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Subgrouping non-specific low back pain based on spinal marker trajectory data: An unsupervised machine learning approach

Hwa-Ik Yoo [a], Ui-Jae Hwang [b], Jong-Gook Choi [c], Oh-Yun Kwon [d,*]

[a] Department of Physical Therapy, Kyungdong University, Wonju 26495, Republic of Korea
[b] Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, 11 Yuk Choi Road, Hung Hom, Hong Kong, China
[c] Department of Disaster and Safety, Kyung Hee Cyber University, Seoul 02447, Republic of Korea
[d] Department of Physical Therapy, College of Software and Digital Healthcare Convergence, Yonsei University, Wonju 26493, Republic of Korea

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ABSTRACT

Background: Non-specific low back pain (LBP) is a heterogeneous condition. Therefore, it is important to investigate whether clinically feasible assessments can identify diverse movement patterns in individuals with LBP.
Purpose: To identify distinct movement-based subgroups among individuals with non-specific LBP using thoraco-lumbo-pelvic marker trajectories during forward bending and to compare the resulting clusters with healthy controls.
Study design: Cross-sectional study.
Methods: Kinematic data were collected from 127 individuals with non-specific LBP and 58 healthy controls during a forward bending task using a smartphone-based video recording system. Three markers were placed over T12, L2, and S2, and their x- and y-axis displacements were extracted using an open-source software. Unsupervised machine learning (K-means clustering) was applied to classify movement patterns within the LBP group based on six kinematic features (the horizontal and vertical displacements of the T12, L2, and S2 markers).
Results: Two clusters were identified within the LBP group: cluster 1 (large-excursion, 54 %) and cluster 2 (small-excursion, 46 %). Both clusters showed significant differences from healthy controls in marker displacement ($p < 0.001$). Cluster 2 reported a slightly higher pain intensity ($p = 0.036$), with no difference in disability scores.
Conclusions: Unsupervised clustering revealed distinct spinal movement subgroups in individuals with non-specific LBP. These findings indicate that both excessive and limited movement may relate to pain-related adaptation, supporting the need for movement-based subgrouping to guide individualized management.

1. Introduction

Approximately 90 % of low back pain (LBP) cases are classified as non-specific LBP, also referred to as mechanical LBP, because no definitive nociceptive source is identified through radiological imaging [1,2]. Therefore, researchers have proposed that habitual postures or movements performed during daily activities significantly trigger non-specific LBP. Specifically, repetitive movements in certain directions may lead to cumulative tensile or compressive stresses on the spinal tissues, ultimately resulting in tissue damage [3]. Supporting this hypothesis, a previous study reported that individuals with LBP demonstrate greater lumbar excursion during the early phase of functional movements [4]. Consequently, various intervention strategies aimed at modifying lumbar-dominant movement patterns and stabilizing the lumbar spine have been developed and implemented in clinical practice [5,6].

However, because individuals with non-specific LBP exhibit heterogeneous movement characteristics, a one-size-fits-all assessment approach often yields inconsistent results. Clinical tests without a classification of LBP may fail to detect the specific movement alterations associated with the condition [7]. Thus, the classification of LBP into subgroups based on control impairment (excessive movement) or movement impairment (restricted movement) during functional tasks has been applied in clinical evaluations to differentiate various

* Corresponding author at: Department of Physical Therapy, College of Software and Digital Healthcare Convergence, Yonsei University, 1, Yeonsedae-gil, Heungeop-myeon, Wonju-si, Wonju, Gangwon-do 26493, Republic of Korea.
E-mail addresses: yoohi@kduniv.ac.kr (H.-I. Yoo), uj.hwang@polyu.edu.hk (U.-J. Hwang), hallaman@naver.com (J.-G. Choi), kwonoy@yonsei.ac.kr (O.-Y. Kwon).

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non-specific LBP subgroups and distinguish affected individuals from those without LBP [8,9]. Based on these classification methods, several studies have identified differences in spinal movement patterns between individuals with LBP and healthy controls during various functional activities [10–12].

Among the functional tasks, forward bending has been identified as a biomechanical risk factor for intervertebral disc injury or herniation [13]. Previous research has classified the movement patterns observed during forward bending into four distinct phenotypes: standard (quicker movement speed and coordinated lumbopelvic motion), lumbar dominant, pelvic dominant, and guarded (slowest movement speed and smallest range of motion) [14]. This suggests the importance of identifying distinct movement-based differences within individuals with the same LBP condition. Such movement pattern phenotypes can be explained by purposeful adaptations to avoid pain [15,16], resulting in greater stiffness and slower movements, or by modified movements of adjacent regions to reduce pain or stress [17].

Recently, efforts have been made to measure these movement characteristics under general clinical conditions [18]. Although forward bending tasks have been frequently examined in previous studies, these assessments typically depend on three-dimensional motion analysis [10, 19–21], which necessitates controlled laboratory conditions or costly instrumentation, thereby limiting practical applications in routine healthcare settings. Therefore, it is necessary to investigate whether spinal (thoraco-lumbo-pelvic) movement patterns in individuals with LBP can be effectively classified using smartphone-based video-tracking methods in combination with open-source analysis software. Moreover, an unsupervised machine learning algorithm (K-means algorithm) enables the classification of individuals with non-specific musculoskeletal pain into distinct subgroups through cluster analysis [18,22]. Accordingly, the purpose of this study was to employ an unsupervised machine-learning algorithm to cluster the movement patterns of individuals with non-specific LBP and compare the resulting movement patterns with those of healthy controls. We hypothesized that individuals with LBP would demonstrate heterogeneous spinal movement patterns.

2. Methods

2.1. Participants

Participants were recruited through musculoskeletal screening examinations aimed at preventing industrial accidents across 11 public service offices. The kinematic data were collected between April and May 2024. These data were originally obtained through an occupational musculoskeletal disorder screening program. The Institutional Review Board of Yonsei University Mirae Campus granted a waiver for the requirement of informed consent. A total of 127 individuals with non-specific LBP and 58 asymptomatic controls participated in this study. The inclusion criteria for the non-specific LBP group were as follows: age between 18 and 65 years, a history of LBP lasting > 3 months; a pain intensity score of at least 2 on the numerical rating pain scale (NPRS), and a pain location between the inferior T12 and the superior buttock line. Healthy controls were recruited based on the absence of LBP in the past three months. The exclusion criteria included a history of spinal surgery, vertebral fracture, or malignancy; hip or knee joint pain or discomfort reported during the test movement; spinal canal stenosis, spondylolisthesis, or ankylosing spondylitis; disc herniation or radiating pain; and current pregnancy or postpartum status within six months.

2.2. Low back pain rating and disability measures

On the screening day, participants completed self-administered questionnaires, including the 11-point NPRS and the Roland-Morris Disability Questionnaire (RMDQ), which is one of the most widely used tools for evaluating functional status in individuals with LBP [23].

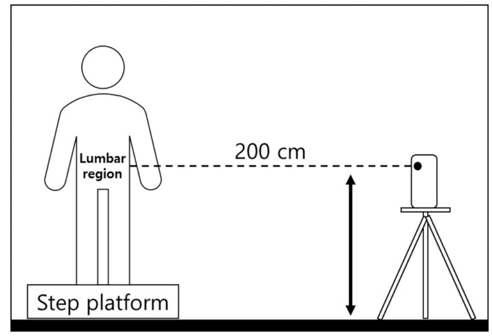

Fig. 1. Smartphone setup for forward bending recordings

Table 1
Intra-rater reliability and measurement precision of marker displacement.

Variables	ICC (95 % CI)	SEM (mm)	MDC (mm)
x-axis displacement of T12	0.984 (0.987–0.987)	7.5	20.7
y-axis displacement of T12	0.991 (0.989–0.992)	4.9	13.5
x-axis displacement of L2	0.976 (0.971–0.980)	8.6	23.8
y-axis displacement of L2	0.990 (0.988–0.992)	3.4	9.5
x-axis displacement of S2	0.984 (0.980–0.987)	7.1	19.8
y-axis displacement of S2	0.986 (0.983–0.989)	3.5	9.6

ICC, intraclass correlation coefficient; CI, confidence interval, SEM, standard error of measurement; MDC, minimal detectable change

The RMDQ consists of 24 items that assess disabilities related to everyday activities such as walking, sitting, dressing, sleeping, and occupational tasks. Each item is scored 1 point if the response is “yes” and 0 if “no,” resulting in a total score ranging from 0 to 24. Higher total scores reflect greater functional impairment associated with low back pain.

2.3. Thoraco-lumbo-pelvic kinematic data measurements

Video data were captured using the smartphone’s built-in camera application set to 4 K resolution (3840 × 2160 pixels) with a frame rate of 60 fps. The smartphone was mounted 200 cm laterally from the center of the step platform and its height was aligned with each participant’s lumbar area (Fig. 1). Three spherical markers (20 mm in diameter) were secured to a Velcro-attached elastic belt and placed over the T12, L2, and S2 spinous processes. The T12 level was determined by identifying the 12th rib and its articulation with the spine. To determine L2, the L4 vertebra was first located using the iliac crest line (Tuffier’s line), followed by moving two vertebral levels superiorly [24]. The S2 spinous process was identified as the midpoint between the posterior and superior iliac spines.

Thoraco-lumbo-pelvic kinematics were analyzed by tracking the trajectories of each marker in the sagittal plane. Marker trajectories were tracked using Kinovea (Kinovea® v. 0.8.15; Kinovea, Bordeaux, France), an open-source motion analysis software. Calibration was performed for each analysis session based on known marker diameters (20 mm). As the Kinovea software tracks trajectories using the RGB values of selected pixels, yellow-colored markers were used, and the background was standardized to white or gray to enhance tracking accuracy. Previous investigations demonstrated acceptable levels of reliability and validity when using Kinovea for kinematic analysis [25,26]. The reliability and accuracy of the marker displacement measures were supported by intraclass correlation coefficients, standard error of measurement, and minimal detectable change values (Table 1), all of which indicated excellent reliability (ICC > 0.9) and strong precision. Following trajectory tracking, the horizontal (x-axis) and vertical (y-axis) displacements were calculated for each marker as the change in position between initial upright posture and the final forward bending

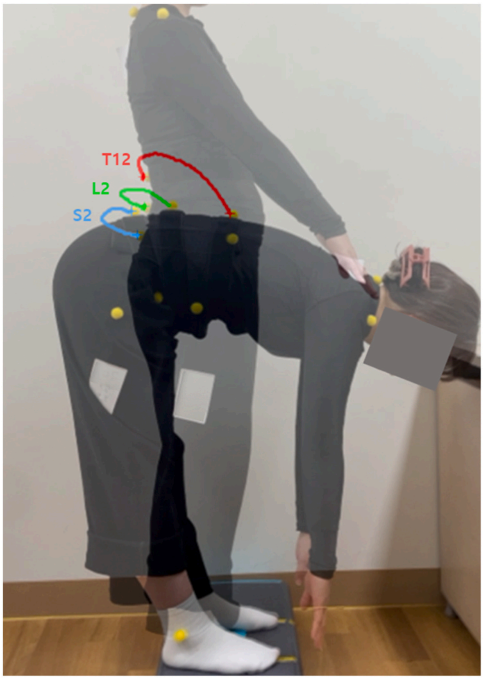

Fig. 2. Three marker trajectories during forward bending task

posture, rather than the total distance. During forward bending, the anterior movement of the marker was recorded as a positive *x*-axis displacement, whereas the posterior movement was recorded as negative. Similarly, superior movement was considered a positive *y*-axis displacement and inferior movement was considered negative (Fig. 2).

To ensure the stable placement of the markers in close contact with the body, all participants were initially instructed to wear a tight-fitting top. A Velcro-based elastic belt with attached spherical markers was fastened around the trunk and positioned in alignment with the spinous processes of T12, L2, and S2. The participants were then asked to stand barefoot in a relaxed, upright position at the center of the step platform, with their feet positioned shoulder-width apart. This posture was recorded as the baseline (zero reference) for kinematic analysis using the Kinovea software. All subsequent thoraco-lumbo-pelvic movements were evaluated in relation to the initial reference position [19]. Following the procedure outlined by Tsang et al., the participants were instructed to perform a reaching-down task from the initial posture, extending their knees and elbows fully while reaching as far as possible [27]. Participants began the movement with their arms comfortably extended forward. The forward bending movement was performed at a consistent pace of 40 beats per minute, which corresponds to the rhythm typically observed in everyday activities [28,29]. Each beat indicated a full phase of the movement; flexion (downward bending) on one beat and recovery (return to upright) on the next. The average and standard deviation of the displacement measurements from the three trials were calculated and used for data analysis.

2.4. Data pre-processing

Participants were categorized into a non-specific LBP group or a healthy control group based on predefined inclusion criteria and self-reported symptoms within the past 3 months. To improve the accuracy and reliability of the machine-learning model, the dataset was examined for missing values and outliers. No missing values were identified during this process. Outliers were handled using listwise deletions. The interquartile range (IQR) method was applied to detect outliers, with values considered outliers if they fell below the first quartile (Q1) minus 1.5 times the IQR, or above the third quartile (Q3) plus 1.5 times the IQR. Using this criterion, 13 of the 185 participants were identified as outliers and were excluded from the machine learning analysis. Thus, 172 participants (57 healthy controls and 115 individuals with non-specific LBP) were included in the final analysis.

Table 2
Participant characteristics.

Variables	With non-specific LBP ($N = 115$)	Without non-specific LBP ($N = 57$)	*p*-value
Sex (male/female)	15/100	8/49	None
Age (years)	38.8 ± 7.7	38.6 ± 7.0	0.849
NPRS (0–10)	5.1 ± 1.5	N/A	
RMDQ (0–24)	10.0 ± 6.4	N/A	
x-axis displacement of T12 (mm)	126.8 ± 50.9	127.1 ± 53.3	0.972
y-axis displacement of T12 (mm)	−40.7 ± 49.9	−47.2 ± 44.8	0.402
x-axis displacement of L2 (mm)	58.1 ± 46.9	56.8 ± 50.5	0.869
y-axis displacement of L2 (mm)	3.6 ± 31.9	0.7 ± 29.6	0.568
x-axis displacement of S2 (mm)	−3.6 ± 48.9	−5.2 ± 54.0	0.850
y-axis displacement of S2 (mm)	38.5 ± 18.7	38.8 ± 22.8	0.993

LBP, Low back pain; NPRS, numerical pain rating scale; RMDQ, Roland–Morris Disability Questionnaire
Data are expressed as mean ± standard deviation

2.5. K-means clustering analysis

A clustering model of thoraco-lumbo-pelvic kinematic patterns during forward bending was constructed using the K-means algorithm, which is an unsupervised machine-learning technique implemented in the Orange data mining software (Orange 3.3.0, Ljubljana, Slovenia). The model was developed based on six input features: the horizontal (*x*-axis) and vertical (*y*-axis) displacements of the T12, L2, and S2 markers during the bending task. Because K-means clustering requires predefining the number of clusters, an exploratory analysis was conducted to determine the optimal cluster count within the range of 2–10. Cluster initialization was performed randomly, with the number of reruns set to 10 and a maximum of 300 iterations [18,22]. The optimal number of clusters is determined using the silhouette method, which evaluates the quality of clustering by measuring how well each data point fits within its assigned cluster relative to other clusters. Silhouette scores range from –1–1, with higher values indicating more coherent and distinct cluster separations [30].

2.6. Comparisons of kinematic data

An independent *t*-test was conducted to examine overall group differences between participants with and without non-specific LBP across all displacement variables (*x*- and *y*-axis displacements of the T12, L2, and S2 markers). One-way analysis of variance was used to compare age and the six-marker displacement data among the clusters and the healthy control group. When a significant difference was observed at the level of 0.05, post-hoc analyses were performed using the Bonferroni correction adjusted for the number of pairwise tests ($p < 0.017$). Additionally, an independent *t*-test was performed to compare the differences in NPRS and RMDQ scores between the clusters. Statistical analyses were performed using IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY).

3. Results

3.1. Participants' characteristics

Table 2 shows the means and standard deviations of all the variables.

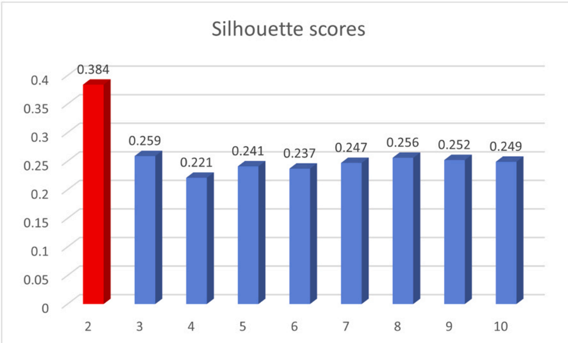

Fig. 3. Silhouette scores according to number of clusters

There were no significant differences in age or marker displacement at T12, L2, and S2 between the participants with and without non-specific LBP ($p > 0.05$).

3.2. Silhouette scores

Fig. 3 shows the outcome of the silhouette analysis applied to K-means clustering. Among the tested cluster counts, ranging from 2 to 10, the silhouette score peaked at two clusters (0.384), leading to the selection of k = 2 as the optimal number of clusters for further comparison. The two resulting clusters, cluster 1 (large-excursion) and cluster 2 (small-excursion), comprised 62 (54 %) and 53 (46 %) individuals with non-specific LBP, respectively.

3.3. Comparison between clustered subgroups and healthy controls

Table 3 presents a comparison of the age and marker displacement data between the healthy control group and the two clusters (C1 and C2). No significant differences were observed between the groups with respect to age. However, all *x*- and *y*-axis displacements of the three markers showed statistically significant differences across the groups (all $p < 0.001$). Post-hoc analysis revealed statistically significant differences ($p < 0.017$) for all pairwise comparisons (C1 vs. healthy, C1 vs. C2, and C2 vs. healthy), except for the *y*-axis displacement of the S2 marker. For S2 marker's *y*-axis displacement, a significant difference was found only between C1 and C2 ($p < 0.001$), whereas the comparisons between C1 and healthy individuals ($p = 0.035$) and between C2 and healthy individuals ($p = 0.023$) were not significant.

In addition, the comparison of pain intensity and disability between C1 and C2 showed a significant difference in NPRS ($p = 0.036$), while no significant difference was found in the RMDQ scores ($p = 0.871$).

4. Discussion

Sub-classification methods for non-specific LBP based on pain-aggravating or pain-relieving postures and movements have provided a reasonable framework for clinical assessment [8,9]. However, these approaches may require refinement because movement patterns can vary depending on individual responses to pain, such as adaptive or avoidant strategies. Unlike previous studies using predefined LBP subgroups (e.g., flexion pattern or active-extension pattern) [10,21,31–34], the present study categorized participants within the non-specific LBP population using clustering of thoraco-lumbo-pelvic marker kinematic parameters, allowing for the identification of distinct movement patterns without relying on a priori subgroup definitions. Greater values along the *x*-axis, representing anterior displacement of each segmental marker, and smaller values along the *y*-axis, representing inferior displacement, suggest greater excursion during forward bending tasks (Table 3). In our study, participants with non-specific LBP were classified into cluster 1, characterized by large-excursion features, and cluster 2, characterized by small-excursion features (Figs. 4 and 5). These findings support the notion that the movement patterns associated with non-specific LBP are fundamentally heterogeneous. Furthermore, the movement patterns of healthy controls were observed to lie between those of clusters 1 and 2, suggesting that the movement characteristics associated with LBP were not linearly defined. Rather, both reduced and excessive movements may serve as indicators of pain-related motor behavior, reflecting a U-shaped relationship in which deviations in either direction may be clinically relevant.

A recent study reported that individuals with non-specific LBP demonstrate a reduced range of motion (ROM) in both the lumbar spine

Table 3
Comparison of *x*- and *y*-axis displacement of T12, L2, and S2 markers during forward bending among three groups.

Variables	C1 (large-excursion) ($N = 62$)	Healthy controls ($N = 57$)	C2 (small-excursion) ($N = 53$)	One-way ANOVA		Post-hoc ($p < 0.017$)[†]	
				F	p		
Sex (male/female)	4/58	8/49	11/42				
Age (years)	39.3 (7.6)	38.3 (7.8)	38.6 (7.0)	0.283	0.754		
x-axis displacement of T12 (mm)	159.9 (33.8)	127.1 (53.3)	88.2 (39.0)	54.355	< 0.001*	(C1 vs. Healthy/C1 vs. C2/C2 vs. Healthy)	
y-axis displacement of T12 (mm)	−76.2 (33.4)	−47.2 (44.8)	0.9 (29.7)	86.406	< 0.001*	(C1 vs. Healthy/C1 vs. C2/C2 vs. Healthy)	
x-axis displacement of L2 (mm)	90.1 (29.8)	56.8 (50.5)	20.7 (33.6)	67.507	< 0.001*	(C1 vs. Healthy/C1 vs. C2/C2 vs. Healthy)	
y-axis displacement of L2 (mm)	−19.0 (21.1)	0.7 (29.6)	30.1 (19.6)	83.307	< 0.001*	(C1 vs. Healthy/C1 vs. C2/C2 vs. Healthy)	
x-axis displacement of S2 (mm)	23.3 (33.3)	−5.2 (54.0)	−35.1 (45.6)	30.513	< 0.001*	(C1 vs. Healthy/C1 vs. C2/C2 vs. Healthy)	
y-axis displacement of S2 (mm)	30.1 (17.3)	38.8 (22.8)	48.3 (15.3)	17.719	< 0.001*	(C1 vs. C2)	
Self-reported measures						*t*	*p*
NPRS (0–10)	4.8 (1.4)	N/A	5.4 (1.5)			−2.124	0.036*
RMDQ (0–24)	10.1 (6.5)	N/A	9.9 (6.4)			0.163	0.871

C1, cluster 1; C2, cluster 2; NPRS, numerical pain rating scale; RMDQ: Roland–Morris Disability Questionnaire; ANOVA: analysis of variance.
* Significant difference ($p < 0.05$).
[†] Bonferroni correction ($p < 0.017$).
Data are expressed as mean (standard deviation).

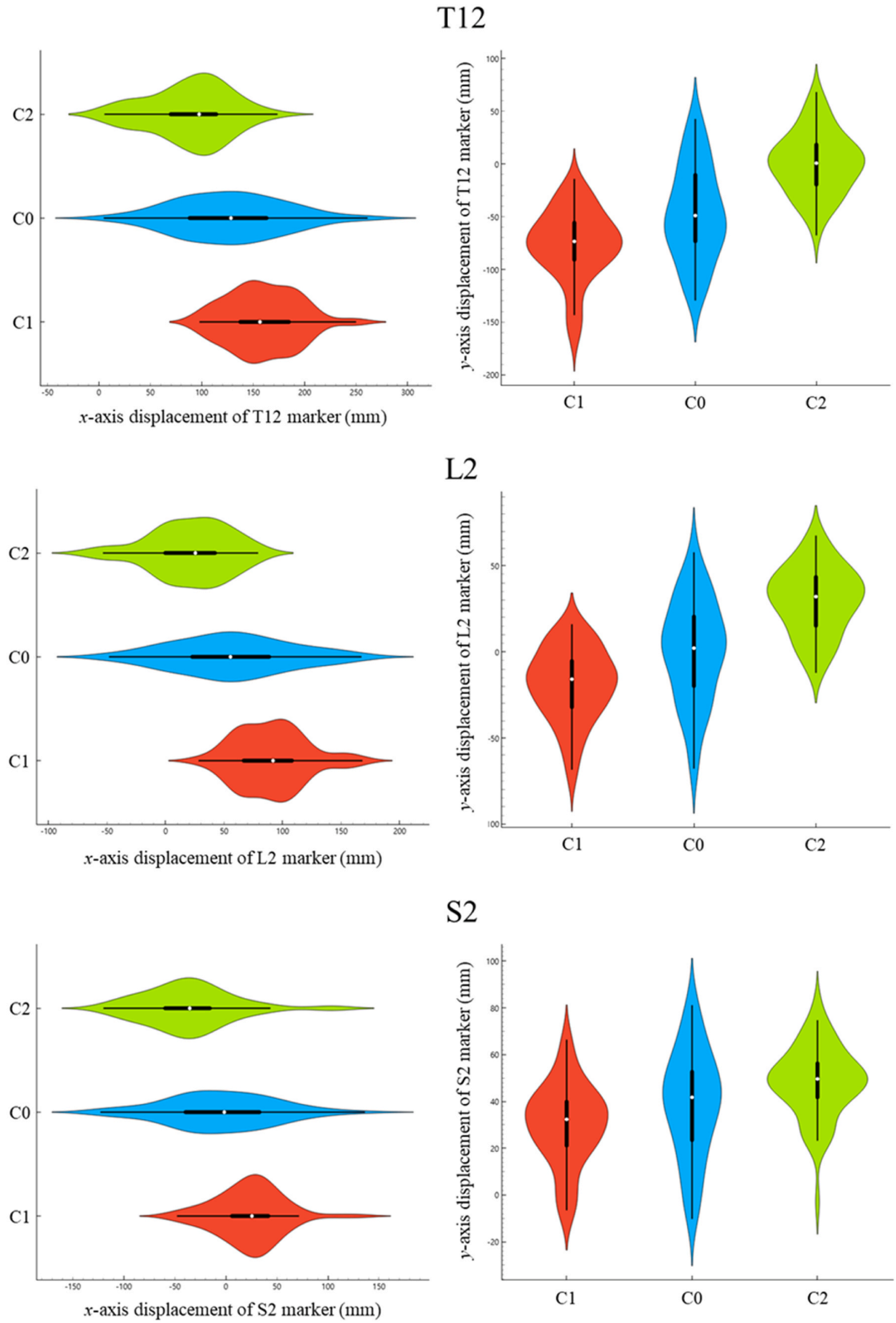

Fig. 4. Violin plot for comparisons of marker displacement data among cluster 1 (large-excursion), cluster 2 (small-excursion), and healthy controls

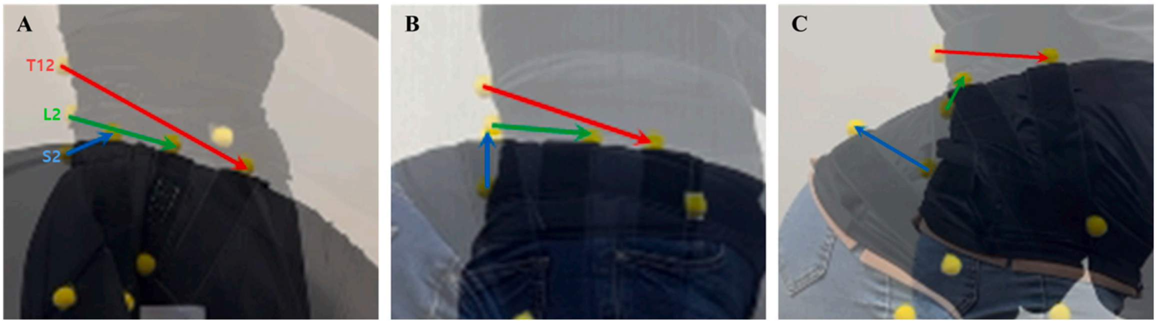

Fig. 5. Representative comparison of marker displacement direction and magnitude between the two clusters and healthy control: (A) illustrates an individual from cluster 1; (B) an individual from healthy control group; (C) an individual from cluster 2

and pelvis during forward bending compared with healthy controls [34]. This finding suggests that in the symptomatic group, a protective mechanism against further pain may be manifested by a reduced lumbar spine flexion moment at the terminal phase of forward bending [12]. In contrast, another study reported no significant difference in the lumbar spine ROM between individuals with and without non-specific LBP during forward bending [11]. When applying LBP subgroup classification, individuals with flexion-related LBP exhibited increased early-phase lumbar flexion ROM [11] and overall greater lumbar spine flexion compared with healthy controls [10,33], whereas those in the extension-related subgroup demonstrated reduced lower lumbar spine ROM [34] accompanied by compensatory increases in hip joint ROM [33]. Although several previous studies have consistently reported an increased lumbar spine ROM in the flexion-related LBP group, contrasting evidence has also emerged, with some investigations documenting reduced upper lumbar spine ROM within the flexion-related subgroup [34]. These conflicting findings indicate that segmental lumbar spine ROM alone is insufficient to accurately differentiate LBP subgroups, as the relationships among these kinematic variables may be inherently nonlinear. Therefore, in the present study, unsupervised machine-learning was applied to an entire cohort of individuals with non-specific LBP, allowing clusters to emerge without relying on predefined group classifications.

The pattern of x-axis movement, where increased values reflect anterior displacement and decreased values reflect posterior displacement, may be influenced by two main factors. First, lumbar and hip flexion naturally shifts the spine forward in the anterior direction. Second, posterior displacement results from a compensatory response to the forward shift of the center of mass during forward bending, which involves ankle plantar flexion and hip flexion [35]. These two factors were likely to interact and determine the resulting x-axis values. Additionally, the pattern of y-axis movement may reflect the extent of flexion during forward bending relative to the medial-lateral axis within the sagittal plane. The T12 marker showed the greatest displacement values, as it encompassed the combined movements of the lower segments along both the x- and y-axes.

Among the 115 participants with non-specific LBP, 62 (54 %) were classified into cluster 1 (large-excursion). Cluster 1 demonstrates a greater degree of flexion during forward bending, as indicated by the highest values along both axes. Developing intervention strategies based on these distinct movement patterns may lead to more effective and individualized management approaches. For example, exercises that enhance spinal stability or promote the use of knee joints to distribute excessive movement may be beneficial for individuals in cluster 1. In contrast, individuals in cluster 2, comprising 53 of the 115 participants (46 %), demonstrated movement patterns similar to the "Guarded group" described by Laird et al. [14]. For those in cluster 2, interventions aimed at improving flexion ROM in the lumbar spine and hip joints were expected to be effective. Although no difference was observed in disability (RMDQ) between clusters 1 and 2, pain intensity was slightly higher in cluster 2. Given the nature of the forward bending task, which is directly related to trunk flexion, this finding aligns previous research reporting that the "Guarded group" exhibited the highest pain score for the patten 'flexion aggravates, extension eases.' Similarly, in our study, the higher pain intensity observed in cluster 2, which was characterized by small-excursion, may reflect a protective mechanism in response to pain.

This study proposes a novel clustering approach based on spinal marker trajectories. However, certain limitations should be considered. First, the participants were office workers, who may have introduced postural biases related to prolonged sitting, thereby limiting the generalizability of the findings. Second, to minimize the relative movement between the skin and clothing, the markers were attached using tensioned Velcro straps. As data collection took place in public service offices, the participants could not remove their clothing for direct skin attachment. Future studies conducted in controlled settings in which markers can be directly applied to the skin may yield more accurate kinematic data. Third, this study did not account for anthropometric characteristics such as body size and limb-to-trunk proportions. Future studies should consider adjusting for these factors to better distinguish spinal mobility from anthropometric effects. In addition, the silhouette score of 0.384 indicated modest cluster separation. This relatively low value likely reflected the continuous spectrum of spinal marker displacements during forward bending, in which no significant differences were observed between individuals with and without pain. Therefore, the current clustering results should be interpreted as exploratory, and future research aiming to achieve stronger cluster validity will be essential before these subgroups can inform clinical decision-making.

5. Conclusions

This study demonstrated that individuals with non-specific LBP exhibit heterogeneous thoraco-lumbo-pelvic movement patterns during forward bending, which can be effectively differentiated using unsupervised machine-learning based on marker displacement trajectories. The identification of distinct large-excursion (cluster 1) and small-excursion (cluster 2) subgroups emphasize the limitations of relying solely on the presence or absence of LBP for classification and supports the need for movement-based phenotyping in clinical assessment. These findings suggest that both excessive and limited motion may serve as relevant indicators of pain-related motor behaviors, reinforcing the U-shaped relationship between movement characteristics and symptom presentation. Integrating such data-driven classification methods into clinical practice may enhance the precision of therapeutic decision making and support the development of individualized interventions tailored to specific movement patterns. Future studies with stronger cluster validity will be necessary before these subgroups can directly inform clinical decision-making.

CRediT authorship contribution statement

Hwa-Ik Yoo: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Jong-Gook Choi:** Visualization, Methodology. **Ui-Jae Hwang:** Validation, Data curation, Conceptualization. **Oh-Yun Kwon:** Writing – review & editing, Supervision, Conceptualization.

Ethics approval

The present study conformed to the ethical guidelines of the 1975 Declarations of Helsinki. The requirement for informed consent was waived by Yonsei University Mirae Campus Institutional review board approval (1041849–202412-SB-267–01) because of the retrospective nature of the study's reliance on preexisting data acquired from investigating the risk factors of musculoskeletal disorders. Ethical approval was obtained from Yonsei University Mirae Campus Institutional review board.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availabilit

The datasets analyzed during the present study are available from the corresponding author on reasonable request.

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