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The Application of Image Texture Analysis Techniques on the Effects of Dry Needling versus Placebo in Low-Back Pain Patients: A Pilot-Study

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Featured Application: This research applied the described methods of image analysis to create a robust analysis model to develop multiple avenues of research of great interest in biomedicine.

Abstract: Low back pain is the leading cause of disability in the world, with a significant socio-economic impact. Deep dry needling is effective in the treatment of this pain, and it is one of the techniques preferred by physiotherapists. In this field, the use of ultrasound provides information of interest such as length, thickness, diameter, cross-sectional area, or muscle volume, among others. Objective: To find out whether the tissue changes (thickness, histogram, and contraction rate) that occur in the lumbar multifidus after application of the deep dry needle are related to changes in the pain and the disability of the patient. Design: Randomized, double-blind, parallel-group clinical trial. Setting: University of Alcalá, Department of Physiotherapy. Subjects: 21 voluntary patients (women and men) with non-specific low-back pain aged 18–65 years. Intervention: Patients were randomly divided into two groups. One group received dry needling and the other group a dry needling placebo. Initial post-needling and one week post-needling assessments were performed by a therapist blinded to the intervention. Variables: Lumbar multifidus thickness measured by RUSI, contraction time measured by M-mode, histograms measured by image analysis, muscle area, pain measured by VAS, pressure pain threshold measured by pressure algometer, and disability measured by Roland–Morris questionnaire. Conclusions: The contraction speed, resting thickness, and pain demonstrated significant differences within each group, but not between groups. There were significant differences in contraction ratio and in PPT between groups. There was excellent intra-examiner reliability in image collection for histogram analysis. Histogram analysis showed no significant differences between groups and measurements, neither for the parameters nor for the parameters combined with the outcome variables. A robust method for the image texture analyses in future histogram muscle analyses has been performed.

Keywords: low-back pain; dry needling; ultrasound; histogram

Citation: Del-Canto-Fernández, A.; Calleja-Martínez, P.; Descalzo-Hoyas, B.; Rodríguez-Posada, S.; Cuenca-Zaldívar, N.; Fernández-Carnero, S.; Naranjo-Cinto, F.; Gallego-Izquierdo, T. The Application of Image Texture Analysis Techniques on the Effects of Dry Needling versus Placebo in Low-Back Pain Patients: A Pilot-Study. *Appl. Sci.* **2022**, *12*, 5556. https://doi.org/10.3390/app12115556

Academic Editors: Jürgen W. Czarske, Lars Buettner and Richard Nauber

Received: 12 April 2022
Accepted: 28 May 2022
Published: 30 May 2022

1. Introduction

Low back pain is the leading cause of disability and loss of productivity worldwide. It has a lifetime prevalence of up to 84% in the adult patient population [1]. The prevalence of low back pain of one month's duration was estimated at 20–26%. Patients with acute

low back pain may resolve their symptoms spontaneously in 70–90% of cases [2]. However, up to 70% of patients may suffer a recurrent episode of low back pain within one year and 54% of them within 6 months [3]. This study is based on an approach to non-specific low back pain, which can be classified according to its duration as acute (<4 weeks), subacute (4–12 weeks), or chronic (>12 weeks) regardless of the etiology [4]. A clinical relationship has been found between non-specific low back pain and pathology in the lumbar multifidus musculature, which has been shown to play an important role in the lumbopelvic complex [5,6]. For the treatment of non-specific low back pain, some authors have proposed the use of dry needling (DN), as there is evidence that it could be effective in pain management [7,8] and the application of deep techniques has been shown to be more effective than its superficial version in improving musculoskeletal pain [9]. For this study, Hong's technique was used to reproduce the patient's symptomatology, to produce the release of antalgic substances as well as the elimination of substance P (SP) and other nociceptive and inflammatory mediators [10,11]. In this study, we approached the lumbar multifidus musculature at L4–L5 level, since there are studies that demonstrate its efficacy in motor-sensory improvement in patients with non-specific low back pain [12,13]. Within the field of rehabilitation, diagnostic ultrasound offers the potential to provide specific tissue information to inform physiotherapy management of low back pain, which as noted is the most frequently encountered condition for physiotherapists mostly for spinal problems, with the lumbopelvic region being the most studied [14,15].

Among the evaluations that can be performed with the ultrasound scanner are the measurement of length, thickness, diameter, cross-sectional area, and muscle volume [16]. In this study, the thickness of the musculature will be measured pre- and post-intervention, muscle contraction time with M-mode, and a histogram analysis post-acquisition.

The M-mode assesses muscle activity, measuring muscle thickness pre- and post-contraction, as it allows visualizing a movement in real time, recording it, and analyzing it with a high spatial resolution. In addition, the location and the angle of the measurement could improve repeatability of the measurement [17,18]. The relationship between the level of isometric activity and the change in muscle thickness must be considered for change in muscle thickness to carry out the study of the level of muscle activity, so this could be a reason why a linear relationship has been described in muscles such as the multifidus muscles ($r^2 = 0.79$; $p < 0.001$), the transverse abdominis ($r^2 = 0.87$; $p < 0.0005$), and the external oblique abdominis (r^2 between 0.7 and 0.8; $p < 0.05$) [19–21].

The histogram is a graphical representation of the grey distribution by assigning a numerical value to each of the micro-pixels within a region of interest (ROI) [22,23]. The mean grey value of the histogram corresponds to the overall hardness, or of the region of interest (in our case muscle), where the value 0 is black (soft) and the value 255 is white (hard) [24,25]. The appropriate size of the region of interest is questionable and some authors, such as Caresio et al. have suggested including as much muscle as possible but avoiding fascia and surrounding bones [26].

The purpose of this research was to analyze the correlation between the application of the dry needling technique, measured with ultrasound and image correlation analysis using software (FIJI) with the improvement of pain, disability, changes in muscle thickness, contraction speed, and histogram in the multifidus musculature in patients with specific low back pain. A further goal of this study was to develop a robust method for muscle histogram analyses relevant for future studies.

2. Materials and Methods

The study is a double-blind, randomized clinical trial (subjects and assessors blinded to allocation) with two parallel groups. One group received DN and the other a placebo. The study was conducted at the Faculty of Medicine and Health Sciences of the University of Alcala (Madrid). It was approved by the ethics committee of the University of Alcala (No. CEIM/2021/1/020), and it was registered at ClinicalTrials.gov, accessed on 1 April 2022 (NCT05067673). All participants were informed, verbally and in writing, about the

procedure to be carried out and that they would have to sign an informed consent form to participate in the study.

The study sample consisted of men and women who met the inclusion criteria and who did not meet any exclusion criteria observed in other studies [12,27]. The inclusion criteria was: 18 to 65 years with non-specific low back pain. The exclusion criteria was: previous lumbosacral spine surgery, osteoporosis, belenophobia, neurogenic pain with positive tests or negative symptoms, red flags: cauda equina syndrome, rapid weight loss, fracture, cancer, infection or systemic diseases, pregnancy, and physiotherapy intervention in the 4 weeks prior to the intervention dry needling too.

A multiple linear regression model was used to calculate the sample size, with a test for an individual regression coefficient (indirect effect). Following a simple mediation model, two predictors were used in the model, an effect size of 0.15, a two-tailed contrast, a value of 0.05 and a statistical power of 80%. After calculations, a sample size of 55 subjects was obtained. Assuming a 20% loss rate, the final sample size was 70 subjects (35 per group). As this protocol was a pilot study, the final sample was 20 patients (10 patients per group). Since the present protocol was a pilot study with 20 patients, two groups of 10 patients were randomized using the statistical analysis program Epidat 4.2 ©.

During ultrasound imaging, no patient was able to look at the ultrasound screen. To ensure blinding of the assessors, neither of them knew to which group each patient belonged.

With the first 10 patients, the intra-observer reliability of the ultrasound measurements (muscle thickness, contraction velocity, and histogram) was assessed, and the process consisted of taking the ultrasound measurements to be carried out in the study in these 10 patients. The ultrasound measurements of each variable were taken three times, and the average was calculated.

The volunteer attended the assigned appointment and inclusion, and exclusion criteria were applied. Demographic data, pain data (Visual Analogue Scale-VAS and Pressure Pain Threshold-PPT with algometer), and disability data were then collected using the Roland–Morris questionnaire. The physiotherapist marked the point of greatest hyperalgesia, where the physiotherapists in charge carried out the intervention in L4–5 segment.

Ultrasound measurements were taken by another of the assessing physiotherapists, both were blinded to the intervention, after which the patient was accompanied to the intervention room where the DN or placebo was performed, depending on the group to which they belonged, information that was only available to the physiotherapists in charge of the intervention. Once the treatment was finished, the pain and disability data were collected again, as well as the ultrasound data from the two blinded physiotherapists, who met with the patient one week after the intervention to collect the study variables again. All ultrasound measurements (except M-mode measurements) were performed using a standardized microphone stand to minimize bias.

DN was performed simultaneously to ultrasound assessment using disposable stainless-steel needles (AGU-PUNT needles of size 0.30 × 50 mm or 0.30 × 60 mm sized to the patient), which were inserted perpendicularly at the point of greatest hyperalgesia using the Hong technique [28], and placebo was carried out with sham DongBang DB100 Acupuncture Needles. The patients were needled just once with only one needle at time.

The ultrasound measurements were made by a VINNO E35 ultrasound device with a linear 5–13 MHz probe and a 48 mm footprint. The measurements were made post-acquisition in a computer with the Fiji software (version 2.3.0, National Institutes of Health, Bethesda, MD, USA) [29]. Two groups of variables were analyzed in lumbar multifidus muscles, morphometrics (thickness, contraction time, and cross-sectional area CSA), and densitometric (histograms). The probe was placed on the patients using a microphone holder (Figure 1), and the position was checked using a clinometer to ensure verticality. The measurements were made on patients and phantoms as a comparator due to the unchanged characteristics.

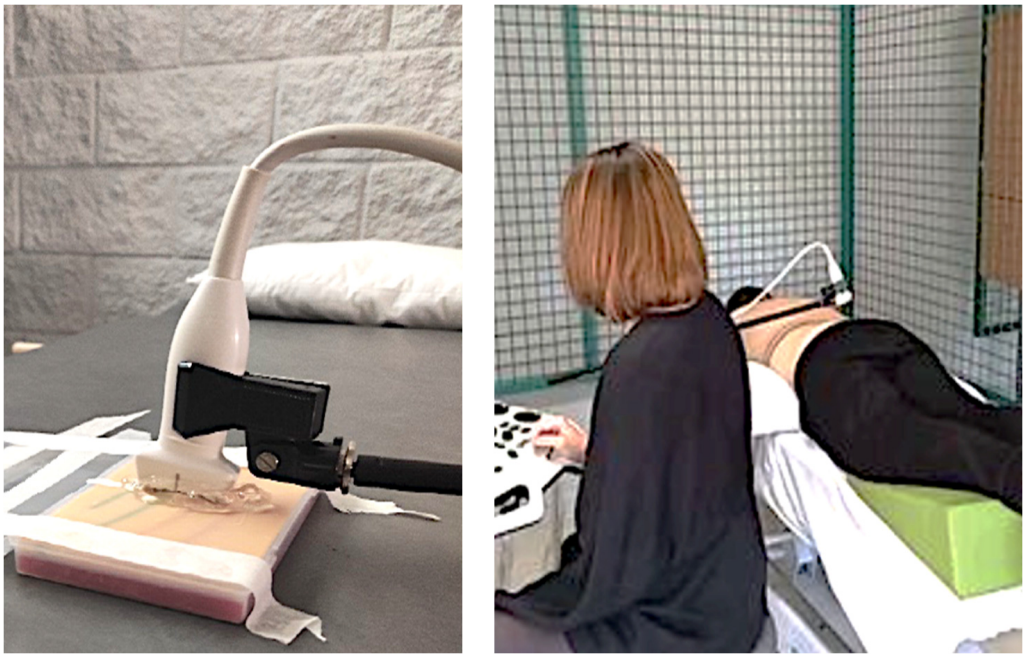
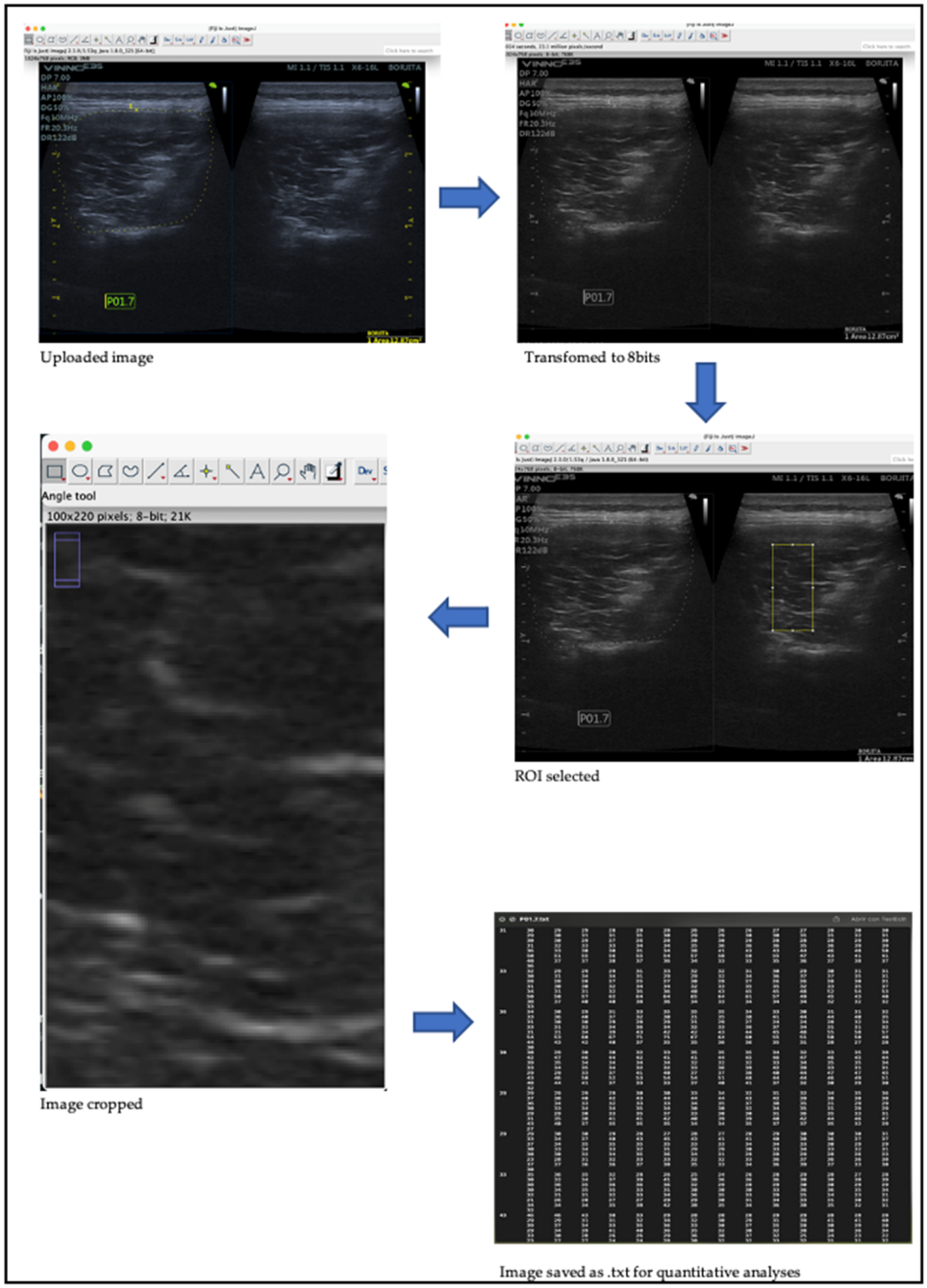


Figure 1. Phantoms and Patients sampling.

A Phantom (AliExpress-Toperfect Teaching Model&Toll Store model CNY 131.54) was used to compare the histogram outcomes with the sampling and to check the validity of the designed procedure because the characteristics of the phantom does not change so it could be a good criterion for comparison.

For the histogram analyses, a manual region of interest (ROI) was cropped in 100 × 220 pixels dimensions in the middle of the multifidus muscles bulk to ensure that it would fit in all samples. The image process is detailed in Scheme 1. The mean, standard deviation, variance, and kurtosis/skewness (of the histogram graph) were calculated, considering this parameter as first-order parameters associated with the grey levels of each pixel. The second-order parameters started by applying a macro (Supplemental Materials), which was made and batched to extract the matrixes for posterior analyses on the spatial distribution of pixel grey levels from different methods as detailed:

- Grey level co-occurrence matrices (GLCM), described by Haralick et al. [30,31], which consist of comparing pairs of pixels separated by a certain distance (by default a value of 1 is used) and in an angular direction (0°, 45°, 90°, and 135°) along the entire matrix, calculating the frequency with which certain grey levels appear in the image and their relationship with each other.
- Run-length matrices (GLRLM) described by Galloway [32] and calculated from the run-length statistic, which represents a set of consecutive pixels having the same grey level in each of the four angular directions described across the entire matrix.
- Local binary pattern (LBP) analysis, described by Ojala et al. [33], compares the intensity of a central pixel, which is taken as a reference value, with the surrounding pixels.
- Blob analysis, described by Nielsen et al. [34], is based on detecting areas close to each other with a similar eco-intensity called “blobs”.



Scheme 1. Imagen processing previous quantitative analyses.

After calculating all the parameters of each image, the correlation between these and the outcome variables was determined using Spearman's correlation coefficient, defined as weak (<0.4), moderate (0.4–0.6), strong (0.6–0.8), and very strong (>0.8). A principal component analysis (PCA) was applied, after checking that the assumptions were met (Kaisermeyer–Odin test for sample adequacy and Bartlett's sphericity test for collinearity) to determine the proportion of variance explained by the parameters in each group and time. Finally, we used a Permanova—a robust version of the parametric Manova—as its assumptions were not met, between groups, both for the parameters and for these combined with the outcome variables. An exact permutation test was used to detect the presence of significant differences in the calculated image texture parameters both between and within groups.

The non-image outcome variables (ultrasound morphology parameters, VAS, Roland–Morris questionnaire, and PPT) were analyzed using a mixed Anova of repeated measures with two factors between (group) and intra (measurements pre- and post-treatment and at 1 week) subjects or its robust version through a Wald-type pseudostatistical (WTS), based on compliance with assumptions (normality and Mauchly's sphericity test for homogeneity of variances). Post-hoc tests with Holm's correction were applied for pairwise comparisons for both main effects and group:time interaction. The effect size was defined with the partial eta squared statistic (η2p), as small (0.01–0.06), moderate (0.06–0.14), and large (>0.14). In the case of the Roland–Morris test that was evaluated before and after 1 week, the Student's *t*-test was applied after checking the homogeneity of variances using the Levene's test; and the effect size was defined with Cohen's D as small (<0.5), medium (0.5–0.8), and large (>0.8).

Histograms were used to evaluate the reliability of the intra-intergroup and intra-group measurements both in the subjects and in the phantom, using the intraclass correlation coefficient (ICC2) defined as poor (<0.5), moderate (0.5–0.75), good (0.75–0.9), and excellent (>0.9).

All statistical analysis was performed with the R version 3.5.1 program. (R Foundation for Statistical Computing, Institute for Statistics and Mathematics, Welthandelsplatz 1, 1020 Vienna, Austria). The level of significance was established at $p < 0.05$.

3. Results

The sample consisted of 20 patients, whose baseline characteristics are shown in Table 1. Significant differences were found in: the contraction ratio in the group:time interaction (F (2, 36) = 3.438, $p = 0.043$), with a higher difference in the placebo group and with a small and significant effect size (0.034 (0.002, 0.201)); the contraction speed variable in the main effect time (WTS (2) = 10.187, $p = 0.006$), with a final difference higher in the placebo group. 002, 0.201)); and the contraction speed in the main effect time (WTS (2) = 10.187, $p = 0.006$), with a final difference of −0.209 (−1.08, 0.678) that pointed higher in the puncture group and with a moderate and a significant effect size (0.04 (0.001, 0.173)). In the variable, PPT in the interaction group:time (WTS (2) = 8.664, $p = 0.013$) with a final difference pointed higher in the puncture group and with a large and non-significant effect size (0.039 (0.002, 0.156)).

Table 1. Baseline characteristics of the participants.

n		**10**	**10**
Age		26.34 ± 10.61	35.83 ± 17.52
Gender, *n* (%)	Female	5 (50.0)	7 (70.0)
	Male	5 (50.0)	3 (30.0)
Height (cm)		172.70 ± 7.20	171.50 ± 7.17
Weight (kg)		68.30 ± 11.64	65.60 ± 13.27

Data expressed with mean ± standard deviation or with absolute and relative values (%).

The rest thickness variable in the main effect time (F (2, 36) = 5.816, $p = 0.006$), with a final difference pointed higher in the placebo group, with a small and significant effect size (0. 038 (0.001, 0.134). VAS in the main effect time (WTS (2) = 44.873, $p < 0.001$), with a final difference 0.144 (−0.263, 0.575), points higher in the placebo group and with a small and significant effect size 0.04 (0.001, 0.157) (Table S1). In the post-hoc tests for the time:group interaction group, no significant differences were found probably because the sample was small, and these tests are not sensitive enough to detect in which specific measurements there are differences between groups (Table S2). In the post-hoc tests for the main effect of time within groups, significant differences ($p < 0.05$) were found in the VAS, Roland–Morris and contraction ratio and lesser in the contraction speed, PPT and rest thickness (see Table S3 for more details).

Histogram Analysis

The ICC2 was found to be excellent in the phantom, good in the patient pretreatment, moderate in the patient post-treatment and patient 1 week (both intra-group and overall), and poor in the patient pretreatment versus phantom average comparison (Table 2).

Table 2. Intraclass correlation coefficient values.

	Dry Needling	Sham	Overall
Patient pre-treatment	0.79	0.782	0.772
Patient post-treatment	0.672	0.701	0.697
Patient 1 week	0.631	0.623	0.637
Phantom			0.981
Patient pre-treatment vs. phantom average	0.216	0.158	0.186

The diagram indicates that in each group and measurement two principal components explain on average 77.554% of the variance (Figure 2).

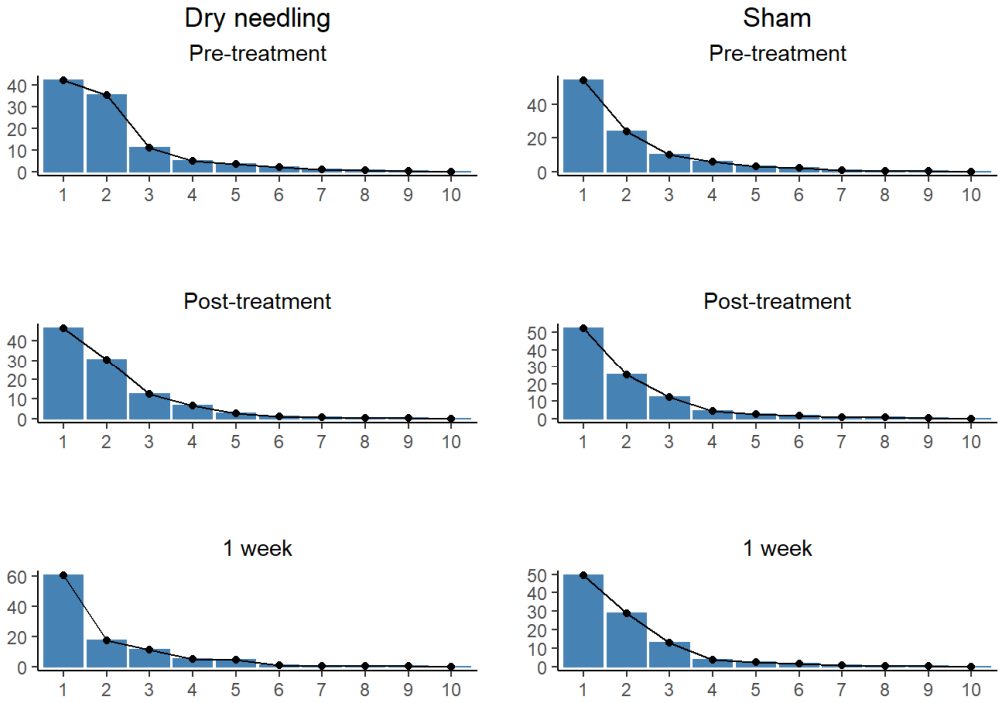


Figure 2. Variance explained by each principal component.

The principal components graph of the subjects in each group and measurement shows how the patients form two distinct clusters, especially at one week. This seems to indicate that the overall differences between the two groups increase over time, and they are more evident after one week (Figure 3).

The principal component graph of the image texture parameters also shows a clustering of them in each group, and the measurement shows how the patients form two distinct clusters, especially at one week, which indicates that the differences between subjects are due to the presence of differences in these texture parameters between both groups, which also increase over time (Figure 4).

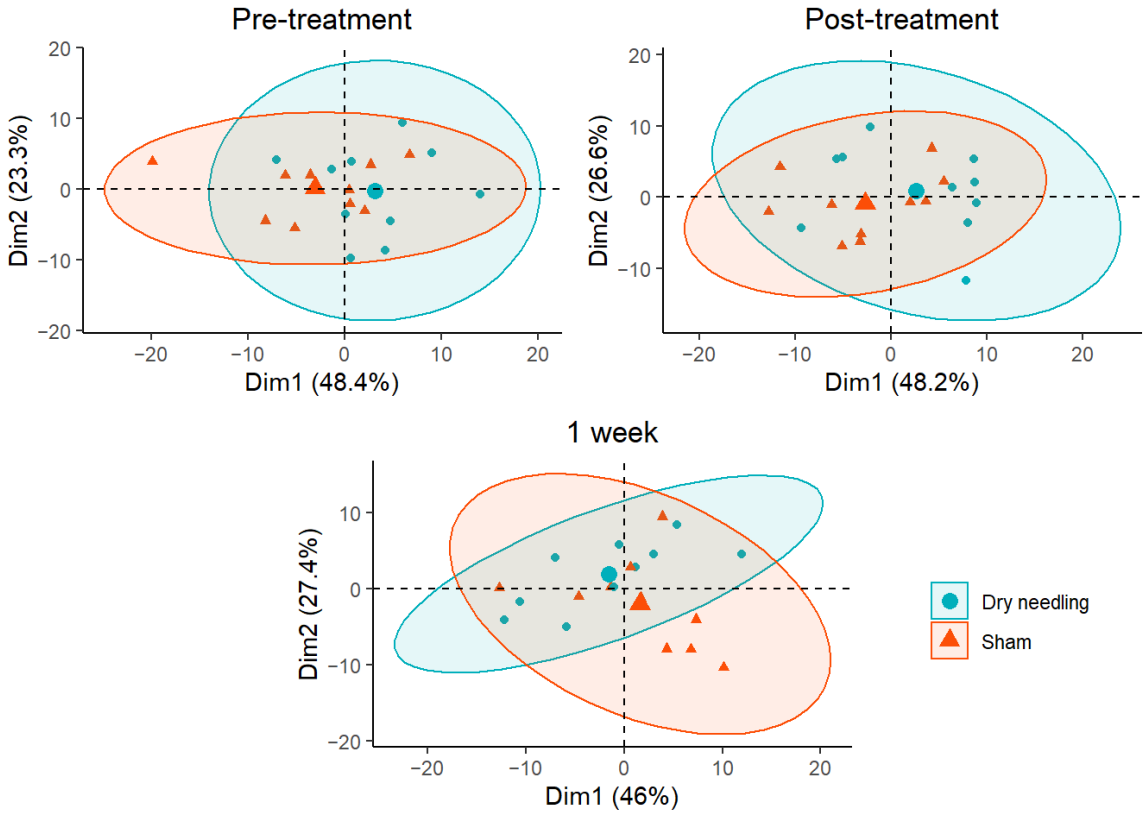


Figure 3. Graphic of individuals in each group.

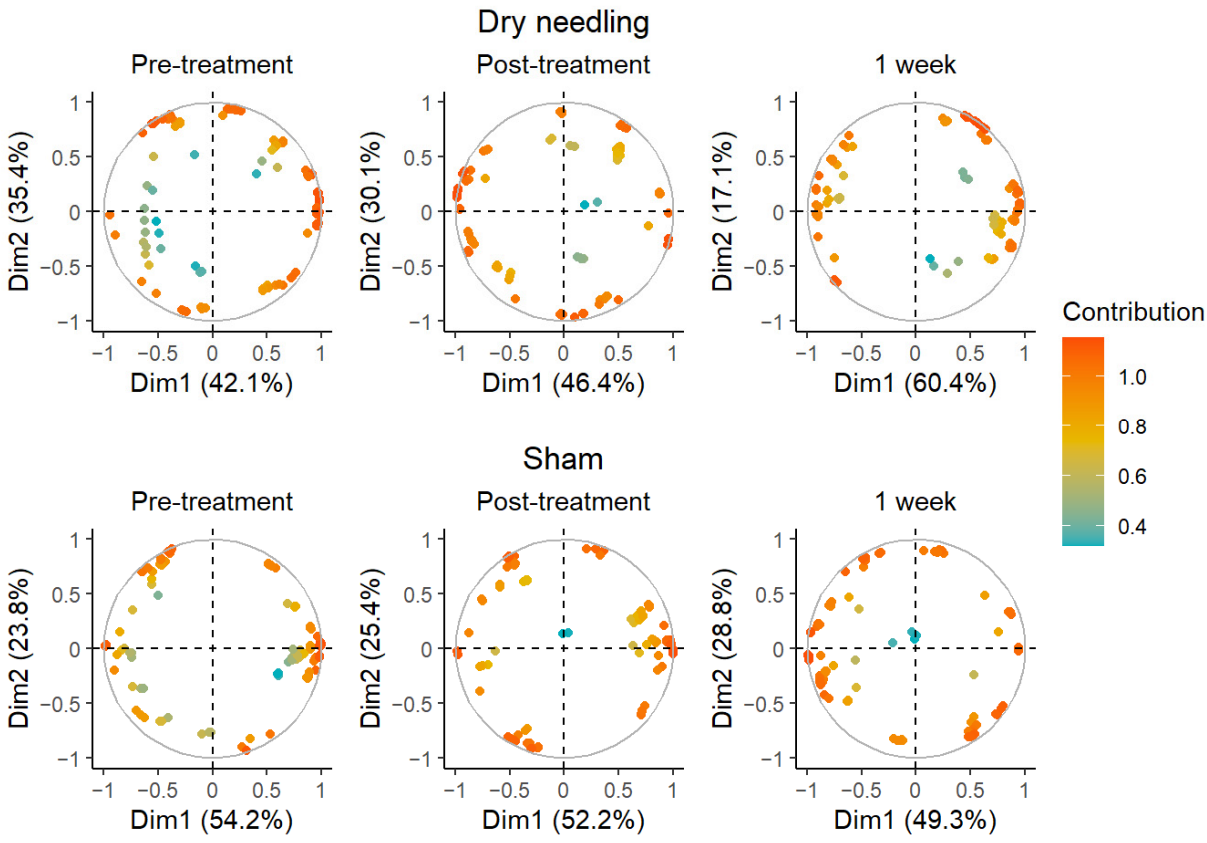


Figure 4. Graphic of variables.

The five image texture parameters that contribute the most to the variance in each measurement group and the principal component are in (Table S4). It is noted how entropy and the sum of entropy are the more important parameters in both groups at every time measurement time.

Permanova shows no significant differences between groups and measurements: neither of the image texture parameters, nor of the parameters combined with the non-image outcome variables (Table 3).

Table 3. Permanova.

	p Value Features	*p* Value Features Plus Outcomes
Pre-treatment	0.074	0.070
Post-treatment	0.334	0.320
1 week	0.106	0.092

The exact permutation test shows significant differences between groups ($p < 0.05$) in multiple image texture parameters post-treatment in both GLCM (autocorrelation at 0°, 45°, 90°, and 135°) and GLRLM (high grey level accentuation at 0°, low grey level accentuation at 0°, 45°, 90°, and 135°), without baseline differences, while non-significant differences were found at 1 week (Table S5).

4. Discussion

The aim of this study was to determine whether there was a correlation between the application of dry needling measured by ultrasound and software image correlation analysis with: an improvement in pain or disability, changes in muscle thickness, contraction velocity, and echointensity (histograms) in the multifidus muscles in patients with non-specific low back pain.

Our results showed that contraction velocity, resting thickness, and pain have significant differences within each group but not between groups and that there are significant differences in the contraction ratio and the PPT between groups despite the small sample size.

Previous studies have examined the efficacy of dry needling on post-intervention low back pain. In the meta-analysis by Liu L et al. [7], there is moderate evidence that dry needling may be recommended to improve the intensity of post-intervention low back pain, but it seems to be unclear in terms of disability or follow-up. However, the meta-analysis by Hu HT et al. [8] concludes that dry needling, compared to acupuncture or placebo needling, is more effective in relieving post-treatment pain and disability in low back pain, but at follow-up it is equal to acupuncture. The meta-analysis by Griswold D et al. [9] also reaches the same conclusion as that of Hu HT et al. for post-dry needling pain. Koppenhaver et al. [12] show that patients with disabilities have a greater improvement in lumbar multifidus contraction and nociceptive sensitivity at one week after dry needling but not immediately after treatment than those without disabilities.

Based on the data obtained in our study, we also found an improvement in lumbar multifidus contraction speed, which increased by 12.6% in the puncture group and 30.37% in the sham group at one week, but post-puncture the increase was 22.1% in the puncture group and only 0.8% in the sham group, so the effect on this variable is just after puncture.

In the study by Dar G et al. [13], the main finding is that after only one dry needling session (application of four needles for ten minutes left in the chosen segment in healthy patients), the multifidus musculature in the L4–L5 segment increases slightly in thickness both at rest and in contraction. If we compare it with our study, we see that there is a significant difference in thickness at rest but within the same group and not between groups. It is noteworthy that in the study by Sadler et al. [35], it was concluded that patients with low back pain have more trigger points and less strength in the gluteus medius than those patients without low back pain. It was also detailed that those with low back pain have a significantly smaller increase in gluteus medius thickness. This information also coincides

with that found in the meta-analysis of De Sousa et al. [36], where there is a decrease in lower limb strength in patients with low back pain.

Thus, it would be interesting for future studies to analyze the strength of the lower limb as baseline data to relate it to the changes caused by dry needling in pain, muscle thickness, contraction speed, etc. The findings of Suehiro et al. [37] indicate that the onset of multifidus activity during prone hip extension is delayed in patients with chronic low back pain compared to the healthy group. Based on the results of our study, we found a significant difference within the same group, but not between groups for the contraction speed study.

Regarding histogram analysis, in the article by Kim et al. [38] they compare the measurement of lumbar multifidus thickness of a fully automatic software versus the analysis of a human expert. The system they propose has a reliability of 72%, however, in our study we obtained a higher reliability in both the phantom analysis and in the pre-treatment patient.

The study of the grey-scale on ultrasound image has been reported of interest in relation to the pathological states [39] as diagnosis criteria; research by Martínez-Payá et al. [40] studied the grey distribution between patients with amyotrophic lateral sclerosis and a healthy group, and four different muscles were analyzed. It has been observed that different characteristics (entropy, homogeneity, and correlation) differ significantly between the patients and the healthy group, which provides information about the progression of the pathology and the differentiation between the musculature of the patient and the healthy subject. In our study, there were no significant differences between groups and measurements, neither of the parameters, nor of these combined with the outcome variables. Although in this study no significant differences were found between groups in variables such as pain, contraction speed or muscle thickness, it would be of interest to increase the sample to obtain more conclusive results, as the evidence and the differences within the same group show.

To the best of the knowledge of the authors, this is the first time a wide histogram analysis as presented has been done in muscle tissue; although it has not had any significant difference in the densitometric histogram analyses, it has opened the door to analyze the characteristics in other pathologies and in individuals with chronic low back pain, or back pain associated with other pathologies such as radiculopathy.

Among the limitations of our study, we must first highlight the smallness of the sample, which reduces the capacity to extrapolate these results to the population; but, because of the results obtained, it offers us a strong reason to extend this sample in successive studies to confirm its conclusions. In addition to increasing the sample size, it would be interesting to include another experimental group where the intervention was carried out on the healthy side of the patient as a prospective study. Another prospective study that would be of great interest—given that the study has been carried out on patients with low back pain, without considering their chronicity—would be to differentiate between subjects with acute and chronic pain. It would also be of great interest to study different dry needling protocols, such as the application of more needles, more sessions, etc. Additionally, it would be of great interest to study the long-term effects of dry needling on the multifidus musculature with more than one punction.

5. Conclusions

This study showed that contraction speed, resting thickness, and pain have significant differences within each group but not between groups. There are significant differences in contraction ratio and PPT between groups despite the small sample size. There is excellent intra-examiner reliability in image collection for histogram analysis. Histogram analysis shows no significant differences between groups and measurements, neither for the parameters nor for the parameters combined with the outcome variables. A robust method for future histogram muscle analyses has been performed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12115556/s1, Table S1: Outcome variables; Table S2: Between groups pairwise comparison p unadjusted and adjusted values; Table S3: Within groups pairwise comparison p unadjusted and adjusted values; Table S4: Top five loading texture features for each component; Table S5: Exact permutations test. A file with the "Macro" for first image processing.

Author Contributions: Conceptualization, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P., N.C.-Z. and S.F.-C.; methodology, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P., N.C.-Z., S.F.-C., F.N.-C. and T.G.-I.; software, A.D.-C.-F., N.C.-Z. and S.F.-C.; validation, S.F.-C.; formal analysis, N.C.-Z., F.N.-C. and S.F.-C.; investigation, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P. and S.F.-C.; resources, S.F.-C. and T.G.-I.; data curation, N.C.-Z., F.N.-C. and S.F.-C.; writing—original draft preparation, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P. and S.F.-C.; writing—review and editing, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P., N.C.-Z., S.F.-C., F.N.-C. and T.G.-I.; visualization, A.D.-C.-F., P.C.-M., B.D.-H., S.R.-P., N.C.-Z., S.F.-C., F.N.-C. and T.G.-I.; supervision, S.F.-C.; project administration, S.F.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and it was approved by the Institutional Ethics Committee of University of Alcala (No. CEIM/2021/1/020 on 19 October 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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