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a cluster analysis approach

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Profiling migraine patients according to clinical and psychophysical characteristics: A cluster analysis approach

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Abbreviation

AROM: active range of motion; BMI: body mass index; CHAID: Chi-squared Automatic Interaction Detection; CMD: cervical musculoskeletal dysfunctions; CM: chronic migraine; EM: episodic migraine; HADS-A: Hospital Anxiety and Depression Scale Anxiety; HADS-D: Hospital Anxiety and Depression Scale Depression; HDI-E: headache-related disability emotional component HDI-P: headache-related disability physical component; ICHD: International Classification of Headache Disorders; IPS: Increased pain sensitivity; N: numbers; NPI: no psychophysical impairment; PPT: pressure pain threshold; SD= standard deviation;

Abstract

Aim

This study aims to profile migraine patients according clinical and psychophysical characteristics.

Method

In this observational study, two cohorts of migraine patients(episodic/chronic) were included. Cohort-1: ictal/perictal phase; Cohort-2: interictal phase.

The following variables were assessed: headache frequency; disability; cervical active range of motion(AROM) in flexion, extension, right/left lateral flexion, right/left rotation; pressure-pain threshold(PPT) over: temporalis, two cervical areas(C1/C4 vertebral segments), and two distal pain-free areas(hand/leg). Cluster analysis was performed using the K-means algorithm. Differences across clusters were investigated.

Results

Cohort-1: 100 patients were included and two clusters were identified. Cluster-1.1(19%), Cluster-1.2(81%). Cluster 1.1 had a higher percentage of men($p=0.037$) and higher disability($p=0.003$) compared to Clusters 1.2. Cluster 1.2 had reduced AROM in flexion, extension, and left/right lateral flexion($p<0.037$), and lower PPT value in all areas($p<0.001$) compared to Cluster 1.1.

Cohort-2: 98 patients were included and three clusters were identified. Cluster-2.1(18%), Cluster-2.2(45%), and Cluster-2.3(37%). Cluster-2.1 had a higher percentage of men compared to clusters-2.2 and 2.3($p=0.009$). Cluster-2.3 had higher headache frequency, and disability compared to Cluster-2.2($p<0.006$), and higher disability compared to Cluster-2.1($p=0.010$). Cluster-2.3 had reduced AROM in all directions compared to Clusters-2.1 and 2.2($p<0.029$). Clusters-2.2 and 2.3 have lower PPT values in all areas compared to Cluster-1.1($p<0.001$).

Conclusion

In the Ictal/perictal phase, two clusters were identified according to clinical and psychophysical characteristics, with one group showing no psychophysical impairment and one with increased pain-sensitivity and cervical musculoskeletal-dysfunctions.

In the interictal phase, three clusters could be identified, with one group showing no psychophysical impairment, one increased pain-sensitivity, and one increased pain sensitivity and cervical musculoskeletal-dysfunctions.

INTRODUCTION

Migraine is a common neurovascular brain disorder affecting around 15% of the population and causing a significant social and economic impact[1]. In recent years, the development of a new class of drugs specifically targeting the CGRP peptide or receptors has shown promising results[2]. This new therapy is inefficient in 24%-66% of migraine patients[3–7] and new tools for identifying responders and non-responders are important for developing personalized treatment regimes[8]. Personalized treatment or precision medicine requires the use of various biomarkers to identify distinct subtypes within the same medical condition and has been used in migraine[9,10]. Among these biomarkers, clinical and psychophysical characteristics have been applied to different pain conditions to identify distinct subtypes[11–13]. In neuropathic pain[14,15] and knee osteoarthritis[16], profiling patients according to psychophysical characteristics has been shown to predict the response to a given type of treatment, leading to the development of a mechanism-based therapy, and enhancing treatment outcomes. Migraine subgroups are currently identified according to one clinical biomarker, headache frequency[17]. However, when it comes to stratifying by migraine severity or identifying responders to non-responders to a given treatment, other classifiers are needed than solely headache frequency[7,18–20]. Among different classifiers, psychophysical characteristics should be considered, due to their relevance in migraine pathophysiology[21–24] and their ability to predict treatment response[19,20,25–28].

The aim of this study was to investigate if different episodic and chronic migraine subgroups could be identified according to clinical and psychophysical characteristics.

Two cohorts were included. Cohort 1 was assessed in the 1) ictal or preictal phases, and Cohort 2 was assessed in the 2) interictal phase. Ten different simple clinical and psychophysical bed-side tools were applied to identify subgroups of episodic and chronic migraine patients.

METHOD

Design

This multicenter, cross-sectional, observational study was based on two cohorts of migraine patients. The study was conducted at the Headache Center of Parma and Genova (Italy) and approved by the Ligurian (244/2018) and "Area Vasta Emilia-Nord" (18305/2019) regional ethic committee. All subjects signed an informed consent form and were assessed between April 2019 and February 2022.

Population

Patients on waiting lists to receive their first visit to the Headache Center were invited to participate in this study. Men and women aged between 18 and 65 with episodic (EM) or chronic (CM) migraine with or without aura for at least 3 months were included and divided into two distinct cohorts according to the migraine phase in which the psychophysical examination was performed. In Cohort 1 EM and CM in the ictal/perictal phase were included. EM patients were considered in the ictal phase if they have headache during the visit and in the perictal phase if they have a headache within the 24 hours before or after the visit[29,30]. CM patients were considered in the ictal phase if they have any type of headache (headache attack with tension-type or migraine characteristics) during the visit. Patients were excluded if they had: any other primary/secondary headache; less than 1 headache attack in four weeks; changes of headache characteristics, or onset of a "new" headache after COVID-19 infection/vaccination; any other neurologic, psychiatric, rheumatologic, or systemic pathology with medical diagnosis; history of head/neck trauma in the previous year; received cervical/head surgery; received manual therapy in the cervical spine, cervical anesthetic block, or botulin injection in the last 6 months; changed the prophylactic treatment in the last 3 months; were unable to speak and understand Italian.

In Cohort 2 EM and CM in the interictal phase were included. EM patients were considered in the interictal phase if they were headache-free during the visit and did not have a headache within the 24 hours before or after the visit[29,30]. CM patients were considered in the interictal phase if they were headache-free during the visit. The exclusion criteria were the same used for ictal/perictal patients (Cohort 1) with the exception that patients that used acute pharmacologic treatment in the 24 hours before the assessment were excluded.

Procedure

The first screening was made by telephone interview where patients were excluded if they presented any signs of red flags[31] or any exclusion criteria. Then, a physical examination was performed in which one physiotherapist for each recruitment center (S.D., M.C.), blinded to the subject's diagnosis, performed the assessment (psychophysical examination, questionnaire, and explanation of how to fulfill a diary for the following four weeks) and recorded the interval between the assessment and the last headache attack. Four weeks following the first evaluation, patients were visited by a neurologist who performed a diagnosis of headache according to the ICHD-3[17]. CM and EM patients with or without aura were included and divided into two cohorts, according to the migraine phase in which the first evaluation was performed, that had undergone two separate analyses:

- Cohort 1: EM and CM in the ictal/perictal phase were included.
- Cohort 2: EM and CM in the interictal phase were included.

Assessment

General and clinical characteristics were assessed for each patient. The Hospital Anxiety and Depression Scale(HADS) was used to assess the impact of anxiety (HADS-A) and depressive (HADS-D) symptoms. A higher score indicates a higher level of anxiety and depression (HADS: 0-21; HADS-D: 0-21) (Table 1).

Headache frequency and disability

A daily updated 4-weeks diary was used to record the headache frequency. The Headache disability index (HDI) questionnaire was used to assess two components of headache-related disability: the emotional headache-related disability (HDI-E 0-52); the physical headache-related disability (HDI-P 0-48). The higher the score, the higher the disability [32]

Psychophysical assessment

- *Active range of motion (AROM):* cervical AROM (extension, flexion, left/right lateral flexion, left /right rotation) was recorded in degrees of movement with the cervical range of motion (CROM) device[22].
- *Pressure pain threshold (PPT):* PPT to hand-held algometry (Somedic AB, sweden), probe area 1cm^2, 30 kpa/s force increase)[21] was assessed over the: trigeminal area (anterior columns temporalis), cervical spine (C1 and C4 vertebral segments, sum left and right articular pillars); distal pain-free areas (second metacarpophalangeal joint and tibialis anterior of the dominant hand). PPT assessment was performed from distal pain-free areas first, then the cervical area, and finally the trigeminal area (symptomatic side in patients with unilateral migraine; dominant side in patients with side/shift or bilateral migraine and controls). The lower the PPT results, the higher the pain sensitivity.

Details of the assessment were fully explained elsewhere[21,22,32].

Statistical analysis

According to variable type and distribution, data were presented as mean (standard deviation), median (interquartile range), or numbers (percentage). For subjects included between April 2019

and August 2021, this was a secondary analysis of these data and previous results were reported elsewhere[21,22,32].

Group allocation

Before the computation of cluster analysis, all variables were standardized, and standard scores (z-scores) were used for subsequent cluster analyses[33]. Clustering was performed using the K-means algorithm, without making any a priori assumptions about the expected number of clusters. K-means cluster analysis was performed for K ranking 2 to 8 and the number of clusters was chosen according to internal and external measurements of accuracy[13].

- Internal measure of accuracy: firstly, all K solutions with at least 1 cluster with a negative mean silhouette width or over 10% of the cases with a negative minimum value of silhouette width were excluded. Then, the number of K was chosen according to the mean silhouette width (the higher the mean silhouette width and the better the K solution) and the number of cases with a negative minimum value of silhouette width (the lower the number of cases with a negative minimum value of silhouette width, the better the K solution)[13].
- External measure of accuracy: each K solution obtained with the K-means algorithm was compared with a K solution obtained with the hierarchical agglomerative clustering method (Ward method). The comparison was performed with the adjusted rand index (ARI)[13]. The ARI assesses the degree of agreement between two partitions of the same set of objects and its values lie on a scale from 0 to 1. The higher the value, the higher the agreement between the two clustering techniques[34].

Difference between clusters

If only two clusters were present, differences across clusters in general and clinical characteristics were investigated with the t-test, Mann-Whitney test, or the Chi-square test (2 x 2 contingency table) according to variable type and distribution. If more than two clusters were present, differences in general characters and frequency were investigated with the ANOVA, Kruskal Wallis test, or Chi-

square test, according to variable type and distribution. The Bonferroni, the Mann-Whitney test, or the Chi-square test (2 x 2 contingency table) were used to run posthoc analyses respectively for ANOVA, Kruskal Wallis test, and Chi-square test respectively, using a Bonferroni corrected p-value. Differences in psychophysical characteristics across clusters were investigated by transforming non-normal distributed variables to fulfill the normality assumption (the normality of the data was assessed with the Shapiro-Wilk test). To avoid the possibility that between-group differences were due to differences in general characteristics, an ANCOVA was performed, including age, gender, body mass index, use of acute treatment 24 hours before the evaluation, hours from the last headache attack, and hours to the next headache attack as covariates in Cohort 1 and age, gender, and body mass index, hours from the last headache attack, and hours to the next headache attack as covariates in Cohort 2. A Bonferroni-adjusted posthoc analysis was performed to make single groups comparisons when more than two clusters were compared. The threshold accepted for statistical significance of the across-groups differences tests was $p<0.05$, and tests of statistical significance were two-tailed.

Clinical predictors of group allocation

Chi-squared Automatic Interaction Detection (CHAID) decision tree analysis was used to identify clinical predictors to be included in each Cluster[20]. To maximize the clinical utility of the assessment procedure, we did not include headache frequency in the model, as to fulfill the diary and assess headache frequency four weeks were needed. The included independent variables were: age, gender, body mass index, HDI-E, HDI-P, AROM (sum all directions), PPT over the trigeminal area, C1, C4, hand, and tibialis anterior. Adjustments were as follows: 1) maximum of 3 levels; 2) minimum number of cases for parent nodes, n=11; child nodes, n=3. The Likelihood ratio was chosen as a statistic and the significance level for splitting nodes was set to $\alpha= 0.05$ applying classical Bonferroni correction to avoid α error accumulation.

Tenfold cross-validation was applied to assess the generalizability of the classification model and the model error for the cross-validated analysis was compared to the model error from the main analysis.

Subjects with any missing data in psychophysical or clinical variables used to compute the cluster analysis were excluded from all analyses. One patient did not fulfill the HADS questionnaire and was excluded from the analysis assessing between cluster's differences in HADS. Statistical analyses were performed using the SPSS (version 24) and SAS (version 9.4) software.

RESULTS

A total of 723 migraine patients were initially screened. In Cohort 1 a total of 100 were included and assessed in the ictal/perictal phase and in Cohort 2 a total of 98 were included and assessed in the interictal phase (Figure 1).

Cohort 1: Ictal/perictal migraine patients

Group allocation

No K solutions presented any cluster with a negative mean silhouette width. According to the number of cases with a negative minimum value of silhouette width, the mean silhouette width, and the comparison with the hierarchical agglomerative clustering method (Ward method) using the ARI, 2 clusters solution were chosen in Cohort 1 (number and % of cases with a negative minimum value of silhouette width= 0(0%); mean silhouette width= 0.28; ARI: 0.54) (Table 2a).

Differences across clusters

General and clinical characteristics

The sample distribution differed across clusters, with 19(19%) patients being included in Cluster 1.1 (no psychophysical impairment = NpI), and 81(81%) patients being included in Cluster 1.2 (Increased pain sensitivity and cervical musculoskeletal dysfunctions =IPS-CMD).

Patients in Cluster 1.1 (NpI) had a higher percentage of males (6(32%) vs 9(11%), p=0.037), worse HDI-E (median (25th, 75th): 12.0(8.0-18.0) vs 24.0(12.0-32.0), p=0.003), HADS-A (mean(SD): 8.3 (3.8) vs 5.4(4.6), p=0.005), and HADS-D (median(25^{th}-75^{th}): 5(3-8) vs 2(1-5), p=0.005) questionnaires compared to Cluster 1.2 (IPS-CMD). No other differences across clusters were observed (Table 1a)

Psychophysical characteristics

Patients in Cluster 1.2 (IPS-CMD) had reduced AROM in flexion (51.1°(12.1°), p<0.001), extension (61.1°(16.2°), p=0.037), right lateral flexion (33.9°(9.7°), p<0.001,), and left lateral flexion (40.4°(11.6°), p=0.019) compared to Cluster 1.1 (NpI) (flexion: 61.4°(9.0°); extension 68.6°(17.9°); right lateral flexion (44.10°(14.5°); left lateral flexion (46.2°(13.7°)).

Patients in Cluster 1.1 (NpI) had higher PPT (lower sensitization) over temporalis (229.3(56.9) kPa), C-1 (756.0(232.9) kPa), C-4 (596.8(190.8), second MCP of the hand (410.1(132.6) kPa), tibialis (587.6(206.0) kPa) compared to Cluster 1.2 (IPS-CMD) (temporalis (112.4(41.2) kPa, C-1 (327.7(122.6) kPa, C-4 (296.3(190.2) kPa, second MCP of the hand (242.2(86.8) kPa, tibialis (300.4(125.0) kPa, p<0.001) (Figure 2a, Table 3a). No other differences were observed between Cluster 1.1 and 1.2 (Figure 2a, Table 3a).

Decision tree

Migraine patients with: 1) PPT C1 > 537 kPa; or 2) PPT C1 > 406 kPa and <= 537 kPa and male gender, were correctly included in the Cluster 1.1 (NpI) with a sensitivity of 95%, a specificity of 94%, a positive predictive value of 78%, and a negative predictive value of 99% (Figure 3a).

Migraine patients with: 1) PPT C1 <= 406 kPa; or 2) PPT C1 > 406 kPa and <= 537 kPa and female gender, were correctly included in Cluster 1.2 (IPS-CMD) with a sensitivity of 94%, a specificity of 95%, a positive predictive value of 99%, and a negative predictive value of 78% (Figure 3a).

The overall accuracy of the model was 94%, and the result of the cross-validation analysis revealed that the average model error for the 10 iterations (mean= 0.07; Standard error=0.03) was similar to that from the main analysis (mean= 0.06; Standard error=0.02)

Cohort 2: Interictal migraine patients

Group allocation

No K solutions presented any cluster with a negative mean silhouette width. According to the number of cases with a negative minimum value of silhouette width, the mean silhouette width, and the comparison with the hierarchical agglomerative clustering method (Ward method) using the ARI, 3 clusters solution were chosen in Cohort 2 (number and % of cases with a negative minimum value of silhouette width= 0(0%); mean silhouette width= 0.18; ARI: 0.36) (Table 2b).

Differences across clusters

General and clinical characteristics

The sample distribution differed across clusters, with 18(18%) patients being included in Cluster 2.1 (NpI), 44(45%) patients being included in Cluster 2.2 (increased pain sensitivity= IPS), and 36(37%) patients being included in Cluster 2.3 (IPS-CMD).

Patients in Cluster 2.3 (IPS-CMD) are older compared to Cluster 2.2 (IPS) (mean years (SD)= 43.4(9.2) vs 31.2 (10.2), p<0.001) with no difference compared to Cluster 2.1 (NpI) (36.9 (13.2), p=0.155). The percentage of male was higher in Cluster 2.1 (NpI) compared to Cluster 2.2 (IPS) (number (%) = 10(56%) vs 8(18%), p=0.009), and to Cluster 2.3 (IPS-CMD) (6(17%), p=0.009). No differences across clusters were observed in body mass index (Table 2b).

Patients in Cluster 2.3 (IPS-CMD) had more duration of the disease (median years (25^{th}, 75^{th}): 19.5(7.0-30.5) vs 8.0(4.3-15.8), p=0.006), higher headache frequency (median day/4 weeks (25^{th}, 75^{th}): 8.5(4.0-11.0) vs 5.0(3.3-7.0) p=0.006), worse HDI-P (mean (SD): 27.4(10.3) vs 20.3 (8.8), p=0.003), HDI-E (mean (SD): 23.6(9.2) vs 15.9 (9.6), p<0.001), and HADS-D (median(25^{th}-75^{th}):

7(5-10) vs 6(3-9), p=0.027) questionnaires compared to Cluster 2.2 (IPS), and higher HDI-E (mean (SD): 23.6(9.2) vs 15.6 (8.0), p=0.010) compared to Cluster 2.1 (NpI). No other differences across clusters were observed (Table 2b)

Psychophysical characteristics

Patients in Cluster 2.3 (IPS-CMD) had reduced AROM in flexion (46.7°(9.5°)), extension (56.2°(10.3°)), right lateral flexion (30.9°(7.1°)), left lateral flexion (31.6°(7.3°)), right rotation (58.2°(9.8°)), and left rotation (59.4°(8.9°)) compared to:

- Cluster 2.2 (IPS) (flexion: 60.8°(1.1°), p=0.002; extension 71.3°(11.0°), p<0.001; right lateral flexion (42.8°(9.5°), p<0.001, left lateral flexion (46.1°(9.7°), p<0.001; right rotation (71.4°(7.3°), p<0.001; left rotation (72.1°(8.9°), p<0.001
- Cluster 2.1 (NpI) (flexion: 59.5°(11.0°), p=0.011; extension 65.8°(12.0°), p=0.029; right lateral flexion (41.2°(5.8°), p<0.001; left lateral flexion (43.2°(7.8°), p<0.001; right rotation (69.0°(8.0°), p<0.001; left rotation (68.4°(9.4°), p=0.007).

No differences were observed between Cluster 2.1 and 2.2 (Figure 2b, Table 3b).

Patients in Cluster 2.1 (NpI) had higher PPT (lower sensitization) over temporalis (230.8(48.9) kPa), C-1 (660.0(158.5) kPa), C-4 (587.0(171.2) kPa); Second MCP of the hand (435.6(122.1) kPa), tibialis (638.9(142.4) kPa) compared to:

- Cluster 2.2 (IPS) (temporalis (128.1(42.0) kPa, C-1 (370.6(115.5) kPa, C-4 (338.9(113.4) kPa, second MCP of the hand (245.9(79.9) kPa, tibialis (358.5(157.8) kPa, p<0.001) (p<0.001),
- Cluster 2.3 (IPS-CMD) (temporalis (150.6(59.7) kPa, C-1 (397.0(146.4) kPa, C-4 (383.5(138.5) kPa, second MCP of the hand (240.5(78.3) kPa, tibialis (357.9(141.1) kPa) (p<0.001).

No differences were observed between Cluster 2.2 and 2.3 (Figure 2b, Table 3b).

Decision tree

Migraine patients with: 1) total AROM > 303° and <= 313° and male gender, or 2) total AROM > 313° and PPT temporalis > 174.3 kPa and PPT C1 > 572.33 kPa, were correctly included in Cluster 2.1 (NpI) with a sensitivity of 77%, a specificity of 99%, a positive predictive value of 93%, and a negative predictive value of 95% (Figure 3b).

Migraine patients with: 1) total AROM > 313° and PPT temporalis <= 174.3 kPa and HDI-P <= 36.0; or 2) total AROM > 313° and PPT temporalis > 174.3 kPa and PPT C1 <= 572.33 kPa, were correctly included in the Cluster 2.2 (IPS) with a sensitivity of 91 %, a specificity of 95%, a positive predictive value of 91%, and a negative predictive value of 95% (Figure 3b).

Migraine patients with: 1) total AROM <= 303°; or 2) total AROM > 303° and <= 313° and Female gender; or 3) total AROM > 313° and PPT temporalis <= 174.3 kPa and HDI-P > 36.0, were correctly included in the Cluster 2.3 (IPS) with a sensitivity of 94 %, a specificity of 92%, a positive predictive value of 87%, and a negative predictive value of 98% (Figure 3b).

The overall accuracy of the model was 90%, and the result of the cross-validation analysis revealed that the average model error for the 10 iterations (mean= 0.19; Standard error=0.04) was similar to that from the main analysis (mean= 0.10; Standard error=0.03).

DISCUSSION

This is to the authors' knowledge the first study applying cluster analyzes to separate distinct subgroups of migraine patients according to their clinical and psychophysical characteristics. Two distinct clusters were found for migraine patients assessed in proximity or during the headache attack, and three distinct clusters were identified for migraine patients when assessed interictally. This information may pave the way for developing targeted prophylactic and symptomatic treatment of episodic and chronic migraine.

Migraine subgroups during the ictal/perictal phase

When migraine patients were assessed in proximity or during the headache attack, two clusters were present: one group (Cluster 1.1, NpI, 19% of the population) showed no psychophysical impairment, and another group (Cluster 1.2, IPS-CMD) showed widespread increased pain sensitivity and reduction in cervical active range of motion. Hypoalgesia over the upper cervical spine and male gender were the main predictors of being included in NpI.

The migraine attack is characterized by increased activation and sensitization of the trigeminocervical complex and higher cortical/subcortical areas that begin before the headache attack, reach their peak during the attack, and gradually restore to baseline level afterward[35,36]. This perictal and ictal enhanced sensitization has been indirectly detected through increased trigeminal and widespread pain sensitivity[30,37,38]. This study's results, in line with others, suggested that this increase in sensitization mechanisms did not occur in 20% of migraine patients[39].

As an increase in neural activation and pain sensitivity could predict treatment response[19,20,25,26,28], these two subgroups may respond differently to the same treatment approach. Preventive treatment whose principal site of action was outside the brain seems to be more effective in migraine patients without increased pain sensitivity and with no neck hyperalgesia[19,26,27]. On the other hand, increased pain sensitivity seems to predict the response to prophylactic treatments with a modulatory effect on the central nervous system, differently according to the headache frequency[20,25]. Thus, future randomized control trials should profile migraine patients according to clinical and psychophysical characteristics to assess if distinct subgroups will have a different treatment response. This would help clinicians identify non-responder to a particular therapy and include them in a more personalized treatment approach.

In line with other conditions, where an increase in pain and sensitization could affect mechanical behavior[40,41], the migraine attack could act as a trigger that impairs active cervical mobility[22]. Our study's results suggest that the same subgroup of patients with increased ictal/perictal pain sensitivity also had ictal/perictal cervical motor impairment, confirming the interaction between

these two psychophysical characteristics[40,42]. As no other study ever assessed the ability of a migraine attack to impair active cervical mobility and the correlation between ictal/perictal cervical motor impairment and increased pain sensitivity, these results should be replicated.

Interestingly, no differences in headache frequency or disease duration were present between migraine patients with or without psychophysical impairments, suggesting that ictal/perictal increased pain sensitivity and impaired cervical active range of motion are not related to the progression of the disease. As the two groups differed in gender, emotional-related disability, and psychological burden, higher psychophysical impairments seem more related to individual characteristics. Different mechanisms in migraine initiation could also occur between these two subgroups. In 80% of migraine patients, the induction of signaling molecules could lead to a migraine attack and increasing in sensitization mechanisms[43–45]. On the contrary, this enhanced sensitization, with concomitant headache induction, did not occur in at least 20% of migraine patients[44,46–48], possibly supporting the presence of migraine subgroups with different mechanisms underlying headache initiation. Thus, future studies should assess if migraine patients with or without psychophysical impairments will respond differently to the experimentally induced migraine attack. This would help researchers to understand if different mechanisms in migraine initiation occur between these two subgroups.

Migraine subgroups during the interictal phase

When migraine patients were assessed interictally, three clusters were present: one group (Cluster 2.1 NpI, 18% of the population) showed no psychophysical impairment, another group (Cluster 2.2, IPS, 45%) showed widespread increased pain sensitivity, and a third group (Cluster 2.3, IPS-CMD, 37%) showed widespread increased pain sensitivity and a reduction in cervical active range of motion.

Cluster 2.1 (NpI, interictal) shares similar prevalence, general and clinical characteristics, and psychophysical values then Cluster 1.1 (NpI, ictal/perictal). Thus, these two subgroups could

represent the same cluster assessed in different phases of the migraine cycle. Interestingly, no reduction in pain sensitivity or cervical active range of motion occurs in Cluster 1.1 (NpI ictal/perictal) compared to Cluster 2.1 (NpI, interictal). This could indicate that in this subgroup of patients, the transitory increase in pain sensitivity that characterized the ictal and preictal phases[30,37,38] did not occur[39]. These results could explain why different studies assessing changes in pain sensitivity across the migraine cycle found heterogenous results[37,49]. However, the hypothesis that these two subgroups could represent the same cluster assessed in different phases of the migraine cycle has to be confirmed/refused by longitudinal studies assessing the same patient across different migraine phases.

Differently from the ictal phase, three clusters were observed interictally. During the ictal/perictal phase, a migraine subgroup with widespread increased pain sensitivity and cervical musculoskeletal dysfunctions accounted for 80% of the sample. On the other hand, when migraine patients were assessed interictally, two subgroups accounted for this 80% of the sample. Both clusters (Cluster 2.2, IPS; Cluster 2.3, IPS-CMD) showed signs of widespread increased pain sensitivity, but only one (Cluster 2.3, IPS-CMD) also had cervical musculoskeletal dysfunctions. We hypothesize that these two subgroups (Cluster 2.2, IPS; Cluster 2.3, IPS-CMD) are a clinical continuum, representing the two extremities in terms of disease progression of a single group (Cluster 1.2 IPS-CMD, ictal/preictal). Cluster 2.2 (IPS) represent the lowers extremity, including younger patients with lower headache frequency, disease duration, and disabilities and no cervical musculoskeletal dysfunctions. On the opposite extremities of this clinical continuum, the progression of the disease in terms of headache frequency, disease duration, and disabilities will be coupled with interictal cervical musculoskeletal dysfunctions (Cluster 2.3 IPS-CMD). The mechanism underlying this progression could be sought in the relationship between increased pain and sensitization and impairment in functionality and mechanical behavior[40,41]. The migraine attack could act as a trigger that cause a transitory reduction in the cervical range of motion[17,22]. In a subgroup of migraine patients, the acute pain and the sensitization occurring during the ictal/perictal phases will lead to

short-term impairment in active cervical mobility (Cluster 1.2 IPS-CMD), which will restore to baseline lever afterward (Cluster 2.2 IPS). However, the perpetuation of this trigger over time, in terms of higher headache frequency, and longer disease duration, could lead to long-lasting changes in cervical mechanical behaviors[41] and interictal impairment in active cervical mobility (Group2.3 IPS-CMD). The fact that these two subgroups (Cluster 2.2, IPS; Cluster 2.3, IPS-CMD assessed interictally) are a clinical continuum and represent the two extremities of a single group (Cluster 1.2 assessed preictally/ictally) have to be confirmed/refused by longitudinal studies that: 1) assessed the same migraine subjects across different phases of the migraine cycle; 2) assessed interictal migraine patient over the years monitoring headache progression.

Clinical application and future research directions

This paper's results suggested that a particular subgroup of migraine patients could have worse clinical and psychophysical characteristics. Thus, clinicians should add to the standard evaluation performed on migraine patients a physical examination aimed to identify impairments of the active cervical range of motion and increased pain sensitivity. Those patients with a reduced cervical range of motion and increased pain sensitivity can be treated with a multidisciplinary approach, including treatment aimed to increase cervical range of motion and reduced pain sensitivity (i.e., physiotherapy intervention[50–52]). Even if, in this study, specific tools were used to assess the active cervical range of motion and pain sensitivity (CROM device and algometer), these aspects can be assessed in a clinical setting without tools. Cervical range of motion reduction could be visually detected (or with the use of a standard rehabilitation goniometer or a smartphone application[53]), and pain sensitivity could be assessed using tenderness to manual palpation. As a lower pain threshold in temporalis muscles and C0-1 vertebral segment were the best predictors of widespread increased pain sensitivity, tenderness should be assessed in these areas.

However, the limit of an assessment without objective measurements is that there are no reference values to identify patients with or without psychophysical impairments. Thus, future observational

trials aimed to replicate the presence of the different migraine subgroups should also include tools that could be easily used in a clinical setting. These bedside tools should be used as predictors to belong to one subgroup or another[12]. Moreover, the fact that patients with worse psychophysical impairments may need to be included in a multidisciplinary approach is a hypothesis that need to be tested. Future clinical trials using a multidisciplinary approach should profile migraine patients according to psychophysical characteristics and identify possible responders/non-responders, and specific therapeutic needs for each group.

Limitations

The population was recruited from specialized headache centers, and over two third of the patients were excluded for age, concomitant pathologies, and concomitant diagnosis of other headache types. Thus, the external validity of these results should be interpreted with caution.

Even if the development of sensitization during the migraine attack seems to be dependent on the time distance from the attack[38,54] to reach the minimal sample needed to perform a cluster analysis[12], ictal and preictal patients were pooled together in Cohort 1. Moreover, the use of medication in the 24 hours before the assessment was allowed in Cohort 1.

However, as the two clusters observed in Cohort 1 did not differ in headache phase, hours from the last/next headache attack, and use of medication in the 24 hours before the assessment and between-group differences were calculated controlling for those variables, the presence of sensitization seems to be related to the different subgroup more than to the headache phase or the medication's use.

CONCLUSION

When assessing migraine patients in the ictal/perictal phase, two distinct migraine clusters were identified according to clinical and psychophysical characteristics, with one group showing no psychophysical impairment (Cluster 1.1, No psychophysical Impairment) and one showing

increased pain sensitivity and cervical musculoskeletal impairments, as well as higher disability (Cluster 1.2, Increased Pain Sensitivity - Cervical Musculoskeletal Impairments).

When assessing migraine patients in the interictal phase, three distinct migraine clusters could be identified, with one group showing no psychophysical impairment, (Cluster 2.1, No psychophysical Impairment), one increased pain sensitivity (Cluster 2.2, Increased Pain Sensitivity), and one increased pain sensitivity and cervical musculoskeletal impairments, as well as higher headache duration, frequency, and disability (Cluster 2.3, Increased Pain Sensitivity - Cervical Musculoskeletal Impairments).

This study highlights specific subgroups of migraine patients in different phases of the migraine cycle providing new insight to pave the way for developing personalized and target prophylactic and symptomatic migraine treatments.

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Table 1: difference in general, clinical and psychophysical characteristics across clusters in M

a: Ictal/peritcal M (Cohort 1)

	Cluster 1.1 (19)	Cluster 1.2 (81)	Between group difference
General characteristics			
Age, median years (25th, 75th) ‡‡	44.0 (25.0-51.0)	40.0(28.0-48.5)	U=706.5, p=0.584
BMI, median (25th, 75th) ‡‡	23.4 (20.9-26.7)	22.9(20.5-26.1)	U=731.0, p=0.918
Gender, N (%) ##			
Female	13(68%)	72 (89%)	Chi^2=4.3, p=0.037*
Male	6 (32%)	9 (11%)	
Acute treatment 24 hours before the evaluation, N (%) ##			
No	13(68%)	65(80%)	Chi^2=1.2, p=0.278
Yes	6(32%)	16(20%)	
Clinical characteristics			
Duration of the disease, median years (25th, 75th)	16.0(11.0-34.0)	14.0(3.0-28.0)	U=607.0, p=0.155
Headache phase, N (%)			
Ictal	8(42%)	46(57%)	Chi^2=5.7, p=0.059
Preictal	4(21%)	24(30%)	
Postictal	7(37%)	11(14%)	
Hours from the last headache attack, median (25th, 75th) ‡‡	5.0(0-0-23.0)	0.0(0-0-21.5)	U=722.0, p=0.654
Hours to the next headache attack, median (25th, 75th) ‡‡	5.0(0-0-44.0)	0.0(0-0-11.0)	U=592.0, p=0.090
Frequency, median day/4 weeks, median (25th, 75th) ‡‡	6.0(5.0-10.0)	9.0(5.5-12.5)	U=613.0, p=0.170
HDI-P, mean (SD)§§	21.8 (10.4)	25.8 (10.5)	t=-1.4, p=0.138
HDI-E, median (25th, 75th) ‡‡	12.0(8.0-18.0)	24.0(12.0-32.0)	U=433.0, p=0.003*
HADS-A, mean (SD)§§[1]	5.4(4.6)	8.3 (3.8)	t=-2.9, p=0.005*
HADS-D, median (25th-75th)‡‡[1]	2(1-5)	5(3-8)	U=446.5, p=0.005*

b: Interictal M (Cohort 2)

	Cluster 2.1 (18)	Cluster 2.2 (44)	Cluster 2.3 (36)	Between group difference	Cluster 1 vs 2	Cluster 1 vs 3	Cluster 2 vs 3
General characteristics							
Age, mean years (SD)§	36.9(13.2)	31.2 (10.0)	43.4(9.2)	F=13.8, p<0.001*	p=0.155	p=0.097	p<0.001*
BMI, median (25th, 75th) ‡	21.5 (19.5-26.2)	21.7 (19.6-23.3)	22.9 (20.6-25.9)	KW, Chi^2=4.4, p=0.109	p=1.000	p=0.723	p=0.114
Gender, N (%) #							
Female	8(44%)	36(82%)	30(83%)	Chi^2=11.6, p=0.003*	p=0.009*	p=0.009*	p=1.000
Male	10(56%)	8(18%)	6(17%)				
Clinical characteristics							
Duration of the disease, median years (25th, 75th) ‡	14.0 (7.8-29.5)	8.0 (4.3-15.8)	19.5 (7.0-30.5)	KW Chi^2=10.1, p=0.006*	p=0.216	p=1.000	p=0.006*
Hours from the last headache attack, median (25th, 75th) ‡	168.0(96.8 -360.0)	144.0(63.0 -240.0)	96.0(48.3-168.0)	KW Chi^2=4.6 p=0.104	p=1.000	p=0.144	p=0.285
Hours to the next headache attack, median (25th, 75th) ‡	88.5(41.3-168.0)	120.0(27.5 -236.5)	49.0(25.0-96.0)	KW Chi^2=5.5 p=0.065	p=1.000	p=0.270	p=0.090
Frequency, median day/4 weeks (25th, 75th) ‡	5.5 (4.0-10.5)	5.0 (3.3-7.0)	8.5 (4.0-11.0)	KW Chi^2=8.8 p=0.012*	p=1.000	p=1.000	p=0.006*
HDI-P, mean (SD)§	21.8(8.6)	20.3(8.8)	27.4(10.3)	F=6.1, p=0.003*	p=1.000	p=0.855	p=0.003*
HDI-E, mean (SD)§	15.6(8.0)	15.9(9.6)	23.6(9.2)	F=8.2, p=0.001*	p=1.000	p=0.010*	p<0.001*
HADS-A, median (25th-75th)‡[2]	5.5(2-9.5)	6(3-9)	7(5-10)	Chi^2=2.8, p=0.245	p=1.000	p=0.630	p=0.387
HADS-D, median (25th-75th)‡[2]	2(0.8-5.3)	2.5(1-5)	5(2-7)	Chi^2=7.8,p=0.020*	p=1.000	p=0.126	p=0.027*

BMI: body mass index, HADS-A: Hospital Anxiety and Depression Scale Anxiety; HADS-D: Hospital Anxiety and Depression Scale Depression; HDI-E: headache-related disability emotional component HDI-P: headache-related disability physical component; N: number; SD: standard deviation;
1: due to missing data 80 patients were included in Cluster 1.2 (IPS-CMI)
2: due to missing data 35 patients were included in Cluster 2.3
§ ANOVA with Bonferroni post-hoc analyses; ‡ Kruskal Wallis with the Mann-Whitney test post-hoc (Bonferroni corrected p-value);
# Chi-square test with 2 x 2 contingency table for post-hoc analyses (Bonferroni corrected p-value)
§§ T-test; ‡ Mann-Whitney test; ## Chi-square test;
* Significant at $p<0.05$; Normality was assessed with Shapiro-Wilk test

Table 2: Determination of the number of clusters in M
a. Ictal/perictal M (Cohort 1)

Clusters ictal/perictal M	Number of clusters with negative mean silhouette width, N (%)	Number of patients with negative silhouettes, N (%)	Mean silhouette width all clusters	Comparison with hierarchical (ARI)
K 2	**0(0%)**	**0(0%)**	**0.28**	**0.54**
K 3	0(0%)	5(5%)	0.17	0.42
K 4	0(0%)	6(6%)	0.17	0.44
K 5	0(0%)	4(4%)	0.18	0.41
K 6	0(0%)	3(3%)	0.21	0.50
K 7	0(0%)	2(2%)	0.20	0.29
K 8	0(0%)	1(1%)	0.22	0.40

b. Interictal M (Cohort 2)

Clusters interictal M	Number of clusters with negative mean silhouette width, N (%)	Number of patients with negative silhouettes, N (%)	Mean silhouette width all clusters	Comparison with hierarchical (ARI)
K 2	0(0%)	2(2%)	0.17	0.23
K 3	**0(0%)**	**0(0%)**	**0.18**	**0.36**
K 4	0(0%)	0(0%)	0.17	0.32
K 5	0(0%)	0(0%)	0.18	0.31
K 6	0(0%)	1(1%)	0.18	0.23
K 7	0(0%)	0(0%)	0.19	0.32
K 8	0(0%)	1(1%)	0.19	0.28

ARI: adjusted rand index; N: number; K= number of clusters using K means cluster analysis; Bold text indicates the number of cluster solutions that were chosen.

Table 3a: ANCOVA including: psychophysical characteristics as the dependent variable; age, gender, body mass index, use of acute treatment 24 hours before the evaluation, hours from the last headache attack, and hours to the next headache attack as a covariate; group as the independent variable (Ictal/pericta M, Cohort 1)

	Cluster 1.1(24)	**Cluster 1.2 (75)**	**Between group difference**
AROM			
Flexion, mean ° (SD)	61.4(9.0)	51.1(12.1)	t=-3.6, p<0.001*
Extension, mean ° (SD)	68.6(17.9)	61.1(16.2)	t=-2.1, p=0.037*
Right lateral flexion, mean ° (SD)	44.10(14.5)	33.9(9.7)	t=-3.9, p<0.001*
Left lateral flexion, mean ° (SD)	46.2(13.7)	40.4(11.6)	t=-2.4, p=0.019*
Right rotation, mean ° (SD)‡	63.5(14.6)	61.8(12.1)	t=-1.2, p=0.222
Left rotation, mean ° (SD)‡	66.0(14.2)	62.4(11.9)	t=-1.7, p=0.085
PPT			
Temporalis, mean kPa (SD)	229.3(56.9)	112.4(41.2)	t=-10.0, p<0.001*
C-1, mean kPa (SD)‡	756.0(232.9)	327.7(122.6)	t=-7.9, p<0.001*
C-4, mean kPa (SD)	596.8(190.8)	296.3(190.2)	t=-9.6, p<0.001*
Second MCP, mean kPa (SD)‡	410.1(132.6)	242.2(86.8)	t=-5.5, p<0.001*
Tibialis, mean kPa (SD)‡	587.6(206.0)	300.4(125.0)	t=-7.1, p<0.001*

Table 3b: ANCOVA including: psychophysical characteristics as the dependent variable; age, gender, body mass index, hours from the last headache attack, and hours to the next headache attack as a covariate; group as the independent variable (Interictal M, Cohort 2)

	Cluster 2.1 (18)	**Cluster 2.2 (44)**	**Cluster 2.3 (36)**	**Between group difference**	**Cluster 2.1 vs 2.2**	**Cluster 2.1 vs 2.3**	**Cluster 2.2 vs 2.3**
AROM							
Flexion, mean ° (SD)	59.5(11.0)	60.8(1.1)	46.7(9.5)	F=7.2, p=0.001*	p=1.000	p=0.011*	p=0.002*
Extension, mean ° (SD)	65.8(12.0)	71.3(11.0)	56.2(10.3)	F=9.1, p<0.001*	p=1.000	p=0.029*	p<0.001*
Right lateral flexion, mean ° (SD)‡	41.2(5.8)	42.8(9.5)	30.9(7.1)	F=16.1, p<0.001*	p=1.000	p<0.001*	p<0.001*
Left lateral flexion, mean ° (SD)	43.2(7.8)	46.1(9.7)	31.6(7.3)	F=14.5 p<0.001*	p=1.000	p<0.001*	p<0.001*
Right rotation, mean ° (SD)	69.0(8.0)	71.4(7.3)	58.2(9.8)	F=16.5, p<0.001*	p=1.000	p=0.001*	p<0.001*
Left rotation, mean ° (SD)	68.4(9.4)	72.1(8.9)	59.4(8.9)	F=13.4 p<0.001*	p=1.000	p=0.007*	p<0.001*
PPT							
Temporalis, mean kPa (SD)	230.8(48.9)	128.1(42.0)	150.6(59.7)	F=19.1 p<0.001*	p<0.001*	p<0.001*	p=0.904
C-1, mean kPa (SD)	660.0(158.5)	370.6(115.5)	397.0(146.4)	F=24.9 p<0.001*	p<0.001*	p<0.001*	p=1.000
C-4, mean kPa (SD)	587.0(171.2)	338.9(113.4)	383.5(138.5)	F=15.8, p<0.001*	p<0.001*	p<0.001*	p=1.000
Second MCP, mean kPa (SD)	435.6(122.1)	245.9(79.9)	240.5(78.3)	F=34.6 p<0.001*	p<0.001*	p<0.001*	p=1.000
Tibialis, mean kPa (SD)‡	638.9(142.4)	358.5(157.8)	357.9(141.1)	F=13.2, p<0.001*	p<0.001*	p<0.001*	p=1.000

AROM: active range of movement; MCP: metacarpophalangeal; PPT: pressure pain threshold; SD: standard deviation; kPa: kilo pascal;
‡ data were log-transformed to fulfill normality assumption. Normality was assessed with the Shapiro-Wilk test
* significant at p<0.05 (in table 3b Bonferroni correction was performed in the postdoc analysis)

Patients recruited and screened for red flags or inclusion criteria: N=723

Excluded (not fulfill inclusion criteria): N=357
- Age= 148
- Not understanding Italian = 30
- Other pathologies= 113
  - Other neurology disease=21
  - Fibromyalgia= 16
  - Other rheumatic disease= 14
  - Anxiety/depression= 36
  - Other psychiatric disorder= 13
  - Oncology disease= 10
  - Cervical / head surgery o WAD in the last year= 3
- Anesthetic cervical block, botulin injection, or manual therapy in the cervical spine in the last 6 months = 66

Patients recruited for psychophysical assessment, questionnaires compilation, and explanation how to fulfill the diary N= 366

DIARY

4 WEEKS

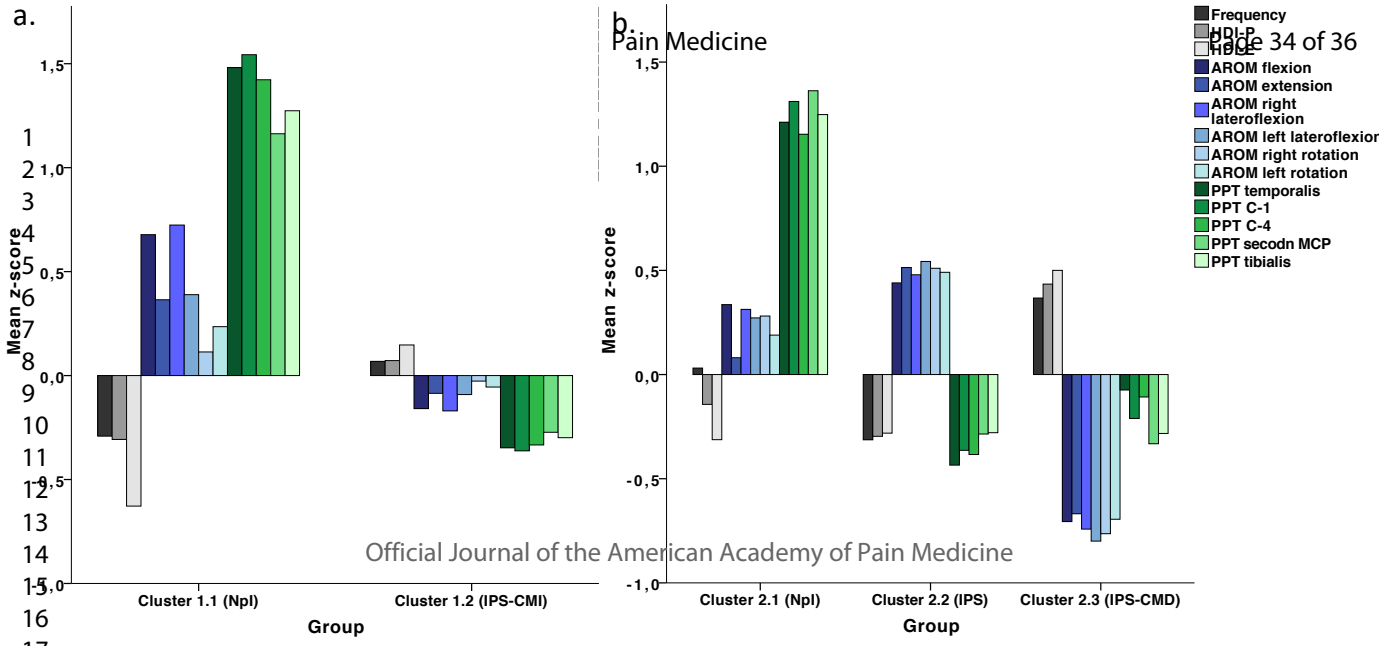
Drop Out: N= 40

Evaluation by a Neurologist (ICHD 3) N=326

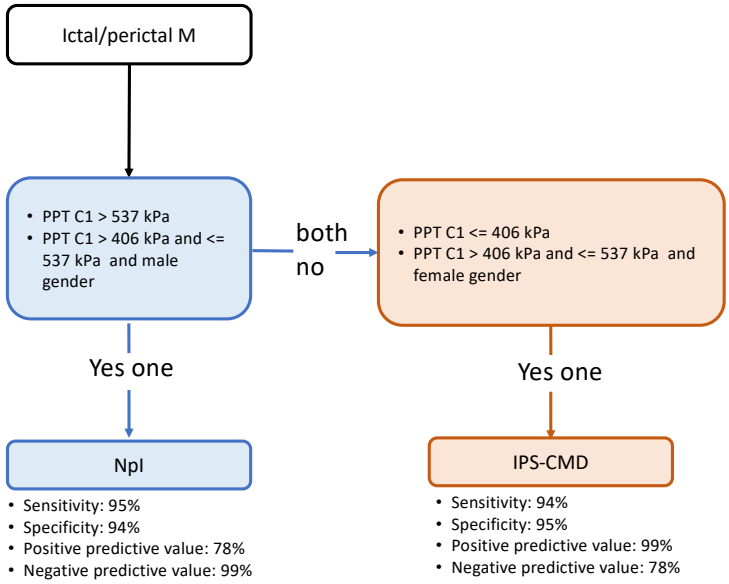
Excluded (not fulfill inclusion criteria): N= 128
- Medication overuse headache= 15
- Tension type headache= 19
- Mixt form headache= 19
- Another headache type= 25
- Headache with < 1 attack for month: 17
- Change prophylactic treatment < 3 months= 15
- Change of headache characteristics or new headache after COVID-19 vaccination or infection= 5
- Acute pharmacologic treatment in the last 24 hours= 6
- Did not fulfill HDI questionnaire= 7

Cohort 1 (M ictal/pericta) N=100

Cohort 2 (M interictal) N=98



a.


Ictal/pericta M
• PPT C1 > 537 kPa
• PPT C1 > 406 kPa and <= 537 kPa and male gender
both
no
• PPT C1 <= 406 kPa
• PPT C1 > 406 kPa and <= 537 kPa and female gender
Yes one
Yes one
NpI
IPS-CMD
• Sensitivity: 95%
• Specificity: 94%
• Positive predictive value: 78%
• Negative predictive value: 99%
• Sensitivity: 94%
• Specificity: 95%
• Positive predictive value: 99%
• Negative predictive value: 78%

b.

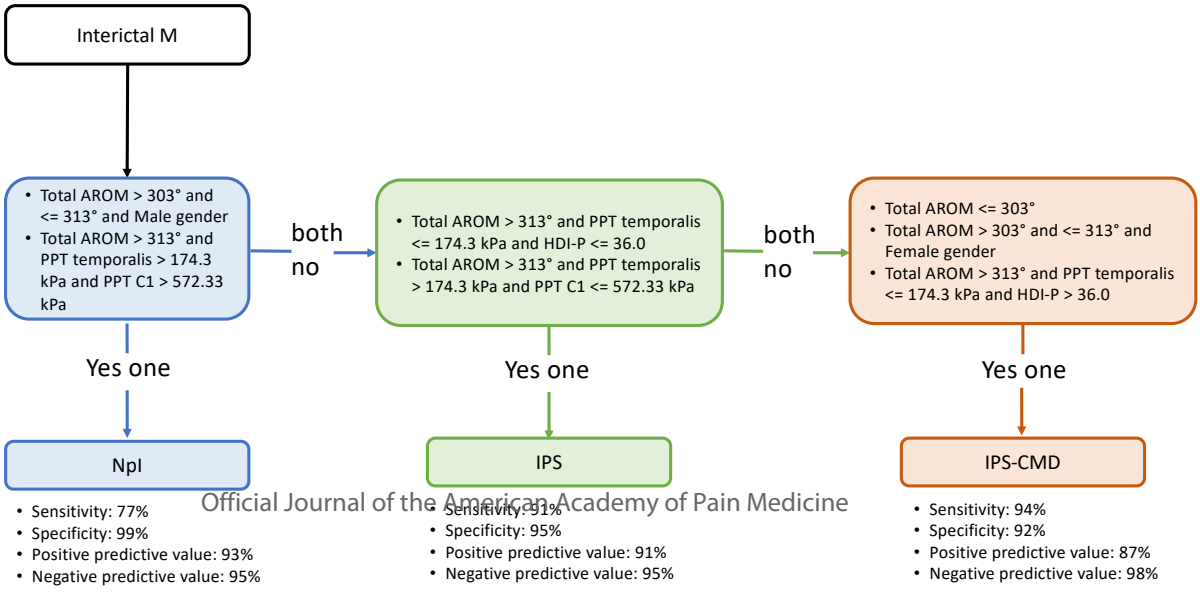

Interictal M
• Total AROM > 303° and <= 313° and Male gender
• Total AROM > 313° and PPT temporalis > 174.3 kPa and PPT C1 > 572.33 kPa
both
no
• Total AROM > 313° and PPT temporalis <= 174.3 kPa and HDI-P <= 36.0
• Total AROM > 313° and PPT temporalis > 174.3 kPa and PPT C1 <= 572.33 kPa
both
no
• Total AROM <= 303°
• Total AROM > 303° and <= 313° and Female gender
• Total AROM > 313° and PPT temporalis <= 174.3 kPa and HDI-P > 36.0
Yes one
Yes one
Yes one
NpI
IPS
IPS-CMD
• Sensitivity: 77%
• Specificity: 99%
• Positive predictive value: 93%
• Negative predictive value: 95%
• Sensitivity: 91%
• Specificity: 95%
• Positive predictive value: 91%
• Negative predictive value: 95%
• Sensitivity: 94%
• Specificity: 92%
• Positive predictive value: 87%
• Negative predictive value: 98%

Figure 1: flow chart

HDI: headache disability index; ICHD: international classification headache disorders; M: migraine; N: number;

Figure 2: Psychophysical characteristics in different clusters of migraine patients assessed in the ictal/perictal phase (a) or in the interictal phase (b)

AROM: active range of motion; HDI-E: headache disability index emotional; HDI-P: headache disability index physical; ICHD: international classification headache disorders; IPS: Increased pain sensitivity; IPS-CMD: Increased pain sensitivity and cervical musculoskeletal dysfunctions; MPC: metacarpophalangeal joint; NpI: no psychophysical impairment; PPT: pressure pain threshold;

Figure 3: predictors to be included in different clusters in migraine patients assessed in the ictal/perictal phase (a) or in the interictal phase (b)

AROM: active range of motion; HDI-P: headache disability index physical; IPS: Increased pain sensitivity; IPS-CMD: Increased pain sensitivity and cervical musculoskeletal dysfunctions; M: migraine; NpI: no psychophysical impairment; PPT: pressure pain threshold; kPa: kilopascal