Cervical Spine Assessment using Passive and Active Mobilization recorded through an optical motion capture

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Abstract

Objective: The purpose of this study was to develop and measure a protocol for evaluation of cervical range of motion (ROM) based on passive mobilization (PM) combined with active mobilization (AM) and recorded through an optical motion capture (MoCap) system.

Method:

Passive and active mobilizations were applied to 24 asymptomatic subjects. Cervical ROM was recorded in 3 anatomical planes (transversal, frontal, and sagittal) using a precision optical system and a set of rigid bodies (RBs) placed on the sacrum, spinous processes of the C7-T1 vertebrae, and the head. Three captures were made for each subject, distributed over 2 days. The characteristics of the PM, the interaction with the AM, and the coherence patterns between tests were analyzed. Reliability was studied for these procedures.

Results:

The reliability results of the PM were high in all analyzed indices; only flexion showed low values. Reliability of AM was greater than PM for flexion-extension and lateralization because of the similarity to rotation. No statistically significant differences were found comparing PM and AM techniques.

Conclusion:

According to the objectives of the study we have developed a cervical ROM assessment based on combined PM and AM protocols at different sessions. It demonstrated high reliability individually and combined; and no differences were detected between PM and AM ROMs. Evaluator, instrumentation, and patient become a set of factors that could influence the results; consequently, to ensure a proper diagnosis they should be used in combined protocols. These protocols could be used to evaluate the functional and structural capacity of patients and they would provide benefits to inform about clinical outcomes in practice.

Key Indexing Terms: Cervical Vertebrae; Range of Motion, Articular; Reproducibility of Results.

INTRODUCTION

Musculoskeletal disorders of the cervical spine have a high incidence and prevalence and are considered a public health problem, especially in developed countries.[1,2] Although there have been significant contributions in different fields to access cervical spine injury, including whiplash associated disorder (WAD), such as diagnostic tests assessing cervical-area alterations, "whiplash severity grading systems," diagnostic imaging tools, and scales, such as the Quebec Task Force, they seem to be insufficient for predicting possible complications of symptomatology.[9,10]

The diagnostic difficulty is because traumatic cervical spine injuries and their associated symptoms are diverse. Variables that have been measured to quantify the degree of dysfunction are isometric muscle strength,[11] motion velocity, smoothness,[12] and cervical range of motion (ROM).[4,13-19] Due to the relationship between joint dynamics and the dysfunction location,[12,16] the ROM is often used to quantify the severity and treatment.[4] This index is also used by the American Medical Association (AMA)[9,20] to assess physical damage or is used in specific legislation in countries such as Spain (Law 35/2015)[21] for the assessment of damage caused by traffic accidents.

One method to evaluate cervical ROM uses voluntary patient movements under the instructions of an evaluator, called active mobilization (AM). This type of mobilization does not require physical interaction between the patient and the evaluator[22] and provides relevant functional information.[23] However, application of AM as an isolated technique is questioned.[22-24] Given the influence of the patient's subjectivity motivated by psychosocial factors,[2,25-27] different types of errors may be observed and the AM technique has high variability of results and a low capacity to predict chronic symptoms.[4,13,23] Likewise, it does not provide clinical information to determine structural function.[25,27]

Another method is passive mobilization (PM).[16,28] In this case, an external force is induced by the examiner to move specific body parts up to the joint limits, while the subject relaxes the joint that is being explored.[23,29] Passive mobilization allows the examiner to assess the "physiological barrier," the structural information of the joint under assessment, which is useful in clinical decision making for treatments.[30-32] It is assumed that this range is not influenced by psychosocial factors as in AM because the captured ROM mainly depends on action and perception of the examiner during the test.[5,27] Therefore, authors have reported a lower variability in the results in applying PM techniques.[15,23]

Most PM techniques use subjective analyses based on the examiner's perception,[22] so is not considered the gold standard.[4,22,23] Consequently, the challenge associated with PM is to provide studies that analyze its properties and characteristics through objective kinematic measurements, enabling its validation as a diagnostic technique.

To validate methodologies based on PM, it is necessary to apply criteria related to accuracy and reliability.[33,34] The criteria involve some standards satisfying reliability, which require that the measurement must be repeatable and invariant to external factors (i.e., the subjectivity of the evaluator, technical-system commitment, and others).

In a systematic review[4] of 46 reliability studies and 21 validation studies where PM and AM techniques were applied, 8 PM studies were found, which described the passive technique used.[35] There is a problem when explaining the characteristics and properties using PM combined with objective measures. In general, PM reliability has not been analyzed in depth[4,13,36] and only a few have used objective measurements for the analysis of PM techniques.[17,22]

If AM and PM were combined for assessment, they could possibly provide greater sensitivity and specific diagnostic information in the clinical-care setting. In addition, the problem concerning the subjectivity of both, which is derived from the evaluator in the PM and is derived from the psychosocial factors of the subject in the AM, might be mitigated by their combination.

Motion capture (MoCap) systems provide precision however they are not exempt from sources of errors, such as those derived from the marker placement on particular anatomical areas that move with respect to the underlying bones and those due to the conditions of application in a specific area or use conditions in a specific field.[37] This is a critical aspect in the measurement of the ROM movements where the maximum range is measured and during the PM where the reflective markers can be hidden or even moved by the evaluator if he or she is not sufficiently trained. Consequently, the variability of the system depends on the design of the set and its degree of integration, that is, the placement of markers, the checks, the understanding of the movements by the subject, the instructions to the patient, or the training of the evaluator in the use of the system. Therefore, the added value provided by MoCap technologies can be diminished in the clinical setting, if the possible sources of error are studied in their practical application, which is the general purpose of this study.

Therefore, the objectives of this study were the following: (1) to develop a cervical ROM assesment protocol based on PM; (2) check the system reliability using the PM and AM, individually and combined; (3) to perform a comparison of both techniques, PM and AM and (4) to understand the influence and interactions between tests when applied together.

METHOD:

Instrumentation

Cervical mobility was recorded through a MoCap system composed of the following components:

1. Set of 8 Optitrack cameras (Flex 13, 1.3PM, 56 ° FOV and 120 FPS) and the OptiTrack Motive 1.9 application (NaturalPoint Inc., 2016).

2. Software motion characterization MoveHuman-Sensors[38,] implemented in Vizard VR Toolkit[39] virtual reality platform and the intellectual property of the University of Zaragoza.

OptiTrack Motive controls the cameras and processes the movement data of certain rigid bodies (RBs), providing 6 degrees of freedom for each of them. The RBs information is read in real time by MoveHuman-Sensors, through a peripheral network communication protocol, and is transferred to a 3D digital human model. At the beginning of each capture, an anatomical calibration process to adjust the digital model to the anthropometry of the subject and associate the position and orientation of each RB with the corresponding body segment by matrix transformation. The software allows visualizing and recording cervical movements in real time while applying the PM or AM techniques.

The RBs correspond to groups of 3 markers (reflective spheres) placed on a rigid support. Each RB was individually designed for an appropriate fit adjustment to its corresponding body part. Three RBs were used to record the cervical kinematics: one on the sacrum, another on the spinous processes of vertebrae C7-D1, and another on the head, Figure 1.

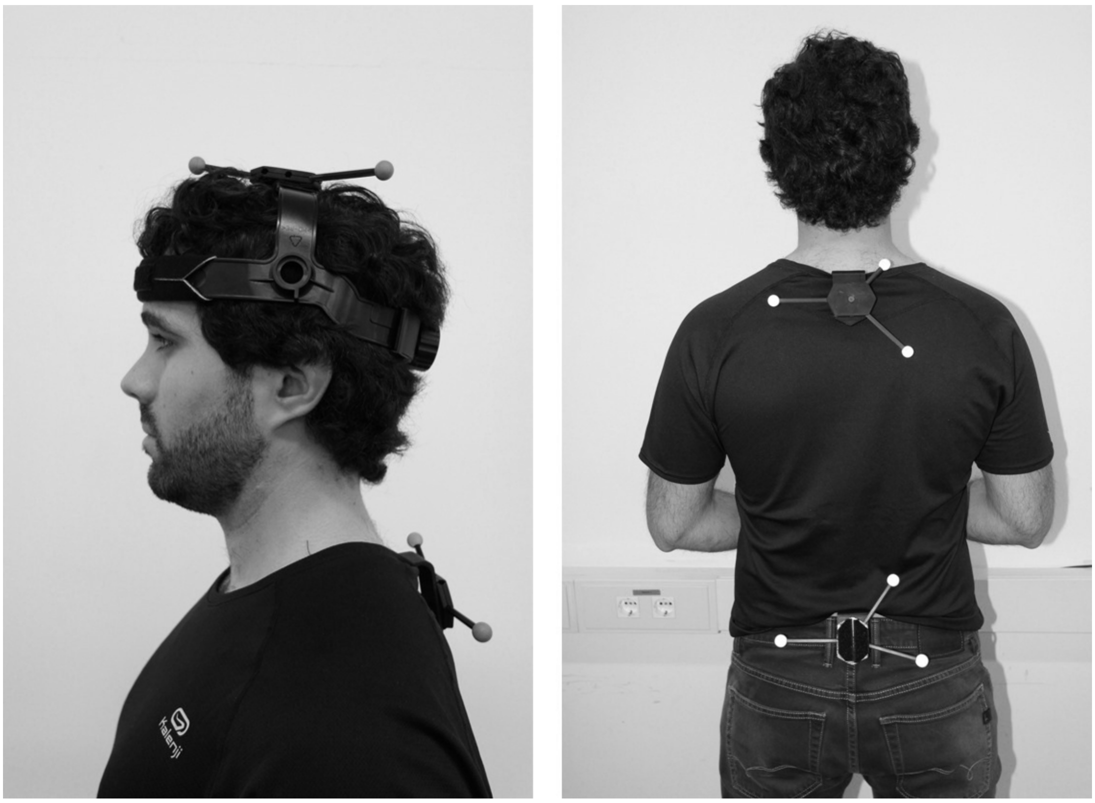


Fig. 1 Location of rigid bodies (RBs) in the sacrum, the spinous processes of C7-D1 vertebrae, and the head.

Participants

Twenty-four asymptomatic subjects (16 men and 8 women) aged 32 ± 11.35 years participated in the study. Anthropometric characteristics from the population are shown in Table 1. The inclusion criteria were the absence of the following: a history of neck or head pain; cervical trauma; vestibular, visual, or nervous problems; or surgeries in the cervical region. These data were collected by interview, which was guided by the same evaluator.

All subjects signed an informed consent to participate in this study and for the filming of the test for research purposes. This study received a favorable dictum on the biomedical research project from the Clinical Research Ethics Committee of Aragón (Spain). This study received authorization from the Aragón Engineering Research Institute of the University of Zaragoza, where the test of this study was conducted.

Table 1. Anthropometric characteristics of the subjects.

	Age (years)	Height (cm)	Weight (kg)
Total (N = 24)	32 (11.35)	179.04 (11.47)	73.03 (7.31)
Male (N = 16)	32 (11.74)	186.20 (8.67)	84.35 (5.45)
Female (N = 8)	35 (10.7)	164.75 (9.99)	57.59 (7.48)

Mean and Standard Deviation (SD). Number of subjects (N). Centimeters (cm).Kilogram (kg)

Process

Figure 2 shows the design of the study. Each participant was evaluated in 3 sessions (S) on 2 different days. A 10-min interval was established between S1 and S2 and more than 24 h between S2 and S3. The citation times to conduct the assessment were assigned to avoid morning stiffness.[35]

In each session, an AM and PM of the cervical ROM were performed in sequential order. The AM test was performed by two operators (biomedical engineers), and the PM was performed by an examiner (therapist specializing in orthopedic manual therapy). There was no contact between the operators and the examiner physiotherapist during the entire study. The operators and examiner were specialists in the operation and instrumentation of the system used.

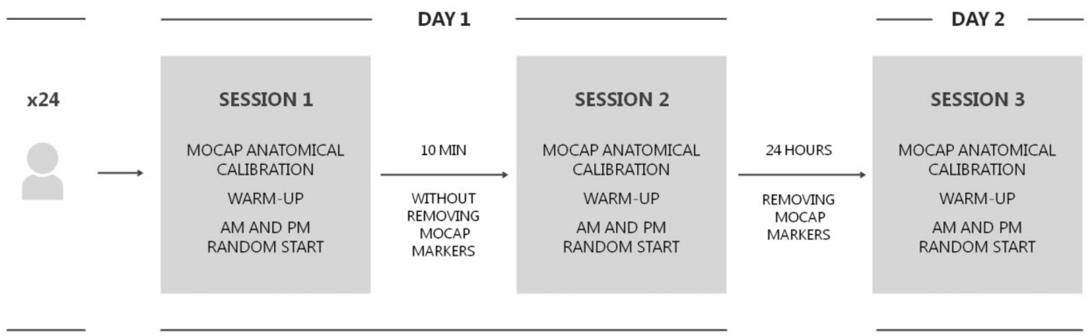


Fig. 2 Method design and process scheme.

At the beginning of each session, the subject was instructed to adopt a natural and upright posture ensuring a 90° angle between hips and knees, with forearms relaxed and resting on the thighs. A blackless, on-swivel and ajustable height chair was used. Next, an anatomical calibration process was performed to adjust the human model subjetc anthropometry, Figure 3.

Fig. 3 Anatomical calibration.

Prior to each evaluation, a series of warm-up exercises was conducted to prepare the cervical muscles and facilitate the subject's familiarity with the movements.

In both ROM evaluations (PM and AM), the following sequence of movements was followed: cervical flexion-extension (F-E), cervical rotation (R) right (RR) and left (LR), cervical lateralization (L) right (RL) and left (LL), measuring the full ranges (F-E, R-R, L-R) and mean ranges (F, E, LR, RR, LL, and RL) with the system.

Figure 4 describes the captured movements and the reference systems considered for the analysis.

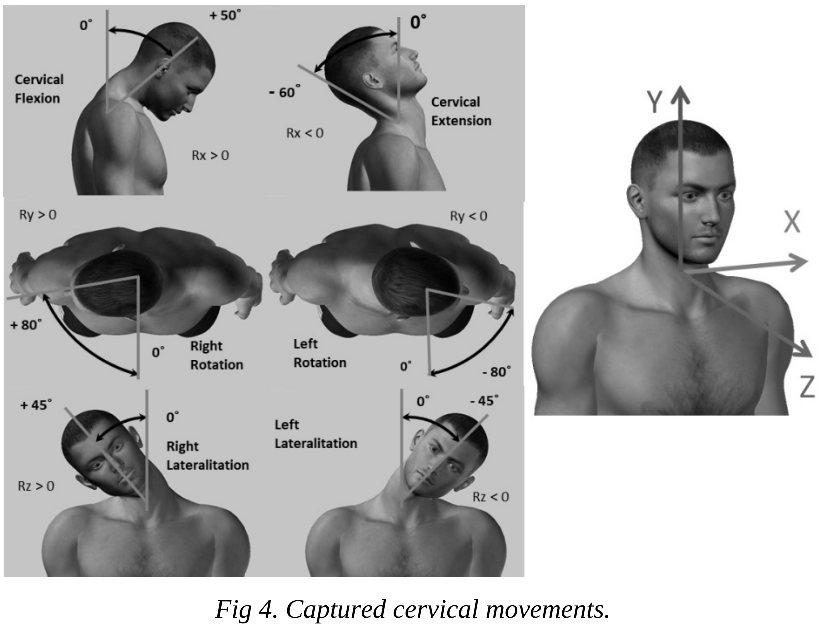


Fig 4. Captured cervical movements.

The AM was executed by continuous and sequential movements performed by the participant without physical assistance from the operator. Each subject was instructed to reach the maximum range without symptoms or pain. The speed of execution was determined by the subject without the influence of any operator.[16]

The PM consisted of anatomical structure mobilization of the subject through the hands and the body by the examiner, avoiding the compensatory movements of the thoracic region. Palpating the spinous processes of the C6, C7, and D1 vertebrae and globally mobilizing the cervical spine to feel movement from C7 to D1 at the base of occipital bone provided movement information. Figure 5 shows the PM technique of F-E.

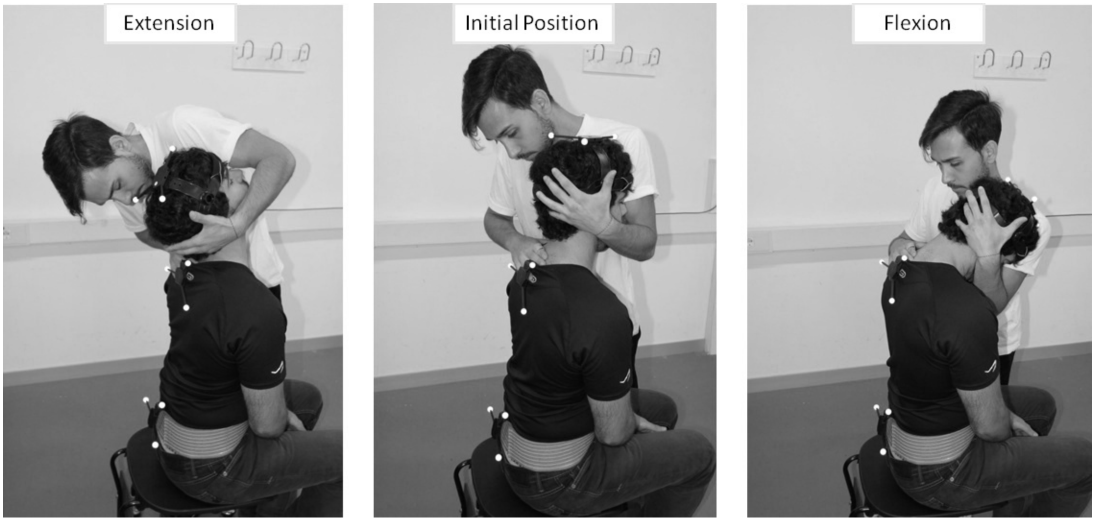


Fig. 5 Passive mobilization for the movement of F-E.

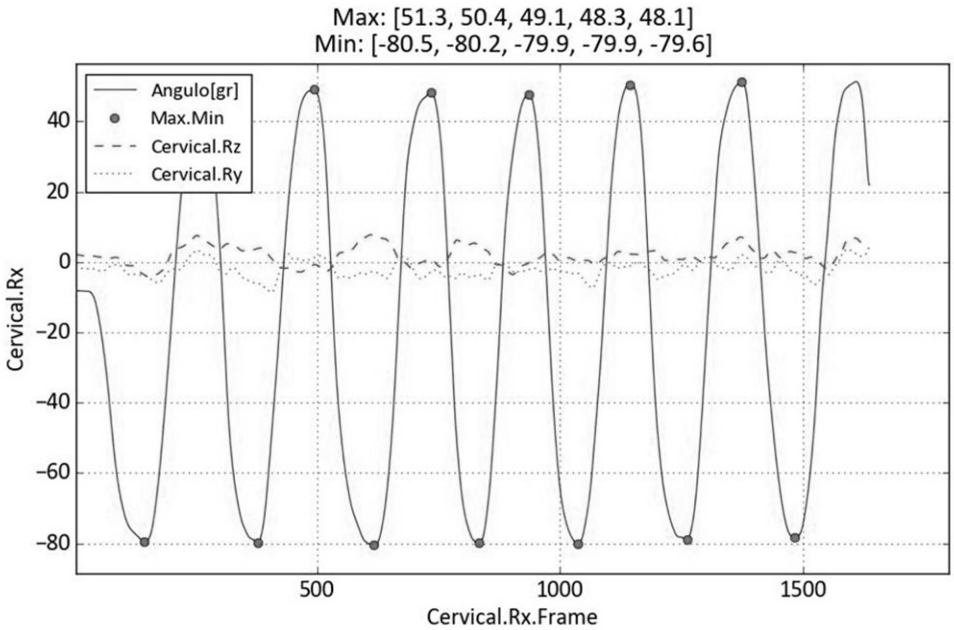
The PM protocol was followed to perform the movements until the "end feel,"[29,40] feeling the final resistance in the palpation hand.[22,23] During the PM, an attempt was made to avoid the appearance of sources of error, both verbal and gestural, toward study subjects that could come from the examiner's prejudices on age, aesthetics, and expressions. In addition, a warm up was held to prevent the muscular protection effect of the subject in performing the movements. The mobilization was conducted by a therapist trained in orthopedic manual therapy.

Statistical Analysis

The variables considered in the statistical study were the average of the three ROM movements, full ranges of F-E, R-R, and L-L and mean ranges of F, E, RR, LR, RL, and LL. The following studies were conducted using Minitab 17 statistical software. First, an exploratory analysis of the data was conducted, including the control of possible outliers and a normality test. The means and standard deviations (SDs) are shown in Table 2. The averages of the three sessions were compared using a repeated-measure analysis of variance (ANOVA) with two fixed factors (subjects and sessions). The influence of the six measures (3 AM and 3 PM) on the ROM was evaluated through ANOVA. When the ANOVA indicated significant differences, the Tukey test was used to determine the pair-wise differences. All factors were defined as fixed. The differences between the two types of evaluations (AM and PM) were analyzed using the Bland–Altman method[41] for interest movements. To evaluate the reliability of the system using both types of mobilizations (PM and AM), three different statistical methods were applied: the interclass correlation coefficients (ICCs),[42] the Bland–Altman method,[41] and the standard error of measurement (SEM).[43] To evaluate important changes in the clinic, the minimal detectable change (MDC) and the coefficient R (95%) were calculated. A comparative analysis of the reliability results achieved in the PM and AM evaluations has been conducted (Tables 4 and 5).

RESULTS

The results are displayed in graphs in Figure 6 (AM) and Figure 7 (PM), which are generated from the MoCap application.



(Fig 6) Graph of Flexion-Cervical Extension Movement during active assesment technique AM (cervical [Rx]). Maximum (Max) and minimum (Min) marked with red dots. The Flexion appears as a positive value, the Extension as negative. The coupled movements of rotation and lateralization (cervical Rz, Ry) are observed.

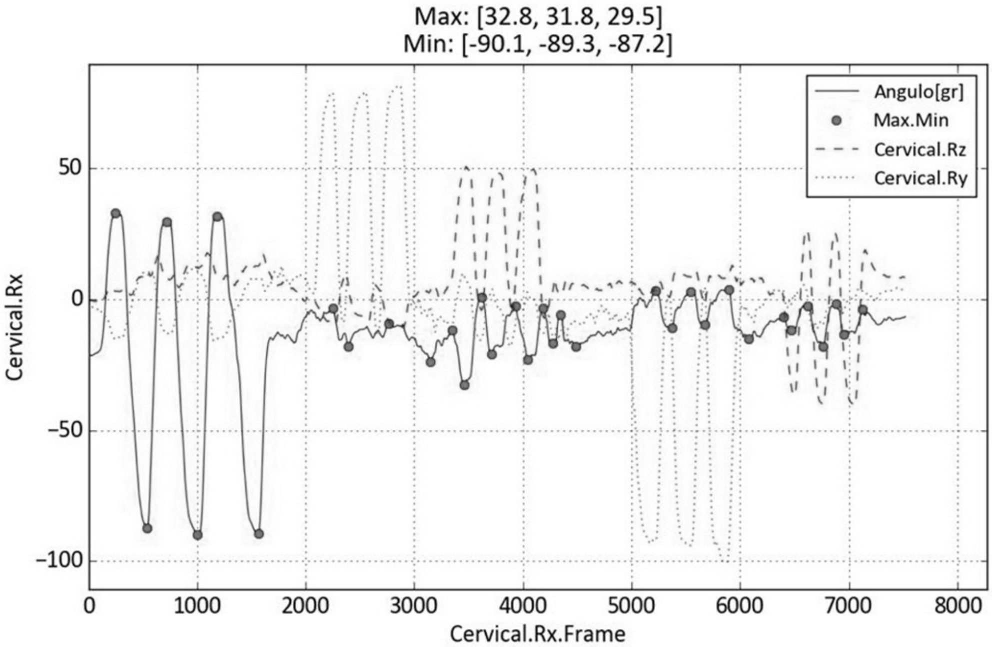


Figure 7. Graph Movement of Flexion-Extension Technique Used for Passive Assessment, PM (cervical Rx). Maximum (Max) and minimum (Min) marked with red dots. Flexion appears as a positive value, the extension as negative. Rotation coupled movements and lateralization (cervical Rz, Ry) are observed.

Statistical analysis (Table 2) shows the results of the exploratory analysis and the average for the movements studied in each session. All movements in both types of assessments, PM and AM, fulfilled the normality hypotheses.

Table 2. Normal study data from active and passive mobilization.

Movement	Technique	μ(SD) S1	μ(SD) S2	μ(SD) S3	Average(SD)	P value
F	**MP**	50.82 (11.1)	48.81 (8.02)	49.80 (9.44)	49.81(9.45)	0.382
	MA	50.37(10.17)	49.33 (9.70)	50.18 (8.89)	49.94 (9.47)	0.392
E	**MP**	68.38 (16.24)	69.53 (14.98)	71.30 (15.16)	69.73 (15.25)	0.282
	MA	66.97 (12.92)	69.07 (13.04)	68.38 (12.47)	68.14 (12.66)	0.220
F-E	**MP**	122.33 (19.79)	122.87 (19.61)	126.74 (17.09)	123.98 (18.68)	0.074
	MA	119.87 (18.58)	120.74 (18.47)	121.06 (16.86)	120.55 (17.75)	0.262
LR	**MP**	80.08 (9.42)	79.42 (10.22)	81.41 (6.92)	80.33 (9.03)	0.604
	MA	75.45 (8.17)	78.75 (10.06)	77.95 (8.98)	77.4 (9.07)	0.008
RR	**MP**	73.05 (9.35)	72.51 (9.78)	74.19 (9.97)	73.25 (9.51)	0.534
	MA	73.76 (7.83)	75.47 (7.71)	76.51 (9.19)	75.25 (8.24)	0.065
LR-RR	**MP**	156.98 (13.80)	155.21 (15.40)	162.79 (14.80)	158.33 (14.52)	0.200
	MA	152.28 (14.63)	157.13 (15.08)	158.09 (17.18)	155.83 (15.63)	0.009
LL	**MP**	39.59 (6.36)	40.35 (5.80)	38.67 (8.45)	39.54 (6.69)	0.680
	MA	39.97 (7.40)	41.54 (6.85)	39.75 (6.47)	40.42 (6.90)	0.590
RL	**MP**	40.96 (7.36)	42.26 (8.49)	44.61 (5.80)	42.61 (7.36)	0.886
	MA	42.42 (5.66)	43.41 (5.86)	43.29 (5.47)	43.04 (5.69)	0.855
LL-RL	**MP**	84.79 (13.50)	88.02 (12.87)	88.31 (11.73)	87.04 (12.69)	0.871
	MA	85.4 (12.01)	87.94 (11.09)	85.92 (11.24)	86.42 (11.45)	0.583

Mean and Standard Deviation (SD) of half and full cycle PM and AM assessments. S1: Session 1; S2: Session 2; S3: Session 3. Average of (S1, S2, S3). ANOVA P value.

Regarding the comparison results between sessions (S1, S2, and S3) by ANOVA, there were no statistically significant differences for the movements studied ($p > .050$) with an exception found for LR and R-R in AM movement.

Regarding the influence between assessments (3 PM and 3 AM), the ANOVA test results (type of evaluation, time [sessions], direction of movement, and subjects) reported no significant ROM difference between (a) the type of evaluation ($p > .05$) or (b) the time of sessions ($p > .05$) for all movements (F-E, L-L, RR, and LR).

The comparison between types of mobilization was studied using the Bland–Altman method. The ROM data corresponding to the average of the three sessions for each subject was used for this comparison (Table 3). In this study, no statistically significant differences ($p > .050$) were found except for R-R, which is of 0.05 order, and LR, which included the value zero in the interval.

Table 3. Bland–Altman method results for the differences between the PM and AM methods

Differences Between Methods (MP-MA)

D.Movement	***Size Sample***	**μd(SD$_d$)**	**R(95%)**	**P***	**μ(95% CI)**
F	Total(n=23)	0.43 (7.13)	14.26	0.290	(-3.51; 2.66)
E	Total(n=23)	1.77 (5.41)	10.82	0.130	(-0.57; 4.11)
F-E	Total(n=22)	3.36 (7.88)	15.76	0.059	(-0.13; 6.85)
LR	Total(n=24)	2.70 (4.36)	8.72	0.006	(-0.86; 4.54)
RR	Total(n=20)	1.15 (5.64)	11.28	0.372	(-3.79; 1.49)
LR-RR	Total(n=20)	3.95 (7.12)	14.24	0.022	(0.62; 7.28)
LL	Total(n=18)	0.67 (5.59)	11.18	0.616	(-2.10; 3.45)
RL	Total(n=19)	1.04 (5.58)	11.16	0.426	(-1.65; 3.73)
LL-RL	Total(n=17)	3.87 (9.29)	18.58	0.105	(-0.91; 8.65)

n: sample size, µ*d*: mean of differences, SDD: Standard deviation of differences, *R* (95%): Bland–Altman coefficient of reproducibility, *p* *: *p*-value Student's t-test for paired samples, (95% CI): Confidence interval for mean of differences.

Tables 4 and 5 show the reliability results. Fleiss et al.[44] considered "excellent reliability" for ICC values to be greater than 0.75 and "fair to good reliability" values to be between 0.4 and 0.75. According to this classification, the values obtained from the ICC demonstrate good-to-excellent reliability for all movements and mobilizations for PM (ICC = [0.621-0.916]) and AM (ICC = [0.729-0.937]). Only the F movement showed lower reliability values for the PM (ICC = 0.428; case intra-examiner S1-S3).

The level of agreement between the three sessions, also called the change in mean, was evaluated using the Bland–Altman method.[41] The t-test for paired samples (between sessions) revealed that there are no systematic differences for all movements analyzed in both techniques ($p > .020$).

Moreover, reliability was assessed by variability measures, such as SEM, R (95%), or MDC. These values assess the errors regarding reliability, following the approach of Strimpakos et al.[35] The following ranges of values were found: PM (R [95%]/average = [0.095-0.329]; SEM/average = [0.034-0.158]; MDC/average = [0.093-0323]) and AM (R [95%]/average = [0.079-0.188]; SEM/average = [0.028-0.066]; MDC/average = [0.077-0.184]).

Table 4. Passive mobilization reliability results.

	INTRA-PHYSIOTHERAPIST (S1-S3)				INTRA-EVALUATOR (S1-S2)				GOBLAL MEAN INTRA-EVALUATOR			
MOVEMENT	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**
F	0.431	0.152	0.422	0.428 (* - 0.87)	0.228	0.163	0.223	0.814 (0.73-0.96)	0.329	0.158	0.323	0.621
E	0.184	0.065	0.180	0.909 (0.86-0.98)	0.196	0.069	0.200	0.911 (0.88-0.98)	0.190	0.067	0.190	0.910
F+E	0.138	0.049	0.135	0.878 (0.81-0.98)	0.102	0.036	0.100	0.954 (0.93-0.99)	0.120	0.042	0.117	0.916
LR	0.106	0.038	0.104	0.855 (0.24-0.92)	0.133	0.047	0.130	0.849 (0.78-0.97)	0.120	0.042	0.117	0.852
RR	0.210	0.074	0.206	0.607 (0.79-0.97)	0.134	0.047	0.131	0.844 (0.81-0.97)	0.172	0.061	0.168	0.726
LR+RR	0.083	0.029	0.081	0.828 (0.75-0.99)	0.107	0.038	0.105	0.78 (0.67-0.95)	0.095	0.034	0.093	0.804
LL	0.135	0.048	0.132	0.91 (0.85-0.99)	0.197	0.070	0.193	0.699 (0.54-0.94)	0.166	0.059	0.163	0.805
RL	0.246	0.087	0.241	0.666 (0.38-0.94)	0.273	0.096	0.267	0.78 (0.65-0.95)	0.259	0.092	0.254	0.723
LL+RL	0.158	0.056	0.155	0.799 (0.21-0.98)	0.136	0.048	0.134	0.89 (0.83-0.98)	0.147	0.052	0.144	0.845

S1: Session 1; S2: Session 2; S3: Session 3. Lower estimate 95% CI (ICC). Zero:*. R(95%): Bland-Altman Method (R95%). SEM: standard error of measurement. MDC: minimal detectable change. ICC: interclass correlation coefficient and 95% Confidence interval (95%CI). Average: mean values of S1, S2, S3.

Table 5. Active mobilization reliability results.

	INTRA-TESTER A (S1-S3)				INTRA-TESTER B (S1-S3)				GOBLAL MEAN INTRA-TESTER (A & B)			
MOVEMENT	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**	**R(95%) / Average**	**SEM / Average**	**MDC / Average**	**ICC**
F	0.161	0.057	0.158	0.864 (0.71-0.98)	0.214	0.076	0.210	0.803 (0.60-0.97)	0.188	0.066	0.184	0.834
E	0.108	0.038	0.106	0.919 (0.90-0.99)	0.113	0.040	0.110	0.955 (0.92-0.99)	0.110	0.039	0.108	0.937
F+E	0.096	0.034	0.094	0.875 (0.83-0.99)	0.071	0.025	0.069	0.967 (0.94-0.99)	0.083	0.030	0.082	0.921
LR	0.142	0.050	0.139	0.709 (0.38-0.96)	0.109	0.039	0.107	0.913 (0.82-0.99)	0.125	0.044	0.123	0.811
RR	0.137	0.048	0.134	0.631 (0.33-0.96)	0.138	0.049	0.135	0.827 (0.64-0.98)	0.137	0.049	0.135	0.729
LR+RR	0.089	0.032	0.087	0.738 (0.64-0.97)	0.069	0.024	0.067	0.953 (0.90-0.99)	0.079	0.028	0.077	0.846
LL	0.141	0.050	0.139	0.834 (0.75-0.99)	0.156	0.055	0.153	0.746 (0.35-0.98)	0.149	0.053	0.146	0.790
RL	0.123	0.044	0.120	0.889 (0.80-0.99)	0.083	0.029	0.082	0.858 (0.62-0.99)	0.103	0.036	0.101	0.874
LL+RL	0.062	0.022	0.061	0.943 (0.80-0.99)	0.116	0.041	0.114	0.822 (0.44-0.98)	0.089	0.032	0.087	0.883

S1: Session 1; S2: Session 2; S3: Session 3. Lower estimate 95% CI (ICC). Zero:*. R(95%): Bland-Altman Method (R95%). SEM: standard error of measurement. MDC: minimal detectable change. ICC: interclass correlation coefficient and 95% Confidence interval (95%CI). Average: mean values of S1, S2, S3.

DISCUSSION

Evaluated Asymptomatic Population

This study is focused on the design of the system and the protocols for cervical spine assessment through ROM measurement. We selected a sample of asymptomatic subjects. This was motivated by the specific objectives of this work, where there are factors that influence AM and PM, either alone or in combination. These factors are the technological and operational system, the placement or possible movement of the markers during capture, the clinical protocol of tests, the environment where they are conducted, the personal factors of the subject, or the factors related to the operator who conducts the evaluation.

Therefore, before assessing patients with cervical spine pathology, it is necessary to study the influence of all the factors not related to the patient's own cervical pathology to analyze the factors related to the system operation and related to the cervical physiological movement of asymptomatic people. This allows laying the groundwork for its real application in clinical settings with pathological subjects, which will allow us to detect the differences with the asymptomatic population of this study. Furthermore, it is useful for the early detection of pathologies, which constitutes the goal of this study.

Considerations of the Applied Experimental Protocol

To address the objectives of this study, a specific experimentation protocol has been designed. A total of three sessions were conducted. In each session, PM and AM were performed in sequential order. The first two sessions (S1 and S2) were made consecutively after a short break without touching the markers or RBs. The third session (S3) was conducted the following day, logically requiring the relocation of the RBs. The applied experimental protocol covers the different possibilities to evaluate the influence of factors that are relevant for the purposes of this study:

1. Placement of the RBs and their possible movement during the capture or after a rest where the subject can be relocated in the seat (between S1 and S2).
2. During the PM, the examiner may inadvertently touch the RBs on the D2 vertebrae or head, which could cause small movements and errors in the capture.
3. Complete relocation of the RBs (between S2 and S3), which can be a source of variability to analyze.
4. The learning effect of the subject between S1 and S2, which would not be expected between S2 and S3.
5. The resting effect between tests, which is short between S1 and S2 and complete between S2 and S3.
6. The effect of performing an AM technique first and then a PM, given the possible influence of one over the other. It is of interest to obtain a protocol for clinical application that combines the use of both techniques, especially for the forensic field, to study the degree of consistency and collaboration of the patient.
7. The effects of the AM applied by the operator (biomedical engineer) that do not require physical interaction with the patient during the capture, and the PM performed by the examiner (chiropractic physician) that requires interaction. The protocol established that there was no interaction between the evaluators.

Given the singularity of PM, where it is necessary to reach the limits of the physiological barrier of the subject's cervical spine, it was considered necessary to block this variability inherent to the knowledge of the technique, training, skill, or experience of the examiner, since, in this paper, we aim to study the effect of the rest of the described factors. However, it is necessary to consider that, in clinical practice, for the follow-up of a patient during a treatment (simulated with the sequence of sessions S1/S2 with S3), it is convenient that the same examiner performs the test, applying the same criteria of PM in the same way. Regarding both considerations, we tried to analyze all the exposed factors, excluding the variability of the examiner that conducts the PM, while the follow-up of a patient in a clinic justifies that only one examiner participated in the three sessions.

Both considerations, on the one hand, seek to analyze all the intervening factors (not including the variability of the examiner that carries out the MP), and on the other, the follow-up of a patient in clinic, justifies that in the three sessions (S1, S2) and S3), a single examiner would intervene. In contrast, two operators participated in the AM. For study purposes, the mean values of both have been considered and collected in Table 5. The statistical analysis corresponding to the inter-operator evaluation is included in a technical note.[45]

This study proposes a method of evaluation for those with cervical trauma (eg, WAD) based on a methodology that combines the PM and AM techniques, providing information on the structural and functional actions, respectively. The validation was conducted through an analysis of the results provided by the MoCap system. Then, the aspects regarding comparison between AM and PM and the reliability of both tests are discussed.

Data Comparison Between Tests

No statistically significant differences ($p > .050$) in the ROM during PM and AM (F-E, RR, and L-L) were found (Table 3), except for the LR motion and R-R range. This last result was more pronounced in S1 and may be motivated by a lack of learning and cervical spine tone before a little training and familiarization with these movements.

Given the predetermined order of testing (AM and PM), it is necessary to analyze the interactions between them to study the possible influence between tests performed in combination. For this purpose, an ANOVA was conducted, which showed an identical ROM in all assessments made (3 AM and 3 PM), which shows that there is no influence between each test. These results are consistent with the fact that both mobilizations (PM and AM) seek to achieve a non-anatomic physiological barrier based on the concepts of manual therapy.[29]

These findings differ from those reported in the literature[13,22,28,36,46,47] by other authors comparing the ROM between types of mobilizations (AM and PM). Rutledge et al.[22] used standardized and predefined PM criteria to reach the cervical movement physiological barrier. Their results show a higher ROM in the AM relative to the PM. In contrast, under the same objective, Castro et al.[47] reported contrasting results.

One explanation for this discrepancy may be associated with the variety of systems and protocols used and the population sample characteristics. In addition, the methodologies are not usually explained in detail, hence the need for studies that analyze these aspects in depth. The fact that psychological, social, and compensatory factors are involved in the WAD etiologic study required the study of the consistency of the measures to assess the degree of patient cooperation in testing.[9] So ensuring an adequate differential diagnosis that reports intentionally or unintentionally exaggerated pain is required. While AM techniques are influenced by these factors, PM techniques are conditioned by the subjectivity of the examiner, which depends heavily on training and experience.[22]

The compromise between evaluator, instrumentation, and patient becomes a set of factors to consider that should not be influenced by the function studied. Consequently, the use of isolated techniques is insufficient to ensure a proper diagnosis.

Reliability: Passive Mobilization

To determine the reliability of both techniques, different reliability coefficients have been calculated, representing various aspects of reliability.[33,41] The intraclass correlation coefficient (ICC) is the preferred retest correlation coefficient.[33] Following the classification proposed by Fleiss[44] for this index, the results show a reliable, good-to-excellent range from 0.621 (RR) to 0.916 (F-E), with the exception for the value of the F movement, which was 0.428 (intra-examiner S1-S3) in Table 4.

Results reported in the literature[23,35,36,46,48,49] show good-to-excellent levels of reliability using different systems and protocols for movements in complete cycles (F-E, R-R, and L-L). Strimpakos et al.[35] discussed PM reliability with an ultrasound system, Zebris CMS20, in different body positions. Their results report ICC values from 0.83 to 0.94 in a sitting position and 0.84 to 0.97 while standing. Morphett et al.[23] obtained similar results using the Cybex 320 electromagnetic system. Assink et al.[36] obtained results of this lower index ranging from 0.73 to 0.77 with the Flock-of-Birds system device. Williams et al.[48] and Stuart Love et al.[49] analyzed the reliability for half-cycle movements. However, these studies work with symptomatic population samples and are not comparable to the results of this study.

The results in the literature for this ICC index are generally high and classified as "excellent reliability." This index considers many factors (sample population, the physiotherapy or tester effects, instrumentation, etc.), which are considered in the reliability.[33,42] However, its isolated application is not sufficient and can lead to misleading results due to the factors on which it depends.[33] Therefore, a complete analysis of reliability requires studying more concepts using statistical methods, which deepen the characteristics and properties of the PM and AM techniques.[4,15,33,41]

Changes in the Mean

The next step in analysis is to calculate the reliability of the possible changes in ROM mean obtained between sessions (intra-examiner S1-S3 and intra-examiner S1-S2). The change in mean is mainly due to a systematic component, which studies differences that are consistently induced between sessions (learning effect, onset of fatigue, or fear of movement), and can be detected through methods such as those developed by Bland–Altman.[41]

Analyzing the results of Table 4, no significant changes were observed in the ROM mean for movements ($p > .020$) except the full range of L-L. We can conclude there are no systematic differences, suggesting the suitability of the designed PM protocol. No pattern has been found among the differences in the obtained measurements between the two sessions and the average of these for the movements studied, thus ensuring that differences do not vary systematically in the measurement interval. A graphic illustration of the results of this is shown in Figure 8.

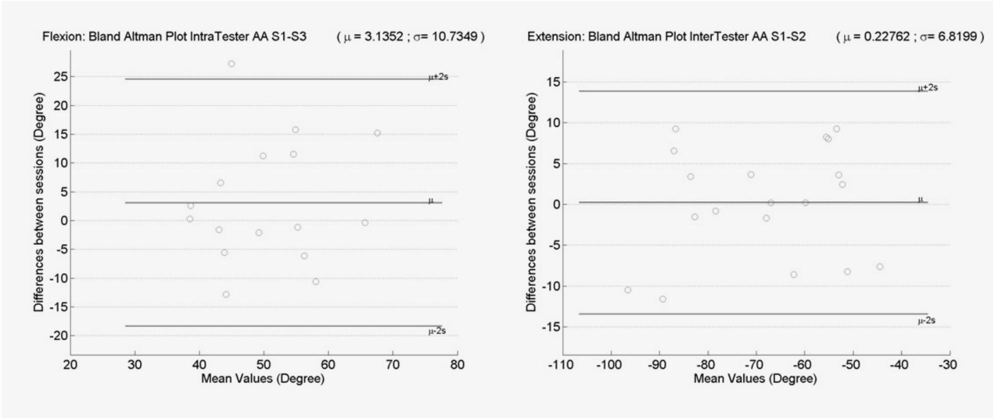


Fig 8. Bland–Altman Graph: Flexion and extension example from passive assessment.

Measurement Variability

To demonstrate the absence of systematic changes, it is necessary to quantify the variability size of the measurements between sessions. Aspects that show real variability given by methodology (technical, protocol, and instrumentation) system factors are called "standard error or typical variation." Accordingly, at this point, less variability is the most sensitive method when discriminating between healthy and pathological subjects to report more reliable measurements.
An index for measuring variability is the SEM. This helps to estimate the measurement dispersion errors when estimating the true measure between the observed measurements in different sessions.[33,43,50]

Reliability of movement with this index has not been studied. Only one author[35] was found to have a well-defined protocol working in an asymptomatic population. The results of the cited study presented an analysis of full ROM (F-E, R-R, and L-L), which ranged from 0.031 to 0.048, which are similar to the results achieved in our work with the range of 0.034 to 0.052 (Table 4).

The use of MoCap systems provided objective measurements of the real capacity of cervical mobility, as stated by Rutledge et al.,[22] and Alqhtani et al.[17] for PM and Inokuchi et al.,[18] Song et al.[19] for AM. This provided an advantage over evaluations based exclusively on the subjectivity of the evaluator and his/her experience in the field. However, the added value provided by these technologies may be impaired or even not useful in the clinical setting, if the possible sources of error considered in this study are not studied and controlled in their practical application.

Interpretation of Results

To facilitate the interpretation of results, the MDC index was obtained. This index is calculated on the variability of the measurements represented by the SEM multiplied by 1.96 and the square root of 2 to include 95% of the observations of differences between measurements.[33] Consequently, the MDC is similar to the "limits of agreement" proposed by Bland–Altman (R [95%]), converging on the same conclusions about the variability of the measurements.

Indices (R [95%] and MDC) are values used by the evaluators to judge significant changes in clinical results as a limit point. Consequently, the lower the value, the more sensitive the technique is required to be to detect these changes. No direct references were found in the literature for the ratio R (95%) relative to PM. Analyzing results of MDC from the mean, we obtained values ranging from 0.093 to 0.144 in full ranges and between 0.117 (LR) and 0.323 (F) for half cycles (Table 4).

These values are in range with those obtained by Strimpakos et al.,[35] who analyzed the full ROM and was the only author who used this index with a well-described protocol and asymptomatic subjects. The reliability results confirm the validity of the PM technique used for assessment of cervical ROM in the movements studied. Only the F movement showed low reliability values for all indices analyzed.

The F movement presents biomechanical and anatomical difficulties. The natural curvature (kyphosis) hinders the general PM, for C0 to C7, and the tendency for the movement focuses on the media segments. If one wants to involve all anatomical structures, making a maximum amplitude mobilization, the upper and lower cervical spine should be technically and structurally distinguished, as their biomechanics are different.[51]

Comparison of Reliability of Passive and Active Mobilization

Considering the overall global reliability values from the ROM means, the F-E movements and L-L in AM show better results regarding PM, which is similar to the case of R-R (Tables 4 and 5). The R-R movement comes to 70% of the C2-C3 cervical segments; therefore, reaching the physiological barrier is easier for the examiner by focusing mainly on this segment to achieve most of the range of amplitude movement.[51]

In the case of F-E and L-L movements, although they involve the same number of cervical segments, they require controlling greater distances of the cervical spine by the examiner because the ROM to achieve the desired barrier is more homogeneously distributed between the vertebrae, which means greater difficulty of execution. In this regard, as claimed by some authors,[51,52] there must be a compromise between the technical and biomechanical motion characteristics when applying the PM technique. Moreover, a correct design of instrumentation is also important to provide a good compromise between technology and instrumentation.[53]

Clinical Appreciations and Future Research

It is suggested that the use of a combined AM and PM test system in one session could provide benefits for clinical practice. This methodology should further evaluate the combination of functional and structural action, that could provide greater information to diagnose WAD.

Given the need for a compromise between the PM evaluation technique and cervical spine biomechanical characteristics, mainly for F movement, greater control is available/achievable if the examiner structurally distinguishes between the upper and lower cervical spine, respecting the structural and biomechanical differences of both sections. This would facilitate the evaluation by obtaining more relevant information and greater reliability.

LIMITATIONS

Although the population sample was sufficient, the accuracy of the study would have been improved with a larger sample. Likewise, the influence of the participation of several examiners to perform PM has not been studied because the purpose was to analyze the rest of the factors that intervene in the conducted tests. However, the characterized and controlled factors of this experimentation and the results of this study that show that the reliability is adequate for the AM and PM, applied in isolation or combination, opens the possibility of studying the influence of combining two operators in future studies.

Future studies should investigate the properties of PM group techniques in symptomatic subjects with diverse levels of disability since the sensitivity and specificity of the techniques studied here could be potentiated in clinical application.

Recently, the use of techniques based on virtual reality ~~(VR)~~ show more functional assessments over conventional methods (AM), obtaining increased ROM for both asymptomatic[24] and symptomatic[26] subjects. Unlike conventional functional techniques (AM), virtual reality replicates the natural cervical sensory stimuli causing spontaneous movements[12,26,54].. Consequently, for a correct evaluation of the functional action, it would be desirable to integrate distraction techniques and emotional modulation ~~(VR)~~, which could positively complement traditional techniques.

Applying MoCap advanced systems, as used in this study, allows the evaluator to obtain cervical movement data dynamically in the three anatomical planes. This allows the evaluator to obtain measures of coupled or secondary movements, knowing the angular velocities or repositioning errors of the patient when they try to return to the starting point.[54] This information may be relevant and meaningful to detect possible cervical spine disorders. Therefore, it would be of interest to delve into the possibilities of this information for diagnostic purposes.

CONCLUSIONS

This study presents a protocol to evaluate cervical ROM using an optical MoCap system. It is based on the combination of AM and PM, through asymptomatic subjects with different operators in several sessions. It demonstrated high reliability individually and combined; and no differences were detected between PM and AM ROMs. Evaluator, instrumentation, and patient become a set of factors that could influence the results; consequently, to ensure a proper diagnosis they should be used in combined protocols. These protocols could be used to evaluate the functional and structural capacity of patients and they would provide benefits to inform about clinical outcomes in practice.

FUNDING SOURCES AND CONFLICTS OF INTEREST

This project was co-funded by the Government of Aragon, the European Regional Development Fund and the University of Zaragoza. It also received co-funding from PREMAP Seguridad y Salud, S.L.U. The I3A - University Institute of Research of Engineering of Aragon, University of Zaragoza, Zaragoza, Spain provided materials in their Biomechanics Laboratory for this study. No conflicts of interest were reported for this study.

ACKNOWLEDGMENTS

The authors wish to thank Dr. J. Manuel Arredondo and Dra. Dolores Ramón from Instituto de Medicina Legal de Aragón for their contributions when defining the study protocols and to Dra Mar Pardos from PREMAP Seguridad y Salud, S.L.U for her support.

BIBLIOGRAPHY

1. Ioppolo F, Rizzo R. Epidemiology of whiplash-associated disorders. In: *Whiplash injuries.* Springer; 2014:13-16.

2. Holm L. Epidemiology of whiplash associated disorders. *Whiplash: evidence base for clinical practice*. 2011;1:1-8.

3. Chiriac M. Creación y validación de un modelo para el estudio de las lesiones cervicales por accidentes de tráfico. . 2009.

4. Williams MA, McCarthy CJ, Chorti A, Cooke MW, Gates S. A systematic review of reliability and validity studies of methods for measuring active and passive cervical range of motion. *J Manipulative Physiol Ther*. 2010;33(2):138-155.

5. Sterling M, Jull G, Carlsson Y, Crommert L. Are cervical physical outcome measures influenced by the presence of symptomatology? *Physiotherapy Research International*. 2002;7(3):113-121.

6. Croft, Arthur C. et al. Comparing 2 whiplash grading systems to predict clinical outcomes. - *Journal of Chiropractic Medicine*. 2016(- 2):- 81.

7. Hartling L, Brison RJ, Ardern C, Pickett W. Prognostic value of the quebec classification of whiplash-associated disorders. *Spine*. 2001;26(1):36-41.

8. Spitzer WO. Scientific monograph of the quebec task force on whiplash-associated disorders: Redefining'whiplash'and its management. *Spine*. 1995;20:1-73.

9. Ramírez C, Ordi G, Fernández S, MI CM. Detección de exageración de síntomas en esguince cervical: Pacientes clínicos versus sujetos análogos. *Trauma*. 2014;25(1):4-10.

10. Arthur C. Croft, PhD, DC, MSc, MPH,a,⍰ Alireza Bagherian, DC,b Patrick K. Mickelsen, DC,c and Stephen Wagner, DCd. - Comparing 2 whiplash grading systems to predict clinical outcomes. - *Journal of Chiropractic Medicine*. (- 2):- 81.

11. Chiu TT, Sing KL. Evaluation of cervical range of motion and isometric neck muscle strength: Reliability and validity. *Clin Rehabil*. 2002;16(8):851-858.

12. Bahat HS, Weiss PLT, Sprecher E, Krasovsky A, Laufer Y. Do neck kinematics correlate with pain intensity, neck disability or with fear of motion? *Man Ther*. 2014;19(3):252-258.

13. Jordan K. Assessment of published reliability studies for cervical spine range-of-motion measurement tools. *J Manipulative Physiol Ther*. 2000;23(3):180-195.

14. Reid SA, Callister R, Katekar MG, Rivett DA. Effects of cervical spine manual therapy on range of motion, head repositioning, and balance in participants with cervicogenic dizziness: A randomized controlled trial. *Arch Phys Med Rehabil*. 2014;95(9):1603-1612.

15. de Koning CH, van den Heuvel, Sylvia P, Staal JB, Smits-Engelsman BC, Hendriks EJ. Clinimetric evaluation of active range of motion measures in patients with non-specific neck pain: A systematic review. *European Spine Journal*. 2008;17(7):905-921.

16. Dall'Alba PT, Sterling MM, Treleaven JM, Edwards SL, Jull GA. Cervical range of motion discriminates between asymptomatic persons and those with whiplash. *Spine*. 2001;26(19):2090-2094.

17. Alqhtani RS, Jones MD, Theobald PS, Williams JM. Reliability of an accelerometer-based system for quantifying multiregional spinal range of motion. *J Manipulative Physiol Ther*. 2015;38(4):275-281.

18. Haruhi Inokuchi Michio. Neck range of motion measurements using a new three-dimensional motion analysis system: Validity and repeatability. [- 12]. 2015(- 1432-0932 (Electronic); - 0940-6719 (Linking)).

19. Young Seop Song, MD,1,2 Kyung Yong Yang, MS,3 Kibum Youn, MS,3 Chiyul Yoon, PhD,2 Jiwoon Yeom, MD,1 Hyeoncheol Hwang, MD,1,2 Jehee Lee, PhD,3 and Keewon Kim, MD, MScorresponding author1,2. Validation of attitude and heading reference system and microsoft kinect for continuous measurement of cervical range of motion compared to the optical motion capture system. - *Annals of Rehabilitation Medicine*. 2016(- 4):- 568.

20. Leanne N. Cupona,* and Warren T. Jahna. Current standards for measuring spinal range of motion for impairment. - *Journal of Chiropractic Medicine*. 2003(- 1):- 8.

21. BOLETÍN OFICIAL DEL ESTADO. JEFATURA DEL ESTADO. Spain. Ley 35/2015, de 22 de septiembre, de reforma del sistema para la valoración de los daños y perjuicios causados a las personas en accidentes de circulación. . 2015;Núm. 228.(Sec. I. Pág. 84473).

22. Rutledge B, Bush TR, Vorro J, et al. Differences in human cervical spine kinematics for active and passive motions of symptomatic and asymptomatic subject groups. *Journal of applied biomechanics*. 2013;29(5):543-553.

23. Morphett AL, Crawford CM, Lee D. The use of electromagnetic tracking technology for measurement of passive cervical range of motion: A pilot study. *J Manipulative Physiol Ther*. 2003;26(3):152-159.

24. Sarig-Bahat H, Weiss PL, Laufer Y. Cervical motion assessment using virtual reality. *Spine (Phila Pa 1976)*. 2009;34(10):1018-1024.

25. Olson SL, O'Connor DP, Birmingham G, Broman P, Herrera L. Tender point sensitivity, range of motion, and perceived disability in subjects with neck pain. *Journal of Orthopaedic & Sports Physical Therapy*. 2000;30(1):13-20.

26. Sarig Bahat H, Weiss PL, Laufer Y. The effect of neck pain on cervical kinematics, as assessed in a virtual environment. *Arch Phys Med Rehabil*. 2010;91(12):1884-1890.

27. Wong A, Nansel DD. Comparisons between active vs. passive end-range assessments in subjects exhibiting cervical range of motion asymmetries. *J Manipulative Physiol Ther*. 1992;15(3):159-163.

28. Dvorak J, Antinnes JA, Panjabi M, Loustalot D, Bonomo M. Age and gender related normal motion of the cervical spine. *Spine*. 1992;17(10S):S393-S398.

29. Kaltenborn FM. Movilización manual de las articulaciones. *Evaluación articular y tratamiento básico.Extremidades.7º ed.España: OMT España*. 2011.

30. Abbott JH, Flynn TW, Fritz JM, Hing WA, Reid D, Whitman JM. Manual physical assessment of spinal segmental motion: Intent and validity. *Man Ther*. 2009;14(1):36-44.

31. Piva SR, Erhard RE, Childs JD, Browder DA. Inter-tester reliability of passive intervertebral and active movements of the cervical spine. *Man Ther*. 2006;11(4):321-330.

32. Hoppenbrouwers M, Eckhardt MM, Verkerk K, Verhagen A. Reproducibility of the measurement of active and passive cervical range of motion. *J Manipulative Physiol Ther*. 2006;29(5):363-367.

33. Lexell JE, Downham DY. How to assess the reliability of measurements in rehabilitation. *Am J Phys Med Rehabil*. 2005;84(9):719-723.

34. Baker R. Gait analysis methods in rehabilitation. *Journal of neuroengineering and rehabilitation*. 2006;3(1):4.

35. Strimpakos N, Sakellari V, Gioftsos G, et al. Cervical spine ROM measurements: Optimizing the testing protocol by using a 3D ultrasound-based motion analysis system. *Cephalalgia*. 2005;25(12):1133-1145.

36. Assink N, Bergman GJ, Knoester B, Winters JC, Dijkstra PU, Postema K. Interobserver reliability of neck-mobility measurement by means of the flock-of-birds electromagnetic tracking system. *J Manipulative Physiol Ther*. 2005;28(6):408-413.

37. Marin J, Blanco T, Marin JJ. Research lines to improve access to health instrumentation design. *Procedia Computer Science*. 2017;113(Supplement C):641-646. doi: https://doi.org/10.1016/j.procs.2017.08.323.

38. J. MZ, M. BP, R. RM, M. MG. Move Human sensors: Sistema portátil de captura de movimiento humano basado en sensores inerciales, para el análisis de lesiones musculoesqueléticas y utilizable en entornos reales. . 2008.

39. WorldViz - Virtual Reality Software [Internet].Available from:. http://www.worldviz.com/.

40. Seffinger MA, Najm WI, Mishra SI, et al. Reliability of spinal palpation for diagnosis of back and neck pain: A systematic review of the literature. *Spine*. 2004;29(19):E413-E425.

41. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Int J Nurs Stud*. 2010;47(8):931-936.

42. Shrout PE, Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. *Psychol Bull*. 1979;86(2):420.

43. Stratford PW, Goldsmith CH. Use of the standard error as a reliability index of interest: An applied example using elbow flexor strength data. *Phys Ther*. 1997;77(7):745-750.

44. - Enderlein G. - Fleiss, J. L.: The design and analysis of clinical experiments. wiley, new york – chichester – brislane – toronto – singapore 1986, 432 S., £38.35. - *Biometrical Journal*. (- 3):-304.

45. Gonzalo Utrilla, J. Javier Marin, M. Belen Sanchez-Valverde, Virginia Gomez , J. Manuel Auria, Maria J.Bone, A .Cristina Royo. Cervical mobility testing in flexion-extension and protraction-retraction to evaluate whiplash syndrome through motion capture. . Universidad de Zaragoza. Zaragoza (España). 2017.

46. Lantz CA, Chen J, Buch D. Clinical validity and stability of active and passive cervical range of motion with regard to total and unilateral uniplanar motion. *Spine*. 1999;24(11):1082-1089.

47. Williams MA, Williamson E, Gates S, Cooke MW. Reproducibility of the cervical range of motion (CROM) device for individuals with sub-acute whiplash associated disorders. *European spine journal*. 2012;21(5):872-878.

48. Love S, Gringmuth R, Kazemi M, Cornacchia P, Schmolke M. Interexaminer and intraexaminer reliability of cervical passive range of motion using the CROM and cybex 320 EDI. *The Journal of the Canadian Chiropractic Association*. 1998;42(4):222.

49. Brown J. Standard error vs. standard error of measurement. *Shiken JALT Testing Evaluation SIG Newslett*. 1999;3:20-25.

50. Cueco RT. *La columna cervical: Evaluación clínica y aproximaciones terapéuticas: Principios anatómicos y funcionales, exploración clínica y técnicas de tratamiento.* Vol 1. Ed. Médica Panamericana; 2008.

51. White A, Panjabi M. Biomechanical considerations in surgical management of the spine. *Clinical Biomechanics of the Spine*. 1990:528-568.

52. Adhia DB, Bussey MD, Ribeiro DC, Tumilty S, Milosavljevic S. Validity and reliability of palpation-digitization for non-invasive kinematic measurement–A systematic review. *Man Ther*. 2013;18(1):26-34.

53. Swait G, Rushton AB, Miall RC, Newell D. Evaluation of cervical proprioceptive function: Optimizing protocols and comparison between tests in normal subjects. *Spine (Phila Pa 1976)*. 2007;32(24):E692-701.