three-dimensional and flexible composite scaffold has already been used in vertebral arthrodesis procedures involving long tracts of the column spine, showing good results and a safety profile. The study was set up as a spontaneous, prospective, observational, post-marketing clinical study, with 12 months follow-up.

☐ MATERIALS AND METHODS

In this prospective observational data collection, we consecutively screened and enrolled patients who had indications for single or multi-level instrumented posterolateral fusion due to symptomatic degenerative disc disease. Primary endpoints were bone regeneration and fusion, intended as the presence of continuous trabecular bone bridge verified by diagnostic imaging (X-ray) and assessed by the Brantigan score; the safety of the medical device, through the incidence of any adverse events, complications, unexpected reactions, accidents.

The Medical Ethic Committee of the Ospedale di Stato della Repubblica di San Marino (Italy) was informed (in conformity to the 1975 *Declaration of Helsinkj*).

The following criteria for inclusion were considered: skeletally mature subjects, at least 18 years of age at the time of surgery, affected by symptomatic degenerative disc disease (i.e. disc herniation, lumbar stenosis, spondylolysis with spondylolisthesis); with indication for posterolateral fusion in the L1-S1 lumbosacral tract.

Exclusion criteria were: alcohol or drugs abuse; active or systemic local infections, drug therapy resulting in impaired bone regeneration (use of cortico-

Brantingan and Steefe classification system		
Fusion score		
GRADE 1	UNFUSED	Obvious radiographic pseudarthrosis, based on collapse of the construct, loss of disk height, vertebral slip, broken screws, cage displacement, or resorption of the bone graft
GRADE 2	PROBABLY UNFUSED	Probable radiographic pseudarthrosis, based on significant resorption of the bone graft, or a major radiolucency or visible gaps in the fusion area
GRADE 3	UNCERTAIN	Bone graft is visible in the fusion area approximately at the same density originally achieved intraoperatively. A small radiolucency or gap may be visible involving a portion of the fusion area, but at least half of the graft area showing no radiolucency between the graft bone and vertebral bone
GRADE 4	POSSIBLY FUSED	Bone bridges the entire fusion area with at least the density originally achieved intraoperatively. No radiolucency between the donor bone and vertebral bone should be present
GRADE 5	FUSED	The bone in the fusion are is radiographically denser and more mature, as compared to the intraoperative phase. No radiolucency detectable between the bone graft and the host bone

Table 1. The Brantingan and Steefe classification system.

steroids, chemotherapeutic drugs, etc.); active malignancy, metabolic or haematic disorders; pregnancy; inflammatory or auto-immune pathologies, hypercalcemia, insulin-dependent diabetic conditions, thyroid function impairment, allergy to equine collagen and calcium phosphate salts.

At pre-operatory, patients' demographic data and clinical history were recorded. X-ray and CT scan were undertaken. The number of vertebral levels fused during surgery was recorded. Patients were followed post-surgery until discharge. X-ray and CT scans were undertaken just after surgery and at 12 months follow-up. Follow-up visits were conducted at 12 months. Any intra-op or post-op adverse event was recorded.

■ **Biomaterial.** The bone graft substitute employed in this study (RegenOss, provided by Fin-Ceramica Faenza S.p.A., Faenza, Italy) is a commercially available, porous, three-dimensional composite bone graft substitute made of type I collagen fibres (of equine origin) in which nano-sized (10-20 nm) crystals of biomimetic Mg-HA are nucleated at a 40-60% ratio. The composite device is manufactured to reproduce the anatomical structure of the bone compartment in the biological processes of neo-ossification. The device is biocompatible. While the bone tissue regeneration proceeds, the device undergoes resorption.

■ **Surgical technique.** All the patients underwent decompression and spinal stabilization using instrumented fixation supports (pedicle screws/rods/cage) in one or more spinal levels between L1 and S1. All the surgical procedures were performed by the same senior surgeon by using the standard open posterior approach to the lumbar sacral spine. Patients underwent intravenous antibiotic treatment 30 minutes before surgery (cefazolin 2 g total amount). Pedicle titanium screws (Expedium system; DePuy Synthes) were used. A bleeding bone fusion bed was obtained through decortication of the posterolateral area from the transverse processes throughout the posterior aspect of the facet joints. The bone graft substitute RegenOss was located by one side of the defect, while autologous bone was placed by the opposite side of the spinal tract to be fused. The wound was sutured in three layers over two suction drainage tubes. Patients were intravenously treated with prophylactic antibiotic therapy immediately after surgery (cefazolin 2 g, total amount) and mobilized for 2 days after surgery. Follow-up visits, including the recording of clinical parameters and radiological analysis, were conducted at 12 months follow-up.

■ **Results assessment.** The degree of fusion was determined using plain radiography and evaluated through the Brantingan and Steefe classification system[3] (Table 1), modified as follows: fusion was considered to be successful with radiographic evidences of mature bony trabecular bridging into the fusion area, no signs of pseudoarthrosis, no signs of interspaces between the bone graft and the host bone. Pseudoarthrosis was defined in case of implant

mechanical collapse, reduction of the intervertebral space, vertebral body sliding, breaking of the screws, or resorption of the bone graft. Pseudoarthrosis (i.e. the lack of fusion) was considered as adverse event. The safety of the medical device was evaluated by recording adverse events or any complication occurring to the patients.

■ **Statistical analysis.** Values are presented as number, mean or percentage, as appropriate. Given the small sample size, no statistical analysis was performed.

□ RESULTS

Twenty patients satisfied the inclusion criteria and were enrolled in the study protocol between 2015 and 2016. There were eleven female (55%) and nine male (45%), mean age 56.4 years (age range: 38-71). All the patients underwent spinal surgery because of degenerative pathologies. Fifteen patients (75%) underwent one level spinal fusion (ten female and five male), whiles the remaining (25%) underwent two fusion levels (one female and four male). All the patients underwent radiographic control at 12 months follow-up.

At follow-up, fusion was assessed by X-ray and evaluated according to the criteria described by Brantigan. Nineteen out of 20 patients (95%) showed a Brantingan score equivalent to Grade 5. Only one patient showed an uncertain radiological outcome (Grade 3). No major complications related to the surgical procedure were recorded in both the treatment groups.

Figure 1 and Figure 2 report due examples of radiographic fusion reached at 12 months follow-up.

□ DISCUSSION

Currently, autologous bone graft is still considered the "gold" standard material for achieving good arthrodesis. However, the relatively limited quantity of local bone makes the need to identify other bone graft sources[20]. Harvesting autograft bone from the iliac crest is a possible solution, and a standard procedure as well, but complications associated with this technique are well known: donor site morbidity, postoperative pain, hematoma, infections and increased blood loss, which may occur in 25%-30% of patients, thus limiting its use[19]. Other alternatives are represented by allograft bone harvested from a cadaveric donor, which is often associated with potential risk of disease transmission, bacterial contamination or host-related reactions[5], or DBM. a kind of highly processed allograft derivative with at least 40% of the mineral component removed by chemical treatments, which negatively impact on the structural integrity and mechanical properties of the material[11,20].

The most common and safe alternative is therefore represented, since years, by synthetic biomaterials such as ceramics.

The use of ceramic-based BGSs in spinal applications has been widely investigated during recent years. In a level I study, Korovessis et al.[12] reported progressive fusion at 12 months follow-up in 60 patients undergoing posterolateral fusion. Yoo et al.[21] showed no statistical significant difference between HA and autologous fusion rate in two different patient groups. The fusion rate in both groups was of about 90% at 2 years follow-up. Nickoli and colleagues[18] reviewed 30 clinical studies using ceramic-based materials as bone graft extenders in the lumbar spine. In 10 studies, involving more than 450 patients, the use of ceramics plus local autograft evidenced a fusion rate of around 90%. Lee and coworkers[14] reported no difference between the patient group treated with HA (87%) and the control group treated with ICBG (89%) in terms of fusion rates. Korovessis et al[12] concluded that HA together with the use of instrumentation and autologous bone provides good performance and a solid dorsal fusion within the expected time. Mashhadinezhad et al[16] evaluated the degree of fusion after applying HA inserted into cages for interbody fusion. The authors reported no difference between fusion rates achieved with HA compared with ICGB at 12-month follow-up, showing that application of HA granules, even inserted in cages, proved to be an effective treatment also for interbody fusion applications. All of these data again confirm the safety and effectiveness of HA-based bone grafts in different spinal applications. In this context, the HA-based device RegenOss, enriched in magnesium and type I collagen, has shown fusion and regenerative properties, as expected. Subjects implanted with RegenOss confirm an improvement of bony fusion at 12 months follow-up and a safety profile, as previously reported by others. Clinical data on the performance of RegenOss in spinal arthrodesis are well known since years. In October 2013, results from a prospective, observational study ("Lumbar posterolateral fusion using a biomimetic and bioinspired bone graft: a prospective

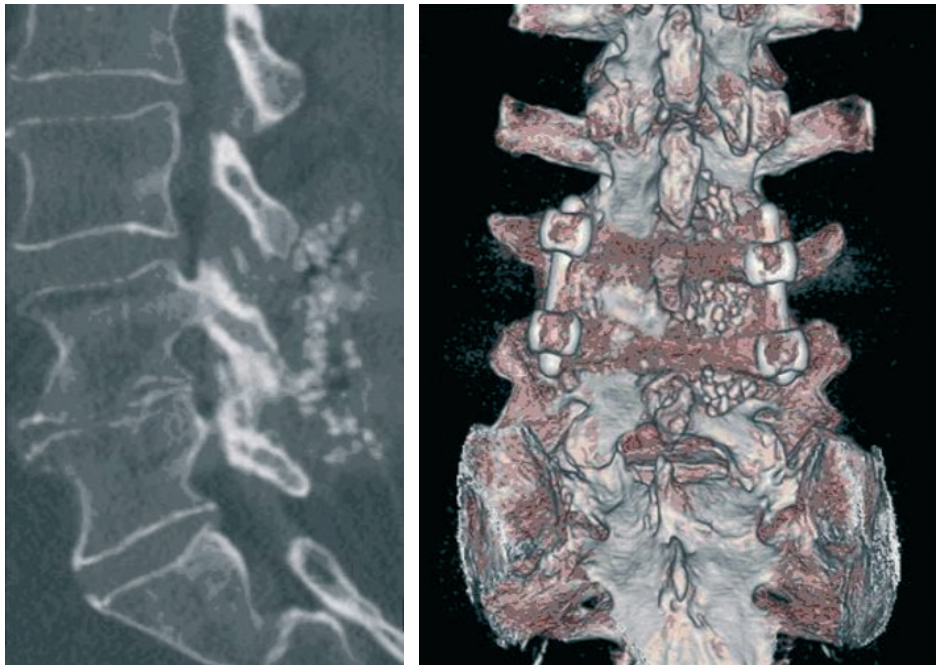

Figure 1. Male, 52 years old, 1 lumbar level treated between L3 and L4. Radiographic and CT scan images showing complete fusion (grade 5 Brantingan scale) at 12 months follow-up.

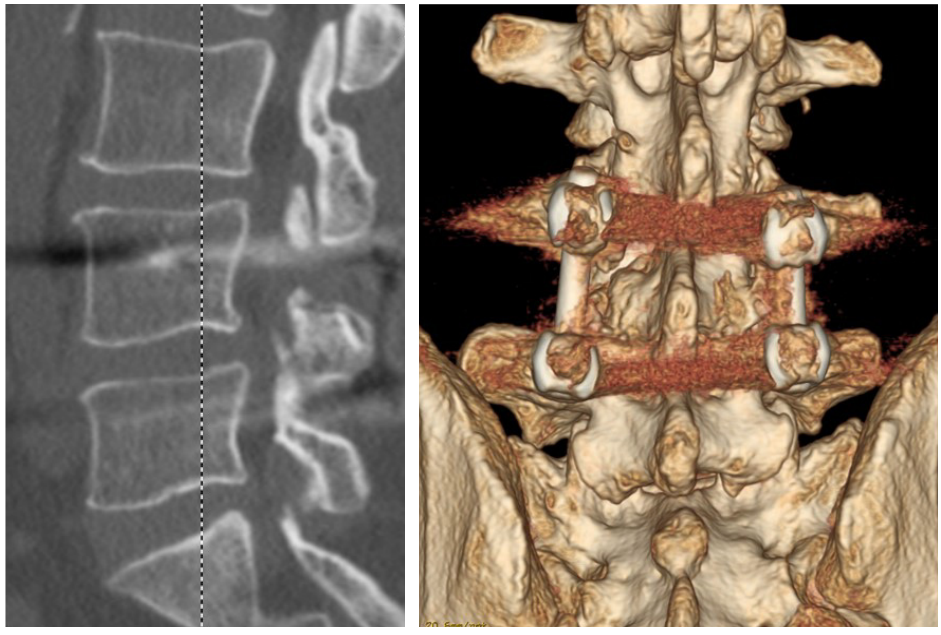

Figure 2. Female, 71 years old, 1 lumbar level treated between L2 and L3. Radiographic and CT scan images showing complete fusion (grade 5 Brantingan scale) at 12 months follow-up.

observational, non-randomized, single arm clinical study") were presented at the "Materials in Medicine Conference" held in Faenza (Italy). The study showed the regenerative properties of the bone graft substitute RegenOss already at six months follow-up, with complete new bone formation and bony fusion was observed in 80% of the cases. Recently, Giorgi and colleagues[7] published the results of a clinical study on the use of RegenOss for long tracts of posterolateral fusion (i.e. scoliosis), showing radiographic evidences of bony fusion in the majority of patients (more than 70%) and reduction of pain at 12 months follow-up, which was improved (95% fusion) at subsequent follow-up periods (36-months). The device showed no complications or side effects even when employed for long tracts spinal tracts. Additionally, in vitro experiments and preclinical tests[6,9] well-documented the ability of the scaffold RegenOss

to support proliferation and differentiation of mesenchymal stem cell, providing a biochemical environment able to promote bone regeneration. A case report by Grigolo and colleagues[8] documented the regenerative properties of the device, together with fast degradation and quick resorption. In this case specifically, the rupture of a metallic bar brought to spinal revision surgery at 14 months follow-up, allowing to document the degree of bone fusion achieved by RegenOss. Histological and immunohistochemical analyses performed on a biopsy sample showed the complete resorption of the device, together with good quality of new bone formation.

☐ CONCLUSIONS

This study has shown that RegenOss, a biomimetic three-dimensional scaffold made of HA enriched in magnesium, provides arthrodesis in posterolateral fusion. The use of the 3D scaffold provides new bone formation, representing a valid solution for the achievement of spinal fusion.

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Review

Epilepsy in Neuro-Oncology: a review with practical management approach

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SUMMARY: *Seizures in oncologic patients represent an important clinical and social complication. Their management needs considering several aspects, among which: (1) seizures recurrence and pharmacoresistance; (2) increased sensitivity to the adverse effects of antiepileptic drugs, (3) changes in clinical response in relation to the progression of the disease, (4) adverse interactions between antiepileptic drugs and chemotherapeutic agents. We can delineate three clinical scenarios in which seizures occur: (1) seizure or epilepsy in patients with a structural "active" neoplastic brain lesion(s) (primary or metastatic), strictly and commonly identified as "brain-tumour associated epilepsy"; (2) seizure or epilepsy in patients without an "active" neoplastic brain lesion, but expression of a structural lesion: peri/post-operative period for any other central nervous system oncologic surgery (e.g. oedema or haemorrhage in pituitary adenoma etc); vascular, paraneoplastic, treatment and infectious complications in systemic cancers; history of a previous central nervous system tumour but not expression of active neoplastic central nervous system disease (e.g. follow-up of meningioma etc); (3) Seizure or epilepsy in other various conditions of any cancer, toxicity of its treatments (mainly acute central nervous system radiotherapy); metabolic and treatment complications in systemic cancers etc. This paper addresses to physicians, in order to choose adequate management and pharmacologic approach in treatment of seizures associated with tumours. The present review refers only to adult patients.*

KEY WORDS: *Anticonvulsants, Brain tumours, Epilepsy, Systemic neoplasms, Treatment guidelines.*

INTRODUCTION

The historical paper of Wroe et al. (1986)[54] describes the differences between neurological and neurosurgical approaches in the management of malignant brain tumour, drawing the conclusion that the neurosurgeon's "interventionist approach was more common, although it did not significantly affect favourably long-term survival". Anyway, surgery in brain tumours is crucial for gaining tumour tissue for histological analysis and relieving symptoms due to neoplastic mass effect and this is much truer as far as the resection of the tumour mass is as extensive as possible.

According to Grisold et al.[16], modern neuro-oncology is a growing new sub-speciality with a strong interdisciplinary character; ranging from internal medicine to radiology, pharmacology and palliative care medicine. Neuro-oncology deals with central (brain and spinal) and peripheral nervous system primary and metastatic tumours and non-metastatic effects of systemic cancers and its treatments. Topics of interest are also drug interferences, neurotoxicity due to cancer treatments, supportive management and neuro-protection, in respect of issues of quality of life. In this context, seizures represent one of the most common clinical features of brain tumour and their

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LIST OF ACRONYMS AND ABBREVIATIONS: **5-FU**= 5-FluoroUracil; **AAN** = American Academy of Neurology; **AANS** = American Association of Neurological Surgeons; **AED** = AntiEpilectic Drugs; **AINO** = Associazione Italiana di Neuro-Oncologia; AP-1 = Alkaline phosphatase 1; **ATLS** = Acute Tumour Lysis Syndrome; **BBB** = Blood-Brain Barrier; **BCNU** = 1,3-Bis(2-Chloroethyl)-1-NitrosoUrea; **BDZ** = BenzoDiaZepine; BID= Bis In Die (Latin: Twice A Day); **BMT** = Bone Marrow Transplant; **CBZ** = CarBamaZepine; CCNU = 1-(2-Chloroethyl)-3-Cyclohexyl-1-NitrosoUrea; **CD4** = cluster of differentiation 4; **CDDP** = Cis-Dichloro-Diamine-Platinum (cisplatinum); **C-Myc** = mRNA Cap Methylation; **CNS** = Central Nervous System; **CT** = ChemoTherapy; **CTD** = ChemoTherapy Drug; **CX** = ConneXins; **DNA** = De-oxyriboNucleic Acid; **DNET** = Dysembryoplastic NeuroEpithelial Tumours; **EEG** = Electroencephalogram; **EIAED** = Enzyme Inducing AntiEpileptic Drugs; **FB**M = FelBaMate; **GABA** = Gamma-AminoButyric Acid; **GBM** = GlioBlastoma Multiforme; **HAT** = Hystone AcetylTransferase; **HDAC** = Hystone De-ACetylase; **HGG** = High Grade Glioma; **HSCT** = Human Stem-Cell Transplant; **IDH** = Isocitrate De-Hydrogenase; **INF** = Interferons; **LCS** = LaCoSamide; **LEAT** = Long-Term Epilepsy-Associated Tumour; **LEV** = LEVetiracetam; **LGG** = Low Grade Glioma; **LGI1** = Leucine-rich Glioma Inactivated 1; **LMT** = LaMoTrigine; **MAB** = Monoclonal AntiBody; **MGMT** = O-6-MethylGuanine-DNA MethylTransferase; **MRP** = Multidrug Resistance Protein; **MTX** = MethoTreXate; **NCSE** = Non-Convulsive Status Epilepticus; **NEIAED**= Non-Enzyme Inducing AntiEpileptic Drugs; **NFKb** = Nuclear Factor Kappa-light-chain-enhancer of activated B cells; **NMDA-AMPA** = N-Methyl-D-Aspartate - α-Amino-3-hydroxy-5-Methyl-4-isoxazolepropionic acid; **OXC** = OXCarbamazepine; **P-21** = P-21 activated kinase; **PB** = PhenoBarbital; **PCNLS** = Primary Central Nervous System Lymphoma; **PCV scheme** = Procarbazine, CCNU and Vincristine scheme; **PHB** = PHenoBarbital; **PHT** = PHenyToin; **PML** = Progressive Multifocal Leukoencephalopathy; **PNS** = Periferic Nervous System; **RANO** = Response Assessment in Neuro-Oncology; **RPLS** = Reversible Posterior Leukoencephalopathy Syndrome; **RT** = RadioTherapy; **SE** = Status Epilepticus; **SIADH** = Syndrome of Inappropriate Antidiuretic Hormone secretion; **TAE** = Tumour Associated Epilepsy; **TMZ** = TeMoZolamide; **TPM** = ToPiraMate; **VPA** = Valproic Acid; **WHO** = World Health Organization.

treatment usually follows general rules with some important warnings.

Neurologists should be the coordinators in the diagnosis and treatment process, because they can synthesize and transfer medical evidence inside the multidisciplinary team.

Epilepsy incidence varies according to tumour location and histological type. In 30-50% of patients, a seizure is the first clinical manifestation of brain tumour and up to 30% will later develop seizures. Occurrence of status epilepticus has been reported to be rare, but its course is more severe, although shorter than the one observed in non-tumoural epileptic patients[1].

Low-grade tumours are more epileptogenic than high grade tumours, with DNET displaying a 100% probability to present with seizures, immediately followed by gangliogliomas and LGG whereas GBM onset with seizure accounts for 68%[10,11].

Solid brain metastases seem to cause seizures less frequently than primary brain tumours. New-onset seizures in patients with known brain metastatic lesions may indicate a haemorrhage in the lesion or tumour progression with oedema[51].

Management of seizures may affect directly and deeply the long-term survival in oncologic patients; many papers have shown significant differences in the use of AEDs as well as the importance to consider concomitant treatment in choosing them[35,39].

As shown in Table 1, seizures or status epilepticus are observed as onset symptom or during the course of disease in various oncological conditions[34].

In this setting, it is useful to maintain the distinction between acute symptomatic seizures, unprovoked late seizures and epilepsy, as suggested more than twenty years ago by the Commission of Epidemiology and Prognosis of the International League Against Epilepsy[20].

□ EPILEPTOGENIC MECHANISMS AND EPIGENETIC PHARMACOLOGIC FEATURES

According to You et al.[55], there seem to be two main explanations about the pathogenesis of tumour-related epilepsy: the former relies upon a biochemical theory (excretion of molecules from the tumour, changes in peri-tumoural microenvironment, changes within the tumour cells), and the latter tends to a mechanical explanation (compression of normal tissue surrounding the tumour with subsequent ischemia and hypoxia, which might increase epileptogenesis). Both processes could potentially cause secondary changes, such as changes in neurotransmitters and their receptors, metabolic changes, and inflammatory responses, eventually leading to epileptic seizures.

Many possible mechanisms to explain epileptogenesis in brain-tumour have been singled out, among which: damage of subcortical network (HGG) or partial deafferentation of cortical regions (LGG), inflammatory changes, blood-brain barrier disruption, morphologic changes (inefficient neuronal migration, changes in synaptic vescicles), gap-junctions alterations through aberrant connexins hyperexpression, ionic changes, molecular genetic changes (low or absent

Causes	Incidence	Remarks
Tumour-Related		
- High-grade glioma	~ 30-40%	Further 30% develop in the follow-up
- Low-grade glioma	> 80%	The most common presenting feature
- Meningioma	~ 20-40%	
- Lymphoma (PCNSL)	~ 10-20%	
- Brain mtastases	~ 20-40%	Particularly frequent in hemorrhagic mets (melanoma, etc)
- Meningeal carcinomatosis	~ 10-15%	
Treatment-Related		
- Chemotherapy	< 1% intravenous systemic CT ~ 4% > if INF or 5-FU > 20% intra-arterial or HSCT/BMT	More frequent when the drugs are given intrathecally/arterially or when BBB is disrupted
- Supportive treatments	Rare, but possible and to be kept in mind	Overdose of the same AEDs, certain antibiotics (quinolones, β-lactames, penicillins) triciclic antidepressants, neuroleptics; ciclosporin A; ondansetron, RPLS
- Acute irradiation	Not determined	Difficult to assess because of several confounding factors
- Late-delayed radionecrosis	~ 20-30%	
Myscellany		
- Metabolic causes	Variable (to severity)	Electrolyte abnormalities, hypoglycemia, SIADH, ATLS, etc
- Vascular (acute or sequelae)	> 10%	
- Infectious	> 20%	Incidence increasing in cancer pts
- Limbic encephalitis	> 60%	Paraneoplastic or viral (in immuno-compromised hosts) and difficult to diagnose if NCSE

Table 1. Main causes of epileptic seizures in cancer patients (not only intracranial structural lesions).

expression of tumour-suppressor gene LGI1), genomic and chromosomal instability, peri-tumoural and tumoural microenvironment imbalance (decreased levels of N-acetylaspartate), changes in amino-acids and neurotransmitter receptors.

It appears then clear that tumors are evolving processes, in which, from an epileptic point of view, different situations can co-exist and contribute to change the seizure frequency as well as condition a different response to the same antiepileptic therapies. The choice of AEDs in neuro-oncology relies on pharmacokinetic characteristics (e.g. enzymatic inducers versus non-enzymatic inducers). Recent studies suggest that, in addition to antiepileptic properties, AEDs have a role as epigenetic modifiers.

Two of the most common forms of epigenetic modification are the acetylation of histones and DNA methylation: the balance between DNA acetylation and deacetylation status is of utmost importance for cell survival and growth. This biochemical process is partially regulated by the activity of hystonedeacetyltransferase and acetyltransferase enzyme. HDACs can modify chromatin structure by removing acetyl groups from histones, resulting in DNA condensation and transcriptional repression.

In tumour cells HDAC activity increases, thus repressing the transcription of genes encoding for antitumour factors, differentiation and cell cycle progression, that is to say, cell growth and proliferation increase, apoptosis reduces.

Glioblastomas meet extensive alterations in methylation of DNA, in addition to alteration of histones acetylation. MGMT is an enzyme that deals with DNA repair: in GBM it undergoes epigenetic modification. The silencing of MGMT via hypermethylation of the promoter represents a positive prognostic factor, be-

cause it promotes the effect of chemotherapeutic alkylating agents by preventing a repair mechanism.
The role of antiepileptic drugs in modulating the acetylation histones state and DNA methylation has also been studied in vitro and in tumour animal cells[7]. Most studies have focused on valproic acid, only a small number were carried out on carbamazepine and levetiracetam.
VPA inhibits HDAC activity in multiple cancer cell lines and animal tumour models. Histone hyperacetilation resulting from VPA exposure appears to be reversible.
VPA shows effects on transcriptions factor such as AP-1 and NFKb, p 21, Cd4, C-Myc as well as on pathways of cell growth and proliferation. VPA has also demonstrated both direct and indirect antiangiogenetic effects inhibiting endothelial cell proliferation in culture and tube formation, in an angiogenesis assay, as well as vascular endothelial growth factor secretion in cultured glioma cells.
In summary, VPA can interfere with the pathways used by tumour cells to escape normal growth restrictions, which would effectively slow the progression of malignancy.
Retrospective studies have shown that adjunct treatment of glioblastoma patients with VPA is associated with an improved survival, in particular in those patients receiving TMZ and radiotherapy[50].
These data have not been confirmed by recent prospective clinical studies, in which valproate use was not associated with improvement of survival[17]. The survival of patients who received VPA did not differ significantly from those with seizure who did not receive VPA. In a multivariate analysis, the association between VPA treatment and survival remained non-significant.
In summary, results of the effective role of VPA on survival in glioblastoma patients are still an open debate. Clinical trials are ongoing on the role of VPA as a HDAC inhibitor.
The epigenetic effect of CBZ and LEV is not yet established, but recent suggestions regarding additional mechanisms of action hold some promises.
For CBZ an inhibitory mechanism on HDAC has been hypothesized, whereas for LEV a modulation of MGMT expression has been speculated. Mechanisms of new AEDs such as lacosamide and perampanel deserve further clinical studies.

☐ ROLE OF HISTOLOGY AND LOCATION OF BRAIN TUMOUR IN SEIZURES

Seizure incidence in brain-tumour patients varies according to tumour location and histology.
Any type of brain tumour can cause epilepsy. The less malignant ones are most involved in the highest rate of seizure recurrence and pharmacoresistance. Smaller tumours and the slow-growing ones are associated with higher rates of seizures than large and rapidly growing neoplasms[10].
The concept of LEAT describes a group of patients affected by low-grade brain tumour and focal chronic epilepsy[11].
Some oncologic subtypes (namely glioneural tumours) are often associated with alterations of cortical development, such as focal cortical dysplasias. In this setting, “dual pathology” (the combination of foreign-tissue lesions, cortical dysgenesias, gliosis or hippocampal sclerosis) represents another strong stimulus contributing to epileptogenesis, particularly in the temporal lobe[11].
The location in cortical areas, obviously, more easily provokes seizures.
HGG causing seizures are mainly located in the temporal lobe, followed by the frontal lobe[23]. LGG are mainly found in the insular, fronto-insular, temporo-insular regions and paralimbic structures[27].
LEAT mainly arises in the temporo-mesial structures (namely limbic lobe) in the site of allo-isocortical transition, where more frequently a neuronal differentiation can be seen.
The incidence of TAE relates to the topographic distribution of brain tumour: previous works suggest that frontal and temporal regions are particularly at risk of seizures or hypothesize that the left hemisphere is more prone to an epileptogenic onset of the disease[19]. Our data taken from a previous study[32,35] do not support this topographic site/side distinction in GBM-patients: in an Epilepsy Onset group there are a right hemisphere-sided prevalence and a slight prevalence of frontal, temporal or carrefour lesions, which however do not reach a statistical significance, but they are worth further studies.
Moreover, the mechanisms of epileptogenesis vary according to the different tumours, because some of them are of intra-axial origin (astrocytoma), whereas others are of extra-axial origin (meningioma).
Epilepsy occurs in > 80% of patients with low-grade glioma and 40-60% of patients with glioblastoma[4,48].

Seizures are less common in patients with brain metastases and incidence varies according to primary tumour pathology. In a retrospective study including 470 patients with brain metastases, 24% of patients had experienced tumour related seizures[10]. While seizures occurred in only 16% of individuals with breast cancer and 21% of those with gastrointestinal metastases, seizure incidence was 29% in lung cancer patients and 67% in those with melanoma, maybe due to the more frequent intracranial haemorrhage in this tumour type.
Genetically subtypes of the same hystologic brain neoplasm show different predisposition to develop epilepsy: newly diagnosed GBM but arising as an evolution of a previous LGG (GBM IDH-mutant), are more epileptogenic lesions than wild-type GBM.
All the previous considerations suggest that the aetiology of TAE is multifactorial, involving host and tumour factors. Therefore, even the response to the various AEDs might be different.

□ CLINICAL AND EEG FEATURES OF SEIZURES

In brain tumours, seizures are always focal, with or without alteration of consciousness and/or secondary generalization. Clinical presentation differs according to the lobar involvement and lesion localization (just like non-tumoural epilepsy) but sometimes the correct characterization of seizures might be more difficult due to adjunctive concurrent symptoms secondary to mass growth and swelling, particularly in HGG. In LEAT focal epilepsy is the most common symptom, often the only one, and pharmacoresistance is quite common. Neurological deficits are uncommon in LEAT group, due to the slow growing, whereas in HGG neurological impairment is more common early or during the course. Secondary generalization can always occur, expecially for LEAT located in extra-temporal sites.
EEG characteristics are not specific. Interictal scalp EEG may disclose epileptiform activity (spikes, sharp waves) generally lateralized to tumour site, with or without focal slow activity of lesional origin, this being particularly true for wide mass or after surgery.
Status epilepticus is a well-known condition that can be observed both in epileptic patients and in general population as presenting symptom of epilepsy.
Convulsive SE is defined as tonic-clonic seizures lasting more than 30', even if recent papers have underlined the importance of starting the treatment as soon as possible, within 5' from onset, seen that a convulsive seizure usually lasts no more than 1-2'[13].
Non-Convulsive SE might occur as well, it requires a careful history collection and EEG registering. SE in general population constitutes a neurological emergency, and has significant associated morbidity and mortality.
Globally, patients with TAE are less likely to develop SE than patients with epilepsy in general population[14]. In most cases, TAE presents earlier in the course of the disease, while SE in TAE patients occurs far later in disease course, sometimes to herald tumour progression, as to mean that there is a progressive impairment of the mechanisms which can terminate seizures. In glioma patients also status epilepticus (defined as a continuous seizure for 30 minutes or more) has more severe course and it is associated (as in all brain tumours) with higher mortality and possibly longer duration than in status epilepticus due to other causes[1]. Even when underlying tumour is stable, status epilepticus in glioma and other tumour patients has longer seizure duration, more and longer postictal neurologic deficits and higher rate of long-term neurological deficits[41].
The treatment replies those used in general population, with a care for drugs that can have inducing or non-inducing enzyme properties, especially in patients who follow chemotherapeutic cycles.

□ PATHOGENESIS AND PHARMACORESISTANCE OF TAE

Various causes of drug resistance in TAE have been identified:
- pathophysiology of brain tumour-related seizures
- progressive course of the disease;
- neurosurgical complications (such as meningitis or brain abscess);
- adverse effects of oncological treatment (radio-necrosis, posterior leukoencephalopathy);
- consequences of pharmacokinetic drug interactions
- various and often higher rates of adverse events by AEDs in this population;
- over-expression of multidrug transporter proteins in brain tumour, that cause reduced brain penetration of AEDs ("transporter hypothesis");
- alterations in drug targets that AEDs normally bind in tumour and peritumoural tissue ("target hypothesis");

Drug resistence mechanisms	
Target hypothesis	**Transport hypothesis** (= low level at site of action)
- mismatch AEDs mechanism of action and TAE pathogenesis - tumor relapse/progression	- serum (because of interactions) - MRP1

Table 2. Summary of drug resistance mechanisms in tumor associated epilepsy.

- altered characteristics of blood-brain barrier in brain tumours.

Despite the common view that voltage-gated ion channels controlling cell excitability and synaptic processes responsible for communication among neurons are involved, the specific events leading to TAE are unknown and comprise many changes above mentioned. We can find several reasons for the clinical inefficacy of AED treatment. First, most AEDs act on excitatory mechanisms by blocking and deactivating Na+ channels and/or Ca^{2+} channels, or they enhance inhibitory mechanisms through an increase of GABA-ergic activity. These two important modes of action of AEDs, however, cover only a few of the pathophysiologic mechanisms of TAE[40]. Second, low levels of AEDs have been reported in 60-70% of patients. This is not related to the pathophysiologic mechanisms of TAE, but it is a consequence of the fact that therapeutic AED levels in patients with brain tumours are difficult to maintain because of frequent pharmaco-dynamic and kinetic interactions with concomitant medications, and from changes in plasma protein (especially albumin) levels. Additionally, the multidrug resistance protein-1 may play a role. Recent hypotheses propose that transport of AEDs by drug efflux transporters MRP such as P-glycoprotein to the blood-brain barrier may play a significant role in pharmacoresistance in epilepsy by extruding AEDs from their intended site of action. Over-expression of proteins that belong to the multidrug-resistance pathway can impact at site of action levels of CBZ/OXC, PHT, PHB, LMT, FBM; or it can exert no effect on LEV; no information is available for TPM[26]. Finally, reappearance of seizures during AED treatment may reflect tumour progression-recurrence or a provoked seizure in a particular phase of the disease.

Table 2 synthesizes mechanisms of AEDs resistance.

At the macroscopic level, slow-growing tumours produce an epileptogenic focus by partial deafferentation of cortical regions, thus causing a denervation hypersensitivity. Recent studies[3] using magneto-encephalography to investigate the functional connectivity between brain regions have suggested that low-grade gliomas, through infiltration of white matter and not only infiltration of the cortex, could modify the natural balance and synchronization of normal networks and cause random networks that might have a lower threshold for seizures generating secondary epileptogenesis[30]. Differently from low-grade gliomas, high-grade tumours, such as GBMs or metastases, induce seizures via abrupt tissue damage due to necrosis, bleeding with subsequent hemosiderin deposition and oedema.

The putative mechanism of epilepsy in extrinsic tumours[24], i.e. meningiomas, or seizures/status epilepticus in the immediate post-operative period for a pituitary adenoma or craniopharyngioma, apart from other complications, is likely related to peritumoural oedema, possibly explaining the high frequency of preoperative seizures in supratentorials and the possible regulating role of H2O flux and uptake exerted by Aquaporin-4[9].

□ EPILEPSY IN GENERAL ONCOLOGY

As specified in Table 1 in routine clinical practice, seizures, *with* a radiographic documentation of the epileptogenic lesion, are encountered as an acute manifestation: vascular (such as ischemic or hemorrhagic stroke, sinus thrombosis, thrombotic thrombocytopenic purpuraor sequelae), paraneoplastic (limbic encephalitis) infectious (meningo-encephalitis, abscess, PML, etc.) and treatment-related (RPLS) complications in systemic cancers[15].

In routine clinical practice, seizures, *without* radiographic abnormalities, are encountered also as a manifestation of treatments (CT, MAB) and metabolic complications in systemic cancers:

- electrolyte abnormalities, hypoglycemia, SIADH, lactic acidosis, hyperammonaemia,

Epilepsy in oncology	
Mechanism of epileptogenesis	- Direct effects on neuronal excitability: altered excitatory NMDA-AMPA or inhibitory GABA pathways - Neurotransmitters : adenosine, glutamate, etc. - Indirect effects via electrolyte disturbances: hypomagnesemia, hyponatremia, hypocalcemia, etc. - Vasogenic oedema: disruption of the BBB - Vascular mechanisms: endothelial damage, mineral microangiopathy, nitric oxide reduction, hyperomocisteine, etc. - Structural lesions: subcortical leukoencephalopathy ("U" fibers), reversible-posterior-leukoencephalopathy, iemporo-mesial lobe atrophy, etc.
Agents of late unprovoked or provoked seizures	- Cyclosporin A, CDDP, MTX - MTX, 5-FU - CDDP, pamidronate - INF- α, 5-FU - Cyclosporin A, tamox, MTX - Cyclosporin A, 5-FU, MTX, in RT cranio-spinal or naso-pharyngeal

Table 3. Main mechanisms of epileptogenesis and agents of late unprovoked or provoked seizures in oncology.

- drug toxicity for instance, following accidental overdosage, or in presence of renal or hepatic disorders (when routine dosages of the agents can lead to toxicity),
- a high dose CT schedule or the administration as part of myeloablative treatment in preparation for human stem-cell or bone marrow transplant[33,36].

Main mechanisms of epileptogenesis and agents of late unprovoked or provoked seizures in these patients are described in Table 3.

□ SUGGESTIONS ON MANAGEMENT

In clinical practice, the neurologist should examine the following items:

1. When should we start antiepileptic drug treatment?
2. Should the approach be different in considering the different tumour histotypes?
3. Which factors should be considered in AED selection?
4. Could we stop the antiepileptic therapy in seizure-free patients?

In patients who manifest seizure as clinical onset of glioblastoma have better prognosis than patients without epilepsy at presentation. Berendsen et al.[4] speculates that GBM patients with epilepsy differ in several oncogenic pathways, again supporting the idea that there is a specific tumour related pathophysiology.

All established anti neoplastic treatment modalities for gliomas are associated with improvement of epilepsy:

- resective glioma surgery decreases frequency of seizure and increases a chance of seizure freedom[28]. Even simple "lesionectomy" can increase chances of seizure freedom, but pre or intraoperative localization and resection of epileptogenic brain region obviously increase this chance. Early surgical intervention showed a strong tendency to predict better seizure outcome;
- in a randomized trial radiotherapy for low grade glioma resulted in decrease of seizure[49]. Conventional radiotherapy in patients with low grade glioma related epilepsy contributes to reduce frequency and severity of seizure by over 75%. In unresectable low grade glioma, stereotactic interstitial radiation (or gamma knife) improves seizure control. However, occasionally seizure frequency increases after surgery and radiotherapy secondary to complication as edema bleeding or radiation necrosis. Onset of epilepsy is the most common complication of brain metastasis treated with stereotactic radio-surgery[53];
- chemotherapy reduces seizure in 50-65% of patients and 20-40% become seizure free[35]. Tumour mass reduction is the postulated mechanism for this clinical improvement. Direct anticonvulsant power of chemotherapic drugs is also present: in particular, temozolomide has an important and significant anticonvulsant effect[22]. de Groot et al.[8] pointed out the effect of the combination of procarbazine, CCNU (lomustine) and vincristine (PCV scheme). Dexametasone, the gold standard treat-

ment for oedema, reduces the risk of seizure but individual cases of increased seizure risk have been reported. Rare cases of increase seizure frequency have been described in course of some chemotherapy with 5-fluouracil, cisplatyin and vincristine, methotrexate and cytarabine. These following CTDs can increase seizure risk: ifosfamide, L-asparaginase, etoposide (intra-arterial), interleukin-2, busulfan (high dose), BCNU, carboplatin (intra-arterial), cytosine-arabinoside (high dose, intra-arterial), bevacizuimab, interferon alpha, cyclophosphamide, anthracyclines and nitrosureas.

In conclusion, as suggested by Avila et al.[2] in a recent review from RANO working group, seizure outcome is an important response criterion of outcome in glioma treatment.

The following suggestions on the management of epilepsy in oncology are the logical and deductive conclusions rising from the analysis of the above-mentioned literature.

Statements are outlined without any strict and intrusive indication of specific drugs.

In our experience[32,35], this particular population of epileptics has to cope with some additional problems:

- peak doses of AED should be reached as quickly as possible after diagnosis;
- the availability of an intravenous formulation which makes the drug likely to be used even in the peri-operative and emergency period;
- a shift to an intramuscular or subcutaneous administrable AED should be provided for in the terminal phase of the disease when the patient is generally unable to swallow;
- sensitisation reactions to AEDs, or seizures, may appear abruptly and dramatically when the steroid is interrupted (e.g. after surgery or awaiting the beginning of radiotherapy) also considering that steroids enhance the GABA inhibitory effect and therefore should protect from epilepsy;
- although phenytoin, carbamazepine, phenobarbital and divalproex are still the most commonly prescribed AEDs for brain tumour patients, the possible leukopenia or thrombocytopenia is a drawback in a patient who will receive cytotoxic chemotherapy;
- every AED shows a specific profile of CNS toxicity, thus complicating the cognitive, behavioural, physical symptoms;
- within the group of malignant neoplastic diseases, epilepsy associated with brain metastases seems to be more easily controllable than TAE in high-grade gliomas.

☐ ANTICONVULSANT PROPHYLASIS

In May 2000, a Panel of Experts of AAN[12] examined twelve studies (four randomized controlled trials and eight cohort studies) to establish the ability of prophylactic anticonvulsants to prevent first seizures in patients with brain tumours: the meta-analysis showed no statistical benefit. Temkin[46] concludes that for patients with brain tumours, regardless of neoplastic type, and with no prior history of seizures:

1. prophylactic therapy with old-AEDs (PHT, VPA, PB, CBZ) is ineffective;
2. tapering and discontinuing anticonvulsants after the first post-operative week is appropriate.

Besides these two well-known recommendations, TAE prophylaxis is currently still characterized by significant behavioural heterogeneity and the literature lacks robust data concerning efficacy and toxicity of new, recently marketed AEDs.

The prophylactic use of AEDs still remains the prevailing practice pattern among members of the AANS[43].

☐ THE COCHRANE LIBRARY AND "AVAILABLE BEST EVIDENCE"

In the Cochrane Library there are 6 reviews on the subject:

- one[31] on the use of AEDs in the SE and two on the timing and on the rapidity[44] of AEDs withdrawal. These do not specifically analyse the clinical query for the oncological population examined and conclude that it is not yet possible to show the best time to withdraw or the rapidity of the optimal rate of tapering of AEDs. More research is therefore needed for both issues;
- two reviews of outstanding interest: "Antiepileptic drugs for preventing[47] or treating[21] seizures in people/adults with brain tumours". Although the former review substantially shares the conclusion of prophylactic inefficacy on the onset of seizures in formerly epilepsy-free brain-tumour patients, inefficacy already expressed by Glantz's, Temkin's and Sirven's meta-analyses, in the discussion it challenges some methodological biases of AAN Practice Parameters. Therefore, this meta-analysis reports how the evidence for seizure prophylaxis with old AEDs is inconclusive, at best. The decision to start an antiepileptic drug for seizure prophylaxis is ultimately guided by the assessment of

individual risk factors and careful discussion with patients.

The latter review shows how only one small, open-label, unblinded, randomised trial met the inclusion criteria for the evaluation of the safety and feasibility of switching from phenytoin to levetiracetam monotherapy or continuing phenytoin for glioma-related seizure control following craniotomy[25]. Levetiracetam appears to have been at least as well tolerated and as effective as phenytoin for the treatment of seizures in people with brain tumours.

In 2008, a panel of experts of the AINO proposed some practical management statements; we refer in particular to the chapter on management in the Emergency setting, not discussed in this present paper[6]. A 2013 revised version is awaiting publication (http://www.neuro-oncologia.eu/news/aggiunti-nuovi-contributi).

In conclusion, if a long-term antiepileptic prophylaxis is not justified in seizure-free patients, the risk of recurrence after a single seizure is considerably higher in patients with structural brain lesion(s) and initiation of treatment should be considered in these patients[35,45].

❒ **A. Seizure or epilepsy in patients with a structural active neoplastic brain lesion(s)**

As stated before, TAE generally refers to seizure or epilepsy in patients with structural active neoplastic brain lesion(s), primary or metastatic.

Despite the well-known fact that TAE differs from other forms of epilepsy for the underlying mechanism, clinical manifestation and response to treatment, epilepsy treatment protocols mostly do not contain specific guidelines for glioma patients.

Gliomas are progressive in nature therefore type and severity of seizures may evolve over time in relation to underlying tumour. A spontaneous worsening of epilepsy can predict a progression of the glioma

In glioma-associated epilepsy, the percentage of patients who become seizure free varies between 23 and 87% depending on subpopulation and study type[18].

In this "chapter A" group we refer (Table 4) to glioma (WHO grade II-IV) patients, brain or dural metastases, atypical and malignant (WHO grade II-III) meningioma, supratentorial anaplastic (WHO grade III) ependimoma etc., in short, all the lesions which require a further radio-chemotherapy program after surgery:

1. seizure-free patients at onset require only peri-operative (± 7 days before and after surgery) profilaxis with the following limitations, after careful discussion in cases selected according to histology or site risk:
   - the extension of AE profilaxis till the end of radiotherapy,
   - a "prudential long-term profilaxis" because of the patient's will, singularity, physical job risk etc.
2. patients with epilepsy at onset require a long-term profilaxis, established as soon as possible.

As far as drug choice is concerned (Table 5):

- in general, new-AEDs (LEV, chronoVPA, TPM, LCS, OXC in order of our preference) present a more favourable profile both in terms of haematological and cognitive efficacy/toxicity and in terms of pharmaco-kinetic and dynamic interactions with the other treatments[37] (in spite of the above-mentioned adverse effects);
- LEV-VPA-LCS-PHB are available also in parenteral formulations particularly useful in fast titration, status epilepticus, general anaesthesia and terminal phase of disease;
- average dosages are similar to what currently indicated in general epileptic population;
- the titration of the most suitable AED (feasible with the cited drugs) should be performed in the pre-operative period;
- in patients without indications for a long-term prophylaxis or with another in range AED, in case of the neuro-anaesthetist's decision of starting PHT in operating room, PHT should be suspended within two weeks and in any case possibly within RT, because of the high risk of sensitisation of this association;
- low-levels, poor compliance and generic substitution are frequent causes of recrudescence;
- in cases of a "certain" acute symptomatic seizures (e.g.: starting of radio-chemotherapy, febrile intercurrent disease, proved low-level of AED in use, etc.) a short treatment (7-10 days, awaiting resolution/removal of the trigger) with a BDZ in monotherapy or in add-on is advisable: clobazam 10-20 or clonazepam 2-4 mg bid (if at the RT starting, adjunct of dexametasone is useful);
- in cases of proved insufficiency of the current AED the add-on of a second AED (inside the above-mentioned list) with different mechanism of action is preferred to a substitution, because of the pharmaco-resistance;

"Best choice" AEDs	
Ideal drug	- Fast titration - Linear kinetic - Intravenous formulation - High absorption - Low protein bound - Reduced interactions (kinetic and dinamic) - Plasma long half-life - Renal escretion - No epatic enzyme induction - No plasma levels monitoring
Ideal management	• *Clinician-related* - Neutral evidences: therefore only "prudential prophylaxis" in absence of seizure because of: - patient's will (singleness, dangerous job) - site ± histology (temporo-mesial or fronto-rolandic site, hemorragic mets) • *Drug-related* - new AEDs = NEIAED (+ os/iv): LVT, VPAch, LCM, BDZ; or TMP, OxCBZ, etc. - major anti-epileptic efficacy - less toxicity - hematologic - neurologic - absence of interaction - poor sensitivity • Timing and/or Host-related - other AEDs (os/ev/im: TPM, OxCBZ, PHT, PB, BDZ, etc.)

Table 4. Criteria for the "best choice" of antiepilectic drugs.

- in cases of convulsive or non-convulsive status epilepticus, see the specific guidelines cited.

❒ **B: Seizure or epilepsy in patients without any active neoplastic brain lesion but due to a structural change**

This group includes patients:

1. with acute symptomatic seizures in the peri/post-operative period for any other CNS oncologic surgery (pituitary adenoma, craniopharyngioma, etc.) because of brain oedema, haemorrhage or any other complication to be treated in add-on or in monotherapy (in absence of a previous prophylaxis) with a fast acting drug such as BDZs;
2. who may develop chronic epilepsy due to treatment (RT), vascular, paraneoplastic or infectious lesions as complications in a systemic cancer (including RPLS) to be treated as indicated in chapter A.

❒ **C: Seizure or epilepsy in other various conditions of any cancer**

This group includes:

1. patients with a history of a previous CNS tumour or during their follow-up (but not expression of active/relapsing neoplastic CNS disease: meningioma etc.) who may develop an unprovoked late seizure (either a brief simply partial seizure, or a prolonged generalized one) which requires a careful diagnostic and treatment evaluation for the risk of repetition;
2. patients with treatments and metabolic complications in systemic cancer without a radiographic presence of epileptogenic lesion. In the other patients, since most seizures are

Table 5. Current available AEDs: peculiar characteristics in neuro-oncologic patients[35]. *Legenda*: AED = AntiEpileptic Drug; BZD = Benzodiazepines; Ca = Calcium channel; CYP = Cytochrome P-450; GABA = γ-AminoButyric Acid; GBP = Gabapentin; inhib. =enzyme inhibition; iv = intravenous; K = Kidney; L = liver; LCM = Lacosamide, LTG = Lamotrigine; LVT = Levetiracetam; Na = sodium channel; NMDA = N-Mehyl-D-Aspartate; OXC = Oxcarbazepine; PGB = Pregabalin; PHB = Phenobarbital (and primidone); PHT = Phenytoin; RUF = Rufinamide; SIADH = Syndrome of Inappropriate Antidiuretic Hormone Secretion; SV = Synaptic Vesicle; TPM = Topiramate; VPA = Valproic Acid; ZNS = Zonisamide.

Anti-epileptic drug	Parental form	Site of action	CYP-inducer / Metabolism	Protein binding (%)	AED (↓ activity) effect on chemotherapy	Chemotherapy (↓ activity) effect on AED	Adverse effects to consider
PHB	iv + im	GABA	1A2, 2A6, 2B6, 2C9, 2C19, 3A4 / L, K	50	Nitrosurea, Prednisone, Methotrexate, 9-aminocampothecin, Thiotepa, Ifosfamide, Doxorubicina, Tamoxifen, Teniposide, Etoposide, Paclitaxel, Procarbazine, Vincristine	Temozolomide	Drowsiness, Stevens-Johnson, shoulder-hand syndrome, cognitive
PHT	iv	Na	1A2, 2B6, 2C9, 2C19, 3A4 / L, K	90	Dexamethasone, Busulfan, Vinblastine, Vincristine, 9-aminocampothecin, Teniposide, Irinotecan, Methotrexate, Paclitaxel, Procarbazine, Sirolimus, Teniposide, 5-fluorouracil	Nitrosurea, Doxorubicin, Carboplatin, Cisplatin, Temozolomide, Vinblastine, 5-fluorouracil, Dexamethasone, Tamoxifen, Teniposide, Doxorubicin, Procarbazine, Bleomycin, Capecitabine	Rash, Stevens-Johnson, incoordination
CBZ	No	Na	1A2, 2B6, 2C9, 2C19, 3A4 / L	75	Methotrexate, Paclitaxel, Vinblastine, Vincristine, 9-aminocampothecin, Sirolimus Procarbazine	Temozolomide	Stevens-Johnson, anemia, SIADH, ↓ cognitive, leukopenia, diplopia
OXC	No	Na	3A4 / L	40	-	Temozolomide	Rash, diplopia, hyponatremia
BDZ	iv/im + rectal/nasal	GABA agonist	= / L	80	-	-	Sonnolence, drowsiness, ↓ cognitive
VPA	iv	Na, GABA	2A6 (inibitor of 2C9, 2C19, 3A4) / L	90	-	Methotrexate, Doxorubicin, Cisplatin	Thrombocytopenia, neutropenia, tremor, pancreatitis, hair loss
TPM	No	Na, NMDA, GABA	3A4 / L, K	30	-	Temozolomide	↓ cognitive, renal calculi, parestestesias
ZNS	No	Na, Ca	(Inhib. 2E1) / L	50	-	-	Drowsiness, headache, renal calculi
LTG	No	Na	No /L	50	Methotrexate	-	Rash, ↓ cognitive, drowsiness, folate reductase enzyme inhibition, very slow titration
GBP	No	GABA, Ca	No /K	< 5	-	-	Drowsiness, ataxia, weight gain
PGB	No	GABA, Ca	No /K	< 5	-	-	Thrombocytopenia, drowsiness, ↓ pain, splenic edema
LVT	iv	SV	No /K	< 5	-	-	Agitation, psychosis, drowsiness
LCM	iv	Na	No /K	< 5	-	-	Drowsiness
RUF	iv	Stab Na channels (?)	No /K	< 5	-	-	Rash, fatigue, drowsiness

acute-symptomatic, the use of a fast acting AED such as clonazepam, clobazam or lorazepam prior to, and until 24 hours (up to 4-7 days) after chemotherapy administration, may be appropriate both in emergency management and as prophylaxis in the follow-up. Lorazepam is used most often and offers the advantages of both lack of any drug interaction and an antiemetic effect. Furthemore BDZs exert an ansiolitic action, particularly useful in these patients. In case of a long-term prophylaxis, new-AEDs are an attractive alternative.

□ CONCLUSIONS

In conclusion, only in recent years have we seen new interest in problems related to anticonvulsant medications in neoplastic disorders mainly for drug interactions, timing of titrations, choice in relation to short survival in patients with glioblastoma or brain metastases. On the other hand, in some settings epilepsy is the major clinical problem (and the only clinical "measurable" efficacy outcome of treatments response), like in patients with low-grade gliomas and in long survivors with high-grade gliomas. This new interest raises the following problems: whether the status of not-receiving AEDs or receiving NEIAEDs versus receiving EIAEDs can affect survival and therefore must be considered a prognostic factor which can influence the outcome and the endpoints, thus justifying the introduction of a further stratification variable in future prospective clinical trials or whether the VPA or LEV may exert an antitumour effect. Up to now, these questions are still unsolved.

In neuro-oncology, a specialized and well-organized team, with the organ specific neurologist as a reference guide, seems to be the best response to the needs of patients with CNS tumours and other neoplastic conditions, who now frequently have to receive care in more than one location. The multidisciplinary approach allows an optimization of the care process, the standardization of treatments, and therefore the collection of conclusive clinical data, the improvement of patients' quality of life and, finally, a cut in social costs.

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Original article

Subtractive magnetic resonance angiography: description of a new diagnostic technique

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SUMMARY: Aims. *The most widely non-invasive technique used for the intracranial circulation's evaluation is magnetic resonance angiography. Particularly 3D time-of-flight sequences are the most useful because they are less susceptible to turbulent flow artefacts compared to phase contrast ones. time-of-flight sequences however have some limitations, among which one of the most important is that it's impossible to suppress the acute - subacute blood's signal. We have developed a technical improvement (denominated subtractive magnetic resonance angiography) of the time-of-flight sequences that will remove the blood's signal and improves intracranial vessels' contrast resolution. The aim of our work was to evaluate benefits and limitations of this new technique.*

Material and methods. *We carried out a comparative evaluation between the conventional technique and the new technique both on twenty healthy volunteers and on twenty-two patients with intracranial vascular disease. Visually comparative evaluation between conventional and subtractive magnetic resonance angiography was carried out.*

Results. *We showed that the subtractive technique improves the cerebral vessels' visualization especially when T1 hyperintense tissues are in the background (for example, blood debris or fat). Moreover, regarding the remaining normal or pathological vessels visualization, the resolution was the same as the conventional technique.*

Conclusions. *In conclusion, the subtractive technique may be usefully adopted for the intracranial circulation's evaluation especially during acute - subacute brain bleeding.*

KEY WORDS: *Angiography, Magnetic resonance, Technique, Time-of-flight sequences.*

INTRODUCTION

Intracranial circulation's MRA evaluation is usually carried out with TOF or PC sequences[15,19,24,27].

MRA is frequently adopted for intracranial vessels' evaluation, mainly for arterial stenosis and aneurysms visualization. Instead the AVM and dural fistulas' studies are difficult with both technique[1,6,18,21,29,30].

Intracranial aneurysms and arterial stenosis are properly displayed with 3D TOF technique because, unlike PC sequences, the former are less sensitive to spin dephasing effect resulting from turbulent flow[8,10,12,13,16].

Then the gold standard technique for noninvasive study of intracranial vessels is 3D TOF one.

We carried out a technical improvement applied to 3D TOF sequences and, in this study, its benefits and limits were evaluated.

One of the most important restrictions of TOF technique is the inability to fully saturate the static

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LIST OF ACRONYMS AND ABBREVIATIONS: **ACoA** = Anterior Communicating Artery; **AVM** = Arterio-Venous Malformations; **CTA** = Computed Tomography Angiography; **DSA** = Digital Subtraction Angiography; **FFE** = Fast Field Echo; **FISP** = Fast Imaging Steady Precession; **FLAIR** = Fluid Attenuated Inversion Recovery; **FOV** = Field Of View; **FSE** = Fast Spin Echo; **ICA** = Internal Carotid Artery; **MCA** = Middle Cerebral Artery; **MIP** = Maximum Intensity Projection; MR = Magnetic Resonance; **MRA** = Magnetic Resonance Angiography; **NEX** = Number Of Excitations; **PC** = Phase Contrast; **PD** = Proton Density; **PICA** = Posterior Inferior Cerebellar Artery; **SE** = Spin Echo; **T** = Tesla; **TE** = Time to Echo; **TOF** = Time-of-flight; **TR** = Time of Repetition; **VENC** = Velocity ENConding.

protons' signal. This limit is especially restrictive when short T1 tissues are in the background. Indeed, because in TOF sequences signal partly depends from T1 relaxation time, such tissues can mimic the signal flow making it impossible the correct vessels' identification[10,16,19,24,29].

Short T1 tissues can be detected in subacute bleeding or in cavernous angiomas (extracellular methemoglobin), in embolized aneurysms (ferromagnetic artefacts) and finally in any calcified or fatty lesion (neoplasms).

The technical improvement we found requires the acquisition, before conventional 3D TOF images, of a sequence exactly the same as diagnostic TOF but with complete flow signal's saturation. This sequence (without flow signal) was used as a "mask" for a digital subtraction process with the conventional TOF; the result was a complete deletion of the static protons' signal, similarly to digital angiography. To obtain the mask we simply acquired a conventional TOF sequence with a saturation pulse placed at the neck, to eliminate the arterial "inflow" signal (Figure 1). After achieving this mask was sufficient to carry out a digital subtraction with conventional sequence to obtain images only with flow signal, completely eliminating static tissues' signal, even if they were hyperintense (Figure 2).

Moreover the fully static protons' signal saturation increases the vessels resolution, thereby improving their visualization.

Aims. The aim of our work was to evaluate benefits and limitations of the new technique, in comparison to the conventional one, both on healthy volunteers and on patients with intracranial vascular disease.

□ MATERIALS AND METHODS

Twenty healthy subjects (12 males and 8 females with an age range between 25 and 68 years, average 43.85 years) and 22 patients with intracranial vascular disease (12 males and 10 females aged between 38 and 82 years, average 60.1 years) were studied in a comparative manner, evaluating the MIP reconstructions derived from subtraction images and those obtained from conventional ones.

As regards the pathological cases in 9 patients a cerebral aneurysm was found: two cases with giant not treated partially thrombosed aneurysm, one small size aneurysm originating from PICA studied after embolization treatment; the remaining six were not treated lesions: five small size aneurysms and one large size lesion; the five small aneurysms originated in two cases from the bifurcation of the MCA, in two cases from ACoA and in one case from the sovraclinoid segment of the ICA. The large aneurysm originated from the basilar artery's apex.

We also studied: 5 patients with acute or subacute intra-axial cerebral hematomas, 2 patients with cavernous angiomas, a patient with previous cerebral ischemic stroke with dystrophic mineralization phenomena due to cortical laminar necrosis which appeared hyperintense in T1-weighted images, 5 patients with AVM, including 2 treated and 3 no and one patient with MCA stenosis.

A 1.5 T magnetic resonance equipment with 23 mt/m gradients (*Philips Gyroscan ACS-NT 3000*) was used for all healthy subjects' studies. The pathologic cases were studied with the 1.5 T unit and partly with a 3 T 45 mt/m gradients unit (*Siemens, Magnetom Verio*).

With both the equipment the following morphological sequences were acquired for each study: axial SE T1-weighted, axial FSE T2-weighted and PD, axial FSE FLAIR T2-weighted. Instead MRA sequences adopted in the 1.5 T studies were 3D TOF FFE (TR 25 ms, TE 6.9 ms, matrix 496x512, FOV 220 mm, 80 slices, 1 slab, thickness 0.7 mm, 1 NEX), while those used in the 3 T unit were 3D TOF FISP (TR 31 ms, TE 3.78 ms, matrix 486x512, FOV 190 mm, 40 slices, 4 slabs, thickness 0.4 mm, 1 NEX).

The volume acquired with angiographic 3D TOF sequences included the intracranial arteries from C3 segment of the ICA, up to A3 section of the anterior cerebral arteries. Thus, this volume includes the main intracranial arteries with exclusion of both PICA and the intracranial tract of vertebral arteries.

The venous signal's deletion has been obtained by a saturation pulse above the acquisition's volume.

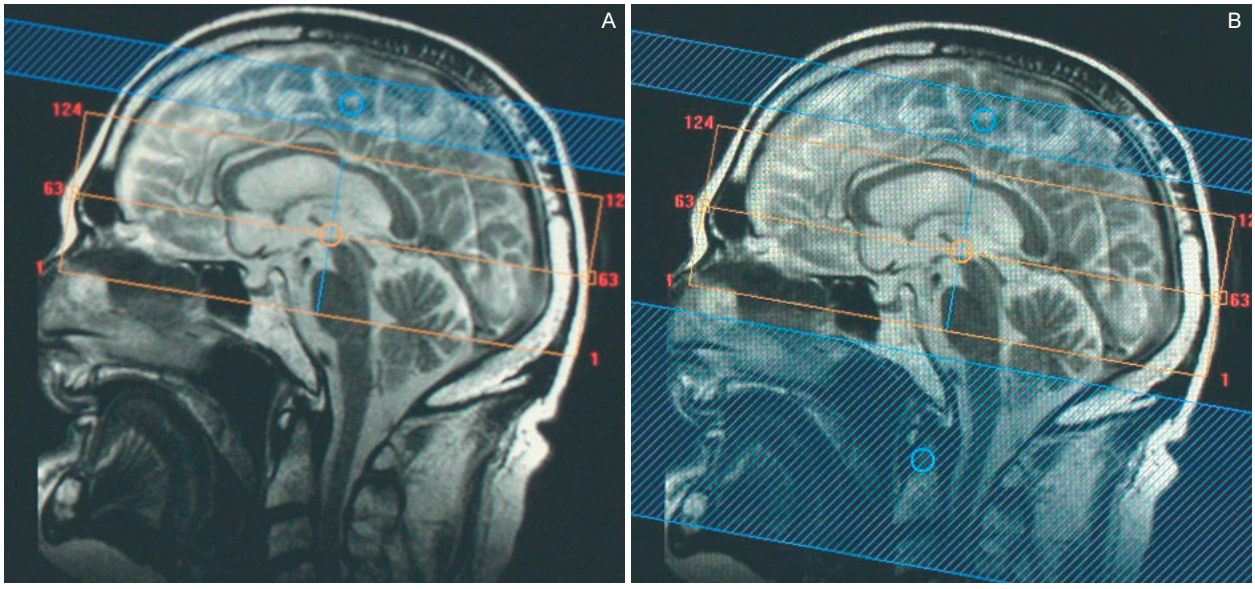

Figure 1. Description of the subtractive technique. Two identical sequences were acquired: the first with conventional technique (A) and the second with a saturation pulse at the bottom that removes the vascular flow's signal (B); the latter was used as a mask.

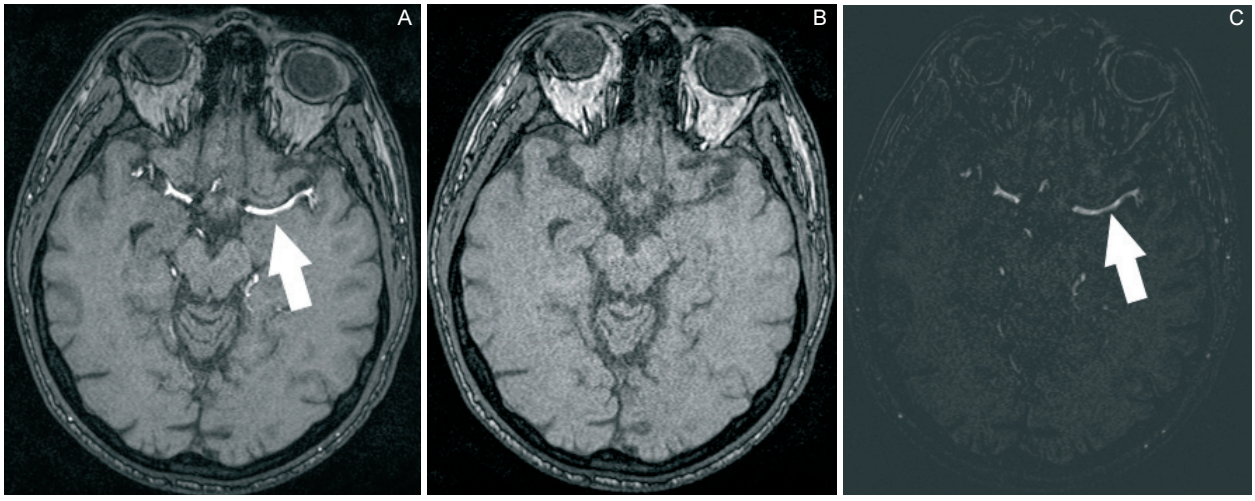

Figure 2. Description of the subtractive technique. A single partition of the conventional sequence (A), the same partition of the mask (B) without flow signal. The third image (C) is the result of the digital subtraction between A and B: only signal flow was displayed with complete removal of the background.

To achieve the mask we have acquired an identical sequence with at most a second saturation pulse below the acquisition volume. The two sequences were sequentially and randomly acquired.

None of the patient was excluded from the series for non-cooperation and therefore inaccurate overlapping of the two angiographic sequences.

Digital subtraction images were subsequently carried. Finally, both conventional and subtracted sequence, were elaborated by means MIP reconstruction, with at least 40 projections, using a dedicated work station (*Easy Vision, Philips*) by a single author.

All patients with cerebral hematoma, aneurysm or AVM were also studied with DSA.

Visually comparative evaluation of both MRA techniques was carried out by two authors. The inter-observer variability was assessed by calculating "inter-observer agreement index".

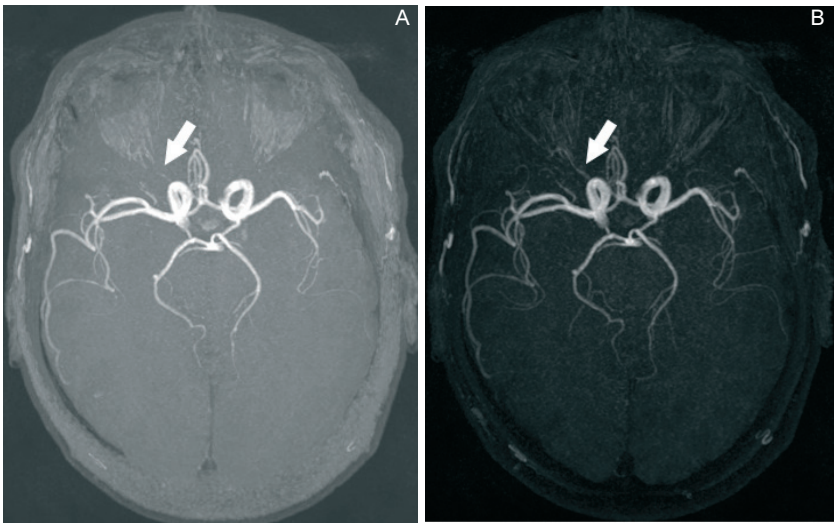

Figure 3. Ophthalmic artery (*arrow*) visualization with conventional (A) and subtractive technique (B). The latter image better displays the artery.

In normal controls number and length of the cerebral arteries displayed were evaluated.
In patients, in addition to the evaluation of normal brain arteries, made as in the controls, visualization of pathological vessels was assessed, always in a comparative manner between the two techniques.

□ RESULTS

Regarding the healthy subjects' evaluation a better visualization of ophthalmic artery with subtractive technique in 28 cases out of 40 was showed, since a large part of this artery's course occurs in orbital fat (Figure 3).
The remaining intracranial arteries were displayed with the same resolution with the two techniques.
Therefore in no case the conventional technique was superior to subtractive one.
Instead as it regards for the patients' evaluation, when background hyperintense tissues were present, it was possible to delete its, obtaining vascular images not disturbed.
This advantage has been achieved in the 5 acute/subacute cerebral hematomas (Figure 4), in the two thrombosed giant aneurysms (metahemoglobin) (Figure 5), in the two cavernous angiomas (metahemoglobin) (Figure 6), in embolized aneurysm, because Guglielmi's coils showed hyperintense signal (Figure 7) and in the patient with cortical laminar necrosis, spontaneously hyperintense in T1-weighted images too (Figure 8).
Regarding patients with small and large untreated aneurysms we founded in all 4 cases a similar lesions' visualization with both sequences without limitations for subtractive technique (Figure 9).
Even in patients with AVM we did not find significant differences between the two techniques (Figure 10).
Finally also in the patient with Sylvian artery's stenosis, we have not found differences between the two techniques as it concerns the lesion's visualization (Figure 11).
The "inter-observer agreement index" was 98.3.
In no case, therefore, the conventional technique proved better of subtractive one to show the pathological processes studied.

□ DISCUSSION

MRA is the method of choice for non-invasive study of intracranial arteries' pathological processes[12,23,27,29].
As we have seen above, two are the angiographic techniques which are used in MRA routine practice: the TOF and PC sequences.
Both are useful in providing good quality angiographic images, but differentiate between them for the acquisition way.
The TOF method is the better for different reasons: it is the most commercially widespread and then used the most, it is more suitable for the fast and turbulent flows (arterial) studies, has shorter acquisition times of that PC and it is characterized by a better spatial resolution each the other[19,24,28].
3D TOF sequences, on the other hand, have some

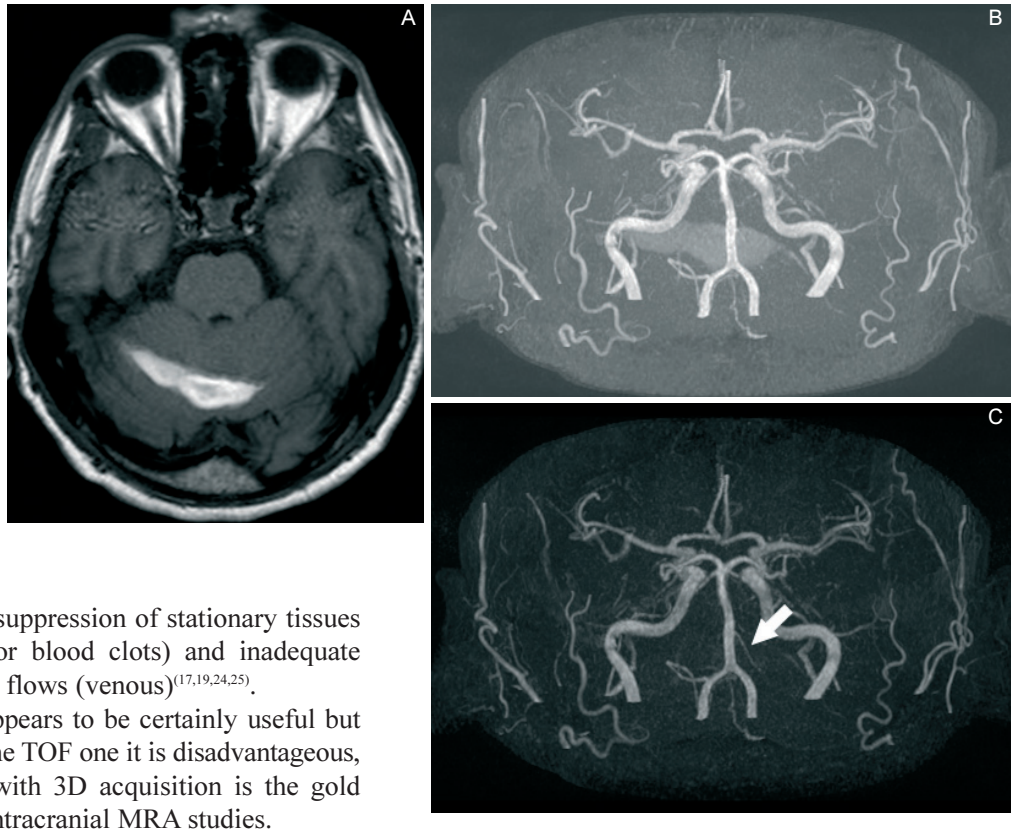

Figure 4. Intra-axial cerebellar haematoma. The lesion is hyperintense in T1-weighted image (A) due to extracellular methemoglobin; in conventional MRA (B) this tissue mimics flow signal, while in subtractive MRA (C) background signal is completely removed and left anterior-inferior cerebellar artery (*arrow*) is displayed. In this patients digital subtraction angiography examination don't show any vascular malformations.

limits: such as poor suppression of stationary tissues with short T1 (fat or blood clots) and inadequate visualization of slow flows (venous)[17,19,24,25].

The PC technique appears to be certainly useful but in comparison with the TOF one it is disadvantageous, and then the latter with 3D acquisition is the gold standard in arterial intracranial MRA studies.

In fact the PC technique although presents some advantages in comparison to TOF as: optimal slow flows visualization without signal's saturation (particularly suitable in intracranial venous circulation's studies) and complete stationary tissue saturation, on the other hand is limited by numerous restrictions than TOF as: long acquisition time, most sensitive to intravoxel dephasing and susceptibility artefacts and the need to optimise the VENC value as a function of the cardiac stroke[10,12,25,27].

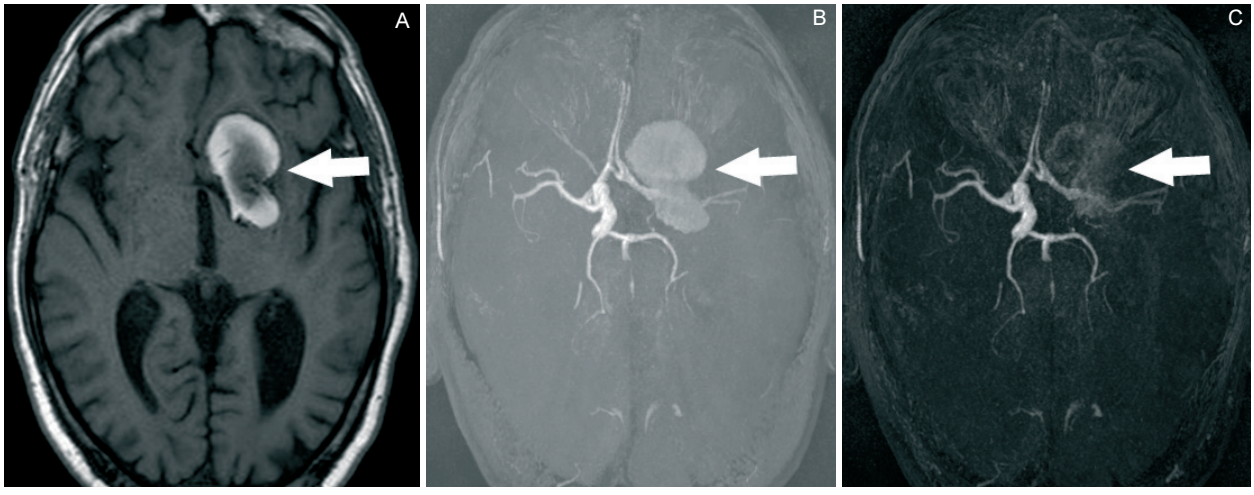

Figure 5. Left middle cerebral artery partially thrombosed giant aneurysm (arrow). The clotted blood shows in T1-weighted image (A) hyperintense signal and then, likewise to the figure 4 haematoma, conventional MRA image (B) is disturbed by extracellular methemoglobin, while subtractive MRA image (C) not.

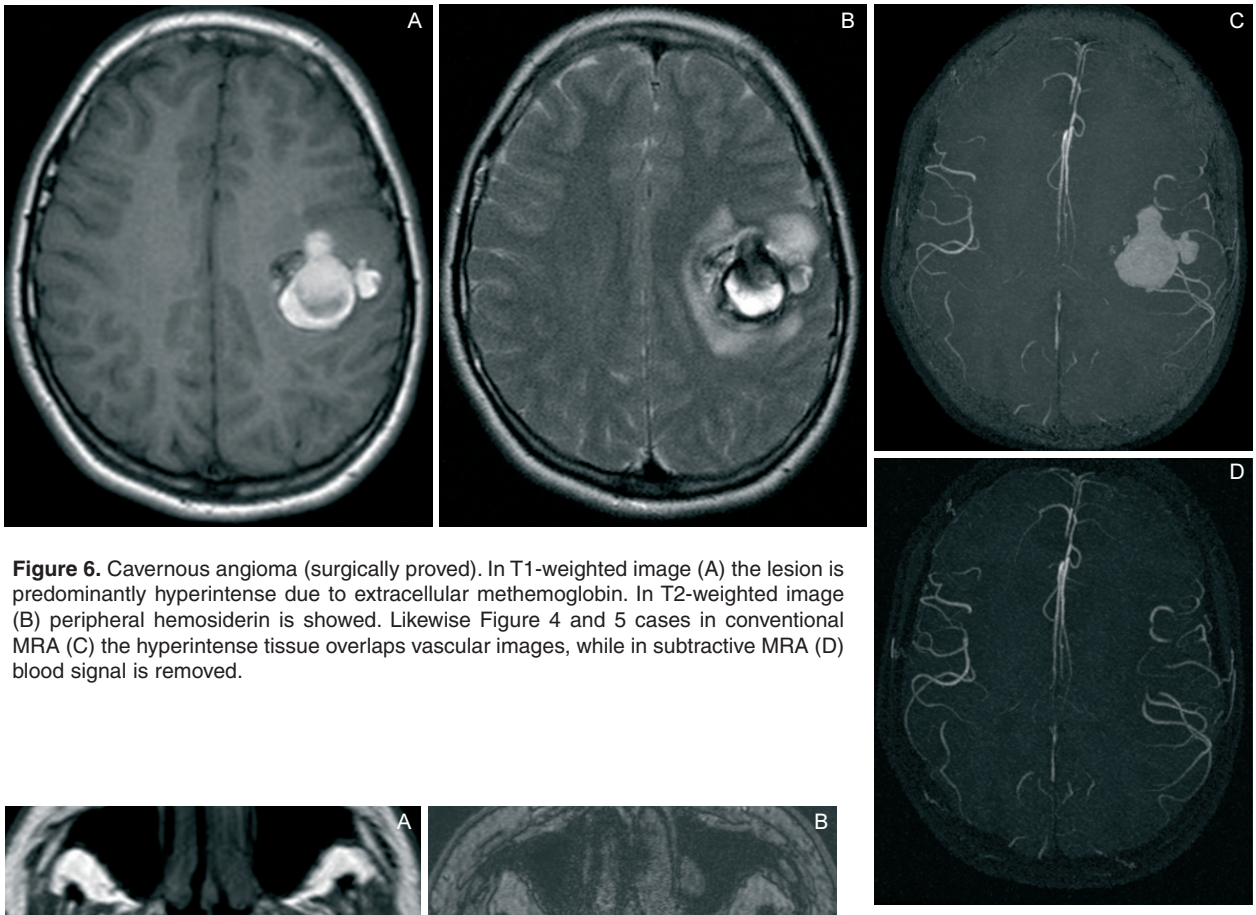

Figure 6. Cavernous angioma (surgically proved). In T1-weighted image (A) the lesion is predominantly hyperintense due to extracellular methemoglobin. In T2-weighted image (B) peripheral hemosiderin is showed. Likewise Figure 4 and 5 cases in conventional MRA (C) the hyperintense tissue overlaps vascular images, while in subtractive MRA (D) blood signal is removed.

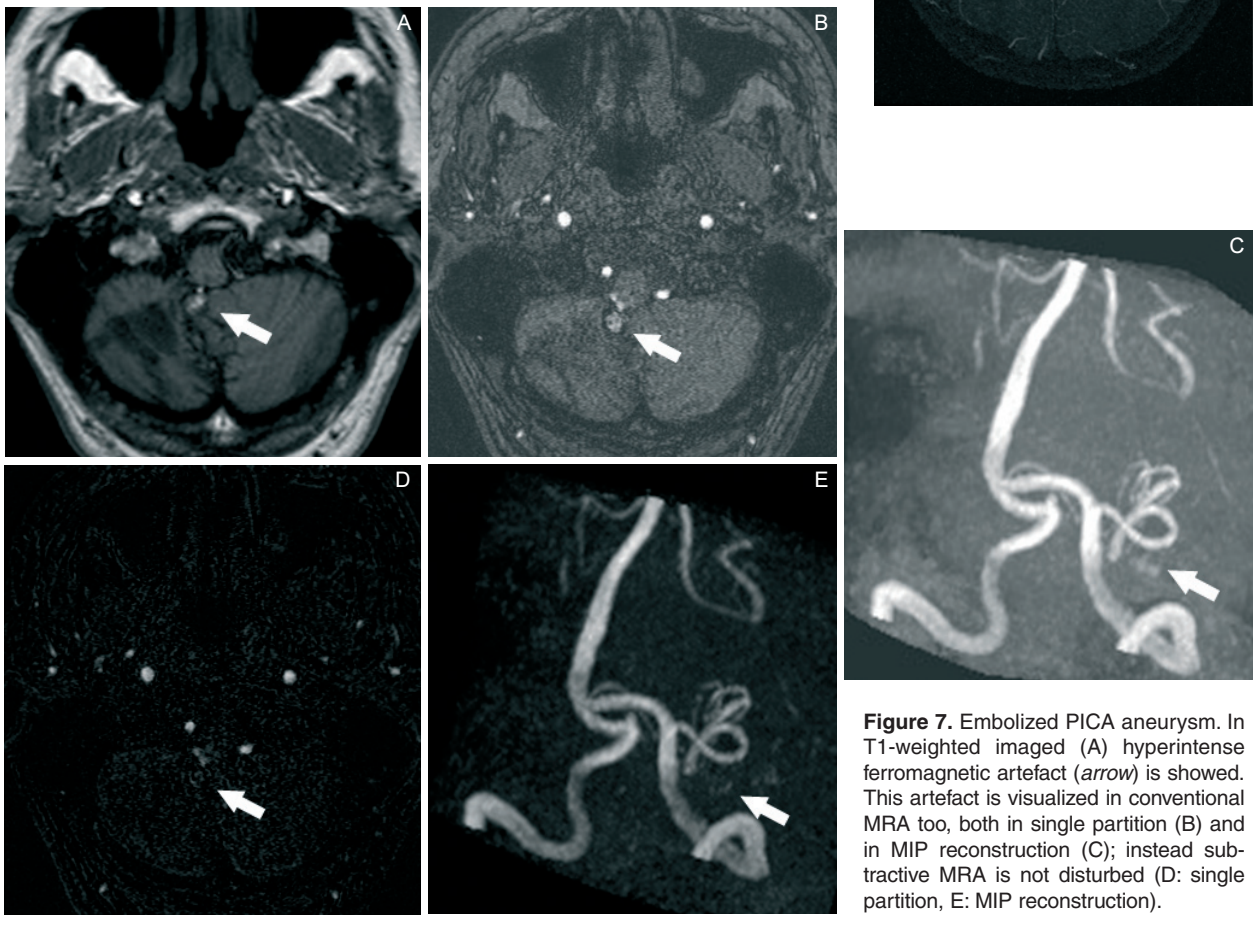

Figure 7. Embolized PICA aneurysm. In T1-weighted imaged (A) hyperintense ferromagnetic artefact (*arrow*) is showed. This artefact is visualized in conventional MRA too, both in single partition (B) and in MIP reconstruction (C); instead subtractive MRA is not disturbed (D: single partition, E: MIP reconstruction).

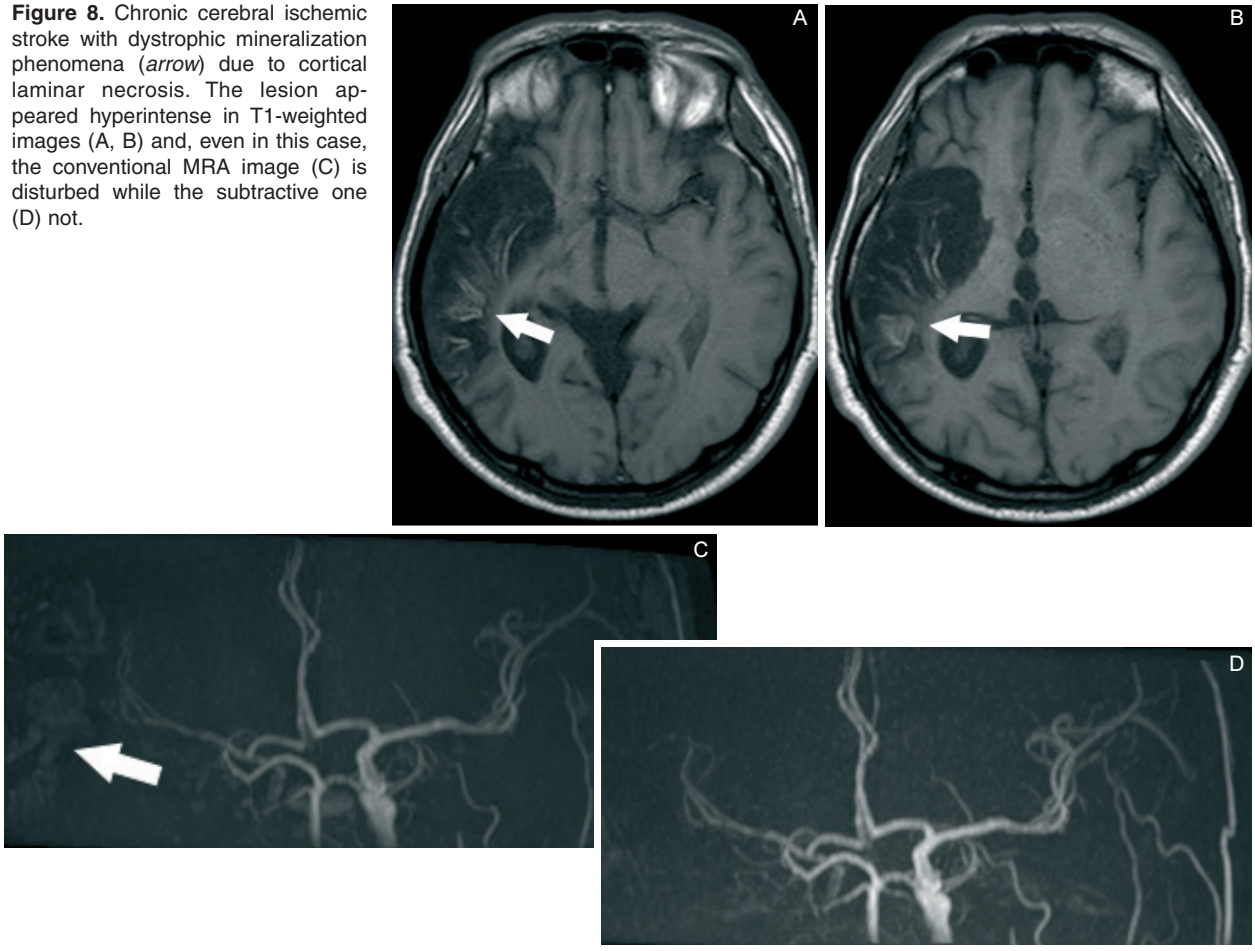

Figure 8. Chronic cerebral ischemic stroke with dystrophic mineralization phenomena (*arrow*) due to cortical laminar necrosis. The lesion appeared hyperintense in T1-weighted images (A, B) and, even in this case, the conventional MRA image (C) is disturbed while the subtractive one (D) not.

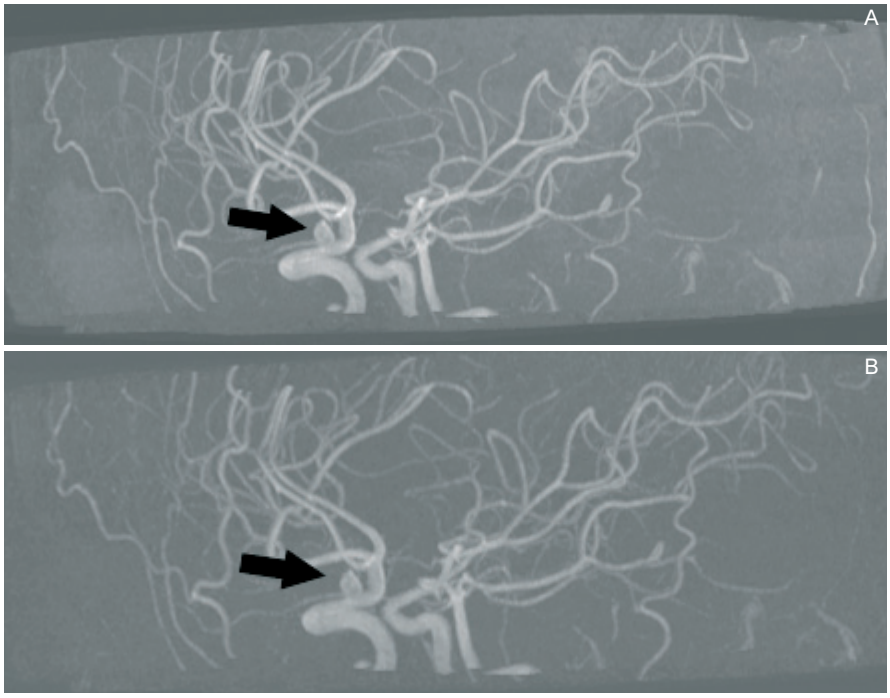

Figure 9. Not treated sovraclinoid internal carotid artery aneurysm (*arrow*). The lesion, both with conventional MRA (A) and with subtractive technique (B), is properly showed.

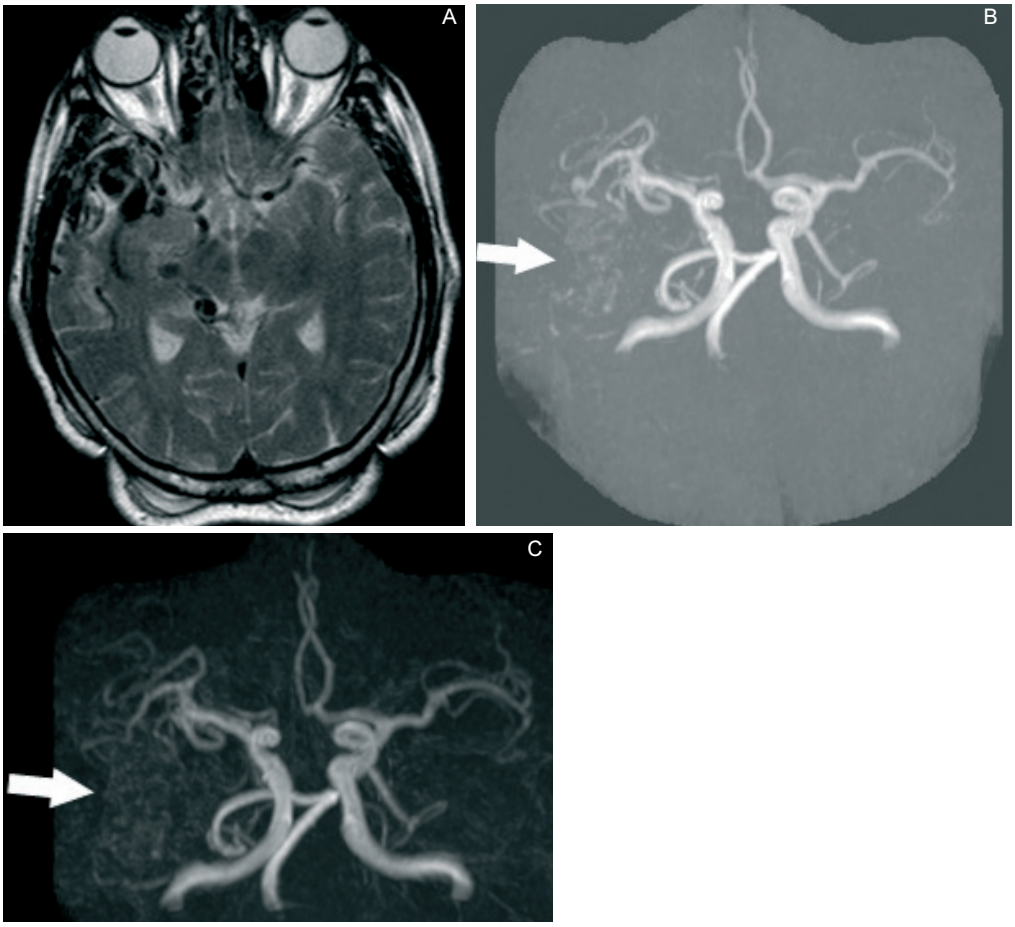

Figure 10. Not treated arterio-venous malformation (*arrow*). T2-weighted sequence (A) with typical signal void images. Also in this case both techniques (conventional: B and subtractive: C) properly showed the lesion.

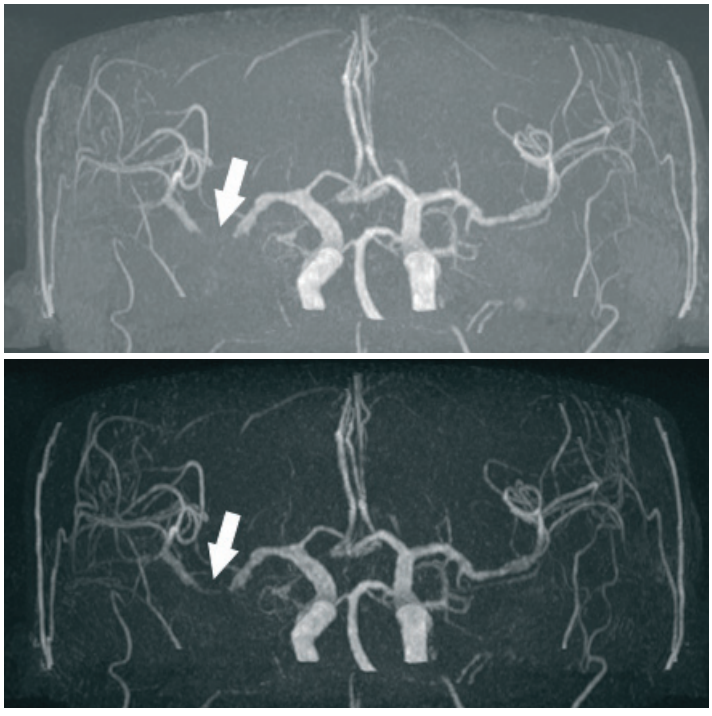

Figure 11. Right middle cerebral artery stenosis (*arrow*). The lesion visualization is the same both with conventional (A) and subtractive MRA (B).

Huston. et al.[11] have carried out a comparative evaluation between the two main techniques (TOF and PC) for the vascular intracranial diseases' evaluation. The study included 27 patients with aneurysms or vascular malformations. The TOF technique superiority for the evaluation of small and medium size aneurysms was demonstrated while the PC sequences were slightly better to evaluate high flow vascular malformations despite a resolution clearly worse respect DSA.

As regards the intracranial vascular stenosis' evaluation, Fujita. et al.[5] identified with high diagnostic sensitivity middle cerebral artery's stenosis with 3D TOF MRA in comparison with DSA, so showing that MRA can be used for a non-invasive evaluation of this pathology.

In recent years CTA, thanks to recent technological developments, enables intracranial circulation's evaluation with quality comparable to MRA.

However CTA has some important limitations such as: high ionizing radiation dose absorbed by the patient, the use of a contrast media presenting risks of adverse reaction and finally the vascular signal not always is dissociable from that of the hyperdense tissues (bone).

Several comparative studies between CTA and MRA confirmed this data[3,4,7,9,14,20,22]. In one of the first published studies Schwartz RB. et al. have shown that, in 21 patients with 30 aneurysms, the diagnostic sensitivity and specificity of the two techniques were similar, however, the MRA is preferred because less invasive[26].

Subtractive MRA was described in a previous our but only as a technical note on a few healthy volunteers and with low field MRI equipment[2].

Our new MRA technique has proven more useful than the conventional one.

Our method presents a significant advantage compared to that conventional: the fully short T1 tissues' signal elimination and particularly the signal of meta-hemoglobin, without the need for PC sequences, that are particularly unsuitable for the turbulent flows' evaluation (which are always present in aneurysms and in vascular stenosis).

This benefit allows an optimal evaluation of all patients suffering from vascular disease and presenting intracranial tissues hyperintense in T1, as long as the patient is collaborating since our method has an acquisition time longer than that of the traditional TOF.

We verified that, both in volunteers and in patients suffering from AVM, aneurysms and arterial stenosis, the visualization of normal and pathological vessels were similar both with conventional and subtractive MRA.

Therefore the subtractive technique has been shown to have a diagnostic potential equal to the conventional one, but with the advantage to be adopted for the intracranial circulation visualization also during cerebral bleeding in acute-subacute stage.

Our technique is simple to apply and presents further advantages.

First of all it can be used with any MRA sequence using the time of flight effect, with any MR equipment and with any field strength and magnet's type.

Moreover it can be used both for arteries or veins evaluation and to study each vascular district, even if we believe that probably the intracranial circle is the most suitable for subtractive method since in this district vessel's significant involuntary movements aren't usually present.

Finally, the subtractive technique enables to visualize the vessels with better contrast resolution respect the conventional one, without signal flow loss.

Our results shows that this novel technique allows to obtain diagnostic results as the conventional one, however, with respect to the latter it has the advantage of eliminating the blood's signal, that is characterized by a short T1 relaxation time, and therefore is equal to flow protons. This implies that, during bleeding, cerebral MRA study is poorly diagnostic because both the arteries and the hematoma are simultaneously displayed by overlapping the signal. Subtractive technique we have proposed removes this limitation because is possible to delete the signal of all the static protons, even those hyperintense in T1. Therefore it can be usefully adopted to eliminate the extracellular meta-hemoglobin's signal (parenchymal acute or subacute bleeding or cavernous angiomas), to remove dystrophic tissue hyperintense in T1 (for example, mineralization phenomena in previous laminar cortical necrosis after ischemia), to eliminate artefacts (for example, in aneurysms embolized with Guglielmi's coils) and finally to improve the visualization of vessels adjacent to adipose tissue (for example, the ophthalmic artery).

Finally it is a technique absolutely risk free and free of additional costs not being necessary to administer the contrast medium.

Only disadvantage is a relative long acquisition time, but acceptable by a cooperative patient. This may be a significant limitation and, for this reason, we don't

recommend the extensive use of the new technique in all patients. Its indications are very robust when a bleeding in the subacute phase is found and thus in this case its adoption is strongly recommended. As for the elimination of ferromagnetic artefacts it can be taken only in cases where these are significant and inhibit a correct vessels' visualization. The optimally visualization of intra-orbital vessels is a minor advantage that can only be used in selected cases.

□ CONCLUSIONS

In conclusion our subtractive technique enables to perform a non-invasive study of cerebral circulation in patients with acute-subacute bleeding with no artefacts or other limitations, which are instead present with conventional TOF technique. The subtractive technique also has other advantages, but of lesser clinical impact, over the conventional one (removal of metallic artefacts in embolized aneurysms and optimally visualization of the ophthalmic artery).

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Original article

3 Tesla three-dimensional Fluid Attenuated Inversion Recovery MR imaging in multiple sclerosis

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SUMMARY: Aims. *Magnetic resonance brain evaluation in multiple sclerosis must be made by sequences that enable you to display the largest possible number of lesions. In our study, carried out with a 3 Tesla equipment, we compared a new three-dimensional fluid attenuated inversion recovery sequence with the conventional ones in order to establish whether it can be used, instead of the other, for the multiple sclerosis patients evaluation.*
Materials and methods. *The sequences used were proton density, fast spin echo T2-weighted, two- and three-dimensional fluid attenuated inversion recovery. Preliminarily, we acquired these sequences in 5 healthy volunteers and subsequently in 40 patients with clinically definite multiple sclerosis. The impact of any artefacts in all 4 sequences was evaluated. Afterwards the contrast resolution between lesions and the surrounding tissue was compared among all the sequences. Finally the number of lesions visualized with the 4 sequences was evaluated: both the total number of plaques and partial subgroups, according to lesions location and size.*
Results. *In some cases (6/40) artefacts in three-dimensional fluid attenuated inversion recovery sequences were present both in volunteers and in patients. The contrast resolution was similar between fast spin echo T2-weighted, two- and three-dimensional fluid attenuated inversion recovery, whereas it was significantly lower in the PD images. Finally, as regards the number of lesions displayed, three-dimensional fluid attenuated inversion recovery sequences proved better than the other in all brain locations and especially as regards the small lesions assessment.*
Conclusions. *The three-dimensional fluid attenuated inversion recovery sequences, in 3 Tesla magnetic resonance studies, may be considered as the best choice for the multiple sclerosis patient study.*

KEY WORDS: *Fluid attenuated inversion recovery sequence, Magnetic resonance, Multiple sclerosis.*

INTRODUCTION

Magnetic resonance evaluation of patients with multiple sclerosis should be more accurate and sensitive as possible, either at the onset of clinical symptoms and in subsequent examinations. It is necessary to ensure the proper differential diagnosis with other diseases as the MS clinical debut can often be vague; while in control studies the aim is to assess the lesions evolution and correlate with the clinical aspect or with any residual neurological deficits and to evaluate the therapy results. The morphological data delivered by MR images are particularly important in experimental studies that evaluate the new drugs effectiveness[1,12,13].

For all these reasons it is extremely important to use an examination technique that identifies the maximum number of lesions with the highest resolution.

Fluid attenuated inversion recovery sequence is considered to be the best technique for cortical and

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LIST OF ACRONYMS AND ABBREVIATIONS: **2D** = Two-dimensional; **3D** = Three-dimensional; **CSF** = Cerebral Spinal Fluid; **CR** = Contrast Resolution; **DIR** = Double Inversion Recovery; **FLAIR** = Fluid Attenuated Inversion Recovery; **FOV** = Field Of View; **FSE** = Fast Spin Echo; **IR** = Inversion Recovery; **MPR** = Multi Planar Reformatting; **MR** = Magnetic Resonance; **MS** = Multiple Sclerosis; **PD** = Proton Density; **ROI** = Region Of Interest; S_B = Signal Background; S_I = Signal Intensity; **TE** = Time to Echo; **TR** = Time of Repetition; **TSE** = Turbo Spin-Echo.

periventricular MS lesions evaluation because signal hyperintensity of cerebral spinal fluid is saturated. The FLAIR sequences adoption enabled to assess that in previous years the number of cortical lesions truly presents in MS patients had been underestimated[8,10].
3D FLAIR sequences have recently been introduced into clinical procedures and they enable to obtain quick high contrast-resolution images and ultra-thin slices in all spatial planes[15].
Lately the use of MR unit with the 3 Tesla static magnetic field is spreading even in the hospital setting for clinical use and not only in research centers; as a matter of fact the diagnostic sensitivity and specificity of these equipments in different neurological applications are still in a developmental phase.
Aim. The aim of our work was to carry out a 3 Tesla MR comparative study among different T2-weighted sequences in patients with certainly established multiple sclerosis, in order to assess the best one to obtain a faithful evaluation of brain lesions number, location and morphological aspect.

□ MATERIALS AND METHODS

In the first part of the study 5 healthy volunteers (3 females and 2 males, aged between 25 and 43 years) without neurological symptoms and with no history of neurological diseases were examined, using the 4 sequences of a protocol in order to evaluate the images characteristics especially with regard to the assessment of any artefacts.
Subsequently a prospective study, including 40 patients with clinically definite MS, was performed (24 patients had a relapsing-remitting course of MS, 13 a secondary progressive one and finally 2 patients were affected of primary progressive type of MS); 24 patients were females and 16 males, aged between 22 and 59 years, average age 44.3 years.
Informed consent was obtained for each patient in each examination.
The study was performed according to the World Medical Association Declaration of Helsinki.
Since the study was performed using sequences perfectly compatible with a clinical protocol for the MS without any harm to patients, no ethics board was needed.
The studies were carried out from March 14th 2012 to November 26th 2012, all of them with a 3 Tesla MR Unit (*Magnetom Verio, Siemens*), with 45 mT/m gradients, 200 mT/m/s slew rate, phased array volume coil.
The following sequences were included in the study protocol:
- axial IR fast FLAIR T1-weighted sequence: (TR 2400 ms, TE 9 ms, TI 1001 ms), FOV 230 mm, matrix 230 x 320, slice thickness 4 mm, acquisition time 3:50 minutes;
- axial TSE T2- weighted and PD sequence (TR 2110 ms, TE 11 and 95 ms), FOV 230 mm, matrix 252 x 448, slice thickness 4 mm, acquisition time 3 minutes;
- axial IR (fast FLAIR) T2-weighted sequence (TR 9000 ms, TE 100 ms, TI 2499 ms), FOV 230 mm, matrix 256 x 256, slice thickness 4 mm, acquisition time 3:20 minutes;
- sagittal IR (fast FLAIR) 3D T2-weighted sequence (TR 5000 ms, TE 395 ms, TI 1800 ms), FOV 230 mm, matrix 256 x 256, slice thickness 1 mm, acquisition time 5:50 minutes. In post processing axial and coronal 1 mm MPR images were reconstructed;
- axial and coronal fast FLAIR T1-weighted sequences after contrast media administration (0.2 mmol/kg) were also acquired in all cases.

The total acquisition time of the entire protocol was 15 minutes and 20 seconds.
Two expert neuroradiologists carried out the image evaluation on a workstation (*Syngo VB17, Siemens*).
The inter-observer variability was assessed by calculating "inter-observer agreement index".
The comparative evaluation was assessed among the following sequences:
1. PD;
2. TSE T2-weighted;
3. 2D Fast FLAIR T2-weighted;
4. 3D Fast FLAIR T2-weighted, including also the axial, sagittal and coronal MPR images.

First of all the artefacts impact in all of the sequences was evaluated.
Then the CR of the lesions and the normal tissue

surrounding them, was calculated with the following formula: $(S_I-S_B)/S_B$, where: S_I was the lesion signal intensity value and S_B is the signal intensity value of the perilesional tissue.

For each patient at least three measurements were carried out, using ROI consisting of at least 24 pixels, all of them placed in the same position for each sequence, using an automatic system.

Then the arithmetic average of the CR values of all the ROIs in all patients for each sequence were calculated and a statistical analysis, using t-test, of the difference among the 3D FLAIR sequences average CR value and the other three was carried out. Statistically significant difference was achieved if $p < 0.05$.

Finally the lesions number, shown with the 4 sequences, was evaluated in order to determine which of these sequences was able to identify the greatest number of MS plaques.

Firstly the total lesions number visualized with each sequence was evaluated. Then 6 subgroups according to the lesions site (cortical, subcortical, periventricular, basal ganglia, centrum semiovale white matter and posterior cranial fossa, i.e. cerebellum and brain stem) were extrapolated from the total. Finally other 4 subgroups, based on the size of the lesions: small (3-5 mm), medium (6-10 mm), large (> 10 mm) and confluent, were considered.

□ RESULTS

Healthy volunteers had well tolerated the MR exam without artefacts in PD, TSE T2-weighted and 2D FLAIR sequences. Instead in 3D FLAIR images posterior cranial fossa artefacts have been identified in 1/5 cases. These artefacts appeared as patchy hyperintense lines.

In PD, TSE T2-weighted and 2D FLAIR sequences artefacts have not been detected in all 40 patients; instead, in 6 out of 40 patients, in the 3D FLAIR sequences posterior cranial fossa artefacts (the same as those seen in healthy volunteers) were present.

The "inter-observer agreement index" was 97.6 for PD images, 97.5 for TSE T2-weighted, 98.0 for 2D Fast FLAIR T2-weighted and 97.4 for 3D Fast FLAIR T2-weighted, while as regards the lesion number evaluation the "inter-observer agreement index" was 97.5 for PD images, 97.3 for TSE T2-weighted, 98.1 for 2D Fast FLAIR T2-weighted and 97.6 for 3D Fast FLAIR T2-weighted.

As concerns contrast resolution, in PD sequences quite low values were showed, instead the other three sequences (TSE T2-weighted, 2D and 3D FLAIR) values were very close to each another and considerably higher than the first one (Figure 1).

The statistical analysis showed a statistically significant difference ($p < 0.005$) only between the contrast resolution means of the 3D FLAIR and PD sequences. Contrarily a statistically significant difference ($p > 0.005$) was not proved between 3D FLAIR and TSE T2-weighted sequences and between 3D FLAIR and 2D FLAIR ones (Figure 2).

Finally regarding the overall lesions number displayed with the 4 sequences superiority of 3D FLAIR images over the other ones was observed, especially towards PD and TSE T2-weighted sequences (Figure 3).

Even regarding the lesions number evaluation, related to the site, 3D FLAIR sequences were more diagnostic than the others (Figure 4). In fact the PD sequences exhibit good diagnostic ability for periventricular and centrum semiovale lesions, while they are less diagnostic for cortical and basal ganglia lesions. TSE T2-weighted sequences showed similar results (good diagnostic ability in periventricular and centrum semiovale lesions and less reliability for cortical and basal ganglia lesions) but with better quantitative values compared to PD. Instead regarding 2D FLAIR sequences the findings were nearly always excellent, except for basal ganglia lesions, even better compared with TSE T2-weighted sequences (Figure 4).

Instead as for the number evaluation related to the lesions size, the 3D FLAIR advantage only concerning small lesions was proved, while regarding the medium, large and confluent lesions visualization, overlapped results among the 4 sequences were showed (Figure 5). The prevalence of 3D FLAIR images for little lesions visualization was clear, especially compared with PD and TSE T2-weighted sequences (Figure 5).

According to these results 3D FLAIR images were superior or equivalent to the other sequences as regards the lesions detected; however, this advantage was more clearly seen in certain types of lesions rather than in others.

□ DISCUSSION

Recently the interest for the cortical gray matter lesions has increased considerably according to a

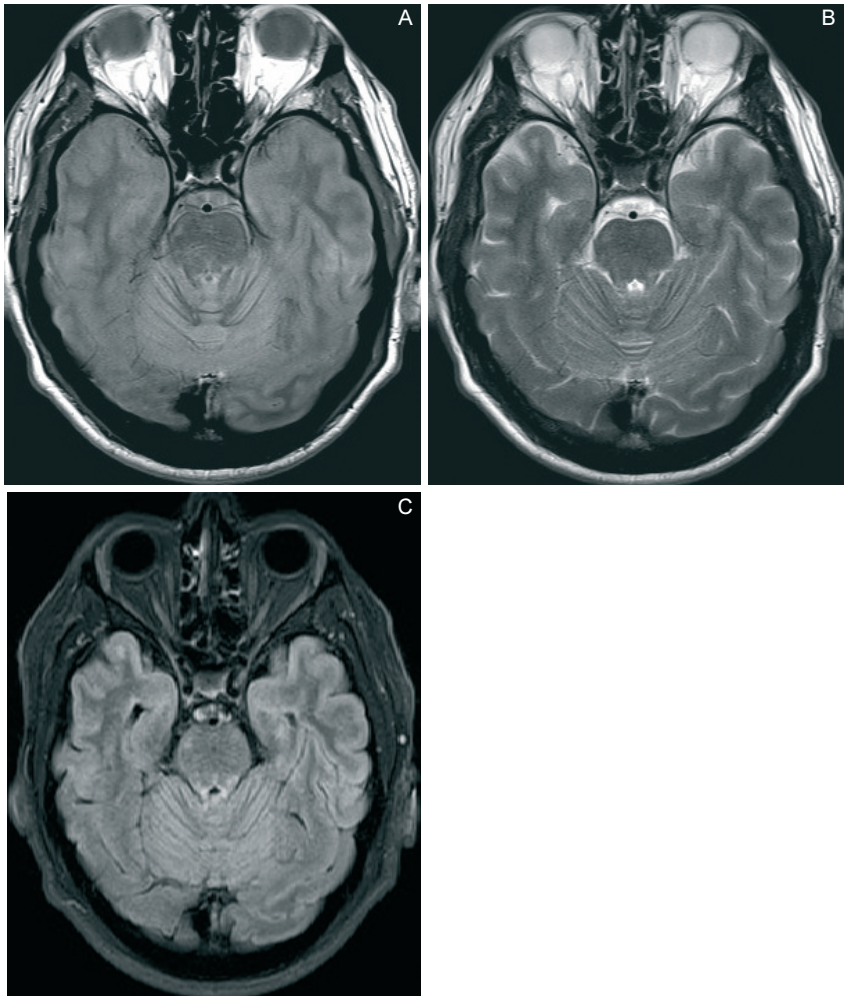

Figure 1. A-D. Artefact. Proton density (A), FSE T2-weighted (B), 2D FLAIR (C) and 3D FLAIR (D) images. Only in 3D FLAIR image the artefact was evident (*arrow*), while the others sequences were free of artefacts.

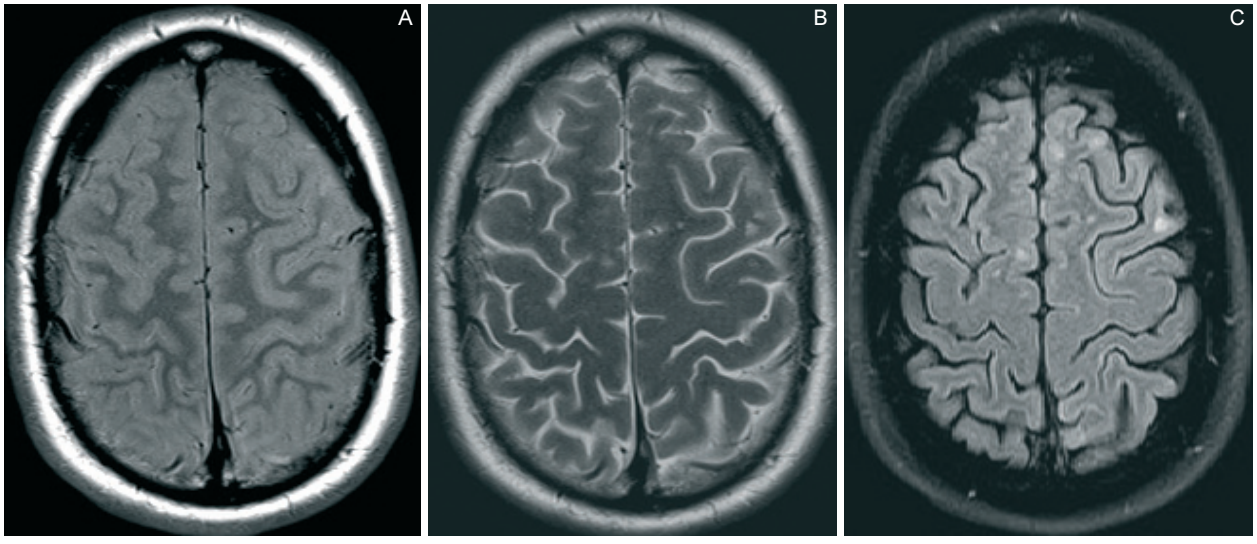

Figure 2. A-D. Cortical MS lesion. Proton density (A), FSE T2-weighted (B), 2D FLAIR (C) and 3D FLAIR (D) images. The cortical lesion, evidenced with the *arrow*, is only visible in the 3D FLAIR sequence (D), while in the other sequences can not be displayed.

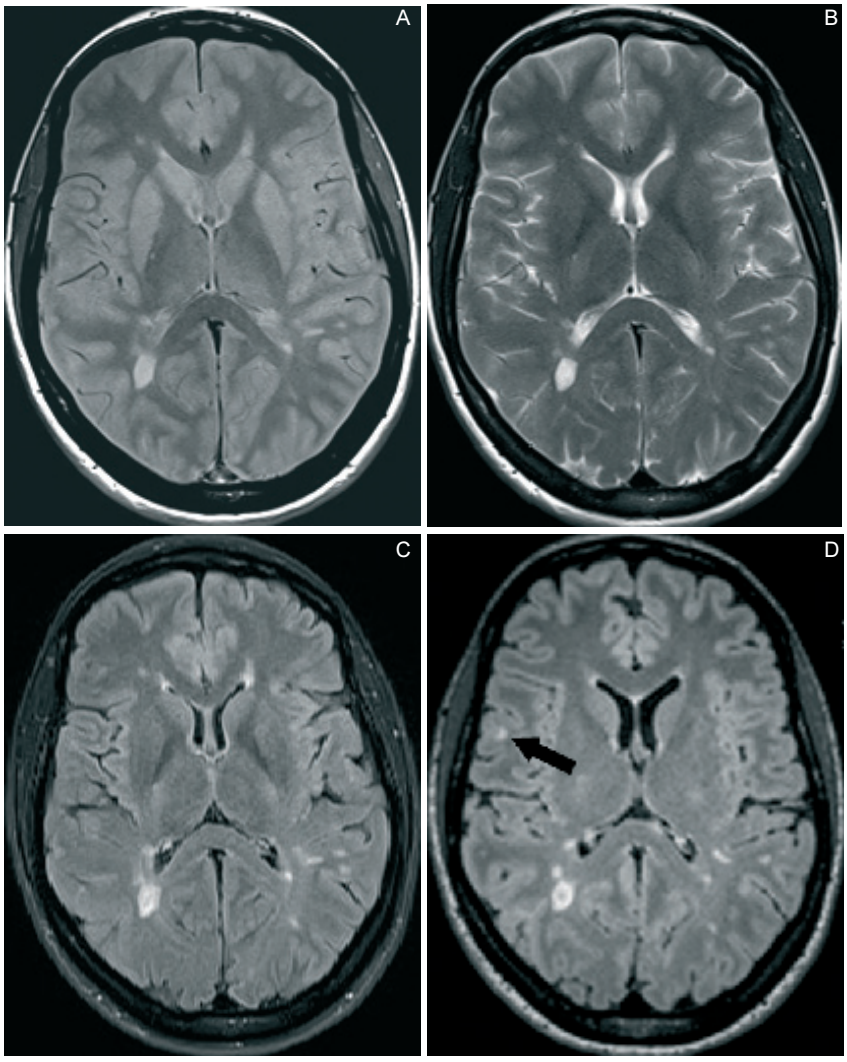

Figure 3. A-D. Subcortical MS lesion. Proton density (A), FSE T2-weighted (B), 2D FLAIR (C) and 3D FLAIR (D) images. Also the subcortical lesion, evidenced with the *arrow*, is only visible in the 3D FLAIR sequence (D), while, even in this case, in the other sequences can not be displayed.

better understanding of MS pathophysiology and evolution and a precise MR visualization of the cortex lesions has become significant[3,9,16].

FLAIR and DIR are the sequences that permit to display better cortical MS lesions, because they are the ones with the best contrast resolution between the lesions and surrounding tissues[8,10,19]. In a recent study, however, it has been shown that the DIR sequences are often inaccurate with regard to the correct identification of cortical lesions[18]. In addition to this, as thin as possible scans must be used because cortical MS lesions are often small. FLAIR sequences, unlike the DIR, can also be acquired with 3D technique, with the advantage to get then ultrathin multiplanar scans (even less than 1 mm.) with high CR; for this reason it is reasonable to assume that this type of sequence is very effective for the detection of gray matter lesions, as well as for the identification of all other lesions located in other brain areas as already well known from previous studies in literature[4,17].

Moreover, since the static magnetic field strength is directly proportional to the signal intensity, generated with a sequence, it is logical to assume that diagnostic gain, using a 3 Tesla MR equipment, can be obtained with lower magnetic fields units, for multiple sclerosis patients' brain lesions evaluation[21].

Furthermore some safety guidelines recommend not to expose the patient to 3 Tesla magnetic field for more than an hour at once. For this reason it is necessary to optimize MR imaging of multiple sclerosis patients in order to obtain relevant information to assess the disease status and its possible evolution in the shortest time possible[14].

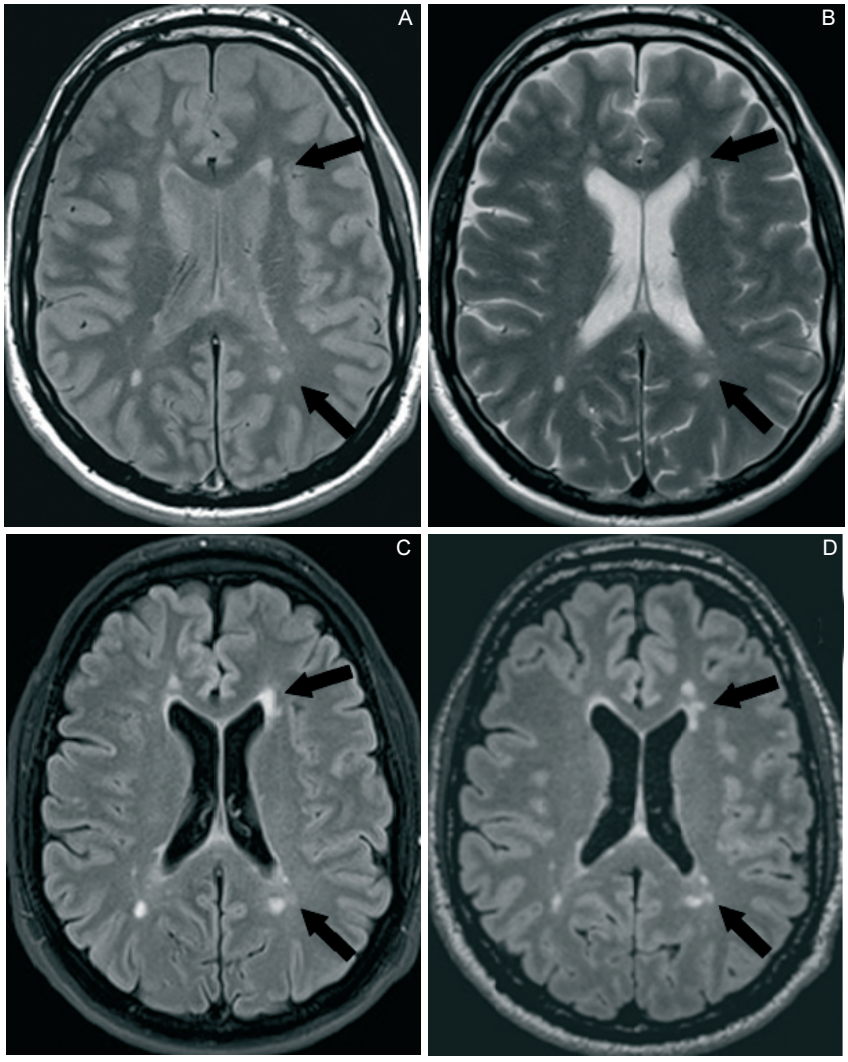

Figure 4. A-D. Periventricular MS lesions. Proton density (A), FSE T2-weighted (B), 2D FLAIR (C) and 3D FLAIR (D) images. Only the thin slice 3D FLAIR image (D) displays optimally periventricular lesions (*arrows*).

Although in the last years many articles were published regarding the use of advanced MRI techniques (for example, spectroscopy and diffusion imaging) the imaging protocols for the study of multiple sclerosis, for the disease diagnosis and for the anatomical evaluation over the time are still based on morphological MRI sequences[1]. FLAIR is the best MRI sequence to define the number, morphology, and lesion load in multiple sclerosis plaques. In fact, due to the CSF signal saturation of both cisters and cerebral ventricles, the FLAIR sequence, is highly sensitive to the detection of brain lesions especially in the periventricular and cortical districts(10). Furthermore, in order to avoid partial volume artefacts and in order to properly define the lesions morphology, which often are contiguous to each other, it is necessary to use images with layer thickness as thin as possible[4,17]. The conventional 2D sequences however have a limit, the signal / noise ratio decreases with the use of thin-layer sequences, so ultra-thin scans cannot be used. On the contrary, the 3D sequences do not have this limitation because the signal is generated from all the acquired volume and the final images are obtained in postprocessing. That's why with the latter technique can be obtained with ultra-thin scans (even a millimetre) without penalizing the signal to noise ratio[15]. Moreover, the 3D sequences allow to obtain multi-planar reconstructions also on coronal and sagittal planes only in postprocessing, with a single data acquisition. For this reason the FLAIR sequences (characterized by high contrast resolution) acquired with 3D technique (characterized by high spatial resolution) might represent the gold standard to evaluate the brain in multiple sclerosis patients, both at

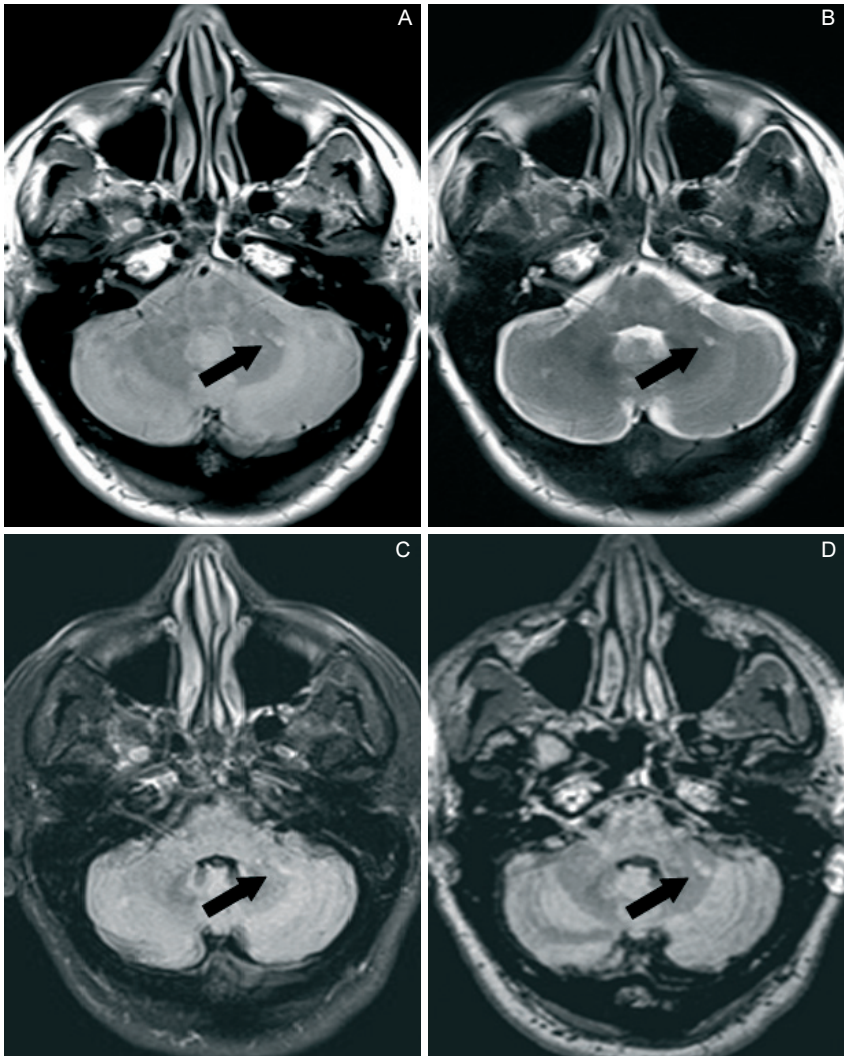

Figure 5. A-D. Cerebellar MS lesions. Proton density (A), FSE T2-weighted (B), 2D FLAIR (C) and 3D FLAIR (D) images. Also in this case only the thin slice 3D FLAIR image (D) displays optimally the lesions (*arrows*).

the time of first diagnosis and in controls over the time. Finally, the third element of our study protocol creates a framework for considering the technique tested by us as optimal for the morphological study of multiple sclerosis using a 3 Tesla MR equipment. It is known that the static magnetic field intensity, in a MR unit, is directly proportional to the signal intensity generated by any sequence[21]. For this reason the use of high field equipment improves the sensitivity and diagnostic specificity in multiple sclerosis and now considered an essential element for the study of the disease, as demonstrated by numerous studies in the literature[7,11,21-23].

So there are all theoretical assumptions to account for the 3D FLAIR sequences, acquired with 3 Tesla equipment, as ideal for morphological evaluation of multiple sclerosis plaques.

Our protocol (lasting 15 minutes) was well tolerated by all patients with a low incidence of voluntary motion artefacts. A check of the 3D FLAIR sequence characteristics was carried out through the comparison with the standard sequences that currently are used for the MS' study.

Our study results have shown that 3D FLAIR sequences are superior to the others to identify MS plaques in all anatomical areas of the brain but especially in cortical areas; furthermore they have been proven to be particularly useful for the detection of small lesions.

The main 3D sequences advantage, is therefore the possibility to obtain, with a single acquisition, high resolution brain images in all planes of space. Also in the longitudinal evaluation of patients the comparison among examinations, carried out at different times, is

easier. In fact, the final images obtained with the 3D sequences can be aligned through post-processing techniques, with a best tolerance to possible scanning planes positioning errors[20].
The possibility to obtain images of studies carried out at different times, with the same spatial orientation and located in the same cranial-caudal position, allows the comparison among them by means of overlapping and digital subtraction techniques that allow to visualize immediately any differences in lesion load in the same patient[20].
The 3D sequences, compared to 2D acquisitions, can also have some disadvantages. The first is the need for a greater space for data archiving. In addition, the larger number of image processing, takes a longer time reporting than 2D sequences. Finally, the 3D sequence acquisition time is a little longer than a 2D one.
It would be in any case easy to overcome disadvantages that do not limit the use of these sequences, if they were accepted as the gold standard for MS.
The available studies in literature agree that the FLAIR sequences (both 2D and 3D) are superior to TSE T2-weighted for the assessment of cortical lesions, while in some of them it is highlighted a limitation with regard to the evaluation of subcortical lesions[2,5,6,10,15]. In fact, the 3D FLAIR sequences are characterized by a lower contrast resolution between gray and white matter compared to the 2D TSE T2w and this may prevent a proper display of the lesions[2,5,6,10,15]. However, in our study we showed that even in the subcortical, 3D FLAIR sequences are superior to others.
Finally an important drawback, related to the use of 3D FLAIR sequences, that we identified in our study is the occasional presence of artefacts in the posterior fossa. However, despite this limitation, also for the evaluation of the posterior cranial fossa lesions, 3D FLAIR sequences have proved better than conventional.

□ CONCLUSIONS

Based on our study results, the 3D FLAIR sequence MRI at 3 Tesla allows to display a greater number of MS lesions compared with conventional sequences. There is no difference among the various brain anatomical areas, as in all areas the superiority of the 3D FLAIR sequences has been demonstrated. This sequence is also characterized by an acquisition time fully tolerated by all patients. It therefore represents an essential diagnostic tool for the MS diagnosis in the first MR examination and is also the optimal sequence for disease temporal evolution evaluation of the brain.

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Article

Interpretation of modern art masterpieces: no motor reflection

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SUMMARY: *In the article we present conceptual counter-arguments to the embodiement role claim, even when motor areas of the brain are activated and, as a pilot case, resume and reproduce the experiment at the base of one of the seminal work about mirror neurons and neuroaesthetics, slightly modifying its measurement protocol and considerably increasing its statistical population. This new study suggests that the aesthetic experience is so strongly affected by cultural and experiential backgrounds of the beholder that somato-motor resonance effects, if any, seem to be undetectable and, so far, unprovable. Recent trends in neuroaesthetics postulate a nexus between dramaticity, sense of movement, in static works of visual art, beholder's aesthetic experience and embodied simulation mechanisms, the rationale being an asserted twofold motor resonance induced in the observer by the dynamic content of the works and by recognizable traces of the artist's creative gestures. Trying to cope with the effects of the subjective cultural conditioning, some pioneering studies have focused on the beholder's differential response to works of abstract art compared to less motor-evocative, computer-made images. Using the same method reported by Umiltà et al. (2012) in Frontiers in Human Neuroscience, as a major result, those investigations don't contradict the embodied simulation hypothesis but they also don't prove it definitively. Here the authors present conceptual counter-arguments to the embodiement role claim, even when motor areas of the brain are activated and, as a pilot case, resume and reproduce the experiment at the base of one of the seminal work, slightly modifying its measurement protocol and considerably increasing its statistical population. This new study suggests that the aesthetic experience is so strongly affected by cultural and experiential backgrounds of the beholder that somato-motor resonance effects, if any, seem to be undetectable and, so far, unprovable.*

KEY WORDS: *Embodied simulation, Experiment, Falsification, Mirror neurons, Neuroaesthetics.*

INTRODUCTION

Apart from their possible top-down relationships, theoretical neuroaesthetics[19,23], embodied simulation[9] and mirror neuron system[20] share several common points as cognitive paradigms in that, they all try to put in relation neurophysiological evidence with superior concepts which, from the bottom up, can be summarized as action goal understanding (assuming neuronal motor resonance), building-up of high level mental constructs like empathy and language (assuming cognitive representations that are bodily rooted in the motor and perceptual system) and aesthetic experience (assuming balanced network cooperation involving functionally specialized areas of the brain). Also, all these three theories are quite

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LIST OF ACRONYMS AND ABBREVIATIONS: **ANOVA** = Analysis Of Variance; **EEG** = ElectroEncephaloGram; **EMG** = Electro-MyoGraphy**; F** = F ratio; **HSD** = honestly significant difference; **MNS** = Mirror Neuron System; **MS** = Mean squares.

recent; they face similar epistemological problems, exemplified by the difficult applicability of the falsification criterion[5,12,15,18]; finally, they are trendy due to the apparent simplicity of the mechanisms they postulate.

In 2007, pivoting on the concept of empathy, a seminal work[7] explicitly connected for the first time neuroaesthetics, embodied simulation and MNS. In that occasion two major ingredients where claimed to participate in the build up of the aesthetic experience in front of visual works of art: first, "the relationship between embodied empathetic feelings in the observer and the representational content of the works" (*sic*); second, "the relationship between embodied empathetic feelings in the observer and the quality of the work in terms of the visible traces of the artist's creative gestures" (*sic*). While that work "did not suggest that the activation of mirror or canonical neurons was sufficient for esthetic appraisal or for judgments about artworks"[2,7], nevertheless it put embodied simulation at center stage, differentiating between "aesthetic appraisal", "aesthetic attitude", "aesthetic experience" (where embodiment should occur) and "aesthetic judgment"[1,6].

In the wake of such claims and in an attempt to uncouple as much as possible cultural and experiential factors from those ones attributed directly to the embodiement mechanism, subsequent investigations concerned the case of non-figurative art or of comparable visual works, for which one could expect a sharpest evidence for at least the second, supposed, ingredient, that is a motor resonance evoked in the beholder by the traces left by the artist in her creative act (affecting, for instance, brushworks style, patterns or trajectories). In this line of research, here are recalled three significant researches that deal with the differential experience that could arise during the observation of both true hand-made visual works and some not human reproductions of them. The first one[22], in the following referred as the "reference work", focused on artworks of the artist Lucio Fontana, compared with some simplified computer-graphics replicas; in this case up to 14 volunteers, exposed to random sequences of originals and simplified copies, were recorded by means of EEG, EMG and an ad-hoc questionnaire; following ANOVA calculations showed significant correlation between originality of the image, activation of motor related area of the brain and subjective perception of "amount of movement" inside the image and its "artistic nature". The second investigation[4] focused on robot-made abstract drawings and their hand-made counterparts made by a sculptor and by a computer-graphics artist; differentiating from images with salient kinematic cues or not (based on the presence of geometrical shapes that are hard to naturally reproduce by hand, as the case of complete circles), ANOVA calculations concerned the answers of 12 volunteers about the guessed human or robotic nature of the sketcher; in this case the correct recognition of the maker type was found to be highly correlated to the absence of geometric salient cues but, even if at a minor extent, also to the presence of subtle kinematics cues (such as smudging in the sketch). In a similar fashion, but in a slightly different context, the third investigation here recalled[16] focused on the recognition of hand-written and typed alphabet letters; in that case, measurements on 11 volunteers clearly showed correlation between changes in the MEG oscillatory activity originating from the motor cortex and changes in the nature of the displayed letters.

All these three investigations appear to show an enhanced activation of motor related areas of the brain when the observer is exposed to clearly hand-made works and they seem not to rule out a possible role for the embodiement mechanism in the aesthetic experience. Nevertheless, till now no satisfactory and uncontroversial explanation has been advanced for the operating details of this mechanism. Even worst, a quite lively scientific community disagrees also with some core claims of the embodied simulation and MNS theories themselves[3,10,14,16].

On the basis of experimental, conceptual and epistemological issues, the author endorses this criticism and he highlights two major problems with embodiement theories. First, low level neural mirroring and high level cognitive experiences belong to different domains that can relate to each other only through matching functions that till now no one has been able to detail. Second, even if many of the pertinent claims seem to rely on experimental results, they appear to fail or at least ignore falsification methods (even when in weak form).

(For a better comprehension of the problem the reader can be see a similar experiment[17] where "The Adoration of the Mystic Lamb" of Jan van Eyck and "Concetto spaziale" of Lucio Fontana are compared on the basis of the theory of mirror neurons, the first, on the basis of simple neuronal plasticity, the second). In order to submit the hypothesis of the embodied aesthetic experience to a falsification test, the author performed an independent verification of the results obtained in the reference work. Pivot of this current investigation is the possibility that the cultural and experiential attitude of the beholder could overwhelm any motor attributable mechanism in her aesthetic experience (rationale: if these were the case, the claim of the embodied simulation applied to art would have been yet to be proven).

In this new research only the questionnaire survey was considered, although in a slightly modified version, while special care was taken of the selection of a wider population of volunteers, differentiated by their personal background. Instead, no EEG or EMG recordings were taken, due to their squareness to the scope of this work and the above cited controversial relationship between such measurements and the true role of mirroring mechanisms. This experiment takes for example in its methods the seminal works of Parma's Group to allow us to falsify them really; otherwise the work would have expressed conclusions but not the falsification of previous ones'. As a major result, this work clearly shows the importance of the cultural and experiential attitude of the beholder in hiding any supposed effect due to empathetic motor resonance with the artwork and, through it, with the creative act of the artist.

□ METHODS

■ **Participants.** Two groups of volunteers participated in the experiment. The first one included ninety-six healthy subjects, equally represented by gender and of comparable age (mean: 18.03 years), coming from different high schools according to an equal partition between art students, building surveyor students, mechanical students and students of professional institutes, the latter ones (vocational students) without specific skills in art and design; in detail: 24 students, twelve female and twelve male, for each school type. The second group included fourteen healthy subjects (seven females and seven males, mean age: 28.28 years) recruited with no explicit care to their cultural background but in analogy with the protocol followed in the reference work.

The study was ethically approved by the managements/ethical commitees of all the high schools involved and of the University of Udine; all experiments were performed in accordance with relevant guidelines and regulations; informed consent was obtained from all participants; all the collected data (questionnaires, recordings, images) was processed and stored in a strictly anonymous way, irreversibly hiding the identity of the involved subjects.

■ **Procedure.** Apart some improvements, highlighted in the following, the experimental protocol was a strict replica of the one exhaustively described in the reference work. Accordingly, participants were exposed to random sequences of abstract images displayed on a 60 cm far, 17-inch size screen. Each image (stimulus) was shown for 1000 ms preceded by a start marker (a sub-sequence consisting of a 4500, 4000 or 5500 ms lasting black background, anticipating a 450, 500 or 550 ms lasting attention symbol) and it was followed by a 500 ms lasting stop marker. After each stimulus was shown, participants were asked to score it according to: "Q1 familiarity" with the image (semantic differential range: [0,10]); "Q2 aesthetic appraisal" of the image (range: [-10,10]); "Q3 amount of movement" perceived in the image (range: [0,10]); "Q4 artistic nature" of the stimulus (that is, is the image a true artwork? - range: ["no","yes"]). In addition to what was done in the reference work, an open-answer question was added to let the subjects freely express their impressions, sensations and comments. In the reference work the images were selected so as to represent two classes of stimulus. The first class (original stimulus) was featured by 3 black and white, high resolution digitized images of different artworks of Lucio Fontana (one, two and three physical cuts on light color canvasses); the second one (control stimulus) was featured by 3 black and white, high resolution digitized images of graphically modified and simplified versions of the original artworks (an example of a paired stimuli concept is depicted in Figure 1). These stimuli (each one displayed 15 times in a randomly shuffled manner) were adopted also in this work but here they were integrated by additional pairs of original paintings of abstract art and control counterparts. The new entries where excerpts from: "Convergence" by Jackson Pollock (1912-1956), coupled with "Excavation" by Willem De Kooning (1904-1997) (pairing criterion: paintings that are similar in colors and

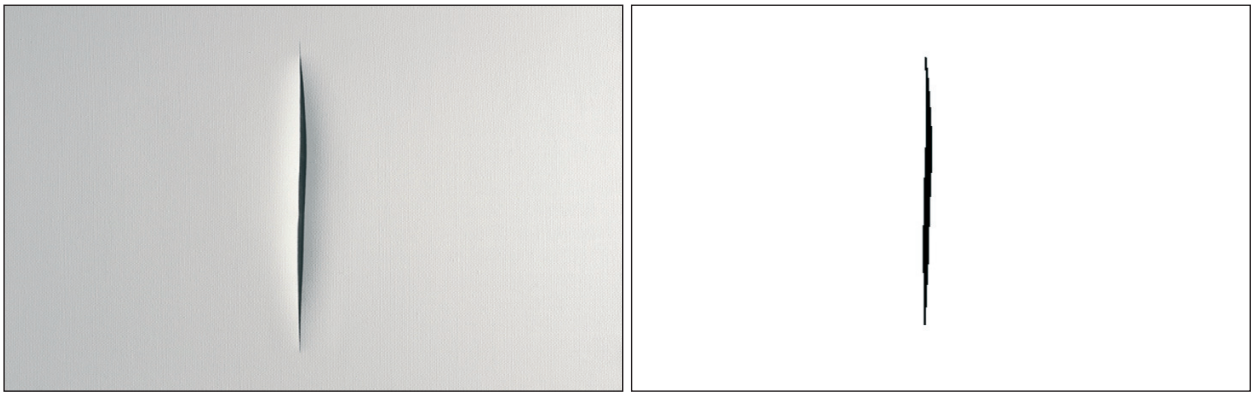

Figure 1. Original and control stimulus. Example of stimuli pair for a Fontana's artwork. *On the left*: original stimulus; *on the right*: smoothed control stimulus.

shapes but made impulsively the first one and quietly the second one); "Number 11" by Jackson Pollock, coupled with a false Pollock (pairing criterion: similar paintings made in different techniques); "Number 14" by Jackson Pollock, coupled with an inkblot pattern by Hermann Rorschach (1884-1992) (pairing criterion: dominance of white and black).

This choice of artworks (Fontana's and Pollock's) was driven by their recurrent pairing within abstract art research and critique, their supposed connection to empathy as stated in one of the seminal works on neuroaesthetics[8] and, as for Pollock, their ability to convey structured information like fractal patterns[11]. The actual stimuli for the Fontana's case are depicted in Figure 1 of the reference work; those one for the Pollock's case are shown in Figure 2 of this work.

■ **Statistical analysis.** After a preliminary tuning analysis, all differential semantic scores were normalized to boolean values, according to the following mappings: for "Q1 familiarity", logical true values were set on scores greater than or equal to 3, as in the reference work; for "Q2 aesthetic appraisal", true values were set on scores greater than 0; for "Q3 amount of movement", true values were set on scores greater than or equal to 3 (answers to "Q4 artistic nature" were already gathered in boolean form). A brief summary of the collected data is given in Table 1 as well as in Figure 3.

Answers to the "Q1 familiarity" question were studied first, also due to the focus given to them in the reference work. While in the present case about 40% of the people declared to be somewhat familiar with the shown artworks, open form remarks provided by the respondents highlighted that, when asserted, this acquaintance was often far from any direct artistic discourse. For instance, Fontana's cuts sometimes evoked female silhouettes (especially in male, aged eighteen, students), blades of grass or simple just another sample of broken fabric: in other words, not really art but somewhat one can experience almost every day. Due to its poor selectivity within the scope of this research, familiarity was thus discharged as a not significant category; instead, in this work the influence of the subjective cultural backgrounds was studied through the lens of the different school specializations.

Accordingly, participants were sorted to form a category (people) explicated by six groups, namely: art students, mechanical students, surveyor students, vocational students (from professional schools), aggregate students (that is, all 96 students) and finally the control, undifferentiated group (14 subjects, aged 28 on average). A second, category (target) was defined according to the nature of the artworks displayed, resulting in four groups: Fontana's original stimuli, synthetic replicas of Fontana's original (control stimuli), Pollock's original stimuli and counterparts to Pollock's originals (control stimuli). A last category (topic) was defined according to which question was asked to the participants, resulting in three groups ("Q2 aesthetic appraisal", "Q3 amount of movement" and "Q4 artistic nature"). Our analysis focused on the role and interactions of these three categories when coupled in a pair-wise fashion as in people versus target and in people versus topic. The statistical analysis consisted in a batch of two-way ANOVA's ($p \leq 0.05$), each one accompanied by pertinent post-hoc Tukey HSD tests (here preferred to the less conservative Newman-Keuls comparisons used in the reference work).

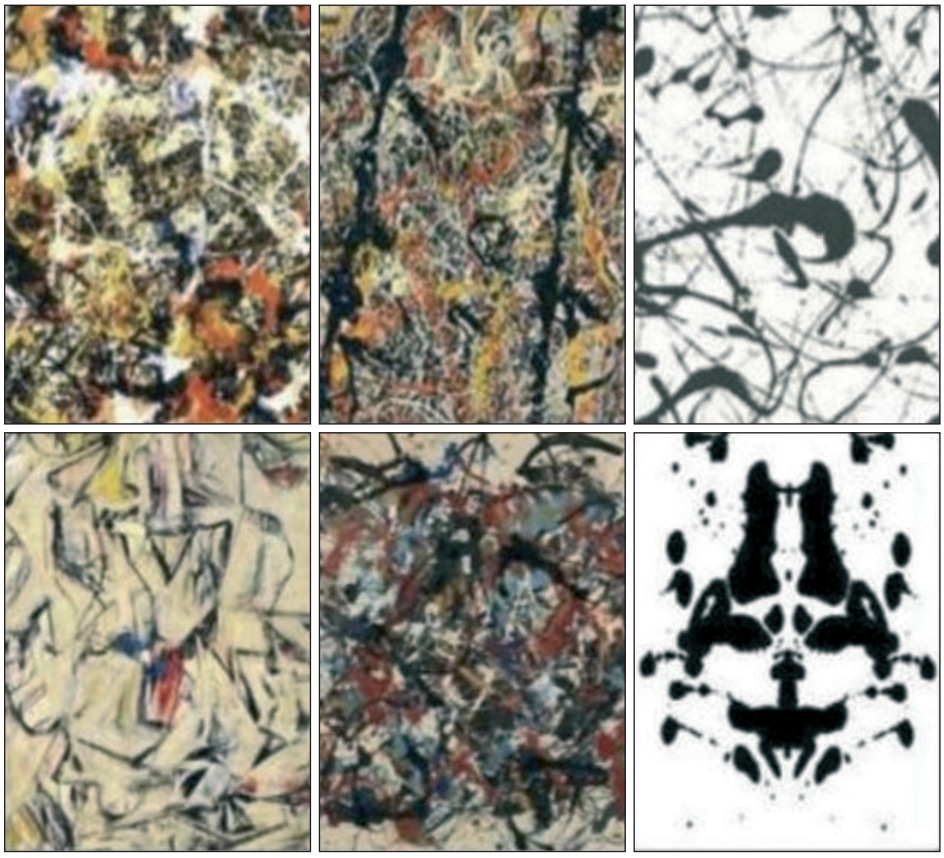

Figure 2. Stimuli around Pollock's artworks. *Upper row:* original stimuli; from the left to the right: details from "Convergence", "Number 11", "Number 14". *Lower row*: control stimuli; from the left to the right: details from "Excavation" by Willem De Kooning, false Pollock, inkblot pattern by Hermann Rorschach.

□ RESULTS

■ **Generality.** For the reader's convenience, this work details only a selection of the obtained results: first, outcomes regarding the aggregate students and the control group are not shown due to their strongly uncorrelated response against the various questions and due to the low nvalue for the control group (here introduced for an assessment of this aspect as addressed in the reference work); second, when people *versus* target is of concern, Tukey test results are reported only when significant variation was obtained for the same people group on different target groups (that is, people intragroup results are not shown in the following); finally, only significant variations ($p \leq 0.05$) are reported; anyway, almost no pvalue was found within the neighboring interval [0.05, 0.10].

■ **Test 1.** ***Amount of movement, Fontana's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target.* Two groups:
- 1 = Fontana's original stimuli,
- 2 = Fontana's control stimuli.

❍ *Q3*. Amount of movement:
- significant variation at: target ($F(1,8632) = 10.02$, $MS = 1.81$, $p = 0.002$);
- significant variation at: people ($F(3,8632) = 414.58$, $MS = 74.81$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 58.86$, $MS = 10.62$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean difference $= -0.05$, $p < 0.001$);
- significant Tukey post-hoc test for: mechanical students group (mean difference $= 0.27$, $p < 0.001$).

■ **Test 2.** ***Sesthetic appraisal, Fontana's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:

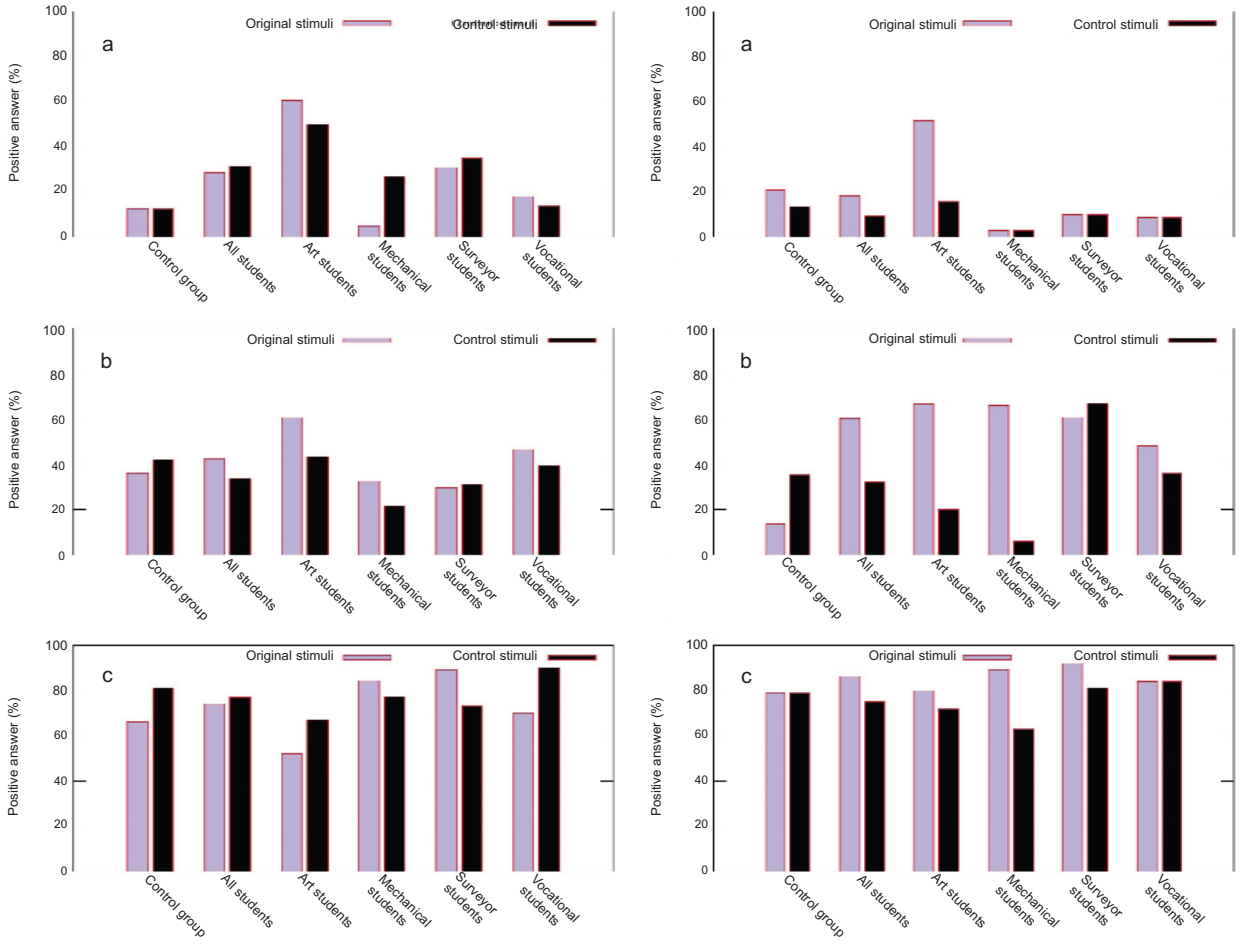

Figure 3. Questionnaire survey summary. Left column: Fontana's case; Right column: Pollock's case. *Legend*: a = perception of movement; b = artistic appraisal; c = recognition of artistic nature.

- 1 = Fontana's original stimuli,
- 2 = Fontana's control stimuli.

❍ *Q2*. Aesthetic appraisal:
- significant variation at: target ($F(1,8632) = 68.41$, $MS = 15.25$, $p < 0.001$);
- significant variation at: people ($F(3,8632) = 129.63$, $MS = 28.90$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 14.61$, $MS = 3.26$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean difference = - 0.11, $p < 0.001$);
- significant Tukey post-hoc test for: mechanical students group (mean difference = - 0.05, $p < 0.001$);
- significant Tukey post-hoc test for: vocational students group (mean difference = - 0.01, $p = 0.013$).

■ **Test 3.** ***Perception of artistic nature, Fontana's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = Fontana's original stimuli,
- 2 = Fontana' control stimuli.

❍ *Q4*. Artistic nature:
- significant variation at: target ($F(1,8632) = 12.37$, $MS = 2.11$, $p < 0.001$);
- significant variation at: people ($F(3,8632) = 145.86$, $MS = 24.86$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 96.04$, $MS = 16.37$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean difference = 0.20, $p < 0.001$);
- significant Tukey post-hoc test for: mecha-

nical students group (mean difference = - 0.02, p = 0.002);
- significant Tukey post-hoc test for: surveyors students group (mean difference = - 0.11, p < 0.001);
- significant Tukey post-hoc test for: vocational students group (mean difference = 0.26, p < 0.001).

■ **Test 4.** ***Amount of movement, Pollock's case.***
❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = Pollocks's original stimuli,
- 2 = Pollocks's control stimuli.

❍ *Q3*. Amount of movement:
- significant variation at: target (F(1,8632) = 175.90, MS = 17.07, p < 0.001);
- significant variation at: people (F(3,8632) = 413.30, MS = 40.10, p < 0.001);
- significant variation at: target&people (F(3,8632) = 175.90, MS = 17.07, p < 0.001);
- significant Tukey post-hoc test for: art students group (mean difference = - 0.31, p < 0.001).

■ **Test 5.** ***Aesthetic appraisal, Pollock's case.***
❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = Pollocks's original stimuli,
- 2 = Pollocks's control stimuli.

❍ *Q2*. Aesthetic appraisal:
- significant variation at: target (F(1,8632) = 844.70, MS = 169.46, p < 0.001);
- significant variation at: people (F(3,8632) = 157.50, MS = 31.59, p < 0.001);
- significant variation at: target&people (F(3,8632) = 252.10, MS = 50.57, p < 0.001);
- significant Tukey post-hoc test for: art students group (mean difference = - 0.41, p < 0.001);
- significant Tukey post-hoc test for: mechanical students group (mean difference = - 0.54, p < 0.001);
- significant Tukey post-hoc test for: surveyors students group (mean difference = 0.12, p = 0.018);
- significant Tukey post-hoc test for: vocational students group (mean difference = - 0.06, p < 0.001).

■ **Test 6.** ***Perception of artistic nature, Pollock's case.***
❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = Pollocks's original stimuli,
- 2 = Pollocks's control stimuli.

❍ *Q4*. Artistic nature:
- significant variation at: target (F(1,8632) = 184.62, MS = 27.34, p < 0.001);
- significant variation at: people (F(3,8632) = 43.52, MS = 6.44, p < 0.001);
- significant variation at: target&people (F(3,8632) = 44.90, MS = 6.65, p < 0.001);
- significant Tukey post-hoc test for: art students group (mean difference = - 0.03, p < 0.001);
- significant Tukey post-hoc test for: mechanical students group (mean difference = -0.21, p < 0.001);
- significant Tukey post-hoc test for: surveyors students group (mean difference = - 0.05, p < 0.001).

■ **Test 7.** ***Amount of movement vs. aesthetic appraisal, Fontana's case.***
❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = amount of movement,
- 2 = aesthetic appraisal (Fontana's originals).

❍ *Q4*. Artistic nature:
- significant variation at: target (F(1,8632) = 205.40, MS = 40.15, p < 0.001);
- significant variation at: people (F(3,8632) = 347.19, MS = 67.87, p < 0.001);
- significant variation at: target&people (F(3,8632) = 76.26, MS = 14.91, p < 0.001);
- significant Tukey post-hoc test for: mecha-

nical students group (mean difference = 0.33, $p < 0.001$);
- significant Tukey post-hoc test for: vocational students group (mean difference = 0.34, $p < 0.001$).

■ **Test 8.** ***Amount of movement vs. perception of artistic nature, Fontana's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = amount of movement,
- 2 = artistic nature (Fontana's originals).

❍ *Q4*. Artistic nature:
- significant variation at: target ($F(1,8632) = 2666.12$, $MS = 444.60$, $p < 0.001$);
- significant variation at: people ($F(3,8632) = 86.27$, $MS = 14.40$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 462.21$, $MS = 77.10$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean difference = - 0.03, $p < 0.001$);
- significant Tukey post-hoc test for: mechanical students group (mean difference = 0.85, $p < 0.001$);
- significant Tukey post-hoc test for: surveyors students group (mean difference = 0.64, $p < 0.001$);
- significant Tukey post-hoc test for: vocational students group (mean difference = 0.57, $p < 0.001$).

■ **Test 9.** ***Amount of movement vs. aesthetic appraisal, Pollock's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = amount of movement,
- 2 = aesthetic appraisal (Pollock's originals).

❍ *Q4*. Artistic nature:
- significant variation at: target ($F(1,8632) = 2202.90$, $MS = 380.90$, $p < 0.001$);
- significant variation at: people ($F(3,8632) = 229.40$, $MS = 39.70$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 129.70$, $MS = 22.40$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean difference = 0.20, $p < 0.001$);
- significant Tukey post-hoc test for: mechanical students group (mean difference = 0.68, $p < 0.001$);
- significant Tukey post-hoc test for: surveyors students group (mean difference = 0.56, $p < 0.001$);
- significant Tukey post-hoc test for: vocational students group (mean difference = 0.45, $p < 0.001$).

■ **Test 10.** ***Amount of movement vs. perception of artistic nature, Pollock's case.***

❍ *People*. Four groups, students only:
- 1 = art,
- 2 = mechanical,
- 3 = surveyors,
- 4 = vocational.

❍ *Target*. Two groups:
- 1 = amount of movement,
- 2 = artistic nature (Pollocks's originals).

❍ *Q4*. Artistic nature:
- significant variation at: target ($F(1,8632) = 8817.30$, $MS = 1002.50$, $p < 0.001$);
- significant variation at: people ($F(3,8632) = 162.60$, $MS = 18.50$, $p < 0.001$);
- significant variation at: target&people ($F(3,8632) = 340.70$, $MS = 38.70$, $p < 0.001$);
- significant Tukey post-hoc test for: art students group (mean diff = 0.33, $p < 0.001$);
- significant Tukey post-hoc test for: mechanical students group (mean difference = 0.91, $p < 0.001$);
- significant Tukey post-hoc test for: surveyors students group (mean difference = 0.86, $p < 0.001$);
- significant Tukey post-hoc test for: vocational students group (mean difference = 0.80, $p < 0.001$).

□ DISCUSSION

Before any comment about our results, it is important to note that the questions was always in the same order: Q1-Q4. We know that is problematic because there could be order effects. Answering the earlier questions may impact one's answering of the later

Artist	Topic	Stimuli	Control group	All students	Art students	Mechanical students	Surveyor students	Vocational students	Mean	Std dev
Fontana	*Perception of movement*	Original	12.5	28.6	60.5	5.0	31.0	18.0	25.9	19.5
		Control	12.5	31.5	50.0	27.0	35.0	14.0	28.3	14.0
	Artistic appraisal	Original	36.0	42.3	60.5	32.5	29.5	46.5	41.2	11.3
		Control	42.0	33.9	43.5	21.5	31.0	39.5	35.2	8.3
	Perception of artistic nature	Original	66.0	74.0	52.0	84.5	89.5	70.0	72.7	13.4
		Control	81.5	77.1	67.0	77.5	73.5	90.5	77.9	7.9
	Mean	Original	38.2	48.3	57.7	40.7	50.0	44.8		
		Control	45.3	47.5	53.5	42.0	46.5	48.0		
	Std dev	Original	26.8	23.3	4.9	40.4	34.2	26.0		
		Control	34.6	25.7	12.1	30.9	23.5	39.0		
Pollock	*Perception of movement*	Original	21.0	18.3	51.5	3.0	10.0	8.5	18.7	17.4
		Control	13.5	9.4	16.0	3.0	10.0	8.5	10.1	4.5
	Artistic appraisal	Original	13.5	60.3	66.5	66.0	60.5	48.0	52.5	20.2
		Control	35.5	32.3	20.0	6.0	67.0	36.0	32.8	20.3
	Perception of artistic nature	Original	79.0	86.4	80.0	89.5	92.0	84.0	85.1	5.2
		Control	79.0	75.1	72.0	63.0	81.5	84.0	75.8	7.6
	Mean	Original	37.8	55.0	66.0	52.8	54.2	46.8		
		Control	42.7	38.9	36.0	24.0	52.8	42.8		
	Std dev	Original	35.8	34.4	14.3	44.7	41.4	37.8		
		Control	33.3	33.4	31.2	33.8	37.8	38.2		

Table 1. Percentage of positive answer to questionnaire survey (after normalization of all semantic differentials to boolean values ["no","yes"]). *Legend:* Mech. = Mechanical; Voc. = Vocational; Std. Dev. = standard deviation.

questions. The order of the questions was not randomized, but they were the criteria used in the paper that we are challenging. We used change position of questions only in the last test (14 participants), to have a correct support for our analysis. Our results from tests T1 and T4 suggest that art students are far more sensitive in decreasing their perception of movement when exposed to the control images instead of the original artworks; conversely, mechanical students show an opposite behavior (at least when Fontana's subjects are of concern); finally, building surveyors and vocational students seem to be quite unconcerned about the nature of the stimuli. This differential outcome, not detectable in the reference work, strongly fades away any apparent effect due to an universal motor resonance between drama expression inside artworks and motor realization in the beholder. Not only at high cognitive levels this claimed resonance appears to be totally undetectable (but still not denied) but it seems that determinant focus should be given to the cultural background of the observer instead. Indeed, art students are specifically educated through theory and exercise in both the recognition and execution (or reproduction) of artworks details and, accordingly, they own a repertoire of techniques that they are also used to embody in form of physical actions and movements. When exposed to original, impetuously made artworks as in the Fontana's or Pollock's case, art students can smartly exploit even the finest details to reverse engineering the artist's creative act; instead, when exposed to more aseptic images, as in the control stimuli case, the same subjects cannot take advantage of landmarks so useful for the expert perception of impressed movements. In a different way, mechanical students are educated to deal with

geometrically exact and clean trajectories as well as to plan and program the operation of devices like Computer Numerical Control routers. For these students, those subtle details so useful to art students are instead likely to be treated as disturbing noise that could obfuscate expected motion patterns inside the image. Among other factors, similar cues could reasonably play a significant role in the recorded differential response: not denied in the reference work, here the author claims their observable preponderance over a somewhat vague, asserted motor resonance between artist and beholder. Furthermore, it should be recalled that also artists get educated through theory and exercise, as pointed out by common sense and pioneering neurophysiological researches[11]. Coherently, if universal mirroring mechanisms are accepted for the comprehension of subtle movements, as impressed in artworks, one should explain how they could keep on operating between eventually diverging neural systems, on the learning artist and on the (not educated) beholder side.

Results from tests T2 and T5 suggest that, when dealing with the artistic appraisal, the transition from the original artworks to the control stimuli induces a coherent variation in the response of all groups (especially the art students one) except the building surveyors students group. In Italy, building surveyors are usually educated to the handling of essential architectural or technical drawings free of smudges and of not geometric decorations. Anyway, in this case the volatility of the concept dealt with, the small amount of variation and the (yet small) size of the statistical population suggest even greater caution in interpreting data.

Results from tests T3 and T6 tests suggest that, when dealing with the artistic nature of the displayed subject, original artworks are better appreciated by all groups, except for the art and vocational students in the Fontana's case. This differential outcome seems to unearth two complementary implications of the subjective cultural background. On one side, personal experience is likely to affect personal sensitivity to expressions of art; on the other one, education could interfere with the understanding itself of the "artistic nature" concept, eventually triggering different mental processes in front of the posed question. While the latter possibility here is only guessed, it seems to be corroborated by the fact that openform remarks given by the participants suggest a strong variability in the perceived (artistic or physical) subjects of the displayed images.

Results from tests T7 and T9 suggest that the perception of movement and the aesthetic appraisal are more correlated for art students than for the other groups (eventually with the exception of the building surveyors students in front of Fontana's originals artworks).

Recalling the considerations just exposed for the outcomes of tests T1, T2, T4 and T5, one can hardly express this correlation in terms of mutual dependency; rather, it seems that, independently, art students show improved attitudes in both movement recognition and aesthetic appraisal.

Tests T8 and T10 suggest similar correlation between perception of movements and recognition of the artistic nature of the subject displayed. Again, the answers of the art students show more coherent variations.

As already mentioned, the aggregate students group and the control group, when compared, have highlighted a variable, different behavior depending on the question that, from time to time, was asked. On one side, the aggregate group synthesizes and averages different scholar backgrounds that have proved to matter; on the other side, the control group, in the image and likeness of that one studied in the reference work, appears to be too much small for any robust statistical investigation. This outcome suggests that further investigation on the topic could take effective advantage by larger statistical populations, carefully categorized in order to better control cultural, emotional and other subjective conditions. Studies suggest, judging by the position and functionality of the premotor cortex investigated with respect to the rest of the cerebral cortex, that, if they exist, mirror neurons could help in the reproduction of works of art depending on the experience of each one rather than in the judgment of the same except in the case in which details such as "the brushstroke" or other similar details of a particular artist are taken. It should be noted, however, that in this case the normal function of the premotor cortex and of the F5 area would be indistinguishable from what passed into literature before the phantom discovery of this new class of neurons[13].

In this case, thinking about an inhibition of the action of the premotor cortex could be sufficient to explain the activation of the areas of the premotor cortex called mirrors both in the precedent study or in the more or less competent evaluation of artworks.

□ CONCLUSIONS

The results obtained throughout this research shed a different light on some claims and results exposed in previous studies about the embodied simulation role in neuroaesthetics. While no neurophysiological measurements have been taken here due to their problematic linkage to the high level perception of impressed movements and the aesthetic experience, attention was paid to isolate critical factors like personal experiences and cultural backgrounds. On this basis it was found that subjective education, in the broadest sense, deeply modulates our individual mental disposition in front of works of visual art, even subverting what one would expect from the application within art experience of debated paradigms like the somatomotor resonance. Strictly speaking, while a possible role for these paradigms cannot be excluded yet, this work suggests the need for finer experimental protocols where affecting factors, like personal culture and actual mood, are better explained and studied over wider statistical populations.

Until today and in the absence of further evidence, what one can reasonably say is that if the artistic experience is a matter of resonance then this resonance should be of cultural, and not motor, nature.

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Case report

Opsoclonus-Myoclonus Syndrome in a COVID-19 positive patient

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SUMMARY: *Opsoclonus-myoclonus syndrome is a rare immune-mediated disease in adults. However the pathogenic role is not well understood. We assessed wether there was an association between Opsoclonus-myoclonus syndrome and the positivity for Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) infection. A 58 years old man with symptomatology suggestive of opsoclonus-myoclonus syndrome was admitted to our hospital; during the hospitalization he developed a typical radiological pattern for COVID-19 pneumoniae. He was studied for possible immuno-mediated causes of the disease or correlation to SARS-CoV-2 infectio. MR imaging, electroencephalography and cerebrospinal fluid examination was normal as the detection of antibodies to neuronal surface antigens. A total body CT scan revealed a typical pattern of ground glass opacities in both lungs. Naso-pharyngeal swab was repeated and revealed a positivity for SARS-CoV-2. Reverse transcriptase-polymerase chain reaction for Sars-CoV-2 RNA in the cerebrospinal fluid was negative. Patient was transferred to a COVID-19 Department. In the following days, a progressive improvement was observed. Number of patients with COVID-19 experiencing different neurological signs and symptoms is rapidly growing in the literature. Opsoclonus-myoclonus syndrome should be added to the spectrum of clinical manifestations associated with this new disorder.*

KEY WORDS: *COVID-19, Myoclonus Syndrome, Opsoclonus.*

INTRODUCTION

Opsoclonus-myoclonus syndrome is a rare immune-mediated disease in adults. The main symptoms include opsoclonus, myoclonus, ataxia, cognitive and behavioural disorders. Opsoclonus is characterized by involuntary, multidirectional saccades with horizontal, vertical and torsional components. OMS is classified as an idiopathic, post-infectious or paraneoplastic syndrome. In adults, small-cell lung cancer, breast and ovarian cancer are the main underlying tumors. Post-infectious brainstem encephalitis, toxic-metabolic disorders and other conditions should also be considered as potential causes. In OMS, several autoantibodies directed against a variety of antigens were found, but diagnostic immunological markers are yet to be discovered[1,2].

CASE REPORT

A 58 years old man presented to emerging department with opsoclonus, myoclonus, ataxia, psychomotor slowing, progressive gait instability and

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LIST OF ACRONYMS AND ABBREVIATIONS: **5-OH** = 5-hydroxy; **AMPA** = α-Amino-3-hydroxy-5-Methyl-4-isoxazolePropionic Acid receptor; **CASPR** =Contactin-ASsociated PRotein; **COVID-19** = COronaVIrus Disease-19; **CV2** = CrossVeinless 2; **CSF** = Cerebrospinal Fluid Examination; **DPPX** = DiPeptidyl-Peptidase-like protein 6; **ED** = Emerging Department; **GABA-B2** = Gamma-AminoButyric Acid B receptor 1; **HIV** = Human Immunodeficiency Virus; **HNK1** = Human Natural Killer 1; **HSV-1** = Herpes Simplex Virus 1; **HSV-2** = Herpes Simplex Virus 2; **MRI** = Magnetic Resonance Imaging; **NMDAR** = N-Methyl-D-Aspartate Receptor; **NPS** = Naso-Pharyngeal Swab; **OMS** = Opsoclonus-Myoclonus Syndrome; **PNMA2** = ParaNeoplastic antigen Ma2; **RT-PCR** = Reverse Transcriptase-Polymerase Chain Reaction; **SARS-CoV-2** = Severe Acute Respiratory Syndrome CoronaVirus-2; **VZV** = Virus Varicella Zoster.

behavioural impairment. Fifteen days earlier, patient developed fever up to 38 °C for 3 days.

His clinical history was unremarkable, no current therapy. In ED NPS for SARS-CoV-2 RNA was negative by real time RT-PCR. He was admitted to our COVID-19 free Department of Neurology.

MRI, performed with contrast fluid, was normal; electroencephalography showed no slowing activity or epileptiform discharges.

CSF showed normal proteins and cells; PCR testing for the DNA of HSV-1, HSV-2, VZV and cytomegalovirus was negative. Cytopathologic examination revealed a mild non-specific inflammation with sediment consisting of some red blood cells, some polymorphs, rare lymphocytes and macrophages.

Blood tests results were normal and excluded Epstein-Barr virus and HIV infections, Lyme-disease and auto-immune disorders; antineuronal cerebellar antibodies (anti-Hu, anti-Yo, anti-Ri, anti-PNMA2, anti-CV2 and anti-amphiphysin) were not detected.

Detection of antibodies to neuronal surface antigens reported in association in OMS: anti-NMDAR, anti-GABA B1, anti-DPPX, anti-HNK1, anti-CASPR 2, anti-AMPA 1 AMPA 2 were all normal as well as the dosing of the protein tau and 14.3.3 in the CSF.

A total body CT scan revealed a typical pattern of ground glass opacities in both lungs, which were highly suggestive for COVID-19 pneumonia.

NPS was repeated and revealed a positivity for SARS-CoV-2.

Patient was transferred to a COVID-19 Department where he received supportive care, and 5-OH Chloroquine.

A second MRI and lumbar puncture were, respectively, repeated 7 and 11 days after admission and were both unrevealing; RT-PCR for SARS-CoV-2 RNA in the CSF was negative.

In the following days, a progressive improvement in both cognitive and behavioural symptoms as well as myoclonus-opsoclonus, trunk and gait ataxia was observed.

□ DISCUSSION

Our patient presented with a prominent neurological syndrome, in the absence of the typical COVID respiratory symptoms .

Although we could not detect SARS-CoV RNA in the CSF, this seems to be the case for most of the reports published to date[3]. Number of patients with COVID-19 experiencing different neurological signs and symptoms is rapidly growing in the literature[3]. OMS should be added to the spectrum of clinical manifestations associated with this new disorder. Further studies are warranted to unravel the mechanisms underlying the pathogenesis of the neurologic manifestations in COVID-19.

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PRESS
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