MR Assessment of Lumbar Disk Herniation Treated with Oxygen-Ozone Diskolysis: The Role of DWI and Related ADC versus Intervertebral Disk Volumetric Analysis for Detecting Treatment Response

A. SPLENDIANI¹, M. PERRI², A. CONCHIGLIA², F. FASANO², G. DI EGIDIO², C. MASCIOCCHI², M. GALLUCCI¹

¹Neuroradiology Unit, ²Radiology Unit, DISCAB University of L'Aquila; L'Aquila, Italy

Key words: lumbar disk herniation, ozone discolysis, MR DWI

SUMMARY – We prospectively assessed the diagnostic criteria of morphologic MRI study (MMS) and the accuracy of DWI and related ADC values (DWI-ADC) versus intervertebral disk volumetric analysis (IDVA) for predicting shrinkage of lumbar disk herniation treated with oxygen-ozone (O₂-O₃) diskolysis. Sixty-eight patients (36 men and 32 women; mean age 39) with lumbosciatica underwent O₂-O₃ diskolysis. The six-month MRI follow-up was performed with FSE-T2 and T2-fat, SE-T1 and DWI-weighted images. IDVA was determined using OsiriX®. Diagnostic criteria and accuracy were evaluated with regards to DWI and related ADC in detecting response to ozone therapy. Fifty-eight of 68 patients had successful outcomes (responders), whereas ten patients showed unsatisfactory outcomes (non-responders). MMS showed that a centrally located herniated disk and grade 1 nerve root compression were more common in the responder group ($p < 0.05$). DWI-ADC and IDVA showed statistically significant shrinkage in the sixth month of follow-up ($p < 0.05$) with a mean ADC value reduction of $2.10 \times 10^{-3} \text{ mm}^2/\text{s} \pm 0.19 \text{ SD}$ in the second month of follow-up ($p < 0.05$). DWI-ADC had an accuracy of 0.81 in detecting response to therapy around the second month of follow-up. DWI-ADC appear to be useful adjuncts to MMS in the follow-up of patients undergoing O₂-O₃ diskolysis.

Introduction

About 80% of adults suffer from low back or leg pain during their lifetime¹. In primary care, 4% of patients with this condition have a lumbar disk herniation², with L5 and S1 nerve roots involved in approximately 95% of cases^{3,4}. However the herniated portion of the disk tends to regress with time after conservative therapy, with partial or complete resolution in two thirds of cases after 11 months⁵. Oxygen-ozone (O₂-O₃) diskolysis is a good alternative to surgical treatment for lumbar disk herniation for patients failing to respond to conserva-

tive treatment, with a success rate of 70-80% without complications^{6,7}. The basic principle of action of intradiscal injection of an O₂-O₃ mixture is the reduction of proteoglycans⁸, consequent dehydration and shrinkage of the disk but no study has been published on an analysis of these changes in MRI. As we previously described, there is a good correlation between high signal intensity in T2-weighted images, type of disk herniation and tendency to regress after non-surgical treatment⁹, but similar evidence has not been demonstrated in subjects treated with percutaneous intradiscal injection of O₂-O₃. Moreover, no published study

Figure 1 MR images of lumbar spine in 36-year-old man with right lumbosciatica. A) Sagittal FSE T2-weighted MR image shows extruded disk herniation with the evaluation of the intervertebral disc cross-sectional area at the level L5-S1. B) Corresponding sagittal DWI-weighted image (b value, 400 s/mm²; 1215/80; slice thickness 5 mm; spacing 1 mm; and NEX 6) with ROI positioned to level L5-S1.

has addressed the possible role of DWI and related ADC values (DWI-ADC) in predicting the benign evolution of disk herniation treated mini-invasively. In fact there is good evidence that DWI has improved diagnostic accuracy for detecting diffusibility water reduction in degenerative disk disease¹⁰. Therefore we assumed that DWI-ADC could help distinguish a possible change in disk water content. On the basis of these results, we undertook a prospective trial to assess the diagnostic criteria of morphologic MRI study (MMS) after O₂-O₃ diskolysis and the accuracy of DWI-ADC versus intervertebral disk volumetric analysis (IDVA) for predicting shrinkage of lumbar disk herniation.

Materials and Methods

Patients

Between September 2009 and June 2011 91 patients with a history of low back or leg pain related to lumbar disk herniation were selected for the protocol of a prospective trial approved by the Medical Ethical Committee of our institution. Preliminary clinical evaluation was performed by one experienced neuroradiologist with 35 years' experience. All participants gave written informed consent prior to enrollment. Inclusion criteria comprised lumbar disk protrusion or herniation documented on CT and MR images, pain for at least eight weeks with

no or poor clinical improvement after conservative therapy, and initial mean visual analog scale (VAS)¹¹ greater than 30%. Exclusion criteria comprised pregnancy, referred allergy to proposed drugs, and syndromes able to mimic the symptoms of a lumbar disk herniation such as facet syndrome, sacroileitis, bone lesions, or previous spine surgery. Twenty-three of 91 patients who did not present at the subsequent follow-up after treatment were excluded from the study protocol. A total of 68 patients (36 men and 32 women, mean age at presentation 39 years) were included in this study and treated (L3-L4, seven patients; L4-L5, 19 patients; L5-S1, 42 patients) with percutaneous intradiscal O₂-O₃ injection performed by two neuroradiologists (with 35 and five years' experience respectively). Thereafter, patients underwent a follow-up period in which control MRI examinations were performed at the second, fourth and sixth month after O₂-O₃ diskolysis. The outcome was measured on the day of the procedure and subsequent follow-up visits, using the VAS¹¹, ranging from 0 to 10 with a cut-off of 2. The questionnaire was administered by another neuroradiologist (with three years' experience).

MR imaging

Each participant underwent MRI examinations of the lumbar spine in our radiology department (using GE Healthcare - Signa EXCITE 1.5T MRI scanner with a phased-array spine coil) before and after O₂-O₃ diskolysis and in the subsequent follow-up at the second, fourth and sixth months. Sagittal FSE-T2 and T2 fat-weighted images were acquired with the following parameters: 4000/108/4 (TR/TE/NEX) (scan time 4.46 min), while for sagittal SE-T1-weighted images 500/20/2 (TR/TE/NEX). Both T1 and T2-weighted images were acquired using the same values of matrix (256 × 192), slice thickness 5 mm, spacing 1 mm and a field of view (FOV) of 26 cm. Axial T2-weighted images were acquired parallel to the disk space with a FOV of 17 cm (scan time 2.38 min). DWI images were obtained in sagittal and axial planes by using isotropic multishot spin-echo-planar MR imaging with followed parameters: 1215/80/6 (TR/TE/NEX), matrix, 128 × 128, with the same values of slice thickness and spacing as T2 and T1-weighted images obtained in sagittal and axial planes (scan time 1.04 min). Total imaging time was about seven minutes. These parameters allowed us to obtain a good

signal to noise ratio¹². The DWI images were acquired without diffusion weighting ($b = 0$ s/mm²) and with diffusion weighting ($b=400$ s/mm²) to minimize diffusion-related loss of signal intensity in disk tissue, which could lead to subsequent inaccuracy in ADC measurement^{13,14}. Post processing time was about two minutes.

Qualitative and quantitative image analysis

Two neuroradiologists (with 25 and three years' experience respectively), blinded to the patients' clinical findings, independently reviewed MR images, and compared them between the responders ($n = 58$) and non-responders ($n = 10$). In case of disagreement between the two primary readers, a third neuroradiologist (with five years' experience) independently examined the image to resolve the disagreement. According to the recommendations of the combined task forces of the North American Spine Society, the American Society of Spine Radiology and the ASNR15, on FSE-T2, FSE-T2 fat and SE-T1-weighted images, the type of herniated disk was classified as protrusion, extrusion and sequestration while the location was classified as central, left/right central, subarticular or foraminal/extraforaminal. Disk degeneration and hydration was assessed on the MR T2-weighted sagittal images by using a five-point scale according to the method of Pfirrmann et al¹⁶. The grade of nerve root compression was classified as contact, deviation, and compression¹⁷. IDVA was obtained on FSE-T2-weighted images using OsiriX[®] 4.1 uploaded on a 2.8-GHz Intel Core 2 Duo 24" iMac. Entire disk and herniated parts were included in the regions of interest (ROIs) on all slices (Figure 1A). The effect of ozone on disk water diffusivity was assessed with the DWI images and corresponding ADC maps. The ADC values were selected through small round ROIs measuring 40-50 mm² within the central portion of the disk on the DWI images (Figure 1B). Both volume and ADC values were calculated and scored as no, small, medium, large and very large disk shrinkage before and after treatment in the follow-up.

Injection technique

All procedures were performed by two neuroradiologists (with 35 and five years' experience respectively) under CT guidance (Somatom Plus 4; Siemens Medical Systems, Erlangen, Germany) and with the patient in the prone

Figure 2 Axial CT images in 45-year-old man with right lumbosciatica. The image shows the paravertebral approach with point of the needle in L5-S1 level. The O₂-O₃ mixture is distributed inside the disk and in the epidural space.

position. Before every procedure, the patients received premedication with intravenously administered 1 g of cefuroxime (CUROXIM; GlaxoSmithKline) and 50 mg of ranitidine (Zantac; GlaxoSmithKline). The O₂-O₃ gas mixture was achieved using an ozone generator (OZO2 Futura; Alnitec, Cremona, Italy). After local anesthesia using 6 mg of mepivacaine (about 2-3 mL of MEPICAIN 2%; Monico SPA, Venice, Italy), a spinal needle (9 or 13 cm 22 gauge) was advanced to the intraforaminal space at an angle between 45° and 60°. Intradiscal and intraforaminal injections were administered with a paravertebral approach in 59 patients and an interlaminar approach in nine patients. Before injection inside the disk, a CT scan was used to confirm that the needle tip was inside the nucleus pulposus. After injection a CT scan was acquired to evaluate O₂-O₃ distribution which was considered satisfactory when the gas was homogeneously distributed inside the nucleus pulposus with diffusion in the epidural and periganglionic space (Figure 2). After this last evaluation, the needle was removed and the procedure was concluded. Overall, the average injected volume of O₂-O₃ in patients was 8-10 mL (28 µg/mL concentration). Every patient additionally received 4 mg of betamethasone in the periganglionic area.

Statistical analysis

To assess the diagnostic criteria of morphologic MRI study (MMS) for predicting shrinkage of lumbar disk herniation after treatment, χ^2 test was used to determine the differences between the responder and non-responder groups in terms of the type of herniation, disk

degeneration and hydration, the location of the herniated disk and the grade of nerve root compression. Student's T test was used to determine the differences between the responder and non-responder groups in terms of reduction of average ADC and whole disks with herniation volume values after treatment. A logistic regression analysis was used to determine any relationship between changes in water diffusivity and herniation size of the disks after O₂-O₃ injection. ROC curves analysis was performed to evaluate the accuracy of DWI-ADC versus IDVA for predicting shrinkage of lumbar disk herniation treated with O₂-O₃ diskolysis.

Results

Qualitative analysis

The final analysis included 68 patients with lumbosciatica and a follow-up of six months. The patients' symptom duration before treatment ranged from three months to two years with no difference between the group of responders and non-responders. Treatments were done at a single level: L3-L4 in seven patients, L4-L5 in 19 patients, L5-S1 in 42 patients. Before and after O₂-O₃ injection, in the subsequent follow-up, no major or minor complications were observed. Fifty-eight of 68 patients (86% of the sample consisting of 40 men and 18 women with a mean age of 37 years) had successful outcomes (responders with a mean VAS below 2) while ten patients (six men and four women with a mean age of 46 years) showed unsatisfactory outcomes (non-responders with a mean VAS above 5) both in a total follow-up

Table 1 Comparison of the diagnostic criteria evaluated with morphologic MRI study between responders and non-responders.

Characteristic		Responders (n=58)	Non responders (n=10)	Total	p value
Type of lumbar disk herniation	Protrusion	21(36%)	1(10%)	22	* $p>0.05$
	Extrusion	24(41%)	4(40%)	28	* $p>0.05$
	Sequestration	13(23%)	5(50%)	18	* $p>0.05$
Disk degeneration and hydration	High	22(38%)	2(20%)	24	* $p>0.05$
	Moderate	24(41%)	3(30%)	27	* $p>0.05$
	Low	12(21%)	5(50%)	17	* $p>0.05$
Location of lumbar disk herniation	Central	10(17%)	1(10%)	11	* $p<0.05$
	Left/right central	46(80%)	7(70%)	53	* $p<0.05$
	Subarticular	0(0%)	1(10%)	1	* $p<0.05$
	Foraminal	0(0%)	1(10%)	1	* $p<0.05$
	Extraforaminal	2(3%)	0(0%)	2	* $p<0.05$
Grade of nerve root compression	1 Contact	17(29%)	1(10%)	18	* $p<0.05$
	2 Deviation	33(56%)	4(40%)	37	* $p<0.05$
	3 Compression	8(15%)	5(50%)	13	* $p<0.05$

Note. Values represent the number of patients; * χ^2 test.

period of six months. Injection level, age, gender or the pre-injection symptom duration did not show significant differences between the responder and non-responder groups ($p>0.05$). The analysis of serial MMS images performed with FSE T2, FSE T2 FAT and SE T1-weighted images, acquired before and after treatments, showed no significant difference between the responders and non-responders in terms of the type, degeneration and hydration of the lumbar disks herniation ($p>0.05$) (Table 1). The location of the herniated disks differed between the two groups ($p<0.05$) (Table 1). A centrally located herniated disk was more common in the responder group. Two cases of extraforaminal disk herniations were also successfully treated while one subarticularly located herniated disk showed an unsatisfactory result. Grade 2 or 3 nerve root compression showed more unsatisfactory results than grade 1 nerve root compression ($p<0.05$). Therefore the grade of nerve root compression also correlated with the clinical outcome (Table 1).

Quantitative analysis

Disk and herniation volume reductions evaluated with IDVA were significantly more frequent in the responder group than in the non-responder group. Student's T test showed a statistically significant reduction at around six months of follow-up ($p<0.05$) after treatment, with an average reduction of whole disks with

herniation volumes around 4-5% (Table 2). Corresponding DWI-ADC analysis of intervertebral disks obtained in sagittal and axial planes before and after treatment showed a statistically significant difference between responders and non-responders in terms of reduction in water diffusion quantified by the corresponding ADC value in particular in the follow-up of the second month ($p<0.05$) (Table 2 and 3).

A logistic regression analysis showed a statistically significant correlation between the reduction in ADC value in the follow-up at the second month and disk and herniation volumes in the responders in the subsequent follow-up at the sixth month (Pearson's correlation coefficient $r = 0.97$).

Diagnostic accuracy

In our study, by plotting ROC curve areas (Table 3) from observed findings (Table 4) in the second month of follow-up, we determined that accuracies for predicting shrinkage of lumbar disk herniation treated with O_2-O_3 discolysis of DWI-ADC and IDVA were 0.81 and 0.68 respectively.

Discussion

Because ozone is an unstable form of oxygen that reacts in water with organic molecules containing double or triple bonds causing an

Figure 3 Axial MR images of a 38-year-old man with severe posterior thigh and lateral leg pain with tingling sensations. A) Axial FSE T2-weighted MR image shows right central sequestered disk herniation at level L5-S1 with a grade II ipsilateral S1 nerve root compression. B) Corresponding diffusion-weighted image (b value 400) showing the average water diffusivity of the annulus fibrosus and nucleus pulposus. Because the distribution of the patient's pain was along the right S1 dermatome, we used a right paravertebral approach to administer intradiscal and intraforaminal injections of ozone. The follow-up MRI assessment 6 months after injection showed resorption of herniated disc (C) with ADC reduction greater than 10%. D) Corresponding DWI image (b value 400) showed low water diffusivity. The outcome was a remarkable improvement with a pain reduction and VAS below 2.

oxide reduction called ozonolysis¹⁸, intradiscal O_2-O_3 mixture injection produces a chemodiskolysis of nucleus pulposus proteoglycans and dehydration followed finally by disk shrink-

age and a direct reduction of root compression^{19,20}. Another reason for using medical ozone to treat disk herniation is its analgesic and anti-inflammatory effects²¹⁻²³. The effect of ozone

Table 2 Comparison of DWI-ADC and IDVA analysis before and after chemodiskolysis with O₂-O₃ performed at the second, fourth and sixth months between responders and non-responders.

Characteristic		Responders (n=58)	Non responders (n=10)	p value
IDVA	Before treatment	16.38 mm ³ ±0.57 SD 1.71 mm ³ ±0.64 SD	16.45 mm ³ ±0.58 SD 1.89 mm ³ ±0.62 SD	*p<0.05
	Second month	16.30 mm ³ ±0.59SD 1.69 mm ³ ±0.62 SD	16.38 mm ³ ±0.50 SD 1.78 mm ³ ±0.67 SD	*p<0.05
	Fourth month	16.25 mm ³ ±0.58 SD 1.67 mm ³ ±0.59 SD	16.36 mm ³ ±0.55SD 1.82 mm ³ ±0.60 SD	*p<0.05
	Sixth month	16.04 mm ³ ±0.41 SD 1.64 mm ³ ±0.22 SD	16.30 mm ³ ±0.40 SD 1.81 mm ³ ±0.25 SD	*p<0.05
DWI-ADC	Before treatment	2.25 × 10 ⁻³ mm ² /s±0.35 SD	2.23 × 10 ⁻³ mm ² /s±0.38 SD	*p<0.05
	Second month	2.10 × 10 ⁻³ mm ² /s±0.19 SD	2.22 × 10 ⁻³ mm ² /s±0.27 SD	*p<0.05
	Fourth month	2.08 × 10 ⁻³ mm ² /s ±0.20 SD	2.20 × 10 ⁻³ mm ² /s±0.25 SD	*p<0.05
	Sixth month	2.03 × 10 ⁻³ mm ² /s±0.25 SD	2.19 × 10 ⁻³ mm ² /s±0.32 SD	*p<0.05

Note. IDVA; Data are mean intervertebral disk and herniated part volume values (mm³) ±standard deviations. DWI-ADC; Data are mean ADC values; * Student's T test.

on chemical radiculitis can also explain the clinical effectiveness of intraforaminal O₂-O₃ injection without intradiscal therapy²⁴. The reported effectiveness of the procedure is promising, with clinical success in 70%-80% of patients²⁵.

In our study, 58 out of 68 patients (approximately 86%) had successful outcomes (the responders) whereas ten patients showed unsatisfactory results (the non-responders) both with a follow-up period of six months. Small differences between our and other studies may be related to patient selection and evaluation methods. Nevertheless, a significant number of patients continue to show symptoms after ozone therapy, and then surgery is required after several months. Therefore, it would be useful for both patients and clinicians to know the likely outcome of nonsurgical treatments and the likelihood of future surgery. In our experience, there was no significant difference between the responders and non-responders to O₂-O₃ diskolysis in terms of the injection level, age, gender or pre-injection symptom duration (p>0.05). In addition, there was no significant difference in terms of the type or hydration of the herniated disk. Furthermore, the present study showed that the location and grade of nerve root compression resulted in significantly different responses to treatment. Only two cases of extraforaminal disk herniation showed a successful result and all the subarticular disk herniations and the disk herniations severely compressing the nerve root showed

unsatisfactory results. It can be assumed that the subarticular location could make the nerve root to be unresponsive to ozone therapy.

Many studies have investigated the prognostic value of MRI in patients treated conservatively, and they have used MRI to evaluate the morphology changes in lumbar disk herniation. Using MRI, Komori et al reported that large sequestered or extruded disk herniations frequently decreased in size after conservative treatment²⁶. As we previously described examining the prognostic value of post-contrast MR enhancement in lumbar disks, the high signal intensity of the disk fragment on the T2-weighted images was related to a reduction of the disk fragment itself^{9,27}. However these data could not predict the response of herniated disks treated with O₂-O₃ injection. Interestingly, we observed a significant reduction in the ADC of the treated disks around the second month, herniation volume reductions around the sixth month of follow-up after O₂-O₃ diskolysis, more frequent in the responder group than in the non-responder group. In our experience, the finding of a statistically significant decrease in the ADC of disks after ozone therapy could be explained by early disk degeneration with a loss of extracellular water and by alterations in the relative proteoglycan content of intervertebral disks. Although we found that mean ADC values in treated disks were lower than those obtained before treatment, previous studies have reported normal or elevated ADC values in severely degener-

Table 3 DWI-ADC and IDVA ROC curves. The accuracies computed from the continuous and dashed ROC curves area are 0.81 and 0.68 respectively. The marked points on the curves are estimated in Table 4. **Table 4** Construction of ROC curves from the results of the interpretative models of images to predict shrinkage of lumbar disk herniation in the second month of follow-up after chemodiskolysis with O₂-O₃.

Neuroradiologists interpretations						
Interpretative models of images		No chance	Low likelihood	Medium likelihood	High likelihood	Very high likelihood
DWI-ADC	Responders (=58)	2	8	12	15	21
	Non-responders (=10)	6	1	1	1	1
HIDVA	Responders (=58)	5	8	14	14	17
	Non-responders (=10)	1	2	2	3	2

Estimations of the points on the ROC curves						
Interpretative models of images		Cut-off point	Sensitivity	1-Specificity	PPV	NPV
DWI-ADC	Between no chance and low likelihood		0.96(56/58)	0.40(4/10)	0.93(56/60)	0.75(6/8)
	Between low and medium likelihood		0.82(48/58)	0.30(3/10)	0.94(48/51)	0.41(7/17)
	Between medium and high likelihood		0.62(36/58)	0.20(2/10)	0.94(36/38)	0.26(8/30)
	Between high and very high likelihood		0.36(21/58)	0.10(1/10)	0.95(21/22)	0.19(9/46)
HIDVA	Between no chance and low likelihood		0.91(53/58)	0.90(9/10)	0.85(53/62)	0.16(1/6)
	Between low and medium likelihood		0.77(45/58)	0.70(7/10)	0.86(45/52)	0.18(3/16)
	Between medium and high likelihood		0.53(31/58)	0.50(5/10)	0.86(31/36)	0.15(5/32)
	Between high and very high likelihood		0.29(17/58)	0.20(2/10)	0.89(17/19)	0.16(8/49)

Note. Values represent the number of patients; PPV, positive predictive value; NPV, negative predictive value.

ated disks^{13,28}. Therefore on the basis of these studies we can affirm that in our experience the disks treated with O₂-O₃ did not show an elevated process of degeneration.

It is pertinent to note the limitations of the present study. First, it was not a double-blind controlled study, which may have affected the clinical outcome measurements following ozone therapy. Some studies also show that

many diffusion MR imaging techniques, such as single shot echo-planar imaging, are not useful for imaging the spine because of the inhomogeneous magnetic environment and the high lipid content of the vertebral bodies that can lead to strong geometric distortions and chemical shift artifacts²⁹. ADC values obtained at 1.5T from corresponding diffusion tensor scans using line scan diffusion imaging is con-

siderably more robust than other common sequences in the presence of global motion and susceptibility differences, giving high quality DWI-ADC and anisotropy maps in the human spine³⁰. In our study, mean ADC values of disks before treatment was $2.25 \times 10^{-3} \text{ mm}^2/\text{s}$ ± 0.35 SD and in disks after ozone therapy $2.03 \times 10^{-3} \text{ mm}^2/\text{s}$ ± 0.25 SD. Nevertheless, it is possible that differences in technique between our study and previous studies³⁰ could account at least in part for the differences in ADC values. Spinal diffusion tensor imaging was not performed, and so we were not able to measure anisotropy within the treated disks. It would be very interesting eventually to cor-

relate anisotropy with the severity of degenerative changes to determine if there are significant changes in diffusion anisotropy in disks at different stages after ozone therapy.

In conclusion, we report a statistically significant decrease in the ADC values of treated disks compared with the ADC values of disks before treatment. In our experience, DWI images with ADC analysis provide additional diagnostic information on water diffusivity in intervertebral disks treated with ozone, completing but not replacing MMS. In particular, the high NPV of DWI-ADC analysis can be useful to select those patients who will require further treatment with ozone.

References

- 1 Andersson GBJ. The epidemiology of spinal disorders. In: Frymoyer JW ed. The adult spine: principles and practice. 2nd ed. Philadelphia: Lippincott-Raven; 1997. p. 93-141.
- 2 Deyo RA, Weinstein JN. N Engl J Med. 2001; 344 (5): 363-370.
- 3 Deyo RA. Early diagnostic evaluation of low back pain. J Gen Intern Med. 1986; 1 (5): 328-338.
- 4 Deyo RA, Rainville J, Kent DL. What can the history and physical examination tell us about low back pain? JAMA. 1992; 268 (6): 760-765.
- 5 Bozzao A, Gallucci M, Masciocchi C, et al. Lumbar disk herniation: MR imaging assessment of natural history in patients treated without surgery. Radiology. 1992; 185 (1): 135-141.
- 6 Guarnieri G, Vassallo P, Pezzullo MG, et al. A comparison of minimally invasive techniques in percutaneous treatment of lumbar herniated discs. Neuroradiol J. 2009; 22 (1): 108-121.
- 7 Andreula CF, Simonetti L, Leonardi M, et al. Minimally invasive oxygen-ozone therapy for lumbar disk herniation. Am J Neuroradiol. 2003; 24 (5): 996-1000.
- 8 Iliakis E, Valadakis V, Vynios DH, et al. Rationalization of the activity of medical ozone on intervertebral disc: a histological and biochemical study. Riv Neuro-radiol. 2001; 14 (Suppl 1): 23-30.
- 9 Splendiani A, Puglielli E, De Amicis R, et al. Spontaneous resolution of lumbar disk herniation: predictive signs for prognostic evaluation. Neuroradiology. 2004; 46 (11): 916-922.
- 10 Kealey SM, Aho T, Delong D, et al. Assessment of apparent diffusion coefficient in normal and degenerated intervertebral lumbar disks: initial experience. Radiology. 2005; 235 (2): 569-574.
- 11 Lee JS, Hobden E, Stiell IG, et al. Clinically important change in the visual analog scale after adequate pain control. Acad Emerg Med. 2003; 10 (10): 1128-1130.
- 12 Holder CA, Muthupillai R, Mukundan S, et al. Diffusion-weighted MR imaging of the normal human spinal cord *in vivo*. Am J Neuroradiol. 2000; 21 (10): 1799-1806.
- 13 Kurunlahti M, Jauhiainen J, Karppinen J, et al. Correlation of diffusion in lumbar intervertebral discs with occlusion of lumbar arteries: a study in adult volunteers. Radiology. 2001; 221 (3): 779-786.
- 14 Kerttula L, Jauhiainen J, Koivula A, et al. Apparent diffusion coefficients and T2 relaxation time measurements to evaluate disc degeneration: a quantitative MR study of young patients with previous vertebral fracture. Acta Radiol. 2001; 42 (6): 585-591.
- 15 Fardon DF, Milette PC. Nomenclature and classification of lumbar disc pathology. Recommendations of the combined task forces of the North American Spine Society, American Society of Spine Radiology, and American Society of Neuroradiology. Spine. 2001; 26 (5): E93-E113.
- 16 Pfirrmann CW, Metzdorf A, Zanetti M, et al. Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine. 2001; 26 (17): 1873-1878.
- 17 Pfirrmann CW, Dora C, Schmid M, et al. Grading of lumbar nerve root compromise with magnetic resonance imaging: a reliability study with surgical correlation. Radiology. 2004; 230 (2): 583-588.
- 18 Richelmi P, Valdenassi L, Berté F. Basi farmacologiche dell'azione dell'ossigeno-ozono terapia. Riv Neuroradiol. 2001; 14 (Suppl 1): 17-22.
- 19 Iliakis E, Valadakis V, Vynios DH, et al. Rationalization of the activity of medical ozone on intervertebral disc: a histological and biochemical study. Riv Neuro-radiol. 2001; 14 (Suppl 1): 23-30.
- 20 Leonardi M, Simonetti L, Barbara C. Effetti dell'ozono sul nucleo polposi: reperti anatomo-patologici su un caso operato. Riv Neuroradiol. 2001; 14 (Suppl 1): 57-59.
- 21 Bocci V, Luzzi E, Corradeschi F, et al. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res. 1993; 12 (2): 121-126.
- 22 Simonetti L, Agati R, Cenni P, et al. Mechanism of pain in disc disease. Riv Neuroradiol. 2001; 14 (2): 171-174.
- 23 Siddal PJ, Cousins MJ. Spine update spinal pain mechanism. Spine (Phila Pa 1976). 1997; 22 (1): 98-104.
- 24 Bonetti M, Fontana A, Cotticelli B, et al. Intraforaminal O(2)-O(3) versus periradicular steroidal infiltrations in lower back pain: randomized controlled study. Am J Neuroradiol. 2005; 26 (5): 996-1000.
- 25 Gallucci M, Limbucci N, Zugaro L, et al. Sciatica: treatment with intradiscal and intraforaminal injections of steroid and oxygen-ozone versus steroid only. Radiology. 2007; 242 (3): 907-913.
- 26 Komori H, Shinomiya K, Nakai O, et al. The natural history of herniated nucleus pulposus with radiculopathy. Spine (Phila Pa 1976). 1996; 21 (2): 225-229.
- 27 Gallucci M, Bozzao A, Orlandi B, et al. Does postcontrast MR enhancement in lumbar disc herniation have prognostic value? J Comput Assist Tomogr. 1995; 19 (1): 34-38.
- 28 Chiu EJ, Newitt DC, Segal MR, et al. Magnetic resonance imaging measurement of relaxation and water diffusion in the human lumbar intervertebral disc under compression *in vitro*. Spine (Phila Pa 1976). 2001; 26 (19): E437-E444.
- 29 Bammer R, Herneth AM, Maier SE. Line scan diffusion imaging of the spine. Am J Neuroradiol. 2003; 24 (1): 5-12.
- 30 Hsu EW, Setton LA. Diffusion tensor microscopy of the intervertebral disc annulus fibrosus. Magn Reson Med. 1999; 41 (5): 992-999.

Prof. Massimo Gallucci
Unit of Neuroradiology
Department of Biotechnological
and Applied Clinical Sciences
University of L'Aquila
67100 L'Aquila, Italy
Tel.: +39 0862368500
E-mail: massimo.gallucci@cc.univaq.it