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Systematic Review

B-mode ultrasound characterization of myofascial trigger points and their response to physiotherapy interventions: a systematic review

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ABSTRACT

Background and objectives: Myofascial pain syndrome, provoked by myofascial trigger points (MTrPs), is a common cause of musculoskeletal pain. Accurate identification of MTrPs remains difficult due to its subjective clinical assessment. B-mode ultrasound (US) offers a promising alternative for characterizing MTrPs, although its clinical utility remains uncertain. This study aimed to evaluate the evidence supporting B-mode US to characterize MTrPs, assess changes in muscle architecture, and analyze the impact of physiotherapeutic interventions.

Methods: A systematic review was registered in PROSPERO following PRISMA guidelines (CRD42024596408). PubMed, Scopus and Web of Science databases were searched in October 2024. Variables of interest included MTrP characteristics (hypo/hyperechoic areas, dimensions, and morphology), muscle architecture (e.g., muscle thickness or pennation angle), and ecotexture analysis (first-, second-, and higher-order features).

Results: From 28 eligible studies and 929 participants, MTrPs were consistently identified as predominantly hypoechoic nodules with heterogeneous echotexture and elliptical shapes, with areas from 0.02 to 3.4 cm^2. Variations in muscle architecture, such as reduced thickness, and echotexture, mainly characterized by reduced echogenicity, were frequent in affected muscles by MTrPs. Physiotherapy interventions showed variable efficacy in altering MTrP characteristics depending on the intervention type and population. Methodological quality ranged from poor/low to good/high, with a moderate risk of bias.

Discussion: This review highlights the potential of B-mode US as an assessment tool for MTrPs. However, inconsistencies in imaging protocols, limited quantitative analyses, and the intrinsic characteristics of the included study designs, make it difficult to draw definitive conclusions.

Conclusions: Although B-mode US shows promise in characterizing MTrPs, methodological limitations highlight the need for further studies of higher methodological quality. Standarizing US assessment protocols for MTrPs could improve diagnostic accuracy and treatment monitoring and benefit clinical practice.

Keywords: Myofascial Trigger Point; Musculoskeletal Pain; Ultrasonography; Physical Therapy; Systematic Review.

1. Introduction

Musculoskeletal pain represents a significant health issue globally, with a notable prevalence in Spain, where approximately 20% of the adult population report some form of pain, and 7% experience it daily[1]. Among various types of musculoskeletal pain, myofascial pain syndrome (MPS) stands out as one of the most frequent causes, affecting more than 85% of the general population at some point in their lives[2]. MPS is provoked by myofascial trigger points (MTrPs), which can be defined as hyperirritable spots within a skeletal muscle taut band that produces local or referred pain[3,4]. These MTrPs can be classified as active, causing spontaneous pain, or latent, generating pain only upon palpation[5]. According to the "*Barometer of Chronic Pain in Spain 2022*", MTrPs are the second most common diagnostic cause of chronic pain, being the source of pain in around 50% of cases[6]. MTrPs are commonly linked with various physical, nutritional, and psychological factors, contributing to their development and exacerbation[7].

Despite their prevalence, diagnosing MTrPs remains challenging due to the reliance on manual palpation and subjective clinical assessment, leading to frequent diagnostic inaccuracies[3]. While widely used, manual palpation is known for its variable inter-reliability and the influence of different factors, including muscle depth[4,8-11]. In response to these limitations, several diagnostic technologies and modalities have been explored, including electromyography, infrared thermography, magnetic resonance imaging (MRI), and elastography, each showing variable diagnostic reliability[12,13]. Unfortunately, these tools have not yet been widely adopted in everyday clinical settings due to their high costs, limited availability, and time-consuming examinations[13]. In this regard, ultrasonography (US), particularly B-mode US, an increasingly accessible tool in clinical settings due to its cost-effectiveness and ease of use[14], is promising but still lacks clear consensus on its ability to detect and characterize MTrPs reliably[13,15,16].

Developing more objective diagnostic criteria is crucial, as accurate identification of MTrPs can guide more targeted therapeutic interventions. Current treatment modalities, such as dry needling or ischemic compression, show effectiveness in alleviating pain temporarily, but their long-term efficacy remains debatable[8]. A deeper understanding of the structural and biomechanical properties of MTrPs, and their behavior under therapeutic interventions is necessary to develop more effective treatments and prevent chronic pain conditions.

Numerous narrative and systematic reviews have investigated the potential of various imaging modalities, including US, for identifying and assessing MTrPs and MPS[12–15,17–20]. Yet, none have specifically focused on the use of B-mode US for the characterization of MTrPs, nor have they examined its utility not only as a guiding tool for invasive procedures but also as a means to quantify the outcomes of these treatments objectively. Therefore, the primary objective of this review was to summarize the current evidence on the use of B-mode US to characterize MTrPs, with secondary objectives that include evaluating the muscle architecture of affected muscles and analyzing changes following physiotherapy interventions. This review aims to summarize the existing evidence on this accessible imaging modality to enhance the diagnosis and treatment of MPS, ultimately improving patient outcomes and reducing the risk of chronicity in musculoskeletal pain conditions.

2. Methods

This systematic review was conducted in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines[21] and is registered with the International Prospective Register of Systematic Reviews (PROSPERO) under the reference number CRD42024596408.

2.1 Eligibility criteria

The PRISMA PICOS framework, which encompasses Participants (P), Interventions (I), Comparators (C), Outcomes (O), and Study Design (S), guided eligibility, and study selection[21].

Population:

- Inclusion: Included studies focused on participants aged 18 years or older. These participants either belonged to a group with MPS, musculoskeletal pain associated with MTrPs, or a healthy group/other condition involving MTrPs (active or latent).
- Exclusion: Studies were excluded if they involved populations where MTrPs (active or latent) had not been previously identified or if they conducted muscle analysis without examining the MTrP within that muscle.

Intervention:

- Inclusion: Studies that performed a US assessment of MTrP using B-mode were included. This could extend to the architecture and echotexture of the muscle containing the MTrP. Studies involving a physiotherapeutic intervention targeting the MTrP were also included, where these characteristics were analyzed.
- Exclusion: Studies that exclusively used alternative assessment tools or US modes other than B-mode (e.g., Doppler mode, elastography) were excluded. Additionally, studies that included any non-physiotherapeutic intervention were not considered.

Comparator:

- Inclusion: No control group was required. Studies were included if they characterized any MTrP with B-mode US, compared between muscles with MTrPs and those without, compared the MTrP with its surrounding or MTrP-free area, or evaluated the same group before and after a physiotherapeutic intervention.
- Exclusion: No specific exclusion criteria were based solely on the comparator conditions.

Outcomes:

- Inclusion: Studies were required to provide qualitative or quantitative analysis of MTrPs and the associated muscles.
- Exclusion: Studies focusing on variables unrelated to the research objectives were excluded.

Study design:

- Inclusion: All types of clinical trials were considered, including randomized controlled trials (RCTs), non-randomized intervention studies, matched controls, cohorts, and case series. Only publications in English or Spanish were included, with no restriction on publication date.

- Exclusion: Non-original research publications, such as book chapters, conference abstracts/papers, letters to the editor, commentaries, protocols, thesis, reviews, or meta-analyses, were excluded.

2.2 Data sources and search strategy

On October 2, 2024, an electronic search was carried out across PubMed, Scopus, and Web of Science databases to identify relevant studies. The search terms were organized into two categories: one focusing on US ("ultrasonography", "ultrasound", "sonography") and the other on MTrPs and MPS ("trigger point", "myofascial", "taut band"). These terms were selected based on a preliminary literature review and keyword identification. A reference search was also conducted to uncover any studies that might have been missed in the database search. The established search terms were then entered into the search engines of the respective databases, combined with Boolean operators, and appropriate filters were applied, leading to the following search strategies:

- PubMed: *("Ultrasonography"[Mesh] OR "ultrasound"[Title/Abstract] OR "ultrasonography"[Title/Abstract] OR "sonography"[Title/Abstract]) AND ("Myofascial Pain Syndromes"[Mesh] OR "trigger point*"[Title/Abstract] OR "myofascial"[Title/Abstract] OR "taut band*"[Title/Abstract])*
- Scopus: *TITLE-ABS-KEY(("ultrasound" OR "ultrasonography" OR "sonography") AND ("trigger point*" OR "myofascial" OR "taut band*"))*
- Web of Science: *((TS=("ultrasound" OR "ultrasonography" OR "sonography")) AND TS=("trigger point*" OR "myofascial" OR "taut band*"))*

2.3 Study selection

To determine whether the studies met the inclusion criteria, two reviewers (ZAB and CAJ) independently assessed each report to minimize bias, adhering to a standardized methodology agreed upon prior to the search. After completing their evaluations, they compared their findings.

All records retrieved from the three databases were initially imported into the bibliographic management software "Mendeley Reference Manager 2.98.0" to eliminate duplicate publications. The articles were then screened, focusing on the title and abstract to identify those potentially meeting the inclusion criteria. This was followed by a second screening phase, where the full texts of the articles that passed the initial screening were thoroughly reviewed. Studies that fulfilled all the inclusion criteria were selected. In cases where the reviewers disagreed, a third researcher (DLH) was consulted to reach a consensus.

2.4 Data extraction process

Two reviewers (ZAB and CAJ) independently extracted data from the studies and subsequently compared the extracted information for consistency. Any reviewer disagreements were resolved by involving a third person (DLH).

During the data extraction process, both evaluators adhered to the same procedure, collecting the following information from each study: author and publication year, study design, sample

characteristics (including sample size, sex, age, study population, and the specific muscle or anatomical region studied), intervention, study variables (related to both MTrP and muscle architecture and echotexture), and the main results.

2.5 Study variables

Data related to MTrP will focus on determining whether they are associated with hypo- or hyperechoic areas within the muscle and on the dimensions and morphology of the MTrP. Key characteristics such as cross-sectional area, muscle thickness, fascicle length, and pennation angle were examined for muscle architecture.

Echotexture analysis involves a detailed statistical evaluation of the pixels within the region of interest (ROI) in the US images. This analysis was classified into three levels: 1) first-order statistical features, which analyze the intensity values of individual pixels (such as echo intensity, variance, standard deviation, skewness, kurtosis, energy, or entropy); 2) second-order texture features, which assess the spatial relationship between pairs of pixels (such as Grey Level Co-occurrence Matrix (GLCM); and 3) higher-order texture features, which examine relationships involving three or more pixels (such as Grey Level Run Length Matrix (GLRLM), Local Binary Patterns (LBP), blob analysis or local texture anisotropy).

2.6 Assessment of methodological quality/risk of bias

Three different tools were employed to evaluate the methodological quality and risk of bias of the articles included in the systematic review, depending on the study design. The two reviewers (ZAB and CAJ) independently assessed the articles, and their results were subsequently compared with the intervention of a third reviewer (DLH).

For cross-sectional, case-control, and case-series studies, the National Heart, Lung, and Blood Institute (NHLBI) tools were applied: the “Quality Assessment Tool for Observational Cohorts and Cross-Sectional Studies”, the “Quality Assessment Tool for Case-Control Studies”, and the “Tool for Assessing the Quality of Case Series Studies”, respectively. The quality of these studies was assessed by answering a series of questions related to the study design, and the articles were categorized as having "good," "fair," or "poor" quality based on their scores.[22]

For RCTs and studies with random assignment, the PEDro scale was used. The PEDro scale consists of 11 items, and each item is scored as one if the criterion is met or zero if it is not. Item 1 assesses whether eligibility criteria were specified (external validity), items 2–9 evaluate the study's internal validity, and items 10 and 11 assess the interpretability of the results. The maximum score is ten points, as the first item is not included in the final score. In terms of score interpretation, articles scoring at least six out of ten were considered of "high quality," those scoring between four and five were deemed of "moderate quality," and articles with a score below four as "low quality".[23]

Finally, for studies involving non-randomized interventions, the ROBINS-I tool ("Risk Of Bias In Non-randomised Studies—of Interventions") was utilized. This tool assesses the risk of bias across seven domains: (1) confounding, (2) selection of participants, (3) classification of interventions, (4) deviations from intended interventions, (5) missing data, (6) measurement of out-

comes, and (7) selection of reported results. Each domain is rated as having a 'low risk,' 'moderate risk,' 'high risk,' 'critical risk,' or 'no information' based on specific questions. The overall risk of bias for each study was then determined by aggregating the scores across all domains.[24]

3. Results

3.1. Study selection

A total of 2,488 studies were retrieved from three electronic databases: PubMed, Scopus, and Web of Science. After removing 989 duplicate entries, the remaining studies underwent an initial screening based on titles and abstracts, resulting in 105 articles eligible for full-text review. Following a comprehensive evaluation of the full texts, 28 studies were ultimately included in the systematic review, meeting all predefined eligibility criteria. The flow diagram (Figure 1) provides a detailed overview of the study selection process, including the specific reasons for exclusion at each stage, adhering to the PRISMA guidelines[21].

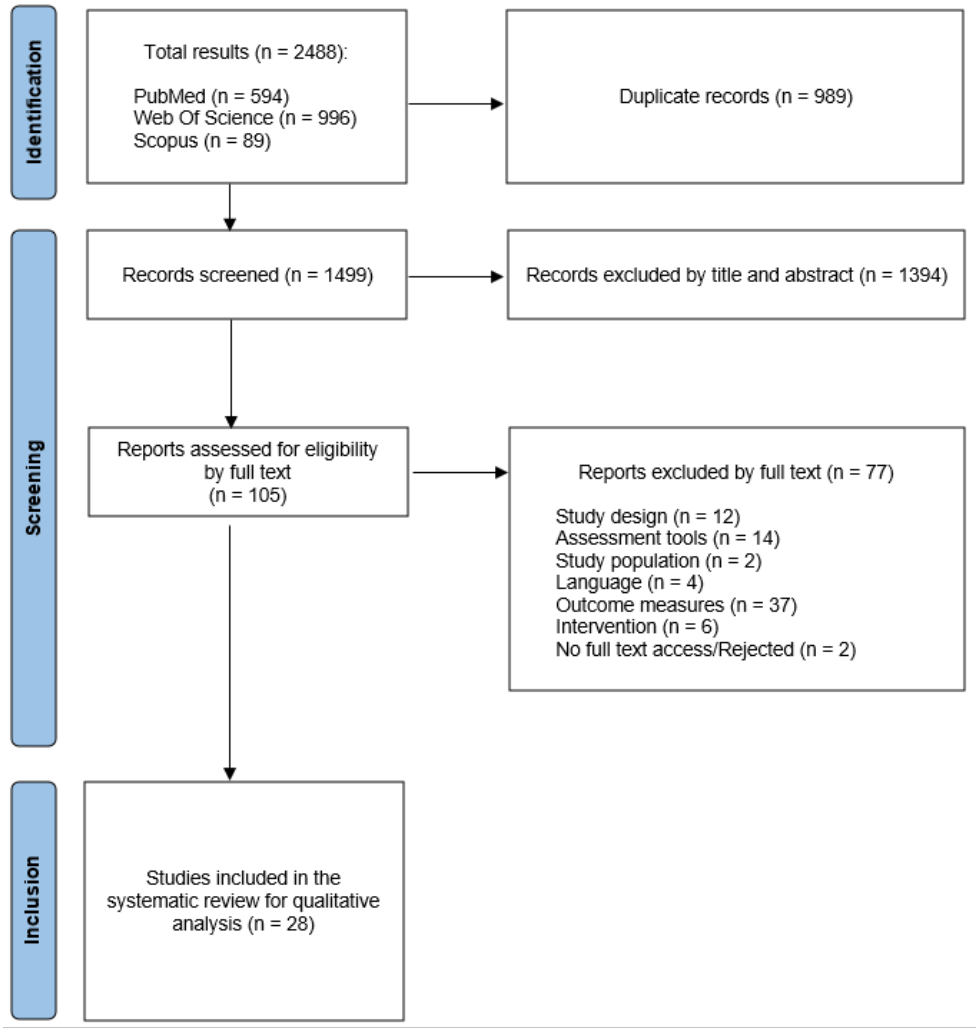

Figure 1. Study screening and selection PRISMA Flow Diagram.

3.2. Study characteristics

Tables 1 and 2 present the main characteristics of the 28 included studies. Table 1 summarizes the 17 observational studies that characterize MTrPs, while Table 2 outlines the 11 studies that include interventions aimed at investigating changes in MTrPs. Both tables are organized alphabetically by the last name of the first author.

3.2.1. Sample characteristics

A total of 929 individuals were included, comprising 151 men (16.3%), 556 women (59.8%), and 23.9% of participants whose gender was not reported. Notably, two participants were reported as dogs[25]. Among the total sample, 181 participants were classified as asymptomatic/healthy individuals (who could present with or without latent MTrPs), while the remaining 748 participants presented with various pathological conditions, including plantar heel pain, temporal headache, neck pain, low back pain, shoulder pain, MPS, ankle/foot pain, and cervicogenic headache. All participants, regardless of their condition, exhibited MTrPs located in muscles such as the upper trapezius, masseter, temporalis, deltoid, quadratus lumborum, longissimus thoracis, piriformis, gluteus medius, sternocleidomastoid, supraspinatus, infraspinatus, and gastrocnemius medialis.

The mean age of the participants was 35.5±6.9 years, ranging from 18 to 75 years. Age data were available in 21 out of the 28 studies included in the review[26–46]. Mean height was reported in only six studies, with an average of 1.7±0.1 meters. Similarly, the mean weight reported in the same six studies was 77.7±16.8 kilograms[28,30,32,34,37,40].

3.2.2. Assessment and intervention

All 28 studies utilized B-mode US to characterize MTrPs, both in studies without interventions and in those assessing the effects of various interventions. All US probes used were linear, with frequency ranges between 4 and 18 MHz. In 6 of the included studies, US image settings, particularly parameters such as time gain compensation, depth (ranging between 2 and 5 cm), and dynamic range, were kept constant[30,31,35,45,47,48]. However, in the remaining studies, it was not specified whether these settings were kept constant or adjusted for each participant. The majority of studies[25,27,29,33,34,36,37,40–44,46,49] based their US evaluation on the findings of Sikdar et al.[16], who first demonstrated MTrPs as hypoechoic regions with heterogeneous textures. In contrast, other studies[26,32,50,51] employed US imaging along the muscle, taut band, and MTrP to visually observe any changes. Lastly, other studies[30,31,35,45,47,48] selected different ROIs within and around the MTrP to assess defining characteristics and identify any differences.

11 studies employed a range of therapeutic interventions to target MTrPs and associated musculoskeletal disorders. The interventions used were: dry needling[37,39,43,46]; exercise therapy focusing on cervical mobility, stretching, and isometric exercises[38,39]; kinesiotaping[38]; manual therapy, targeting scapular and shoulder mobilization[40]; ischemic compression[40,42,43]; intramuscular electrical stimulation[41]; acupuncture[44]; electroacupuncture[44]; shockwave therapy[25]; and cold spray stretching[46]. Some studies combined interventions, such as kinesiotaping with exercise[38]; dry needling with exercise[39]; and a multimodal approach including warm heat packs, transcutaneous electrical nerve stimulation, and US therapy[45]. Control or sham groups were also included in several studies[40,42–44].

Table 1. Observational studies focused on myofascial trigger point characterization using B-mode ultrasound without interventions.

Author and year	Study design	Sample characteristics	Study variables	Main results
Ball et al., 2022	2 case reports	Case 1: Plantar heel pain MTrP in MG, and deltoid n = 1 (1 M); Age = 53.0 Case 2: Temporal headache MTrP in UT n = 1 (1 F); Age = 26.0	US imaging	In all muscles, a large hypoechoic contracture knot (109 mm × 47 mm in size in MG, two ~75 mm × ~75 mm in the deltoid and one 61 mm × 22 mm in UT) with smaller hyperechoic 'speckles' within (approximately 1 mm × 1 mm in UT) was identified
Cankurtaran et al., 2023	Observational, cross-sectional	Neck pain for at least 3 months n = 52 (43 F, 9 M); Age = 38.0 (21.0-63.0)	Muscles in which MTrPs were present and the number of MTrPs Thickness (mm) at rest of posterior cervical muscles, UT and MT Presence of hypoechoic areas	Positive correlation between the presence of MTrPs on physical examination and those detected by US in multifidus, UT and MT ($p<0.02$) A negative correlation was observed between MTrP detection and muscle thickness in all muscles ($p<0.05$) The median values of muscle thicknesses ranged from 0.2 to 1.9
Cojocaru et al., 2015	Observational, cross-sectional	Low back pain n = 8	US imaging in lumbar region	Ellipsoidal hypoechoic area in the muscle with an area ranging grom 1.2 to 3.4 cm^2
Da Silva et al., 2020	Observational, cross-sectional	Unilateral shoulder pain for at least 3 months MTrPs in UT n = 40 (26 F, 14 M); Age = 41.8±12.9; Height = 1.7±0.1; Weight = 74.6±14.6	MTrP area (cm^2) Thickness (cm) at rest and at contraction of UT	Area: 0.1±0.1 Thickness at rest: 1.0±0.2 Thickness at contraction: 1.4±0.3
Elbarbary et al., 2023	Observational, cross-sectional	Temporomandibular disorder MTrPs in masseter and temporalis n = 57 (46 F, 11 M); Age = 38.2±15.6	MTrP: cross-section size (mm), depth within the muscle (mm), and area (mm^2)	Cross-section size (transverse view): • Temporalis: 1.6±1.1 • Masseter: 1.7±0.7 Depth: • Temporalis: 5.4±3.0 • Masseter: 8.7±2.8 Area: • Temporalis: 1.9±1.3 • Masseter: 2.4±1.5 MTrPs were identified as hypoechoic nodules
Kumbhare et al., 2017	Observational, cross-sectional, case-control	Neck pain group n = 15 (10 F, 5 M) Healthy group n = 15 Total sample Age = 31.3±4.1; Height = 1.7±0.9; Weight = 81.2±20.9	ROIs were manually selected (UT) EI (mean, standard deviation, kurtosis, skewness) and blob analysis	A statistically significant difference in EI ($p<0.05$), being lower in the neck pain group Neck pain group showed an increase in blob area (2.7 vs. 0.1 mm^2) and a decrease in the number of blobs (0.02 vs. 0.1/mm^2 tissue)
Kumbhare et al., 2018	Observational, cross-sectional, case-control	Regional neck pain group Active or latent MTrPs in UT n = 37 (18 active MTrPs, 19 latent MTrPs) Healthy group n = 24 Total sample Age = between 20 and 65	18,300 ROIs of 150x80 pixels within the fibers of UT were obtained from a 10-second video in each participant For each ROI, 92 feature descriptors were extracted (76 GLCM, 7 GLRLM, 5 first-order texture, 3 blob, 1 LBP)	All identified features provided different information for each group ($p<0.001$) 9 of the identified features were different between healthy individuals and patients with latent or active MTrPs, while the remaining 14 features were only different between 2 groups
Kumbhare et al., 2020	Observational, cross-sectional, case-control	MPS group MTrPs in UT n= 15 (10 F, 5 M); Age = 31.8±7.3 Healthy group n = 15 (7 F, 8 M); Age = 22.7±7.1	ROIs were manually selected from the B-mode images in longitudinal view SVM was trained with the set of 13 higher-order features, including mean blob area, mean blob count, entropy and ten LBP features	The features with the highest loading factors were LBP2, LBP6, LBP10, Blob area and Blob count A significant association was found between the identified features and the clinical designations (MPS or healthy) ($p < 0.001$) All variables were statistically different between groups ($p<0.001$), except Blob area ($p=0.11$)
Lewis and Tehan, 1999	Observational, cross-sectional	Active MTrPs n = 11 (7 F, 4 M); Age (F) = 53 (30-74); Age (M) = 22 (21-36)	US imaging	There was no correlation between clinical identification of MTrPs and US imaging In 9 participants (81.82%), no abnormality or asymmetry was reported In 1 participant, a 5mm diameter hyperechogenic area related to the superficial fascia of the ES was observed, which was absent on the contralateral side

Author and year	Study design	Sample characteristics	Study variables	Main results
Shankar and Reddy, 2012	Case report	MPS n = 1 (1 M); Age = 58.0; Height = 1,8; Weight = 112.0	US imaging to localize the taut band in right shoulder in longitudinal and transversal view	Transversal view: • Localized areas of high EI in UT and supraspinatus at a depth of 3 cm • Loss of white spot pattern on a dark background Longitudinal view: • Discontinuous, wavy or clumped fascicular pattern
Sikdar et al., 2009	Observational, cross-sectional	Acute neck pain Active MTrP in UT n = 9 (7 F, 2 M); Age = 23-55	MTrPs area (cm^2) Tissue imaging scores were assigned from 0 (normal, uniform EI) to 2 (abnormal structure with multiple focal hypoechoic)	MTrPs appeared as hypoechoic, elliptical-shaped focal areas with a heterogeneous ecotexture and appeared to coexist with multiple nodules in close proximity Area: 0.2±0.1, with no significant difference between active MTrPs (0.2±0.1) and latent MTrPs (0.2±0.1)
Taheri et al., 2016	Observational, cross-sectional	Symptoms suggestive of MPS Active MTrPs in UT n = 15 (11 F, 4M); Age = 40.6±5.7	MTrPs area (cm^2) and EI (histogram) in longitudinal view EI of muscle with MTrPs in longitudinal and transversal views Thickness (mm) at rest of UT Pennation angle (degree) of UT	Area: 0.5±0.1 EI: • MTrPs: 41.6±2.8 • Muscle (longitudinal view): 55.4±3.5 • Muscle (transverse view): 54.7±3.6 Muscle thickness: 11.5±0.4 Pennation angle: 14.6±0.5 MTrPs apparead as focal hypoechoic areas with heterogeneous echotexture
Takla et al., 2016	Observational, cross-sectional, case-control	Low back pain for at least 3 months Active MTrPs in QL, LT, piriformis, and GM n = 25; Age = 34.3±6.2; Height = 1.6±0.1; Weight = 66.6±9.9 Healthy group Latent MTrPs in QL, LT, piriformis, and GM n = 25; Age = 35.8±5.9; Height = 1.7±0.1; Weight = 70.2±10.6	MTrP and surrounding tissue. Tissue imaging scores were assigned as '0', for uniform EI; '1', for a focal hypoechoic region with a stiff nodule; and '2', for multiple hypoechoic regions with stiff nodules	MTrPs (active or latent) appeared as one focal hypoechoic region, whereas the normal, immediately surrounding myofascial tissue showed uniform echogenicity (p<0.001)
Tsai et al., 2024	Observational, cross-sectional	MPS n = 25	Location, depth, height and shape of the MTrPs of the low back muscles	MTrPs can exist at all depths within muscle tissue, however about 70% are found near the muscle surface The mean size of MTrPs was 0.6±0.03 cm. 0.2 cm. increase in MTrP size for every cm. increase in muscle tissue size Groups exhibiting twitching had larger MTrP thickness (0.64±0.04 cm), but not significant (p=0.65), tan groups with no twitching (0.58±0.03 cm)
Turo et al., 2013	Observational, cross-sectional, case-control	Neck pain for at least 3 months Active MTrPs in UT n = 14 (10 F, 4 M); Age = 36±12 Healthy group n = 15 (6 F, 9 M); Age = 28±8	ROI was manually marked on each US image Additionally, ROI was divided into two parts: muscle belly and fascial border Entropy and areas with entropy <4 (threshold assigned to locate MTrPs)	Whole trapezius and fascia border analyses demonstrated a significantly (p<0.05) lower mean entropy and a significantly larger size of regions with entropy less than 4 in 'active' compared to 'normal' sites In the muscle belly analysis, only one statistical difference was observed between 'asymptomatic normal' and 'normal' sites (p<0.05), but none between 'active' and 'normal' sites
Zadeh et al., 2023	Observational, cross-sectional	No symptoms or history related to neuromuscular disease Each UT (right and/or left) was labeled as active MTrPs (n = 30), latent MTrPs (n = 30), or healthy control (n = 30) n = 63	For each participant, 4 images were manually selected from the 300 images generated from a 10-second video, capturing from lateral to medial each muscle Texture feature analyses through statistical features (entropy, contrast, correlation, homogeneity, energy, mean and variance) in different approaches: LBP, Gabor features, SEGL method and B-mode	Statistical differences (p<0.05) were observed in almost all features for all methods except B-mode (i.e. entropy, contrast and energy) and Gabor (i.e. mean and correlation) Machine learning could not classify the three groups sufficiently
Zale et al., 2015	Observational, cross-sectional, case-control	Ankle/foot pain group n = 17 Healthy group n = 8 Total sample n = 25 (14 F, 11 M); Age = 28 (18-63)	8 muscles were diagnostically evaluated: QP, soleus, TA, TP, EDL, EDH, PL, and PB An MTrP scoring system was used, assigning 0 (negative, no pain and no palpation of MTrP), 1 (patient felt pain, but no nodule was palpated), 2 (patient felt pain and nodule was palpated) or 3 (palpable, painful MTrP seen in US) MTrPs area (cm^2)	Of 500 total MTrPs areas that were palpated in symptomatic and asymptomatic participants, 441 (88.2%) had a score of 0, 16 of 1, 15 of 2 and 18 of 3 Area: ranged from 0.05 to 0.21, with a mean of 0.09 MTrPs appeared as inhomogeneous hypoechoic areas

EDH: extensor digitorum hallicus; EDL: extensor digitorum longus; EI: Echo intensity; ES: erector spinae; F: female; GLCM: Grey Level Co-occurrence Matrix; GLRLM: Grey Level Run Length Matrix; GM: gluteus medius; LBP: local binary patterns; LT: longissimus thoracis; M: male; MG: medial gastrocnemius; MPS: myofascial pain syndrome; MT: middle trapezius; MTrPs: myofascial trigger points; PB: peroneus brevis; PL: peroneus longus; QL: quadratus lumborum; QP: quadratus plantae; ROI: region of interest; SVM: support vector machine; TA: tibialis anterior; TP: tibialis posterior; US: ultrasound; UT: upper trapezius. Data is expressed as mean±standard deviation. Age is expressed as mean±standard deviation or median (min-max). Height is measured in metres. Weight is measured in kilograms. Statistically significant differences correspond to a p-value<0.05.

Table 2. Studies assessing myofascial trigger point post-intervention changes using B-mode ultrasound.

Author and year	Study design	Sample characteristics	Study variables	Follow-up	Intervention	Main results
Adigozali et al., 2019	Case series (intervention)	Neck pain for at least 12 weeks Active MTrP in UT (defined as hypoechoic nodule) n = 13 (13 F); Age = 28.9±7.7; Height = 1.6±6.1; Weight = 60.1±11.7; BMI = 22.3±3.6	MTrP area (mm^2) Thickness (mm) at rest of UT	Before and after treatment (1 session)	Dry needling was performed for 1 session, using 0.25x50 mm needles. The needle was manipulated rapidly back and forth until after 10 continuous repetitions no more LTRs were visible	Area: • Before treatment: 20.8±6.1 • After treatment: 21.9±4.8 (p=0.42) Thickness: • Before treament: 11.7±1.9 • After treatment: 13.0±2.5 (p=0.26)

Ceylan et al., 2022	RCT	Neck pain for at least 12 weeks
		n = 57 (45 F, 12 M); Age = 33.6±7.9
			MTrP diameter (mm)
			Thickness (mm) at rest of UT
				Before treatment, after treatment (4 weeks) and after 8 weeks	Kinesiotape + exercise group (n = 29): It was applied in 'I' strip using the space correction technique for a total of 4 sessions, 2 days a week. In addition, this group performed the same exercise programme as the following group
					Exercise group (n = 28): The programme included cervical mobility, isometric chin-in, UT stretching, upper trunk extension with chin-in, scapular retraction, bent over row and reverse flies. Each exercise was to be performed in 3 sets of 10 repetitions, 3 times per week for 4 weeks. Participants were required to complete an exercise diary
						Diameter:
						• Before treatment: 0.160 (between groups)
						  • Kinesiotape: 4.9±1.1
						  • Exercise: 5.1±1.5
						• After treatment: p<0.001 (between groups)
						  • Kinesiotape: 3.5±1.2 (p<0.001)
						  • Exercise: 4.6±1.4 (p=0.006)
						• 8 weeks: p<0.001 (between groups, vs before); p=0.075 (between groups, vs after)
						  • Kinesiotape: 3.2±1.1 (p<0.001)
						  • Exercise: 4.5±1.2 (p=0.004 vs before, p=0.196 vs after)
						Thickness:
						• Before treatment: p=0.430 (between groups)
						  • Kinesiotape: 11.2±1.9
						  • Exercise: 10.9±2.1
						• After treatment: p=0.008 (between groups)
						  • Kinesiotape: 9.4±1.1 (p<0.001)
						  • Exercise: 10.2±1.9 (p<0.001)
						• 8 weeks: p=0.006 (between groups, vs before); p=0.02 (between groups, vs after)
						  • Kinesiotape: 9.1±1.1 (p<0.001 vs before, p=0.001 vs after)
						  • Exercise: 10.1±1.8 (p<0.001 vs before, p=0.391 vs after)

Da Silva et al., 2023	RCT	Unilateral shoulder pain for at least 12 weeks
		Active MTrPs (identified by hypoechoic focal regions with heterogeneous echotexture) in the symptomatic shoulder
		n = 60 (32 F, 28 M); Age = 46.6±10.7; Height = 1.7±0.1; Weight = 78.7±14.7; BMI = 27.5±4.5
			MTrP area (cm^2)	Before treatment, after treatment (6 weeks) and after 10 weeks	Mobilization group (n = 20): mobilisation with movement (posterior sliding of the humeral head + active shoulder elevation in the scapular plane) and passive scapular mobilisation in all directions were performed. Three sets of 10 repetitions were performed for each of these, with an interval of 30 seconds between each set
					Compression group (n = 20): same joint mobilisations with the addition of ischaemic compression on the MTrPs of the UT. Pressure was maintained for 1 minute, repeated 3 times with 30 second intervals between repetitions
					Sham group (n = 20): in addition to the joint mobilisations received, sham isquemic compression was applied, without pressure on the identified points, but with the same intervention time
					2 sessiones per week, for 6 weeks
						Area:
						• Before treatment:
						  • Mobilization: 0.1±0.1
						  • Compression: 0.1±0.1
						  • Sham: 0.1±0.0
						• After treatment:
						  • Mobilization: 0.0±0.1
						  • Compression: 0.0±0.0
						  • Sham: 0.0±0.0
						• 10 weeks:
						  • Mobilization: 0.0±0.0
						  • Compression: 0.0±0.0
						  • Sham: 0.0±0.0
						• No differences within groups.
						• No effect for the group x time interaction.

Author and year	Study design	Sample characteristics	Study variables	Follow-up	Intervention	Main results
Hadizadeh et al., 2023	Case series (intervention)	MTrPs in UT (confirmed as hypoechoic area) n = 12 (12 F); Age = 27.3±5.4	MTrP diameter (mm) and area (mm^2) Thickness (mm) at rest of UT	Before treatment and after treatment (1 week)	Intramuscular electrical stimulation intervention was applied in 3 sessions during 1 week. The cathode was connected to the needle using a pincer electrode, and the anode was placed on the spinous processes of the C7 vertebra using an adhesive electrode. An electric burst current with a frequency of 2 Hz and a pulse width of 200 ms was applied for 10 min	Diameter: • Before treatment: 8.5±0.6 • After treatment: 5.9±1.0 (p<0.001) Area: • Before treatment: 25.7±4.7 • After treatment: 13.3±3.8 (p<0.001) Thickness: • Before treament: 10.5±2.0 • After treatment: 9.5±2.2 (p=0.016)

Jafari et al., 2017	RCT	Cervicogenic headache originating from MTrPs in sternocleidomastoid (defined as hypoechoic focal points with heterogeneous echotexture)
		n = 9 (9 F); Age = 38.7±13.2
		Healthy group
		n = 10 (10 F); Age = 35.7±11.9
			MTrP area (mm^2)	2 weeks before the baseline assessment and 2 weeks after treatment	Ischemic compression (n = 9): the maximum tolerable pressure was applied for 30-60 seconds. This process was repeated 3 times at 30-second intervals. If the headache pattern was reproduced or the pain disappeared, the intervention was interrupted. Intervention was managed by 4 sessions within 8 days, with one-day interval between each session
					Control group (n = 10): no treatment
						Area:
						• Baseline:
						  • Ischemic compression: 5.3±1.9
						  • Control: 5.4±1.0
						• Final: p = 0.017 (between groups)
						  • Ischemic compression: 3.4±1.3
						  • Control: 5.4±1.0

Korkmaz and Ceylan, 2022	RCT	Neck pain for at least 12 weeks
		MTrPs in UT
		n = 62 (50 F, 12 M); Age = 34.6±7.4
			MTrP diameter (mm)
			Thickness (mm) at rest of UT
				Before treatment, after treatment (3 weeks) and after 12 weeks	Dry needling plus exercise group (n = 33): once a week for a total of 3 sessions. 0.25x25 needles were used. Two additional needles were inserted 1-2 mm away from the first needle, using a rapid in-and-out technique until the LTR was obtained. Once obtained, the needles were held for 10 minutes. In addition, this group performed the same exercise programme as the next group
					Exercise group (n = 29): 3 times per week, 20 repetitions of each exercise in one set for 12 weeks. The exercises were taught in one 30-minute session, and the remaining sessions were done at home. Participants were required to complete an exercise diary. Exercise therapy included cervical range of motion and active stretching of the cervical paravertebral and trapezius muscles
						Diameter:
						• Before treatment: 0.260 (between groups)
						  • Dry needling plus exercise: 5.4±1.8
						  • Exercise: 5.3±1.5
						• After treatment: p=0.002 (between groups)
						  • Dry needling plus exercise: 3.6±1.4 (p<0.001)
						  • Exercise: 4.8±1.7 (p=0.001)
						• 12 weeks: p=0.021 (between groups, vs before); p=0.122 (between groups, vs after)
						  • Dry needling plus exercise: 3.3±1.4 (p<0.001 vs before, p=0.176 vs after)
						  • Exercise: 4.4±1.4 (p<0.001 vs before, p=0.046 vs after)
						Thickness:
						• Before treatment: 0.712 (between groups)
						  • Dry needling plus exercise: 11.4±1.8
						  • Exercise: 10.6±1.7
						• After treatment: p=0.004 (between groups)
						  • Dry needling plus exercise: 9.9±1.4 (p<0.001)
						  • Exercise: 10.1±1.6 (p=0.001)
						• 12 weeks: p=0.04 (between groups, vs before); p=0.343 (between groups, vs after)
						  • Dry needling plus exercise: 9.8±1.5 (p<0.001 vs before, p=0.377 vs after)
						  • Exercise: 9.8±1.5 (p<0.001 vs before, p=0.009 vs after)
						MTrPs was seen as a focal hypoechoic area with a heterogeneous echotexture

Müller et al., 2015	RCT (pilot)	Head, neck, and/or upper back pain for at least 24 weeks
		Active MTrP in UT
		n = 24 (24 F); Age = 27.3±5.1; BMI = 22.6±3.1
			MTrP area (pixels)	Before and after treatment (evaluations were fixed between the second and fifth day of the menstrual period)	Acupuncture group (n = 9): the points selected were GB20, GB21, LI4, LV3 and two Ashi points in each UT
					Electroacupuncture group (n = 7): 0.25x30 mm needles were used, connected to points GB20 and GB21 as well as the two Ashi points in each UT. It was applied with equipment with eight isolated outlets producing asymmetric biphasic nunpolarised waveforms. The frequency was adjusted to alternate between 2 and 100 Hz, with a total treatment time of 30 minutes
					Sham group (n = 8): sham acupuncture was performed, placing the needles superficially 1 cm from the acupuncture group points
					8 treatment sessions were scheduled at the same time of day for 24-26 days, with two 30-minute sessions per week
						Area:
						• Before treatment:
						  • Electroacupuncture: 1911.9±499.2 (right side), 1761.1±613.1 (left side)
						  • Acupuncture: 1693.6±617.5 (right side), 1553.1±477.1 (left side)
						  • Sham: 1520.1±312.6 (right side), 1549.8±497.0 (left side)
						• After treatment:
						  • Electroacupuncture: 1252.0±330.5 (right side, p=0.003), 1324.6±620.6 (left side, p=0.005)
						  • Acupuncture: 1070.2±411.3 (right side, p<0.001), 1054.6±400.22 (left side, p<0.001)
						  • Sham: 1397.8±253.9 (right side, p=0.117), 1396.3±263.7 (left side, p=0.093)

Author and year	Study design	Sample characteristics	Study variables	Follow-up	Intervention	Main results
Owen, 2022	2 case reports	Case 1: MTrP in supraspinatus and infraspinaturs n = 1 (1 M); Great Pyrinees mix; Age = 10 Case 2: MTrP in infraspinatus n = 1 (1 M); Neutered labrador; Age = 9	Fiber pattern and hypoechoic areas	Before treatment, after treatment and after several weeks	Piezoelectric shockwave	Case 1: Supraspinatus and infraspinatus had an irregular fiber pattern and contained hypoechoic areas. Two sessions were required for resolution of the MTrP. After 8 weeks the fibrillar pattern and EI were normal Case 2: Infraspinatus had an irregular fiber pattern. Four sessions were required for resolution of the MTrP. After 8 and 12 weeks the fibrillar pattern was normal
Sancar et al., 2021	Non-randomized intervention	Neck pain for at least 4 weeks Active MTrPs in UT n = 63 (63 F); Age = 35.6±11.2; BMI = 25.0±4.5 Healthy n = 20 (20 F); Age = 35.6±11.2; BMI = 24.4±4.1	Blob size and blob count difference	Before treatment, after treatment (2 weeks) and after 12 weeks	10 consecutive sessions over 2 weeks of conservative physiotherapy consisting of the application of a warm heat pack (74.5°C, 20 minutes), transcutaneous electrical stimulation (60 mA, 70-80 Hz, 20 minutes) and therapeutic US (1.25-1.6 W/cm2, 5 minutes per zone. The duration of each session ranged from 70 to 80 minutes	Blob size: • Before treatment: p<0.005 (both sides, between groups) • Neck pain: 30.8± 5.0 (right side), 31.7± 5.5 (left side) • Healthy: 13.8± 9.3 (right side), 12.5± 8.0 (left side) • After treatment: • Neck pain: 25.9±5.7 (right side, p<0.001), 28.1±5.5 (left side, p<0.001) • 12 weeks: • Neck pain: 33.8±10.1 (right side, p=0.25), 35.4±11.2 (left side, p=0.28) Blob count: • Before treatment: p<0.005 (both sides, between groups) • Neck pain: 7.7± 1.3 (right side), 7.9± 1.6 (left side) • Healthy: 9.5± 2.7 (right side), 9.7± 2.7 (left side) • After treatment: • Neck pain: 8.0±1.8 (right side, p<0.01), 8.1±1.8 (left side, p=0.58) • 12 weeks: • Neck pain: 8.1±1.6 (right side, p=0.06), 8.4±1.9 (left side, p=0.15)
Togha et al., 2020	RCT	Same sample as Jafari et al., 2017 but including 10 more participants with cervicogenic headache n = 10 (10 F); Age = 32.0±12.7	Same as Jafari et al., 2017	Same as Jafari et al., 2017	The interventions of the ischemic compression group and the control group were the same as in Jafari et al., 2017 Dry needling (n = 10): 0.25x40 mm needles were inserted in the anteroposterior direction while the LTRs were extinct	Area: • Baseline: • Ischemic compression: 5.3±1.9 • Dry needling: 4.7±1.3 • Control: 5.4±1.0 • Final: • Ischemic compression: 3.4±1.3 (p=0.042 vs control) • Dry needling: 2.1±0.7 (p=0.002 vs control, p=0.815 vs ischemic compression) • Control: 5.4±1.0
Ustun et al., 2024	RCT	Neck and/or back pain for at least 12 weeks Active MTrPs in UT n = 60 (51 F, 9 M); Age = 39.7±11.6; BMI = 25.5±4.7	MTrP was assessed with US to detect the typical pattern characterised by a focal hypoechoic area with heterogeneous internal texture EI (histogram)	Before and after treatment (3 weeks)	Dry needling group (n = 30): this was performed with 0.25x25mm needles and the needle had to remain in the MTrP for 1 to 3 minutes and was withdrawn after regression of LTR Cold spray stretching group (n = 30): cooling spray was sprayed onto the surface simultaneously with the application of passive stretching Both groups received one session per week for 3 weeks	EI: • Before treatment: p=0.392 (between groups) • Dry needling: 51.7±17.0 • Cold-spray-stretching: 48.4±12.0 • After treatment: p=0.329 (between groups) • Dry needling: 57.2±13.1 (p<0.017) • Cold-spray-stretching: 53.9±12.8 (p<0.001)

BMI: body mass index; EI: Echo intensity; F: female; LTR: local twitch response; M: male; MTrPs: myofascial trigger points; RCT: randomized controlled trial; US: ultrasound; UT: upper trapezius. Data is expressed as mean±standard deviation. Age is expressed as mean±standard deviation or median (min-max). Height is measured in metres. Weight is measured in kilograms. Statistically significant differences correspond to a p-value<0.05.

3.2.3. Outcomes and follow-up

The included studies collected a range of variables related to MTrP characteristics, muscle architecture, and echotexture analysis. Depending on the study, these variables were measured using various techniques and units, and they provide critical insights into both the anatomical and physiological properties of MTrPs and surrounding muscle tissue.

Several studies investigated different dimensions and properties of the MTrPs. The MTrP area, reported in either mm^2, cm^2, or pixels, was a frequently measured variable, appearing in 14 studies[16,26,29,32,33,36,37,40–44,50]. The MTrP diameter was another metric (measured in mm or cm), recorded in four studies[29,38,39,41]. Additionally, MTrP depth within the muscle, which describes how deep the MTrP lies beneath the muscle surface, was documented in two studies[29,49]. MTrP height was explicitly assessed in the study by Tsai et al.[49], offering another dimension to describe the three-dimensional structure of the MTrP. MTrP shape was qualitatively evaluated in six studies[16,26,32,49,51]. Finally, MTrP location was examined in two studies[27,49], specifying the precise anatomical location of the MTrP within the muscle tissue.

In addition to MTrP-specific variables, studies also examined muscle architecture characteristics. Muscle thickness at rest (measured in mm or cm) was the most commonly reported muscle architectural parameter, appearing in seven studies[27,28,33,37–39,41]. Muscle thickness during contraction was measured in the study by Da Silva et al. (2020)[28] and provides a dynamic measure of muscle architecture. The pennation angle, representing the angle between the muscle fibers and the line of force generation, was reported in the study by Taheri et al.[33].

The echotexture of the muscle tissue surrounding MTrPs was another focus area, providing quantitative data on tissue composition and structure through US imaging. First-order statistical features were reported across seven studies[30,31,33,35,46–48]. These features are derived from the histogram of pixel intensities in the US images and provide information about the distribution of brightness and texture within the muscle. Additionally, several studies described regions of hypo- or hyperechoic tissue without using histograms[16,25,26,32–34,36,39,50,51]. Second-order texture analysis, which explores more complex relationships between pixel intensities, was also evaluated. The GLCM was reported exclusively in the study by Kumbahre et al. (2018)[48]. Lastly, higher-order texture features were employed to investigate even more intricate texture patterns within muscle tissue. Specifically, the GLRLM was documented in Kumbhare et al. (2018)[48]; LBP was used in three studies[31,47,48]; and blob analysis (blob area/size, blob count) was applied in four studies[30,31,45,48] to quantify the size and distribution of distinct areas of differing echo intensity within the muscle tissue.

All observational studies included were cross-sectional, which precluded the possibility of follow-up over time. Follow-up assessments varied significantly across studies, with time points ranging from immediate post-intervention assessments to extended periods of several weeks or months. In most studies, initial post-intervention assessments were conducted at one to eight weeks. Several studies conducted additional follow-up evaluations at 2, 6, 8, 10, and 12 weeks[25,38–40,42,43,45].

3.3. Assessment of methodological quality/risk of bias

The methodological quality of the included studies varied according to the designs. Firstly, for observational cohort and cross-sectional studies (Table 3), quality ranged from poor to good, with 55.5% rated fair. Common shortcoming included lack of justification of sample size, failure to assess exposure prior to outcome measurement, unclear definitions of outcome measures, and inadequate control for confound variables. Secondly, all case-control studies (Table 4) were classified as fair, with key methodological issues involving insufficient justification of sample size, lack of exposure assessment prior to results, recruitment of different population groups, lack of concurrent controls, and lack of reliable and valid exposure/risk measures. Moreover, case series studies (Table 5) were rated as poor or fair due to inherent limitations in their design. The main concerns related the absence of information on consecutive inclusion of cases, comparability of subjects and adequacy of follow-up duration.

Table 3. Assessment of methodological quality by NHLBI Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.

Author and year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Quality Rating
Cankurtaran et al., 2023	YES	YES	CD	YES	NO	NO	CD	NO	YES	NA	YES	NR	NA	YES	Fair
Cojucaru et al., 2015	YES	YES	NO	NR	NO	NO	CD	NO	YES	YES	NO	NR	NO	NO	Poor
Da Silva et al., 2020	YES	YES	YES	YES	YES	NO	CD	NO	YES	YES	NO	YES	NA	NO	Good
Elbarbary et al., 2023	YES	YES	CD	YES	YES	NO	CD	NO	YES	NA	NO	YES	NA	NO	Fair
Lewis and Tehan, 1999	YES	YES	NO	NR	NO	NO	CD	NO	NO	NA	NO	YES	NA	NO	Poor
Sikdar et al., 2009	YES	YES	NO	NR	NO	NO	CD	YES	YES	NA	NO	YES	NA	NO	Fair
Taheri et al., 2016	YES	YES	CD	YES	NO	NO	CD	NO	YES	YES	NO	YES	NA	NO	Fair
Tsai et al., 2024	YES	YES	CD	YES	YES	NO	CD	YES	YES	NA	NO	NR	NA	NO	Fair
Zadeh et al., 2023	YES	YES	CD	NR	NO	NO	CD	YES	YES	NA	NO	NR	NA	NO	Poor

CD: Cannot determine; NA: Not applicable; NR: Not reported. Q1: Research question; Q2-Q3: Study population; Q4: Groups recruited from the same population and uniform eligibility criteria; Q5: Sample size justification; Q6: Exposure assessed prior to outcome measurement; Q7: Sufficient timeframe to see an effect; Q8: Different levels of the exposure of interest; Q9: Exposure measures and assessment; Q10: Repeated exposure assessment; Q11: Outcome measures; Q12: Blinding of outcome assessors; Q13: Follow-up rate; Q14: Statistical analyses.

Table 4. Assessment of methodological quality by NHLBI Quality Assessment Tool for Case-Control Studies.

Author and year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Quality Rating
Kumbhare et al., 2017	YES	YES	NR	NO	NR	YES	YES	NR	NR	NO	CD	NR	YES	Fair
Kumbhare et al., 2018	YES	YES	NR	NO	NR	YES	YES	NR	NR	NO	CD	YES	NO	Fair
Kumbhare et al., 2020	YES	YES	NR	NO	NR	YES	YES	NR	NR	NO	CD	NR	NO	Fair
Takla et al., 2016	YES	YES	NR	NO	YES	YES	YES	NR	NR	NO	CD	YES	YES	Fair
Turo et al., 2013	YES	YES	NR	NO	NR	YES	YES	NR	NR	NO	CD	YES	NO	Fair
Zale et al., 2015	YES	YES	NR	NO	YES	YES	YES	NR	NR	NO	CD	YES	NO	Fair

CD: Cannot determine; NA: Not applicable; NR: Not reported. Q1: Research question; Q2: Study population; Q3: Target population and case representation; Q4: Sample size justification; Q5: Groups recruited from the same population; Q6: Inclusion and exclusion criteria prespecified and applied uniformly; Q7: Case and control definitions; Q8: Random selection of study participants; Q9: Concurrent controls; Q10: Exposure assessed prior to outcome measurement; Q11: Exposure measures and assessment; Q12: Blinding of outcome assessors; Q13: Statistical analyses.

Table 5. Assessment of methodological quality by NHLBI Quality Assessment Tool for Case Series Studies.

Author and year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Quality Rating
Adigozali et al., 2019	YES	YES	NR	CD	YES	YES	CD	YES	YES	Fair
Ball et al., 2022	YES	YES	NR	NO	YES	YES	CD	NO	YES	Poor
Hadizadeh et al., 2023	YES	YES	NR	CD	YES	YES	CD	YES	YES	Fair
Owen, 2022	YES	YES	NR	CD	YES	YES	CD	NR	YES	Poor
Shankar and Reddy, 2012	YES	YES	NA	NA	YES	YES	CD	NO	YES	Poor

CD: Cannot determine; NA: Not applicable; NR: Not reported. Q1: Research question; Q2: Case definition; Q3: Consecutive cases; Q4: Comparable subjects; Q5: Intervention; Q6: Outcome measures; Q7: Follow-up; Q8: Statistical analyses; Q9: Results.

In relation to intervention studies, RCTs assessed with the PEDro scale (Table 6) demonstrated moderate to high methodological quality. However, significant limitations were identified, in particular the inability to blind therapists and, in some cases, the absence of between-group comparisons prior to treatment, which could introduce bias into the results. Regarding non-randomised intervention studies, only the study by Sancar et al.[45] was assessed using the ROBINS-I tool (Table 7), revealing a moderate risk of bias.

Table 6. Assessment of methodological quality by PEDro scale.

Author and year	1	2	3	4	5	6	7	8	9	10	11	Total	Quality
Ceylan et al., 2022	YES	YES	NO	YES	NO	NO	YES	YES	YES	YES	YES	7	High
Da silva et al., 2023	YES	YES	YES	NO	YES	NO	YES	YES	YES	YES	YES	8	High
Jafari et al., 2017	YES	YES	YES	YES	NO	NO	NO	YES	YES	YES	YES	7	High
Korkmaz and Ceylan, 2022	YES	YES	NO	YES	NO	NO	YES	YES	NO	YES	YES	6	High
Müller et al., 2015	YES	YES	NO	NO	YES	NO	YES	NO	NO	YES	YES	5	Moderate
Togha et al., 2020	YES	YES	YES	NO	NO	NO	YES	YES	YES	YES	YES	7	High
Ustun et al., 2024	YES	YES	YES	YES	NO	NO	YES	YES	NO	YES	YES	7	High

1: Eligibility criteria were specified (this item is not taken into account for the final score); 2: Subjects were randomly allocated to groups; 3: Allocation was concealed; 4: The groups were similar at baseline regarding the most important prognostic indicators; 5: There was blinding of all subjects; 6: There was blinding of all therapists who administered the therapy; 7: There was blinding of all assessors who measured at least one key outcome; 8: Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups; 9: All subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analyzed by "intention to treat"; 10: The results of between-group statistical comparisons are reported for at least one key outcome; 11: The study provides both point measures and measures of variability for at least one key outcome.

Table 7. Assessment of risk of bias in studies with non-randomized intervention by ROBINS-I.

Author and year	D1	D2	D3	D4	D5	D6	D7	Overall risk of bias
Sancar et al., 2021	Moderate	Moderate	Moderate	Low	Moderate	Moderate	Moderate	Moderate

D1: Bias due to confounding; D2: Bias in selection of participants into the study; D3: Bias in classification of interventions; D4: Bias due to deviations from intended interventions; D5: Bias due to missing data; D6: Bias in measurement of outcomes; D7: Bias in selection of the reported result. Overall risk of bias: low (low risk of bias for all domains); moderate (low or moderate risk of bias for all domains); serious (serious risk of bias in at least one domain, but not at critical risk of bias in any domain); critical: critical risk of bias in at least one domain); no information (there is no clear indication that the study is at serious or critical risk of bias and there is a lack of information in one or more key domains of bias).

3.4 Synthesis of results

3.4.1. MTrP characteristics

Cross-sectional studies reported substantial variability in the MTrP area across different muscles and populations. The largest areas were observed in individuals with low back pain, ranging from 1.2 to 3.4 cm^2[50], notably larger than those in other groups. In chronic neck pain and chronic unilateral shoulder pain, the MTrP area in the upper trapezius ranged from 0.1 to 0.2 cm^2[16,28,37,40], while in MPS, between 0.2 and 0.5 cm^2[33,41]. Similar values were observed in indi-

viduals with ankle/foot pain[36]. In contrast, smaller areas were found in temporomandibular disorders and cervicogenic headache, ranging from 0.02 to 0.05 cm^2 in muscles such as the temporalis, masseter, and sternocleidomastoid[29,42,43]. Comparable results were noted in a healthy population, where the mean area in the sternocleidomastoid was 0.05 cm^2[42]. Furthermore, no significant differences were observed between active and latent MTrPs[16].

Post-treatment, significant changes in MTrP area were observed depending on the intervention, with differences ranging from 0.01 to 0.1 cm^2. Dry needling showed no significant reduction of the MTrP area in individuals with chronic neck pain (p=0.42)[37], but was significantly more effective in reducing MTrP area than no intervention in cervicogenic headache (p=0.002)[43]. In chronic unilateral shoulder pain, none of the interventions produced any significant intra- or inter-group changes[40]. When comparing ischemic compression to no intervention in cervicogenic headache, significant improvements were observed in favour of ischemic compression (p=0.017)[42], although no significant difference was found when compared to dry needling (p=0.815)[43]. Lastly, intramuscular electrical stimulation, acupuncture, and electroacupuncture demonstrated significant reductions in the MTrP area in the upper trapezius within treatment groups[41,44].

The diameter of MTrPs also varied across muscle groups and study populations. In chronic neck pain, the mean diameter of the MTrPs of the upper trapezius was about 0.5 cm[38,39], whereas in individuals with temporomandibular disorders, smaller diameters were recorded, such as 0.2 cm in the temporalis and masseter[29]. Among interventions for chronic neck pain, kinesiotaping and dry needling combined with exercise resulted in the most substantial reductions, with mean decreases of 0.2 cm, while exercise alone appeared to be less effective, particularly in the short-term[38,39]. Intramuscular electrical stimulation, meanwhile, produced a statistically significant reduction of 0.3 cm in the upper trapezius (p<0.001)[41].

MTrPs were predominantly characterized as hypoechoic focal areas or nodules with heterogeneous internal echotexture across several studies[25,29,33,34,36,37,39–43,46]. The shape of these nodules was generally described as ellipsoidal or elliptical[16,50], and in some instances, multiple nodules were observed coexisting nearby[16]. Additional features included the loss of white spot pattern on a dark background in the cross-sectional view[32], as well as discontinuous, wavy, or clustered fascicular patterns in the longitudinal view[25,32]. These MTrPs were found at various depths within the muscle tissue, with a predominance near the muscle surface[49], though reported depths ranged from 0.5 cm[29] to 3 cm.[32] However, in some studies, MTrPs were identified as hyperechoic regions[32,51] or as smaller hyperechoic “speckles” within a larger hypoechoic contracture knot[26]. Only one study evaluated post-intervention changes, specifically following shock wave therapy, demonstrating that by 8 to 12 weeks after treatment, the fibrillar pattern and hypoechoic regions had returned to normal[25].

3.4.2. Muscle architecture

Variations in muscle thickness at rest were observed depending on the muscles harbouring MTrPs. All subjects included in the studies analyzing this parameter suffered from chronic neck pain, chronic unilateral shoulder pain, or MPS, with values ranging from 0.1 to 1.9 cm in the posterior cervical, upper trapezius, and middle trapezius muscles[27,28,33,37–39,41]. Notably, mus-

cles containing MTrPs demonstrated significantly lower muscle thickness ($p<0.05$) compared to those without MTrPs[27]. Interventions such as kinesiotaping and exercise significantly reduced upper trapezius thickness in individuals with chronic neck pain, with decreases ranging from 0.08 to 0.2 cm[38,39]. Kinesiotaping produced more significant reductions in muscle thickness compared to exercise alone[38]. In contrast, dry needling as a standalone treatment did not result in significant changes ($p=0.26$)[37]; however, when combined with exercise, it yielded more significant reductions compared to exercise alone in the short-term[39]. Intramuscular electrical stimulation also significantly reduced muscle thickness, decreasing 0.1 cm ($p=0.016$)[41].

Additional aspects of muscle architecture examined included muscle thickness at contraction, which was 1.4 cm in the upper trapezius compared to 1.0 cm at rest in individuals with unilateral chronic shoulder pain[28], and the pennation angle in the upper trapezius, reported to be 14.6 degrees in the MPS population[33]. However, no direct comparisons were made between different groups or post-intervention outcomes for these parameters.

3.4.3. Echotexture

Among the first-order statistical features, mean echo intensity was the most frequently reported feature in all cases in the upper trapezius. In populations with chronic neck/back pain and MPS, the echo intensity of the upper trapezius, where MTrPs were located, ranged from 48.4 to 55.5 values in histogram[33,46], while the echo intensity of the MTrPs themselves was 41.6[33]. In individuals with neck pain, echo intensity was significantly lower ($p<0.05$) compared to healthy controls[30]. Furthermore, this group exhibited reduced mean entropy and larger regions with entropy values below 4 ($p<0.05$)[35]. When comparing active and latent MTrPs, and healthy controls, significant differences were observed in correlation, homogeneity, mean, and variance; however, no differences were found in entropy, contrast, or energy[47]. Both dry needling and cold spray stretching interventions in populations with chronic neck/back pain resulted in a significant increase in echo intensity ($p<0.017$ and $p<0.001$, respectively), though no significant difference was found between the two interventions ($p=0.329$)[46].

With regard to second-order and higher-order texture features, studies were exclusively conducted on the upper trapezius, yielding similar results across different features. Various metrics of the GLCM significantly distinguished between healthy individuals and those with active and latent MTrPs in a population with neck pain[48]. Similarly, both blob area and blob count demonstrated significant differences between healthy controls and those with neck pain, with larger blob areas and reduced blob counts observed in the latter group[30,31,45,48]. The LBP approach also revealed significant distinctions between healthy individuals and those with active or latent MTrPs[31,47]. Post-treatment, a combination of warm heat pack, transcutaneous electrical stimulation, and therapeutic US led to significant reductions in blob size ($p<0.001$) and blob count ($p<0.01$), although these effects were observed only in the short-term[45].

4. Discussion

The primary objective of this systematic review was to synthesize the current evidence on the use of B-mode US for characterizing MTrPs, with secondary objectives focused on evaluating

muscle architecture and assessing changes following physiotherapy interventions. Based on the inclusion of 28 studies, of which 17 were observational, primarily dedicated to MTrP characterization, and 11 studies including a physiotherapeutic intervention to assess post-treatment changes, the review objectives have been largely met. The findings varied substantially depending on the specific muscles affected by MTrPs, the population studied, and the type of intervention applied.

A common observation across most studies was the characterization of MTrPs as predominantly hypoechoic areas or nodules with heterogeneous internal echotexture, often presenting an ellipsoidal or elliptical shape. However, there is still controversy since some studies described the MTrP as hyperechoic areas[19,51,52], which were considered by renowned experts such as Jan Dommerholt and Robert D. Gerwin as possible fibrous tissue, fascia or collagen rather than MTrPs[53]. The most remarkable limitation of the review is that few studies performed quantitative image analysis or included a control group. In fact, of 929 individuals analysed in the review, only 181 were healthy controls, highlighting an area for future research. Another significant limitation was the suboptimal methodological quality and high risk of bias present in most of the studies analyzed in the review.

As mentioned above, most studies have described MTrPs as hypoechoic regions. However, a notable issue is that many of these studies are based on the findings of Sikdar et al.[16], who first demonstrated this characteristic of MTrPs. The validity of this study is subject to the fact that it is a preliminary observational study lacking quantitative analysis of echotexture. This limitation may bias the whole body of evidence on this topic, as most studies assume that MTrPs are presented this way without further examination of the US images. In line with this, and despite the lack of quantitative image analysis in these studies, some studies included in our review[30,33,46] examined the echotexture of MTrPs and suggested that these exhibit lower echo intensity compared to surrounding tissue and healthy control muscles. Furthermore, interventions such as dry needling and cold spray stretching were found to increase echo intensity, which was considered a normalization in relation to the surrounding tissue[46].

From an etiological perspective, MTrPs are described as clusters of electrically active foci, each associated with a contracture knot and a dysfunctional motor endplate within skeletal muscle[54]. This dysfunction results in an excessive acetylcholine (ACh) release and a concomitant reduction in acetylcholinesterase activity, initiating action potentials and sustained muscle contraction[55]. The painful region within the MTrP represents a complex histo-anatomical architecture and should not be considered merely a simple muscle contracture knot[3]. This distinction is crucial, as specific histopathological alterations within MTrPs, such as the elevated concentration of proteoglycans, which attract water, may explain the observed reduction in echo intensity in these areas[56]. Concerning this, structural changes in muscle fibers and the endomysium are considered primary histopathological characteristics responsible for abnormal muscle echotexture, leading to the development of hypoechoic areas/nodules[14]. These changes may be associated with local fluid accumulation or tissue edema resulting from acute inflammatory exudates combined with blood or residual byproducts[57]. Biochemical analyses further support this perspective, which have identified elevated levels of inflammatory mediators, neuropeptides, catecholamines, and cytokines near MTrPs[58].

More advanced image analysis techniques, such as second-order or higher-order features (e.g., blob analysis, LBP analysis, or GLCM), have shown potential in distinguishing between asymptomatic individuals and those with MTrPs, as well as between active and latent MTrPs[30,31,47,48]. Despite the promise of these methods, further research with higher methodological quality is needed, as some of the results remain contradictory and inconsistent. Additionally, machine learning has been explored for MTrP characterization, although the high variability in US images presents significant challenges[47].

One of the key reasons for the discrepancies found in the literature, compared to other musculoskeletal structures with well-defined US evaluation protocols, is the lack of reliable, standardized guidelines for MTrP characterization across various US modalities (e.g., B-mode, Doppler, elastography). As a result, diagnostic parameters such as accuracy, sensitivity, and specificity range significantly from 33% to 100%[12]. This has led to considerable confusion among clinicians and researchers, calling into question the accurate identification of MTrPs and increasing the risk of image misinterpretation[17,52]. However, Doppler US and elastography findings appear more conclusive in symptomatic MTrPs, showing significant alterations in blood flow, including retrograde blood flow, elevated pulsatility index, and changes in peak systolic and minimum diastolic velocities[59]. Furthermore, elastography has reduced local vibration amplitude, asymmetry in muscle fiber orientation, and increased shear wave velocity[60–62]. Other diagnostic techniques, such as electromyography, infrared thermography, and MRI, have yielded mixed and complex results. Among them, MRI appears to be the most reliable for detecting focal signal alterations in MTrP regions[63–65]; however, like elastography, it faces limitations in clinical application.

In relation to the area of MTrPs, our review identified ranges from 0.02 to 3.4 cm^2, with larger muscles showing larger mean areas. Recent reviews by Elbarbary et al.[12] and Mazza et al.[13] reported values between 0.03 and 0.5 cm^2, demonstrating the variability across different muscle groups. Specifically, values as low as 0.02 cm^2 were observed in muscles such as the masseter and temporalis[29], while larger areas, up to 3.4 cm^2, were reported in lower back muscles[50]. Similarly, the diameter of MTrPs followed this trend, ranging from 0.2 to 0.5 cm, with Tsai et al.[49] highlighting a positive correlation between MTrP size and muscle size, showing an increase of 0.2 cm in MTrP diameter for every 1 cm increase in muscle tissue. Muscles containing MTrPs tend to exhibit reduced thickness[27], and interventions often led to even greater muscle thickness reduction, which was interpreted as a positive outcome. However, this reduction requires further investigation, as muscle inhibition due to pain could also decrease in muscle thickness. The reduction in both the MTrP area and the muscle thickness after interventions was significant, though the clinical relevance of these changes, particularly in relation to muscle thickness restoration as a potential positive outcome, remains to be fully understood. In this context, US can be considered a dual-purpose tool: first, as a diagnostic tool for the characterization of MTrPs; and second, as a therapeutic guide, in particular for minimally invasive techniques such as US-guided dry needling, which are widely used in clinical practice[66].

The present systematic review is the first to not only review all the existing literature on the characterization of MTrPs using B-mode US, but also to incorporate a detailed analysis of the muscle architecture in which these MTrPs are located and provides an examination of the changes that

occur in these characteristics following a physiotherapeutic intervention. However, the review has its limitations, which must be considered in interpreting the findings.

First, the studies included in this review vary significantly their level of evidence and methodological quality, with considerable heterogeneity among them. Observational studies predominate, but the review also includes case series and non-randomized, uncontrolled intervention studies, many of which have small sample sizes. Several factors further limit the generalizability of the findings: (1) Most studies only report p-values without corresponding effect sizes, which restricts the potential for robust conclusions and limits the replicability of the research; (2) Only six studies mentioned maintaining consistent US imaging settings, and many did not report whether they standardized US probe pressure or angle, which could significantly affect the results; (3) Several studies did not report the time lag between physical examination and subsequent US imaging, a delay that should ideally be minimized to ensure accuracy of assessment; (4) A notable limitation is the lack of clarity in some studies regarding the diagnostic criteria used to identify MTrPs, which may introduce inconsistencies and limit the reproducibility of the results; (5) Furthermore, current methods used in US imaging, mainly when applied as a follow-up tool, may not provide definitive conclusions; (6) Lastly, most of the included studies focused exclusively on superficial muscles, largely due to the reliance on manual palpation as the reference standard to facilitate accurate US assessment. However, this approach is inherently limited, as manual palpation cannot reliably assess deeper muscles, and therefore, the conclusions of this review cannot be generalized to all muscle groups, particularly those located in deeper anatomic regions.

For future research, several aspects deserve to be explored: (1) Echotexture analysis has so far only been performed on the upper trapezius muscle, which limits the generalizability of the results to different muscle groups. Future research should prioritize comparative studies that include a wider range of muscles, especially those that are clinically relevant and impactful, incorporating not only healthy control groups but also individuals with other pathological conditions. Such studies would help to establish whether these US imaging features consistently serve as reliable indicator for muscle tissue characterization, allowing this analysis to be extend to deeper muscle structures where manual palpation is less feasible, and US presents greater precision; (2) It is crucial to investigate to what extent the lack of standardization of US imaging settings within the same study population affects echotexture analysis, as well as to determine the feasibility of comparing studies that analyse the same variables but use different settings, US probes, ROI selection and other technical parameters; (3) Agreement between US imaging and manual palpation for the detection of MTrPs is a critical issue that requires further study to increase diagnostic accuracy and improve management of MPS and other conditions; (4) Research is also needed to develop a reliable methodology to establish US imaging as a indicator capable of objective monitoring of treatment efficacy and patient outcome, prioritizing longitudinal studies to capture dynamic changes in MTrPs during and after treatment; (5) Finally, progress in this field requires more high-quality studies, along with greater standardization in imaging protocols and diagnostic criteria, as well as methodological homogeneity in terms of study designs and populations. These steps are essential to enable future meta-analysis, which could generate conclusive evidence on the utility of B-mode US in characterizing MTrPs. Such studies are essential to translate these findings into clinical practice, given its ease of use and accessibility.

5. Conclusions

Although most studies identify MTrPs as hypoechoic structures with heterogeneous internal echotexture, controversy remains regarding their characterization. The same applies to changes in this structure following physiotherapeutic interventions, as the current definition of MTrPs in US imaging cannot detect reliable changes. This uncertainty highlights the need for further studies using quantitative methods to accurately measure MTrPs and draw valid conclusions. The lack of standardized measurement protocols introduces important bias, making comparing results between different studies difficult. Therefore, objective measures are needed to effectively monitor MTrP changes, which could then be applied in clinical practice to better understand the effects of interventions in these areas.

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