Research Square

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Expression and Distribution of M1 and M2 Macrophages in the Degeneration Process of Human Lumbar Intervertebral Disc Herniation: A Histological and Clinical Efficacy Analysis

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Research article

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Abstract

Background: Macrophages were previously proved to participate in the progression of lumbar disc herniation (LDH), but the phenotypic subtypes of macrophages and the association between M1/M2 positivity and clinical efficacy in LDH are not understood. This study aimed to determine the expression and distribution of M1 and M2 macrophages in LDH and investigate the association between M1/M2 positivity and outcome of LDH therapy.

Methods: Immunohistochemical analyses of M1 and M2 markers were used to identify M1/M2 macrophages and their prevalence and distribution in LDH patients. The association among prevalence, clinical characteristics, and clinical efficacy was evaluated. Differences in the presence of M1 and M2 macrophages with or without modic changes (MCs) and those in the high-intensity zone (HIZ) were also analyzed.

Results: The induced nitric oxide synthase (iNOS) and CD206 were expressed in all 79 LDH patients tested. The morphology and distribution of iNOS+ and CD206+ cells varied at different degenerative LDH stages. iNOS+ cells significantly decreased with increasing Pfirrmann grade and age, whereas CD206 cells increased. iNOS+ cells showed a positive correction with visual analog scale scores, while CD206+ exhibited positive correction with the Oswestry disability index on pre-operative day 3. A significant increase in iNOS+ cells was observed in the HIZ, whereas more CD206+ cells were found in MC tissues.

Conclusions: Differences in morphology and distribution of M1/M2 cells in LDH suggested that these cells originated from both recruited and resident macrophages. Significantly different expression in MCs and the HIZ and significant correlation with clinical efficacy indicated the important role of the M1/M2 transition in the immune and inflammatory response in LDH. Management of the M1/M2 transition may be a feasible approach for preventing LDH.

Introduction

Lumbar disc herniation (LDH) is a highly prevalent and wide spread health problem, which imposes a substantial economic burden on individuals and society [1]. LDH is the leading cause of lower back pain and neurological deficit, according to physical examination scores carried out using the visual analog scale (VAS), and the Oswestry disability index (ODI) [2]. The homeostasis of various physiological systems, such as those associated with nutrition, mechanical stress and oxygen supply in intervertebral discs (IVDs) are negatively affected by LDH [3, 4]. However, the specific mechanisms underlying these effects remain unclear. Particularly in the early stages of LDH, treatment is directed at relieving pain and symptoms, rather than addressing the pathological process itself [5]. Currently, immune homeostasis is attracting considerable attention in LDH, owing to its role in tissue degeneration and regeneration.

The IVD, an immune-privileged organ, comprises a central nucleus pulposus (NP), a surrounding annulus fibrosus made of collagen fibrils, and adjacent cartilaginous endplates [6]. IVDs promote the exclusion of NP tissue from immunologic tolerance during development [7]. A previous study reported that the

apoptosis-associated gene *FasL*, which is predominantly expressed in normal NP cells (NPCs), induced the destruction of infiltrating Fas-bearing immune cells, thereby ensuring the continuance of the IVD as an immune-privileged organ [8]. However, if the immunologic balance is damaged via annulus rupture or end plate micro-fracture due to IVD degeneration, an auto-immune reaction occurs, leading to the infiltration into the tissue, and activation, of various types of immune cells, including macrophages [9–11].

Macrophages play a major role in activating the innate immune system and contribute to the healing of tissue injuries and wounds [12]. Pro-inflammatory M1 and anti-inflammatory M2 macrophages help maintain the homeostasis of several tissues [13–15]. Studies have indicated that inflammatory and immune responses in LDH may stem from macrophage infiltration of NP tissues [16–18]. *In vitro* experiments have established the existence of interactions between NPCs and macrophages under both healthy and unhealthy culture conditions, resulting in the upregulation of inflammatory factors encoded by the inflammation-related genes *IL-1β*, *IL-6* and *Ccl3*, and the extracellular matrix metabolism genes *Adamts4*, *Mmp13* and *Acan*, in co-cultured NP cells.[21,30,31] Studies in animal models of IDD have demonstrated that the presence of M1 macrophages is enhanced during the LDH process [19]. A study in cadaver demonstrated the accumulation of M1 and M2 in degenerative disc tissue [20]. However, the questions of whether these macrophages are involved in human LDH in a similar manner, and whether the presence of these macrophages is associated with degeneration due to LDH remain unanswered.

In the present study, the cell surface marker induced nitric oxide synthase (iNOS) was selected to represent the M1 macrophage phenotype, and CD206 was used to represent the M2 macrophage phenotype in LDH [21]. The levels of expression and distribution of iNOS+ (M1) and CD206+ (M2) macrophages in LDH were measured using immunohistochemistry. Our objective was to analyze the presence of M1 and M2 macrophages in LDH, and investigate the association between these macrophages, the clinical characteristics of patients, and the efficacy of treatment, to better understand the roles of these macrophages in LDH.

Materials And Methods

Inclusion and exclusion criteria of patients with LDH

The study was performed at the Department of Orthopedics of Gaozhou People's Hospital, Guangdong, China, and was approved by Hospital ethics committee (GZPH-2019-016). Patients with LDH were retrospectively and consecutively reviewed from January 2017 to January 2019. The inclusion criteria were as follows: (a) the patients were diagnosed with LDH and underwent lumbar disc discectomy surgery, (b) NP paraffin specimens, lumbar spine radiography results, and magnetic resonance imaging scans were available, and (c) the patient provided informed consent. The exclusion criteria included LDH caused by tumor, infection, or other disorders that may impact "normal" spinal aging and degeneration, as well as the patients with medical conditions that potentially affect macrophage polarization. The flow diagram for the inclusion of patients with LDH is shown in Fig. 1.

Human NP specimens

NP tissues were harvested during lumbar disc discectomy surgery of LDH patients, and immediately washed with physiological saline at least three times, to ensure the blood was removed within 30 min. After fixation with 4% paraformaldehyde for approximately 24 h, the NP was dehydrated and embedded in paraffin. Finally, 5 μm thick serial sections were cut and stored at 4 °C.

Immunohistochemistry

The sections were deparaffinized, rehydrated, and soaked in citric acid antigen repair solution buffer (10 mM citric acid, pH 6.0) overnight at 60 °C, to expose the iNOS and CD206 epitope. The samples were then soaked in 3% hydrogen peroxide for 15 min to inactivate endogenous peroxidase. After washing with PBS three times, the sections were blocked with 1% goat serum at room temperature for 1 h, and incubated with anti-iNOS (ab15323, Abcam, Cambridge, UK) and anti-mannose receptor antibody (ab64693, Abcam) overnight at 4 °C. Finally, the sections were stained with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and then 3,3-diaminobenzidine was used to visualize the chromogen with hematoxylin used for counterstaining [22]. After dehydration and clearing with dimethylbenzene, the sections were sealed with neutral gum, and images were obtained using a Leica inversion microscope (Leica, Wetzlar, Germany).Ten fields were randomly selected under 2 × 10 magnification, and the amount of positive staining of NP macrophages was measures, and a mean value obtained. The percentage of positively staining macrophages in each section was analyzed and counted by two observers.

Evaluation of Clinical Outcome

The Oswestry disability index (ODI) was used to evaluate patients' daily life activities, and the visual analog scale (VAS) was used to assess the intensity of low back and leg pain. The ODI and VAS scores were retrospectively collected by telephone for 3 d pre-operation, 1 month post-operation, and 12months post-operation, and were evaluated by two of the authors (Liang DM and Lai LY).

Statistical methods

The data are presented as the mean ± standard deviation, and as frequency distributions where appropriate. Spearman non-parametric correlation analysis was used to determine the associations between percent of positive-staining cells and patient clinical characteristics. The Mann-Whitney U test was conducted to compare non-parametric data, and the Chi-square test was used to evaluate frequency data. Statistical analyses were performed using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). The level of significance was set to $P < 0.05$.

Results

Demographic summary

Seventy-nine NP tissue samples were harvested from 53 male patients and 26 female patients. The mean age of the patients was 47 ± 13 years. Patients presented with Pfirrmann grades as follows: Grade I:1, Grade II:2, Grade III: 17, Grade IV: 49, and Grade V: 10. MCs were observed in 32 cases but were not seen in47 cases, while a high-intensity zone (HIZ) was observed in17 cases and was absent in62. The baseline and clinical characteristics of the LDH patients are shown in Table 1.

Table 1
Characteristics of the study population

Gender(M/F)	**53/26**
Mean age (M ± SD)	47.5 ± 13.5
LBP duration (M ± SD)	
Leg pain duration (M ± SD)	
Pfirrmann grades	
I	1
II	1
III	18
IV	49
V	10
MCs	
Yes	35
No	44
HIZ	
Yes	15
No	64
Stenosis sites	
L3-L4	5
L4-L5	53
L5-S1	21
LBP: Lower back pain; HIZ:High-intensity zone;	

Cell morphology and distribution of M1 and M2 macrophages in LDH patients

During the progression of LDH, NP tissues develop structural abnormalities such as cracks and tears, reduced cell counts, and the formation of cell clusters [20]. In this study, we detected iNOS + and CD206 + in “normal” (Figs. 2A and 2E) or mild degenerative NP tissue (Fig. 2B and 2F), as well as in moderately (Figs. 2C and 2G), to heavily degenerative LDH (Figs. 2D and 2H). A mouse IDD model and cadaver specimens also produced the same result. Variability in the shapes of cells expressing iNOS and CD206 was observed. Some formed clusters with multiple nuclei (Figs. 2B and 2F), and some differentiated into hypertrophic chondroid-like cells (Figs. 2C and 2G),while others evolved into resident-like NPCs with or without a surrounding matrix (Figs. 2D and 2H), This diversity in cell morphology probably indicates that these cells originated fromboth resident and recruited macrophages.

Correlations between expression of iNOS + and CD206 + and Pfirrmann grades or ages in LDH patients

The Pfirrmann classification was used to assess the degree of disc degeneration, from Grade I to Grade V, from T2-weighted images [23]. Both iNOS + and CD206 + cells were detected in all 79 LDH specimens of all grades. The proportion of iNOS-positive cells ranged from 10–97%, with an average of 34 ± 14%, whereas that of CD206 + ranged from 16–88%, with a mean of 56 ± 16%. The correlation between iNOS positive cells and Pfirrmann grades were significantly negative ($r=-0.28$; $p = 0.012$; Fig. 3), while that between CD206positive cells and Pfirrmann grade was significantly positive($r = 0.53$, $p < 0.001$; Fig. 3). Patient age, which ranged from 21 to 81 years, displayed a similar correlation as Pfirrmann grades, as indicated by a negative relationship with iNOS ($r=-0.31$; $p = 0.01$; Fig. 3) and a positive relationship with CD206 ($r = 0.32$; $p = 0.004$; Fig. 3).

Correlation between clinic efficacy (VAS score and ODI) and iNOS positivity in LDH patients

The LBP and leg pain (LP) VASs were retrospectively recorded on preoperative day3, and post-operative months 1 and 12. Subsequent analysis of the correlation between the two VAS scores, LBP and LP, and iNOS positivity on preoperative day 3 indicated that both scores were significantly positively correlated with iNOS ($r = 0.25$, $p = 0.03$, and $r = 0.25$, $p = 0.04$, respectively); (Fig. 4). However, the LBP and LP VAS scores for postoperative months 1 and 12 were not significantly correlated with iNOS positivity (LBP: $r = 0.15$, $p = 0.22$, and $r = 0.05$, $p = 0.68$; LP: $r = 0.18$, $p = 0.16$, and $r = 0.17$, $p = 0.14$; Fig. 4).No Oswestry disability index (ODI) scores on preoperative day 3,or postoperative months 1 and 12,showed a significant correlation with iNOS positivity($r = 0.1$, $p = 0.87$;$r = 0.34$, $p = 0.69$; and $r = 0.23$, $p = 0.61$; Fig. 4).

Correlation between clinical efficacy (VAS score and ODI) and CD206 positivity in LDH patients

No significant correlation was observed between CD206 positivity and the VAS scores—LBP or LP—on pre-operative day 3, or post-operative months 1or 12 (LBP: $r = 0.00$, $p = 0.98$; $r = 0.16$, $p = 0.35$; $r = 0.07$, $p = 0.51$; and LP: $r = 0.00$, $p = 0.99$; $r = 0.11$, $p = 0.37$; $r = 0.14$, $p = 0.22$; Fig. 5). The percentage ODI at post-operative months 1 and 12 also showed no correlation with CD206 positivity ($r = 0.03$, $p = 0.74$; and $r = 0.08$, $p = 0.45$; Fig. 5). However, percentage ODI showed a significant positive correlation with CD206 positivity on pre-operative day3 ($r = 0.24$, $p = 0.03$; Fig. 5).

Expression of iNOS + and CD206 + in NPs from LDH patients with or without MCs

MCs, which are categorizes as type I–III, are vertebral endplate and bone marrow injuries detected by magnetic resonance imaging [24]. All 79 LDH were divided into MC and N-MC groups using the same patient baseline characteristics.LDH samples with and without MCs had no differences (Table 2). Staining revealed that the mean proportion of positive iNOS in the MCs group was 32 ± 11%, compared to 38 ± 17% in the group without MCs, but this difference was not significant (p = 0.23, Fig. 6). However, 60 ± 15% of cells were CD206-positive in the MCs group, significantly higher than the 50 ± 15% seen in the N-MCs group (p = 0.018; Fig. 6).

Table 2
Comparison of characteristics of LDH patients with or without MCs

Total		**MCs**		
		Yes	**No**	**p**
	79	47	32	
Gender(M/F)	53/26	31/16	22/10	0.07*
Age (M ± SD)	47.5 ± 13.5	41.±13.46	51.7 ± 12.11	0.06△
Pfirrmann grades				0.11*
III	18	3	15	
IV	49	38	11	
V	10	6	4	
Stenosis sites				0.73*
L3-L4	5	3	2	
L4-L5	53	30	23	
L5-S1	21	14	7	
HIZ				0.06*
Yes	15	12	3	
No	64	35	29	
*: chi-squared test; △: Mann–Whitney U-test; HIZ: High-intensity zone; MCs: Modic changes; LDH: Lumbar disc herniation;				

Expression of iNOS + and CD206 + in LDH patients with or without HIZ changes

Most studies have shown that changes in the HIZ are involved in the degenerative cascade, and are strongly associated with LDH [25]. Patient gender, age, Pfirrmann grade, MCs, and stenosis site were not

significantly different (Table 3).The two groups were LDH patients with HIZ changes, and LDH patients without HIZ changes. iNOS positivity was observed in39 ± 9% of samples from the HIZ group compared to34 ± 14% of samples from the non-HIZ group. CD206 + was expressed in59 ± 14% of samples of the HIZ group and 0.55 ± 0.16% of the non-HIZ group. The HIZ group showed significantly higher iNOS + expression than the non-HIZ group (p = 0.04, Fig. 7), whereas no significant difference inCD206 expression was detected between the two groups (p = 0.39; Fig. 7).

Table 3
Comparison of characteristics of LDH patients with or without HIZ changes

Total		**HIZ changes**		
79		**Yes**	**No**	**p**
		15	64	
Gender(M/F)	79	8/7	45/19	0.21*
Age (M ± SD)	47.5 ± 13.5	43.6 ± 9.7	48.9 ± 14.1	0.14△
Pfirrmann grades				0.26*
III	18	3	15	
IV	49	12	37	
V	10	0	10	
Stenosis sites				0.43*
L3-L4	5	1	4	
L4-L5	53	12	41	
L5-S1	21	2	19	
MCs				0.06*
Yes	47	12	35	
No	32	3	29	

*: chi-squared test; △: Mann–Whitney U-test; HIZ: High-intensity zone; MCs: Modic changes; LDH: Lumbar disc herniation

Discussion

In the current study we collected patient histology and clinical data from LDH patients over a period of two years, and performed immunohistochemistry analyses to detect the proportion of M1and M2 macrophages positive for the phenotype markers iNOS and CD206.The associations between M1/M2 and patient baseline criteria, including Pfirrmann grade, age, MCs, and HIZ, and clinical efficacy, including

VAS and ODI score, were analyzed. To the best of our knowledge, ours is the first clinical study to investigate the expression of different macrophage subtypes in LDH patients. iNOS+ (M1) and CD206+ (M2) were expressed in all 79 LDH samples.

The morphology of many iNOS + or CD206 + cells was similar to that of NPCs, having a round or oval shape with or without a surrounding pericellular matrix [18, 20].The morphology of M1 and M2 macrophages in the same or different LDH samples was diverse. Specimens from mouse IDD models and cadavers indicated that the presence of resident macrophage-like cells in the IVD was associated with its immune-privileged organ status, and that these cells probably originated due to a transition of NPCs in LDH [20]. The increased proportion of iNOS-positive cells (34 ± 14%) and CD206-positive cells (56 ± 16%) probably indicates the transition of NPCs to resident macrophage-like cells. Our study is the first to show that resident macrophages originate via transition of NPCs in degenerative LDH samples, and that this pathological transition probably leads to a new phenotype, associated with the process of degeneration in LDH.

The cadaver study revealed the presence of mixed macrophage phenotypes [20]. In some LDH samples, over 50% of cells were positive for M1 and M2, indicating colocalization of M1/M2 in LDH. This phenomenon may be due to M1 and M2 both originating from M0 macrophages, so that both types of cells should be colocalized with M0 [26]. Such colocalization of M1/M2 macrophages leads to a dynamic balance between M1/M2 in the LDH microenvironment, and consistent M1/M2 transition results in precise regulation of LDH progression. This work may help achieve a better understanding of the pathological changes and mechanisms underlying the degenerative processes associated with LDH.

The key finding in our study was the significant negative correlations between M1/M2 positivity and Pfirrmann grades. This observation revealed a predominantly pro-inflammation process during early degeneration, followed by an anti-inflammation state during later degeneration in LDH, as seen in some other tissue injury processes [21, 27–30]. Aging was positively correlated with the presence of M1/M2, as was Pfirrmann grade, indicating that aging may be a newly identified harmful factor for immune homeostasis in LDH. As an IVD ages, its immune balance is affected by recruited macrophages as well as resident macrophages, thereby facilitating the M1/M2 transition. Hence, regulation of immune homeostasis by M1/M2 plays an important role in LDH, and modulating this process may delay, inhibit, or even reverse degeneration.

Two significant correlations were detected in our study. First, M1 positivity was correlated with VAS scores—LBP and LP—on pre-operative day 3, indicating the occurrence of inflammation in LDH. Second, the positive correlation between ODI and M2 positivity on pre-operative day 3, indicated that a process of tissue repair and reconstruction occurs in a high ODI percentage LDH patients. Although these correlations were weak, they implied that M1/M2 participates in LDH to some extent, and that regulating this transition may help relieve pain and promote regeneration in LDH patients [31]. No other associations tested were significant, at either post-operative time point. Possible causes for this finding may be: (i) samples may not represent the true state of the M1/M2 ratio, as they were harvested at the time of

surgery; (ii) ODI percentage is similar to the cumulative results of M1/M2 transition, although the proportions in the study were collected at specific time intervals; and (iii) the immune response or inflammation in postoperative LDH patients is usually reduced or restrained by the administration of drugs [32].

MCs are a specific degenerative phenotype associated with LBP, and generalized inflammation caused by macrophages is considered to be a key factor linked to the genesis of MCs [33]. However, to date, the role of M1/M2 macrophages in generating MCs remains unknown. In this study, the expression levels of the M1 macrophages tested indicated no difference between the MCs and N-MCs groups, but a significantly higher expression of the M2 macrophage marker was detected in the MC group than in the group without these changes. All MCs were of type II, produced by a chronic degenerative process with fatty replacement [34]. To date, although studies have reported an association between macrophages and MCs, to the best of our knowledge, our results are the first to suggest that subtypes of M1 macrophages do not participate in the progression of type II MCs. The increasing expression of M2 macrophages may be explained by their infiltration into the replacement fatty tissue of type II MCs. Anti-inflammatory, rather than pro-inflammatory, changes are likely to occur during the development of type II MCs.

HIZ is identified by a high-intensity signal on T2-weighted magnetic resonance images, suggesting IVD disruption, annular fissures and vascular granulation of tissue in IDD [35]. Although HIZ is strongly associated with LBP [25], the role of M1/M2macrophages is unknown. A previous study reported that CD68-positive macrophages were prevalent in the HIZ zone [36]. In this study, both M1 and M2 macrophages were first detected in LDH. The higher expression of iNOS + cells in the HIZ group demonstrated that M1, rather than M2, macrophages contributed more to the HIZ. M1 macrophages, which normally secrete large amounts of pro-inflammatory cytokines and mediators in the early stages of tissue damage to promote inflammation, may show potential as therapeutic targets for LDH.

This study has certain limitations. Firstly, few type I and III MCs were collected in our study over two years, so only type II MCs were analyzed. Future studies including MC I and III are needed to confirm and extend our results. Secondly, only two types of macrophage were tested. Thus, a comprehensive analysis of many different macrophage subtypes may still be required. However, M1 and M2 are typical subtypes of polarized macrophages known to play important pro-inflammatory and anti-inflammatory roles in tissue injury. Examination of the M1/M2 transition may allow its role in LDH to be clarified to a certain extent. Thirdly, only iNOS was used to identify M1 cells, and CD206 to identify M2 cells. Dual-staining based immunofluorescence analysis of M0/M1 and M0/M2 may strengthen our results. However, M1/M2 phenotypic plasticity poses a challenge to the identification of exact phenotypes, as both iNOS and CD206 are widely used in other tissues [37], as previously shown in human cadaver studies [20]. Finally, there may be some other factors that may potentially affect macrophage polarization in the study. Although we have strictly enforced the inclusion and exclusion criteria, it may be hard to totally avoid the influence of extraneous factors.

Conclusions

To the best of our knowledge, this study is the first to identify an association between M1/M2positivityand the development of LDH, and to analyze the correlation between M1/M2 positivity and clinical efficacy on pre-operative day 3 and post-operative months 1 and 12. The wide distribution and positivity of M1/M2 expression, and the diversity of cell morphology, implies that both resident and recruited macrophages participated in the process of LDH. Both iNOS + and CD206 + cells showed significant correlations with Pfirrmann grade and age, indicating a predominant role of M1/M2 transition in the LDH process. The higher M1 concentration seen in relation to HIZ changes, as well as M2 positivity in MCs, was the first evidence to indicate the involvement of the M1/M2 transition in the complex, but incomplete, healing process of LDH. Finally, the significant correlation between iNOS positivity and VAS, and the significant correlation between CD206 and ODI on pre-operative day 3may imply the participation in LDH of inflammatory processes initiated by M1/M2. This phenotypic transition should not be disregarded, as the obvious interplay and balance that occurs between macrophage phenotypes and other cell types may contribute to their regenerative or protective capacity. Overall, our study may provide insight into the pathogenesis of this condition, and lead to the optimization of pharmacological intervention and treatment for LDH.

Abbreviations

LDH Lumbar disc herniation

IVD Intervertebral discs

IDD Intervertebral discs degeneration

MC Modic change

HIZ High-intensity zone

iNOS induced nitric oxide synthase

ODI Oswestry disability index

VAS Visual analog scale

LP Leg pain

LBP Lower back pain

NP Nucleus pulposus

NPC Nucleus pulposus cell

Declarations

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Authors' contributions

Xiaochuan Li carried out most of the experiments, participated in the analysis of data, and drafted the manuscript. Chunming Huang, Shaojian Luo, Tianli Zhou, Wen Chen and Wu Fan participated in the design of the study, data analysis, and interpretation and drafted the manuscript. Wu Fan performed the statistical analysis. Maosheng Wang and Xiaochun Bai participated in the design and coordination of the study and finalized the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was performed at the Department of Orthopedics of Gaozhou People's Hospital, Guangdong, China, and was approved by Hospital ethics committee (GZPH-2019-016).

Consent for publication

All authors consented to the publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

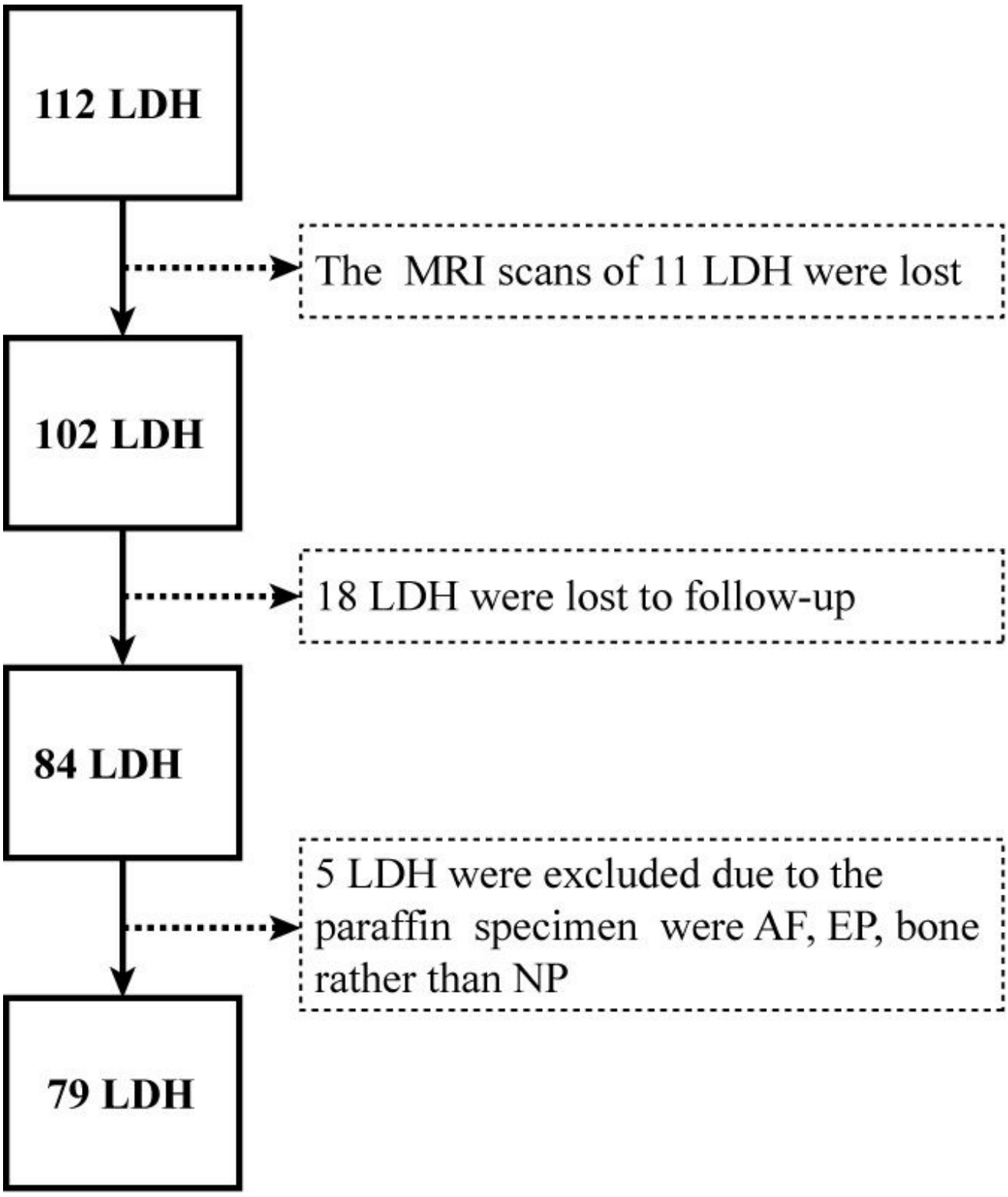

Figure 1

Flow diagram design of selection of the LDH patients in the study

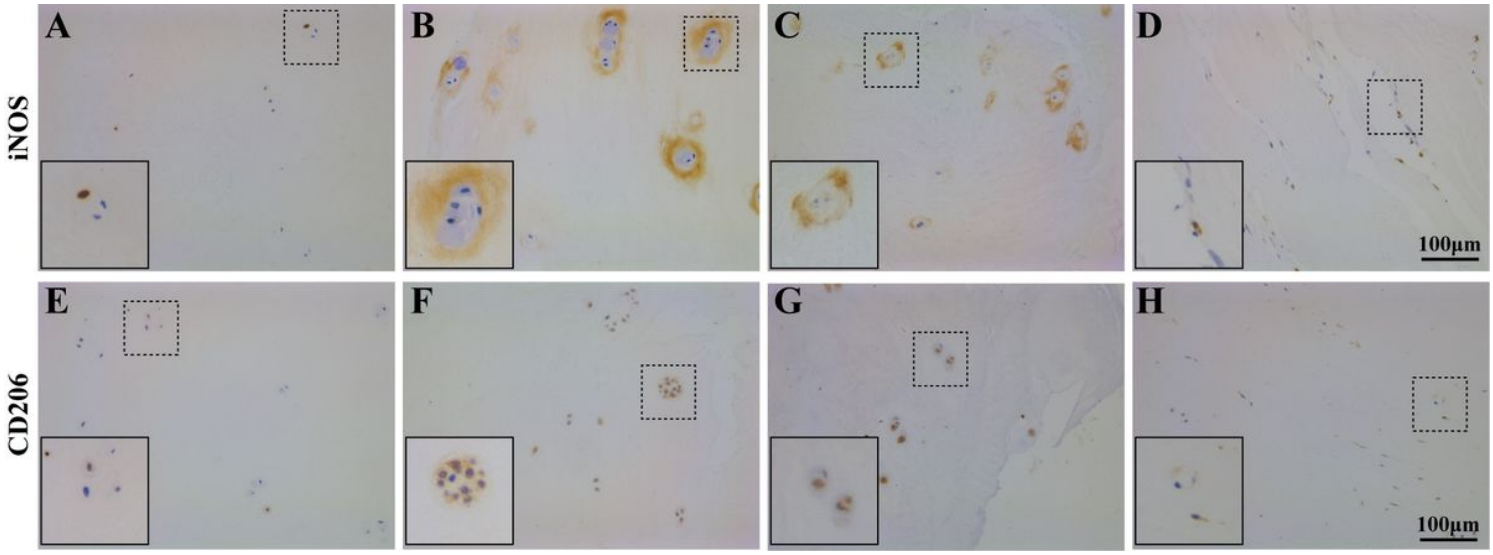

Figure 2

Positive expression and distribution of iNOS+ (M1) and CD206+ (M2) macrophages with diverse cell morphologies in LDH samples were shown, ranging from the “normal” (a and e), to mildly degenerative NP tissues (b and f) to moderately (c and g), and heavily (d and h) degenerative LDH. Bar=100 μm.

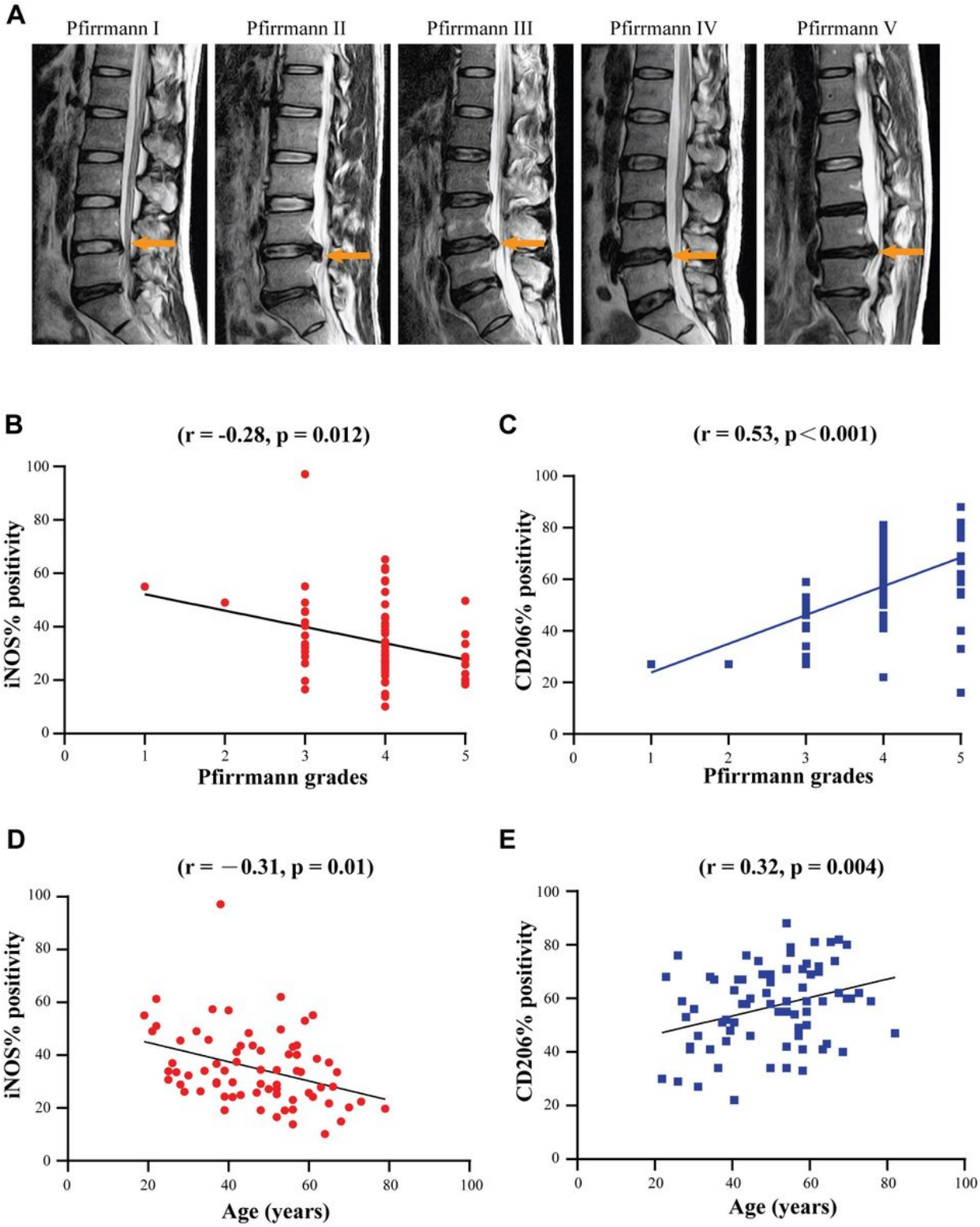

Figure 3

Both iNOS+ and CD206+ exhibited significant correlations with Pfirrmann grade and age. (a) T2W1 MRI images illustrate Pfirrmann grades from I to V, with the IVD segment indicated by a yellow arrow. The correlation between Pfirrmann grades and iNOS (b) as well as CD206 (c) positivity was significant. The negative correlation between age and iNOS+ (d), as well as the positive correlation between CD206+ and age (e), were significant.

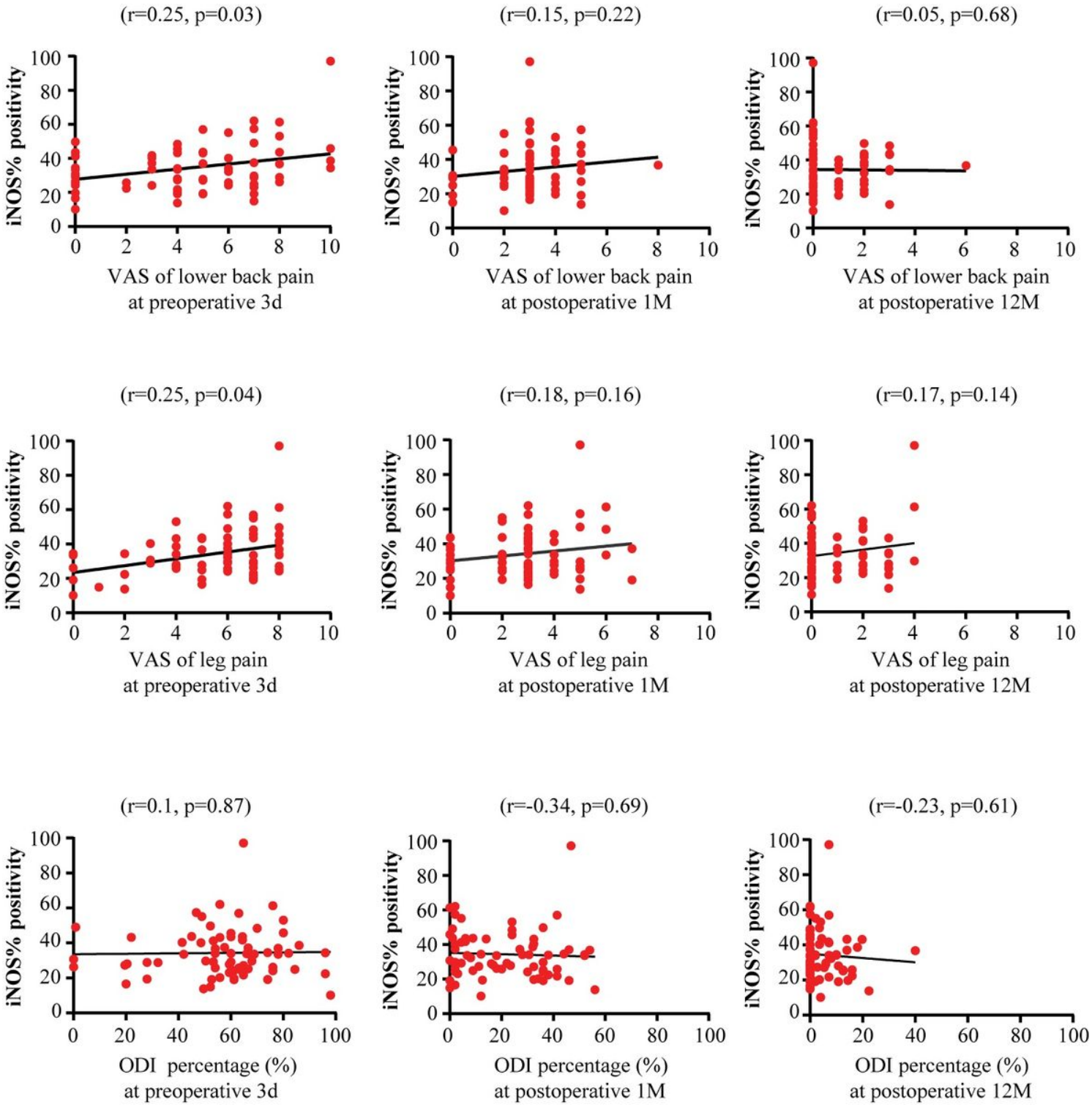

Figure 4

Correlation between iNOS positivity and clinical efficacy (VAS score or ODI) analyzed on pre-operative day 3, as well as post-operative months 1 and 12.

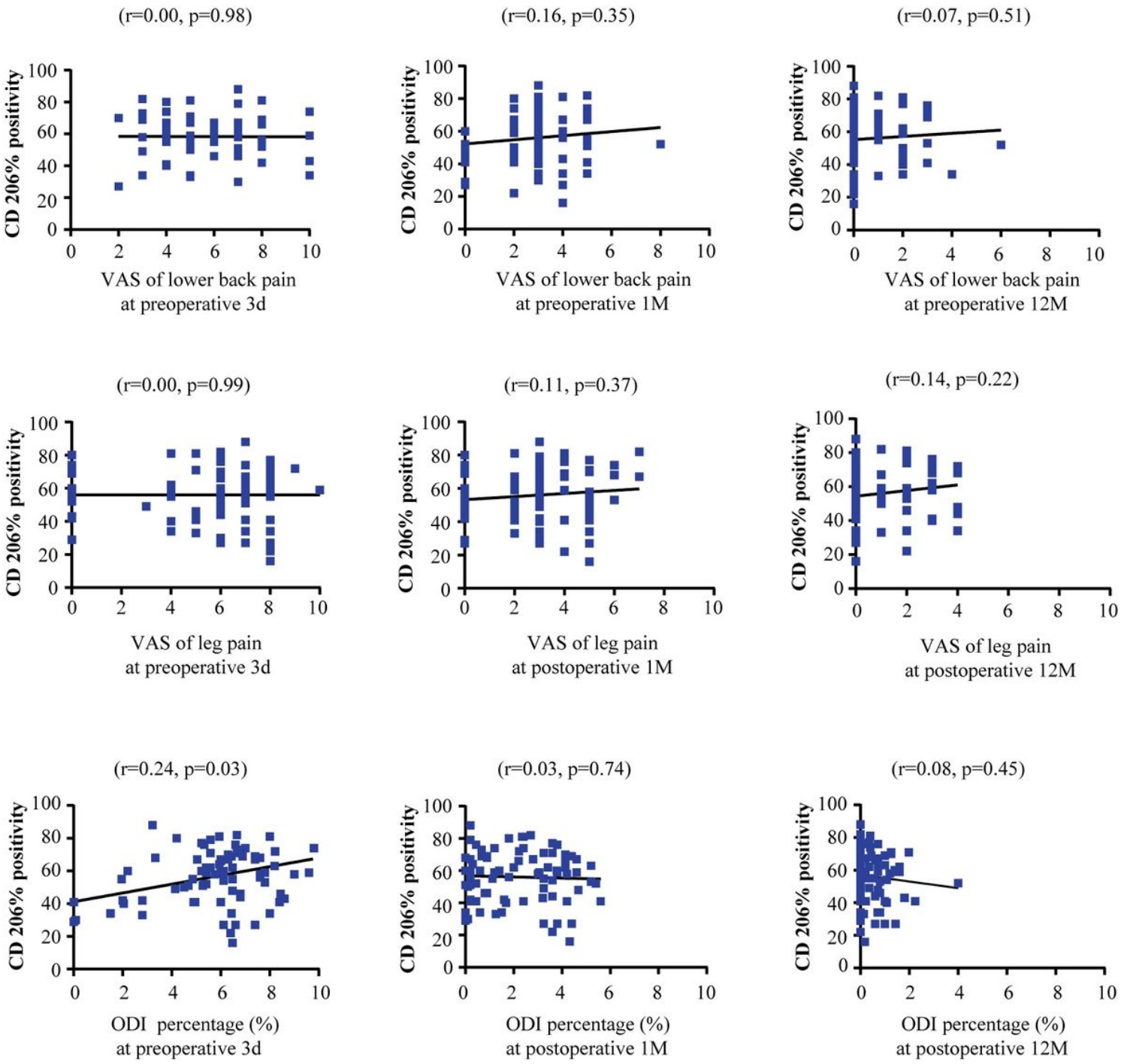

Figure 5

Analyses of the correlation between CD206 positivity and clinic efficacy (VAS score or ODI) were performed on pre-operative day 3, as well as post-operative months 1 and 12.

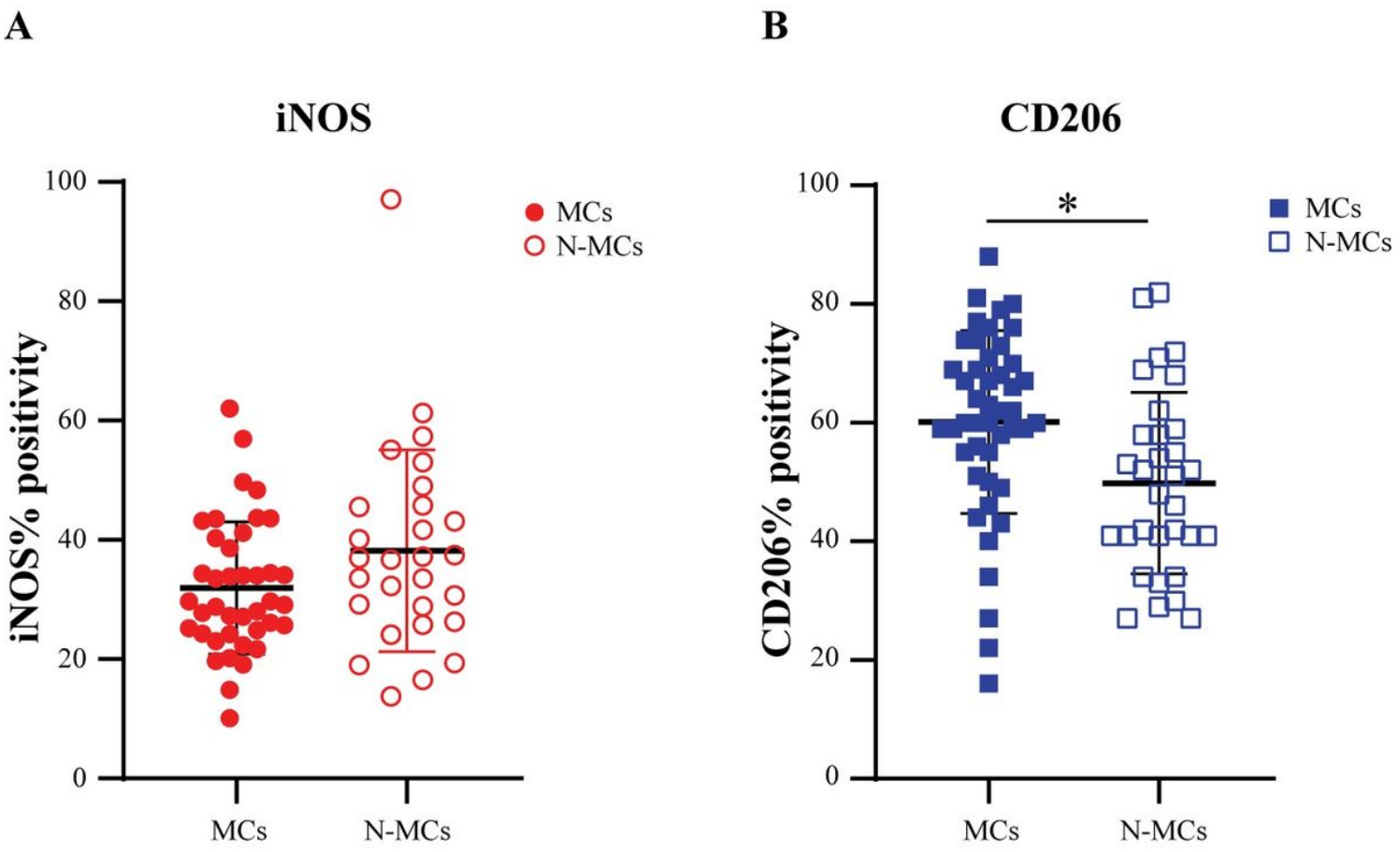

Figure 6

Comparison of iNOS+ (a) and CD206+ (b) expression in LDH patients with or without MCs.

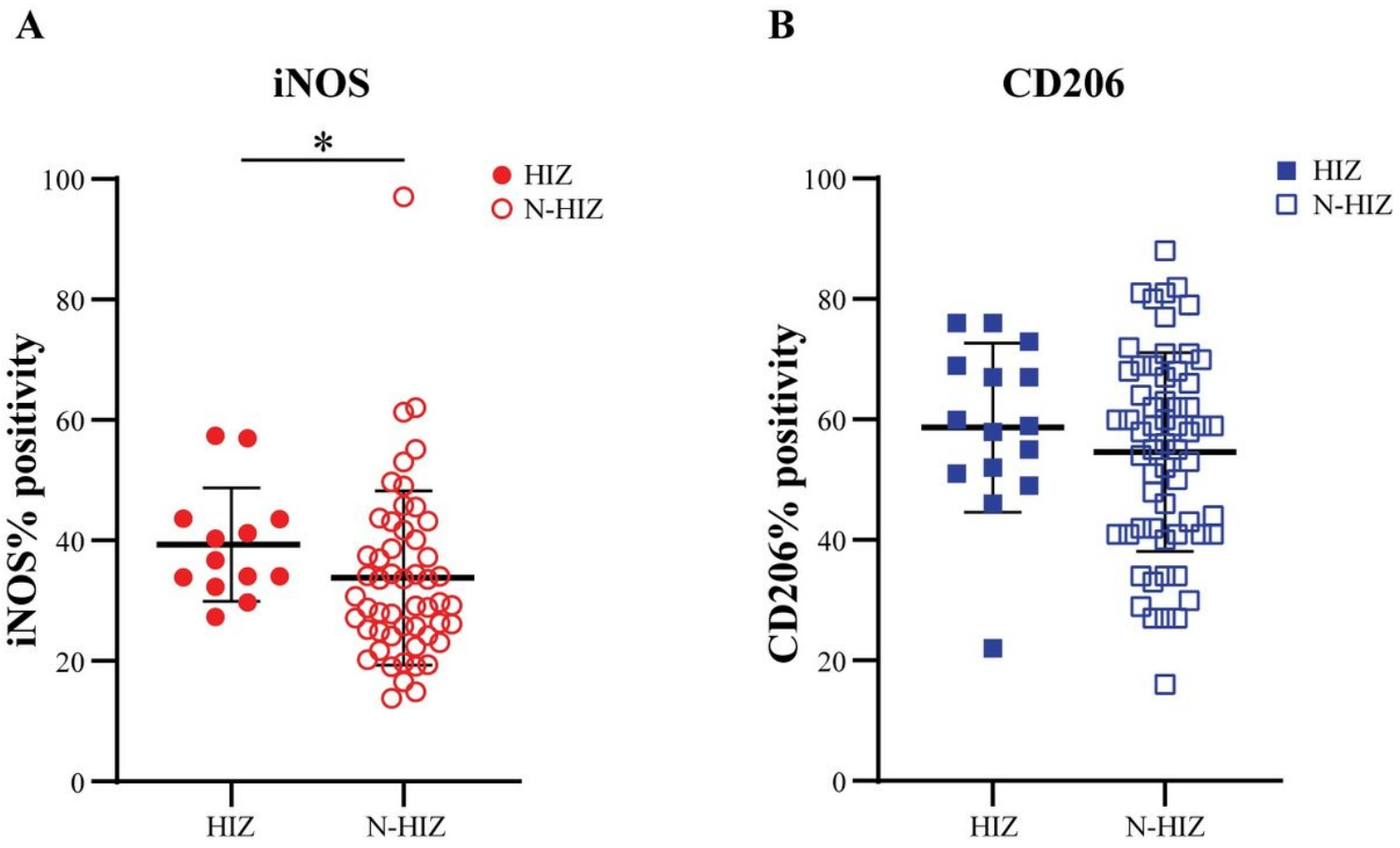

Figure 7

Comparison of iNOS (a) and CD206 (b) positivity in NPs from LDH patients with and without HIZ changes.