Serum CGRP, VIP, and PACAP usefulness in migraine: a case–control study in chronic migraine patients in real clinical practice

Sara Pérez-Pereda1 · María Toriello-Suárez1 · Gonzalo Ocejo-Vinyals2 · Sandra Guiral-Foz2 · Jesús Castillo-Obeso3 · Silvia Montes-Gómez3 · Rosa M. Martínez-Nieto3 · Fernando Iglesias4 · Vicente González-Quintanilla1 · Agustin Oterino5

Received: 17 June 2020 / Accepted: 28 August 2020 / Published online: 20 September 2020 © Springer Nature B.V. 2020

Abstract
Calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide-38 (PACAP-38) have relevant roles in migraine pathophysiology. Their serum levels have been proposed as biomarkers for migraine. Our aim was to assess their diagnostic value in real clinical practice in a cohort of chronic migraine (CM), episodic migraine (EM) and healthy controls (HC). We recruited subjects with CM, EM and HC at two medical centers. Blood samples were drawn under fasting conditions in the interictal period, immediately centrifuged and stored at −80 ºC. Serum levels were determined by ELISA. Neuropeptide levels, the effect of preventatives, correlations with clinical and demographic variables, and their diagnostic value were studied among clinical categories. 296 age- and sex-matched subjects (101 CM, 98 EM and 97 HC) were included. All three neuropeptide serum levels were higher in CM [median and IQ for CGRP = 18.023 pg/ml (14.4–24.7); VIP = 121.732 pg/ml (48.72–186.72) and PACAP = 204.931 pg/ml (101.08–597.64)] vs EM [CGRP = 14.659 pg/ml (10.29–17.45); VIP = 75.603 pg/ml (28.72–107.10); and PACAP = 94.992 pg/ml (65.77–128.48)] and vs HC [CGRP = 13.988 pg/ml (10.09–17.87); VIP = 84.685 pg/ml (35.32–99.79), and PACAP = 103.142 pg/ml (59.42–123.97)]. Using multinomial modeling, only VIP (OR 1.011, 95% CI 1.003–1.018, p = 0.005) and PACAP (OR 1.003, 95% CI 1.001–1.005, p = 0.002) increased the risk for CM, but not for EM. CGRP did not predict CM or EM. This model could correctly classify only 62/101 (61.38%) of CM, 75/98 (76.53%) of EM, and 5/97 (4.12%) of HC [globally 147/296 (49.8%)]. Individually, PACAP performed the best for classifying clinical categories [global accuracy 150/296 (50.67%)]. In CM, neuropeptide levels were higher in those OnaBT-treated than in no-treated patients. Although interictal serum CGRP and VIP were higher in CM than both EM or HC, their utility to discriminate migraine categories was low. Contrary to other studies, PACAP serum levels were also higher in CM than in EM or HC and had more discriminative capability to distinguish CM from EM and HC. Further investigation is needed for determination technique standardization.

Keywords CGRP · VIP · PACAP · Migraine · Neuropeptides

Abbreviations
TVS Trigemino-vascular system
CGRP Calcitonin gene-related peptide
VIP Vasoactive intestinal peptide
PACAP Pituitary adenylate cyclase activating polypeptide
CM Chronic migraine
OnaBT Onabotulinum toxin
TTH Tension type headache
HC Healthy controls
EM Episodic migraine
MOH Medication overuse headache

Agustin Oterino
agustin.oterino@gmail.com

1 Neurology Department University Hospital Marqués de Valdecilla and IDIVAL, Cantabria, Spain
2 Immunology Department University Hospital Marqués de Valdecilla and IDIVAL, Cantabria, Spain
3 Primary Care Camargo Costa Health Center, Cantabria, Spain
4 Neurology Department University Hospital of Burgos, Burgos, Spain
5 Neurology Department University Hospital Marqués de Valdecilla and IDIVAL, Avda Valdecilla s/n, 39008 Santander, Cantabria, Spain
Background

Migraine is a complex neurovascular disorder with lacking fully understood pathophysiology. There is enough evidence on the activation of the trigeminal-vascular system (TVS) that leads to the release of vasoactive peptides at their nerve terminals leading to the phenomenon known as neurogenic inflammation, that has been hypothesized to be responsible for migraine throbbing pain [1, 2]. Despite the high frequency of this disorder, affecting 15% of the general population and around 2% in its chronic form [3, 4], diagnosis is based on clinical criteria due to the absence of validated biological markers [5]. Accuracy of migraine diagnosis is troublesome since different types of headaches and comorbidities frequently overlap, especially in chronic headaches [6], something that would be improved with biochemical, radiological and neurophysiological biomarkers.

Searching for useful migraine biomarkers, several authors have investigated plasmatic or serum levels of migraine-involved neuropeptides, such as calcitonin gene-related peptide (CGRP) [2, 7–19], vasoactive intestinal peptide (VIP) [11, 19–21], and pituitary adenylate cyclase activating polypeptide-38 (PACAP-38) [21–26] (Table 1). CGRP, so far the best known migraine neurotransmitter, is a powerful vasodilator of cranial vessels, released by sensory fibers of the trigeminal nerve, that also exerts neuromodulatory effects on central pain circuits [2]. It is known to be released during migraine attacks [2] and this has been reflected in several studies as an elevation of plasma levels in the external jugular vein and/or peripheral blood in migraine patients, both during and outside attacks [9–13]. It was also seen that these CGRP levels decreased as pain was relieved with triptans [13]. CGRP role in migraine pathophysiology is strongly supported by the fact that its infusion in migraineurs has shown to trigger migraine attacks [27], and that new drugs that antagonize CGRP or its receptors could abort crises [28–30]. It might also have some role in the persistence of pain as has been suggested by the finding of significantly elevated plasma levels in chronic migraine (CM) patients outside attacks [18]. Onabotulinum toxin (OnaBT), a most effective treatment for CM, has proved to reduce plasmatic CGRP levels [16], and higher levels predicted a better response to treatment [17, 19]. All these data have led to proposing its usefulness as a biomarker for CM. However, this hypothesis could not be validated by others [7], addressing the need for further studies. VIP and PACAP-38 are two parasympathetic peptides released by the efferent arm of the trigeminal-facial arch that also have vasoactive functions. VIP serum levels have been found higher during migraine attacks in patients who had parasympathetic activation [11], and also higher interictally in episodic and CM [19–21]. Peripheral blood VIP levels also help, though to a lesser degree than CGRP, in predicting response to OnaBT [19]. Nevertheless, VIP intravenous infusion to migraineurs, despite causing marked dilation of cranial arteries, does not induce migraine [31]. In view of these results, it seems that VIP could be a marker of parasympathetic nervous system activation, but VIP induced vasodilation is by itself not enough to cause a migraine attack. PACAP-38 has been found higher in plasma from the cubital vein [23], as well as from the external jugular vein [24], during attacks, with plasmatic decrease in responders after sumatriptan administration [24]. Interictal measurements were lower in migraineurs as compared with healthy controls (HC) [23, 24], and tension type headache (TTH) [25], suggesting a difficult to explain PACAP-38 role in migraine pathogenesis. Administered intravenously, it induces migraine-like attacks in 58% of migraineurs [26]. However, unlike CGRP and VIP, interictal PACAP-38 has shown no changes in peripheral blood in CM [21], suggesting a low utility as a CM biomarker. Nevertheless, PACAP-38 plays an important role in migraine, not only in premonitory phase, but also in activation and propagation of trigemino-vascular reflex and neurogenic inflammation, which could exacerbate migraine phenotype [32]. The finding that over 80% of CM patients recognized at least one cranial autonomic parasympathetic symptom [33] could be the manifestation of a more complex phenotype of CM.

Therefore, there are some data pointing to a possible usefulness of these neuropeptides as biological markers of CM, but more validation studies are needed in order to solve some pending unknowns such as optimal detection method, sensitivity, specificity, reproducibility [34], and behavior of these neuropeptides under real conditions. We hypothesized that CGRP, VIP, or PACAP-38 (from now only PACAP) serum levels, or a combination thereof, could serve as biomarkers for migraine, especially for CM. To do this, we determined and compared CGRP, VIP and PACAP interictal serum levels in a case–control study of CM, EM, and HC, and assessed their possible diagnostic value in daily clinical practice, including patients treated or not with oral preventatives and OnaBT.

Methods

We conducted a case–control study approved by the Ethics Committee of Cantabria, Spain. We prospectively recruited migraine patients between 18 and 65 years old in two different medical centers in the Northern Spain (one tertiary center and one primary care center). In consideration of real clinical practice, we gathered patients treated with OnaBT and other preventatives, and also naïve patients to seek the
| Study | Sample size (N) | Samples | Technique | Main interest finding (data are presented as mean ± standard deviation or median and IQ range) |
|-------|----------------|---------|-----------|------------------------------------------------------------------------------------------------|
| Goadsby et al. Ann Neurol 1990 [11] | 22 migraineurs (10 MA vs 12 MWA) vs: 31 HC for the CF group 12 HC for the EJV group. | Peripheral blood (EJV and CF) | RIA | During migraine attacks, there were higher levels in all studied patients in EJV CGRP (92 ± 11 pmol/l in MA; 86 ± 4 pmol/l in MWA) but not in CF (40 ± 11 pmol/l in MA; 43 ± 6 pmol/l in CF) |
| Tvedskov et al. Ann Neurol 2005 [14] | 21 patients (all MWA) | EJV and CF blood | RIA | No differences between EJV or CF CGRP levels during or outside attacks |
| Sarchielli et al. Cephalalgia 2006 [13] | 20 migraine patients [10 responders vs 10 nonresponders to rizatriptan] | EJV | RIA | Higher CGRP levels in responders (12.2 ± 3.2 pmol/l) vs nonresponders (7.4 ± 2.4 pmol/l) |
| Gallai et al. Cephalalgia 1995 [8] | 75 patients (30 MA and 45 MWA) and 30 HC | Antecubital vein | RIA | CGRP levels in MA and MWA were significantly higher during attacks (MA 51.4 ± 7.8 pmol/l; MWA 50.3 ± 6.7 pmol/l) compared with the interictal period In the pain free period there were no differences with the control group (MA 34.7 ± 7.2 pmol/l; MWA 39.3 ± 8.6 pmol/l; HC 38.2 ± 6.5 pmol/l) |
| Rodríguez-Osorio et al. Neurology 2012 [9] | 47 EM and 23 controls | Brachial artery | ELISA | Patients with migraine had higher levels of CGRP (164.2 ± 139.1 pg/ml) vs controls (37.1 ± 38.5 pg/ml), which further increased during migraine attacks (298 ± 100.3 pg/ml) |
| Fusayasu et al. Pain 2007 [10] | 41 MA, 54 MWA and 52 HC | Cubital vein | Enzyme immunoassay (spectrophotometric method) | CGRP interictal plasma levels were higher in MA (18.8 ± 8 pg/ml) and MWA (19.1 ± 9.4 pg/ml) compared to controls (13.4 ± 4.4 pg/ml) |
| Ashina et al. Pain 2000 [12] | N = 40 (6 MA; 14 MWA; 20 controls) | Cubital vein | RIA | Interictal CGRP levels were significantly higher in migraineurs (75 ± 8 pmol/l) vs controls (49 ± 3 pmol/l) Patients suffering exclusively MWA had significantly higher levels (82 ± 10 pmol/l) CGRP levels were higher in female patients (76 ± 9 pmol/l) than in female controls (48 ± 4 pmol/l) |
## Table 1 (continued)

| Study                                         | Sample size (N) | Samples | Technique | Main interest finding (data are presented as mean ± standard deviation or median and IQ range) |
|-----------------------------------------------|-----------------|---------|-----------|------------------------------------------------------------------------------------------------|
| Cernuda-Morollón et al. *Neurology* 2013 [18] | 103 CM women, 43 EM, 31 HC and 14 episodic cluster headache (ECC) | Antecubital vein | ELISA | Interictal CGRP levels were significantly higher in CM (74.90 pg/ml) as compared with HC (33.74 pg/ml) and EM (46.37 pg/ml) and ECC (45.87 pg/ml). Within the CM group, CGRP were significantly higher in those with MA (81.29 ± 27.11 pg/ml) vs MWA (69.65 ± 28.39 pg/ml) (p = 0.013). A CGRP concentration of 58.22 pg/ml correctly classified 85.7% of CM. |
| Cernuda-Morollón et al. *Pain* 2015 [16]     | 83 CM, before and one month after OnaBT treatment | Antecubital vein | ELISA | CGRP levels after OnaBT treatment (51.89 pg/ml, range 199.4–10.2) were significantly lower as compared with CGRP levels before treatment (74.09 pg/ml, range 241.0–11.4). One month after treatment, CGRP levels did not change in nonresponders (51.89 pg/ml), but significantly decreased in responders (52.48 pg/ml). |
| Cernuda-Morollón et al. *Headache* 2014 [19] | 81 CM, 33 HC | Antecubital vein | ELISA | Interictal CGRP was significantly higher in CM (64.9 ± 31.0) vs HC (33.3 ± 15.7). CGRP were significantly higher in responders to OnaBT (70.4 ± 31.9) than in nonresponders (48.3 ± 21.2). Probability of being a responder to OnaBT was 28 times higher in patients with a CGRP level above the threshold of 72 pg/ml. |
| Domínguez et al. *Headache* 2018 [17]        | 62 CM under OnaBT treatment [47 responders, 15 nonresponders] and 24 HC | Antecubital vein | ELISA | Baseline CGRP levels were significantly higher in responders (133.1 ± 86.6 ng/ml) vs nonresponders (58.2 ± 91.7 ng/ml). CGRP > 50 ng/ml (AUC 0.800; 95% CI 0.652–0.947) was associated with good response. |
| Lee et al. *Head and Pain* 2018 [7]          | 156 migraineurs (106 EM, 50 CM) and 27 HC | Antecubital vein | ELISA | There was no higher interictal levels of CGRP levels in CM (64.9 ± 15.32 pg/ml) compared to EM (67.0 ± 20.70 pg/ml; p > 0.999) and HC (75.7 ± 20.07 pg/ml; p = 0.104). Higher serum CGRP concentration did not predict treatment response in CM. |
Table 1 (continued)

| Study | Sample size (N) | Samples | Technique | Main interest finding (data are presented as mean ± standard deviation or median and IQ range) |
|-------|----------------|---------|-----------|------------------------------------------------------------------------------------------------|
| VIP   |                |         |           |                                                                                                 |
| Goadsby et al. Ann Neurol 1990 [11] | 22 migraineurs (10 MA vs 12 MWA) vs 31 HC for the CF group, and 12 HC for the EJV group | EJV and CF | RIA | During migraine attacks, VIP was not higher in migraineurs in EJV or CF than HC. Only two patients in the MA group, with prominent lacrimation and rhinorhea, had marked elevation in EJV VIP (37 and 36 pmol/l) |
| Cernuda-Morollón et al. Headache 2014 [19] | 81 CM, 33 HC | Antecubital vein | ELISA | CGRP and VIP were significantly higher in CM (173.7 ± 150.7 pg/ml) vs HC (88.5 ± 62.3 pg/ml) |
| Cernuda-Morollón et al. Headache 2016 [21] | 86 CM, 32 HC, 35 EM | Antecubital vein | ELISA | Interictal VIP levels were significantly higher in CM (136.0 ± 111.5 pg/ml) as compared to HC (88.6 ± 61.0 pg/ml) and EM (103.0 ± 56.7 pg/ml) |
| PACAP-38 |                |         |           |                                                                                                 |
| Tuka et al. Cephalalgia 2013 [23] | 87 migraineurs and 40 HC | Cubital vein | RIA | Significant lower PACAP-38 in interictal plasma of migraineurs (24.60 ± 3.59 fmol/ml) as compared with HC (26.54 ± 4.43 fmol/ml) |
| Zagami et al. Ann Clin Transl Neurol, 2014 [24] | 15 (2 MCA and 13 MSA) | External jugular vein | RIA | Reduction in PACAP levels with improvement of migraine headache (from 36 ± 3 pmol/L to 26 ± 3 pmol/L 1 h postsumatriptan) |
| Han et al. Clin Chim Acta, 2015 [25] | 133 migraineurs (95 EM and 38 CM), 106 patients with TTH, 50 HC | Antecubital vein | ELISA | Significantly lower PACAP-38 levels in migraineurs (32.81 ± 12.78, range 10.62–77.16 pg/ml) than TTH (39.96 ± 15.90, range 15.22–74.24 pg/ml) and HC (42.45 ± 13.57, range 18.00–93.28 pg/ml) Lower levels in EM (34.14 ± 13.12 pg/ml) and CM (29.48 ± 11.39 pg/ml) than in HC (p = 0.001) |
effect of preventatives in serum levels of CGRP, VIP, and PACAP.

**Study participants**

All participants signed written informed consent before study entry. Subject selection followed a pre-specified scheme 1:1:1, age- and sex-matched for CM, EM, and HC. At the end of the inclusion period, some subjects were excluded for analysis because they did not fit matching criteria. We classified subjects according to the International Classification of Headache Disorders 3β [35] in CM for at least the last year, EM and HC. HC were recruited among healthy volunteers in the primary care clinic and among family members (8 male patients’ spouses), patients’ friends, and healthy personnel. HC were asked for any type of acute headache or chronic pain. All subjects were instructed for blood drawing under fasting conditions and having fulfilled headache diary. Among patients, blood samples were drawn from those who had not had a migraine attack in the previous 72 h nor they had consumed acute anti-migraine medication (triptans or NSAID). Exclusion criteria included headache not fitting migraine criteria and insufficient headache information from the previous month. Patients having CM or EM could also have previous medication-overuse headache (MOH) or TTH. Psychiatric comorbidities were not an exclusion criterion. We collected patients’ information on demographic variables (age, age at migraine onset, sex and body mass index), clinical variables (migraine with or without aura, MA, migraine without aura, MWA, healthy controls), contraceptive intake and MIDAS and HIT-6 scales.

**Laboratory procedures**

All blood samples were drawn at the Headache Clinic of the University Hospital Marqués de Valdecilla, in order to minimize timing for sample handling. A total of 5 ml blood samples was obtained in tubes without anticoagulant and with separating gel, under fasting conditions. Samples were immediately centrifuged, put on ice, aliquoted, and stored at -80°C until analyses, taking a total time of ten minutes. Peripheral blood samples were taken from the antecubital vein from 296 age- and sex-matched subjects (30 males: 12 CM, 9 EM, and 9 HC; and 266 females: 89 CM, 89 EM and 88 HC). Data from seven subjects were discarded because of age-matching failure. ELISA-based assays were performed using commercially available competitive ELISA kits: BlueGene Biotech Co kit (Pudong New District, Shanghai) for PACAP and Cloud-Clone Corp kit (Wuhan, China) for CGRP and VIP, strictly following manufacturers’ instructions. Kit detection ranges were 12.35–1000 pg/
ml for CGRP, 6.17–500 pg/ml for VIP and 0–1000 pg/ml for PACAP. All ELISA were performed by the same experienced technician who was blinded for the clinical diagnosis. All samples were analyzed continuously in the same laboratory, under the same environmental conditions, and using the same batch for samples from different clinical groups (CM, EM and HC), in order to avoid a possible batch effect that could condition differences in results in the different groups.

Statistical analysis

Data are reported as the median and interquartile range (IQ), or mean and standard deviation (SD). The t-test for independent samples or Kruskal–Wallis (or Mann–Whitney U-test for comparison of two categories) were used depending on the normal distribution for continuous variables by Kolmogorov–Smirnov test. Pearson’s “r” statistic was used for bivariate correlation analysis for normal variables, Spearman’s “rho” test for non-normal variables, Chi2 for categorical variables. All tests were considered significant for two-tailed p < 0.05.

To evaluate the relationship among clinical groups and CGRP, VIP and PACAP, individually, we used a general linear model analysis. A multinomial logistic regression analysis, with clinical groups as polychotomous dependent variable, was performed to evaluate the risk of having CM or EM using serum levels of CGRP, VIP and PACAP as independent variables, adjusted for sex and age. Other confounding variables such as migraine aura, hypertension, diabetes mellitus, alcohol, smoke, hyperlipidemia, or previous ischemic heart disease had been discarded for logistic regression as they had no effect on neuropeptide levels (data not shown). A p value < 0.05 was considered to indicate statistical significance. Odds ratios (ORs) are expressed with their 95% confidence interval (CI). Additionally, we evaluated the power of CGRP, PACAP, and VIP to correctly classify clinical groups, obtaining proportions for the global sample and for specific clinical group. We have taken into consideration the potential collinearity of independent variables analyzing the variation inflation factor and tolerance. In a second model, we used only migraine categories, serving EM as reference.

All tests were performed with the SPSS package (IBM SPSS Statistics for Windows, v22.0. Armonk, NY: IBM Corp.).

Sample size calculations

For this purpose, we used published data for CGRP, PACAP, and VIP means and SD [18, 20, 21]. We calculated sample size necessary to discriminate CM and non-CM groups in the worst scenario. Thus, we obtained a power of 90% for CGRP (n = 90; alpha = 0.01) and PACAP (n = 81; alpha = 0.01) for mean differences of 20%; and a power of 80% (n = 87; alfa = 0.05) for VIP mean differences of 30%.

Results

Clinical results

This is the primary analysis of present data. A total of 296 subjects, 30 male and 266 female, were recruited from March 2016 to May 2019; 101 had CM (mean age 41 ± 10 y), 98 had EM (mean age 41 ± 10 years) and 97 were HC (mean age 41 ± 10 years). A total of 48 patients had migraine with aura (MA) (26 CM and 22 EM); a total of 8 CM patients and 5 EM had exclusively MA. Clinical and demographic characteristics are summarized in Table 2. A total of 81 patients (69 CM, 12 EM) were on preventive treatments; of these, 42 CM were on OnaBT, and 39 on other preventatives (27 CM).

Univariate analyses

For all neuropeptides, we had no missing data. We found that CGRP, VIP and PACAP were significantly higher in CM [median and IQ = 18.023 pg/ml (14.4–24.7), 121.732 pg/ml (48.72–186.72) and 204.931 pg/ml (101.08–597.64), respectively] vs EM [CGRP = 14.659 pg/ml (10.29–17.45); VIP = 75.603 pg/ml (28.722–107.10); and PACAP = 94.992 pg/ml (65.77–128.48)] and vs HC [CGRP = 13.988 pg/ml (10.095–17.87); VIP = 84.685 pg/ml (35.32–99.79), and PACAP = 103.142 pg/ml (59.42–123.97)] (p < 0.001 for all comparisons). There were no differences between EM and HC for all these neuropeptides. We observed that ranges were wider in CM than EM and HC, especially for PACAP (Fig. 1).

Bivariate analyses

We observed weak correlation of all these neuropeptides with some headache outcomes, such as MIDAS (rho = 0.337, p < 0.001 for PACAP; rho = 0.214, p = 0.009 for VIP; and rho = 0.250, p = 0.002 for CGRP), but only PACAP had some correlation with total headache days (rho = 0.266, p = 0.002). Age had a marginal effect on CGRP levels (rho = -0.152, p = 0.011) and none on VIP (rho = - 0.027, p = 0.654) or PACAP levels (rho = 0.094, p = 0.115). We observed that sex could have some effect on CGRP [for male, OR 1.097 (1.01–1.19; p = 0.025] and VIP [for male, OR = 0.993 (0.98–1.0; p = 0.037)] serum levels. CGRP had a moderate correlation with VIP (r = 0.558, p < 0.001), and
PACAP (r = 0.504, p < 0.001), VIP and PACAP also correlated, but in an even lesser degree (r = 0.435, p < 0.001).

**Effect of preventatives on CGRP, PACAP, and VIP serum levels.**

We have also investigated the relationship of CGRP, PACAP, and VIP with preventatives in CM and EM patients. In CM patients, median and IQ differed from those under OnaBT treatment (n = 42) and other preventatives (n = 27) or no treatment (n = 32. Table 3). Univariate analysis disclosed that all three neuropeptides had higher levels in OnaBT treated patients having CM [OR = 1.088 (1.03–1.16) for CGRP, p = 0.006; OR = 1.003 (1.001–1.005), for PACAP, p = 0.002; OR = 1.018 (1.009–1.03), p = < 0.001] than no treated patients. However, age had some effect on the risk of CM and having OnaBT treatment [OR = 1.067 (1.02–1.12), p = 0.010], but sex did not (Table 4). Modeling preventative treatment as dependent variable, age, sex and neuropeptides as independent variables, we observed that the relationship of CGRP and PACAP is lost comparing OnaBT treatment with no treatment, adjusted for sex and age, but also that VIP and age increased the risk for being under OnaBT treatment; but PACAP still increased the risk for having other treatments against no treatment [OR = 1.002 (1.000–1.004), uncorrected p = 0.044]. In this model, the risk for being on OnaBT treatment versus other treatments in CM patients is only age-dependent (Table 4).

For EM patients, only PACAP distribution varied between subjects with other preventatives [n = 86; median and IQ = 98.991 pg/ml (69.16–168.23)] and those with no treatment [n = 12; 91.919 pg/ml (64.99–126.74); p = 0.019, using Mann–Whitney U test].

**Multinomial logistic regression modeling**

We also evaluated the association of CGRP, PACAP and VIP and clinical categories, using a model adjusted for age and sex. This model could explain a third of the total variance [Pearson’s goodness-of-fit χ² = 554.617 (p = 0.344), and Nagelkerke’s pseudo R² = 0.313]. Only VIP [OR = 1.011 (95% CI 1.004–1.018, p = 0.005)] and PACAP [OR = 1.003 (95% CI 1.001–1.005), p = 0.002] increased the risk for CM, but not for EM (Table 5). CGRP did not influence CM or EM clinical diagnosis. This model could correctly classify only 62/101 patients (61.38%) of CM, 75/98 (76.53%) of EM, and

### Table 2 Demographics and clinical characteristics

|                          | Chronic migraine n = 101 | Episodic migraine n = 98 | Healthy controls n = 97 | p value |
|--------------------------|--------------------------|--------------------------|-------------------------|---------|
| Female n (%)             | 89/101 (89.9)            | 89/98 (90.8)             | 88/97 (90.8)            | 0.773   |
| Age ya                   | 41 ± 10                  | 41 ± 10                  | 41 ± 10                 | 0.998   |
| Age at onset ya          | 15 ± 9                   | 19 ± 10                  | N/A                     | 0.038   |
| HIT-6a                   | 61.86 ± 13.0             | 58.44 ± 9.8              | N/A                     | 0.105   |
| MIDASa                   | 68.79 ± 65.79            | 17.17 ± 26.5             | N/A                     | < 0.001 |
| Days with headache/termb | 48 ± 2                   | 17 ± 22                  | N/A                     | < 0.001 |
| MAc n (%)                | 26/101 (26%)             | 22/98 (22%)              | N/A                     | 0.743   |
| Only MA n                | 8/101 (8%)               | 5/98 (5%)                |                          |         |
| Refractory migraine n (%)| 18/101 (17.8%)           | 19/101 (1.02%)           | N/A                     | < 0.001 |
| Co-existing tensión-type headache n (%) | 34/101 (33.7%) | 22/98 (22.4%) | 0/97 (0%) | 0.079<sup>d</sup> |
| Previous MOH n (%)       | 20/101 (19.8%)           | 3/98 (3.06%)             | N/A                     | < 0.001 |
| Hypertension n (%)       | 8/101 (7.9%)             | 4/98 (4.08%)             | 2/97 (2.06%)            | 0.315   |
| Hyperlipidemia n (%)     | 10/101 (9.9%)            | 12/98 (12.2%)            | 14/97 (14.4%)           | 0.621   |
| Ischemic heart disease n (%) | 0/101 (0%) | 1/98 (1%) | 0/97 (0%) | 0.99   |
| Smoker n (%)             | 25/101 (24.7%)           | 23/98 (24.5%)            | 26/97 (26.8)            | 0.845   |
| Alcohol n (%)            | 27/101 (26.7%)           | 19/98 (19.4%)            | 15/97 (15.5%)           | 0.145   |
| Contraceptives n (%)     | 8/101 (7.9%)             | 24/98 (24.5%)            | 20/97 (20.6%)           | 0.001   |
| BMI (n)<sup>a</sup>      | 25.49 ± 5.1 (83)         | 25.16 ± 4.3 (65)         | 25.30 ± 4.8 (73)        | 0.768   |
| Fibromyalgia n (%)       | 5/101 (4.9%)             | 3/98 (3.06%)             | 0/97 (0%)               | < 0.001 |
| Alodynia n (%)           | 51/101 (50.5%)           | 18/98 (18.4%)            | N/A                     | < 0.001 |

<sup>a</sup>Mean ± SD

<sup>b</sup>Term = 90 days

<sup>c</sup>Refers to predominantly migraine with aura

<sup>d</sup>It refers to comparison between CM and EM

HIT headache impact test, MIDAS Migraine Impact Disability Scale, BMI body mass index

© Springer
5/97 (4.12%) of HC [globally 147/296 subjects (49.8% was correctly classified)].

Individually, CGRP correctly classified 133/296 patients (44.93%) [60/101 (59.4% of CM); 60/98, (61.22% of EM), and 8/97 (8.3% of HC)], PACAP 150/296 (50.67%) [53/101 (52.47% of CM), 91 (92.85% of EM), and 0/97 (0.0% of HC)], and VIP 137/296 (46.28%) [62/101 (61.38%) of CM; 69/98 (70.4%) of EM, and 0/97 (0.0% of HC)].

Although all three neuropeptides showed moderate correlation in the covariance matrix among them (CGRP-PACAP, r = 0.504, p < 0.001; CGRP-VIP, r = 0.558, p < 0.001; and PACAP-VIP, r = 0.435, p < 0.001), the degree of collinearity was low (variance inflation factor = 1.145 for CGRP-VIP and CGRP-PACAP, and 1.226 for PACAP-VIP) and tolerance was higher than 0.8 for all correlations.

Discussion

To our knowledge, this is the first time that a joint assessment of CGRP, VIP and PACAP serum levels and correlations among them have been studied in patients with CM and EM. There is a long debate about whether serum levels in peripheral blood of these neuropeptides reflect the activation of the TVS and whether they can help us to differentiate migraine clinical categories (CM vs EM) [7, 18, 20, 21, 34, 36]. This latter question has been the rationale for our study.

Neuropeptide levels in chronic migraine

Firstly, we found that interictal serum CGRP, VIP and PACAP were significantly higher in CM compared to EM and HC (Fig. 1). Regarding CGRP, these findings concur with some previously reported [18], but disagree with others [7]. Some authors have found higher CGRP levels in MA than in migraine without aura [12, 18], so a lower prevalence of MA within the CM group has been suggested to condition no differences between CM and EM [7]. However, we found no effect of MA on CGRP serum levels. Actually, mean CGRP values for the different clinical categories varied throughout published studies. Our observed median values of CGRP ranged from 14 pg/mL in HC to 20.4 pg/mL in CM; those observed in Lee et al. [7] from 75.7 pg/ml in HC to 64.9 pg/ml in CM; and those in Cernuda-Morollón et al. [18] from 33.74 pg/ml in HC to 74.9 pg/ml in CM. Given that the procedure and ELISA-based assay kits used were the same, it is possible that differences in ELISA performance, instability of the detecting antibody, or other, unreliable conditions could render disparate results across laboratories.

Regarding VIP, according to our results, other researchers also found higher interictal serum levels in CM [20]. Nevertheless, the present study is the first to show elevated interictal serum PACAP levels in CM [21].

In the univariate analyses, we also observed that ranges of the concentrations of neuropeptides in the CM group were strikingly wider than in EM and HC groups, especially for PACAP (Fig. 1). These data suggest that there is a large

---

**Table 3** Neuropeptide serum levels according to active preventive treatment in chronic migraine patients

| Treatment          | CGRP pg/mL | PACAP pg/mL | VIP pg/mL |
|--------------------|------------|-------------|-----------|
| OnaBT treatment    | 20.726 (16.56–30.11) | 141.502 (217.90–622.50) | 167.070 (120.45–210.30) |
| Other preventatives| 17.596 (14.9–21.9)    | 134.797 (91.4–753.4)    | 94.496 (74.9–151.6)    |
| No Preventatives   | 13.479 (9.6–19.72)    | 114.143 (79.90–156.4)   | 80.872 (52.7–128.1)    |

Values are given as median and interquartile range

*Kruskal–Wallis test

Point estimates are given as median (inter-quartile range)
interindividual variation to the detriment of accurate clinical diagnosis. Such a variation of these neuropeptide serum levels does not conform to the hypothesis of a persistent TVS activation in CM, as previously suggested [18], against the explanation that frequent migraine attacks increase the probability for detecting higher neuropeptide levels. But again, there is variability among studies. The ranges of CGRP, VIP and PACAP concentrations are also wider in CM in Cernuda-Morollón et al. studies [18, 21], while Lee et al. [7] found greater variation in CGRP levels in the EM group.

**Neuropeptide levels in episodic migraine**

In the present study, serum CGRP, VIP and PACAP were not significantly higher in EM than in HC. These results support an episodic and less frequent activation of the TVS in these patients. Regarding CGRP, this concurs with Lee et al. findings [7], but disagree with two other studies that determined CGRP also in peripheral blood outside episodes in EM patients [10, 12]. These studies recruited subjects solely from specialized clinics, which could condition a selection bias of patients with more frequent migraines than those recruited in our study, both from a specialized clinic and primary care. However, stratified analysis according to headache frequency, performed by Lee et al. [7], as well as by Ashina et al. [12], did not find any such correlation. In the present study, only PACAP showed some correlation to total headache days.

Our results reflecting no higher PACAP levels in EM than HC, concur with the preceding ones [21, 23, 24], unlike those of VIP, whose levels have been reported higher in EM compared to HC [20]. It should be noted that VIP may preferentially rise in those patients who experience marked autonomic symptoms [37], and the proportion of patients with autonomic symptoms was not assessed in this study.

### Table 4 Results of univariate analysis and multinomial logistic regression modeling for preventive treatment groups and neuropeptide serum levels

|                | OnaBT treatment OR (95% C.I.) | p value | Other preventatives OR (95% C.I.) | p value |
|----------------|-------------------------------|---------|----------------------------------|---------|
| **Univariate analysis** |                               |         |                                  |         |
| CGRP           | 1.088 (1.03–1.16)             | **0.006** | 1.048 (0.98–1.12)              | 0.155   |
| PACAP          | 1.003 (1.001–1.005)           | **0.002** | 1.003 (1.001–1.005)           | **0.011** |
| VIP            | 1.018 (1.009–1.03)            | **<0.001** | 1.006 (0.99–1.02)            | 0.203   |
| AGE            | 1.067 (1.02–1.12)             | **0.010** | 0.983 (0.94–1.03)             | 0.486   |
| SEX            | 1.167 (0.3–4.5)               | 0.824   | 0.560 (0.1–3.3)              | 0.523   |

**Model 1**

|                | OnaBT treatment OR (95% C.I.) | p value | Other preventatives OR (95% C.I.) | p value |
|----------------|-------------------------------|---------|----------------------------------|---------|
| CGRP           | 1.021 (0.94–1.11)             | 0.609   | 0.992 (0.91–1.08)              | 0.853   |
| PACAP          | 1.002 (1.000–1.004)           | 0.124   | 1.002 (1.000–1.004)           | **0.044** |
| VIP            | 1.013 (1.003–1.02)            | **0.011** | 1.003 (0.99–1.014)            | 0.589   |
| AGE            | 1.066 (1.01–1.13)             | **0.022** | 0.978 (0.93–1.03)             | 0.385   |
| SEX            | 1.459 (0.25–8.5)              | 0.676   | 0.541 (0.08–3.8)             | 0.537   |

**Model 2**

|                | OnaBT treatment OR (95% C.I.) | p value |
|----------------|-------------------------------|---------|
| CGRP           | 1.064 (0.96–1.17)             | 0.227   |
| PACAP          | 0.999 (0.99–1.001)            | 0.324   |
| VIP            | 1.008 (0.99–1.02)             | 0.119   |
| AGE            | 1.086 (1.03–1.15)             | **0.005** |
| SEX            | 2.875 (0.3–25.1)              | 0.340   |

*This model was constructed using preventive treatment categories as dependent variable -no active preventive treatment as reference, sex as factor -using female as reference-, and age, CGRP, PACAP, and VIP as independent variables

*Same as Model 1, but dependent variable had two categories, OnaBT treated patients, and, as reference, no preventatives

**OnaBT** Onabotulinum toxin

Significant p values are highlighted in bold
We found moderate correlations between VIP and PACAP, VIP and CGRP, and PACAP and CGRP. The activation of the TVS is believed to result in the release of CGRP by the trigeminal nerve terminals, and VIP and PACAP by the efferent arm of the trigeminal-facial arch. Although in some individuals the release of one peptide may predominate over another, there seems to be a certain correlation among their levels, indicating that all of them may participate somewhat in most migraine pain.

The inverse correlation obtained between age and CGRP, though marginal, could correspond to changes in headache severity in migraine attacks that occur with aging, with decreasing throbbing, pressure and stabbing [38], which might mean less TVS activation during attacks and lower neuropeptide release. This differs from the studies by Cernuda-Morollón et al. and Lee et al. in which CGRP concentrations were not influenced by age [7, 18]. From our explanation, we would have expected to find an inverse correlation with PACAP and VIP as well, which, like others [21], we did not. In the case of VIP, its levels may depend on the presence and intensity of autonomic symptoms [37]. A further study is warranted to analyze the correlation between VIP and the presence of autonomic symptoms.

### Neuropeptide levels and preventive treatment relationship

We have observed that CGRP, VIP, PACAP and age were all higher in CM patients under OnaBT treatment than those with no preventative in univariate analysis, but only age and VIP increased the risk of being in the OnaBT treatment group after adjustments. CGRP levels decreased from 76.85 pg/mL to 52.48 pg/mL in CM patients considered responders in one study [16]. It is noteworthy that even after OnaBT treatment, CGRP still remained higher than that observed in controls in a previous study of the same group [18]. There are no previous reports analyzing PACAP or VIP as treatment response. Our study was not specifically designed for studying treatment effect in CGRP, PACAP, or

---

**Table 5** Results of univariate analysis and multinomial logistic regression modeling for clinical categories and neuropeptides levels

|                  | Chronic migraine OR (95% C.I.) |   | Chronic migraine OR (95% C.I.) |   |
|------------------|---------------------------------|---|---------------------------------|---|
| CGRP             | 1.125 (1.07–1.18)               | p<0.001 | 1.020 (0.97–1.07)               | 0.439 |
| PACAP            | 1.005 (1.003–1.007)              | p<0.001 | 0.998 (0.99–1.001)              | 0.207 |
| VIP              | 1.018 (1.01–1.02)                | p<0.001 | 1.001 (0.99–1.008)              | 0.734 |
| AGE              | 1.001 (0.98–1.03)                | 0.965  | 1.001 (0.98–1.03)                | 0.961 |
| SEX              | 1.318 (0.529–3.28)               | 0.553  | 0.989 (0.38–2.61)                | 0.982 |

**Model 1**

|                  | Chronic migraine OR (95% C.I.) |   | Chronic migraine OR (95% C.I.) |   |
|------------------|---------------------------------|---|---------------------------------|---|
| CGRP             | 1.052 (0.992–1.115)             | 0.992  | 1.027 (0.97–1.09)               | 0.376 |
| PACAP            | 1.003 (1.001–1.005)             | **0.002** | 0.998 (0.995–1.001)             | 0.180 |
| VIP              | 1.011 (1.003–1.018)             | **0.005** | 1.001 (0.993–1.008)             | 0.818 |
| AGE              | 0.998 (0.968–1.032)             | 0.992  | 0.998 (0.97–1.027)              | 0.914 |
| SEX              | 1.904 (0.595–6.091)             | 0.278  | 1242 (0.409–3.767)              | 0.702 |

**Model 2**

|                  | Chronic migraine OR (95% C.I.) |   |
|------------------|---------------------------------|---|
| CGRP             | 1.025 (0.97–1.08)               | 0.375  |
| PACAP            | 1.005 (1.002–1.008)             | p<0.001 |
| VIP              | 1.007 (1.0–1.014)               | **0.041** |
| AGE              | 0.997 (0.987–1.03)              | 0.856  |
| SEX              | 1.466 (0.475–4.5)               | 0.506  |

CGRP: calcitonin gene-related peptide, PACAP: pituitary adenylate cyclase activating polypeptide-38, VIP: vasoactive intestinal peptide

a This model was constructed using clinical categories as dependent variable, sex as factor (using female as reference), and age, CGRP, PACAP, and VIP as independent variables

b Same as Model 1, but dependent variable had two categories, chronic migraine, and, as reference, episodic migraine

Significant results are highlighted in bold

---

Correlations

We found moderate correlations between VIP and PACAP, VIP and CGRP, and PACAP and CGRP. The activation of the TVS is believed to result in the release of CGRP by the trigeminal nerve terminals, and VIP and PACAP by the efferent arm of the trigeminal-facial arch. Although in some individuals the release of one peptide may predominate over another, there seems to be a certain correlation among their levels, indicating that all of them may participate somewhat in most migraine pain.

The inverse correlation obtained between age and CGRP, though marginal, could correspond to changes in headache severity in migraine attacks that occur with aging, with decreasing throbbing, pressure and stabbing [38], which might mean less TVS activation during attacks and lower neuropeptide release. This differs from the studies by Cernuda-Morollón et al. and Lee et al. in which CGRP concentrations were not influenced by age [7, 18]. From our explanation, we would have expected to find an inverse correlation with PACAP and VIP as well, which, like others [21], we did not. In the case of VIP, its levels may depend on the presence and intensity of autonomic symptoms [37]. A further study is warranted to analyze the correlation between VIP and the presence of autonomic symptoms.
VIP serum levels; then our findings with PACAP and VIP must be taken cautiously.

**Diagnostic value**

No other study has performed a multinomial regression modeling including these 3 neuropeptides to assess their overall effect on migraine, which was globally as low as 49.8%. Individually, CGRP was the worst at classifying clinical groups, being 44.93% of subjects correctly classified, and only 59.4% of CM patients. Using ROC curves, Cernuda-Morollón et al. found that a CGRP concentration of 58.22 pg/ml correctly classified 85.7% of CM [18]. Although in the latter study, diagnostic value of CGRP would be higher, both show a non-negligible percentage of CM patients (40% and 15%) that would not be correctly classified by CGRP. It has been hypothesized that CM patients whose CGRP levels are in the range of HC may suffer from other headaches mimicking CM, or that the pain in these cases is secondary to the release of other substances [36]. Our opinion is that these values depend on the moment in which they are determined and on the procedure of determination. Surprisingly, in the present study PACAP levels correctly classified more than 92% of EM, a diagnostic value that had not been previously reported. None of the neuropeptides was useful to discriminate HC from migraine patients.

This study has several limitations. Although we fitted with preliminary sample size calculations, present sample size maybe needs to be increased to get enough power for multiple comparisons and stratified analyses. Regarding sampling, there were 81 patients under preventative treatments who were not withdrawn as they formed part of our secondary objectives. This could have influenced neuropeptide levels in the EM and CM groups. Though this effect has not been demonstrated for oral preventatives [18], CGRP has shown to decrease in good responders to OnaBT treatment [16]. However, in this study, as we already mentioned, serum levels of OnaBT-treated patients continued to be higher than those untreated patients in the CM group. Regarding laboratory procedures, sample handling and peptide determination are unsolved issues that need further studies to ensure uniformity analyzing these neuropeptides. In the present study, samples were taken and stored at -80°C in less than 10 min as recommended. Although degradation time after centrifugation is not known [7], we have made, as previous researchers, great efforts to minimize the time until analysis. Finally, some of the kits used are not specific for the peptide under analysis. For instance, PACAP kit, measures both PACAP-27 and PACAP-38, but PACAP-38 is the most prevalent isoform in mammals [22], and this kit is the same that previous researchers used [21].

The present study also has some strengths, such as a bigger sample size than most similar previous studies, age- and sex-matched samples in order to minimize possible age and sex variations [39] and the joint analyses of three neuropeptides, something which allowed us to study correlations to help better understand the process of TVS activation. A subset of subjects was recruited in a primary care center, thereby avoiding any possible selection bias of only the most severely impaired patients recruited in specialized clinics. Additionally, unlike some previous studies [18], we did not exclude patients with psychiatric comorbidities, something which makes our sample more representative of the reality in a Headache Clinic.

**Conclusions**

We found that interictal serum CGRP, VIP, and, for the first time PACAP levels, were significantly higher in CM than both EM and HC, regardless of preventative treatments. These findings support the role of the three neuropeptides in migraine, but more specifically in CM. However, with currently available techniques, CGRP, VIP and PACAP serum levels are hardly useful individually or jointly to discriminate migraine clinical categories. Only PACAP showed a significant capacity to correctly classify the EM. Although there is a prevailing need for a migraine biomarker feasible worldwide using standardized techniques, nowadays, being CGRP the best candidate as a migraine biomarker [36], there is no consistent method for its analysis. Sample handling and determination process of these neuropeptides needs to be standardized. Our results regarding the PACAP role in CM need further replication.

**Acknowledgements** We are indebted to John Hopkins for style revision.

**Author contributions** SPP, MTS and AOD conceived and designed the study. SPP, MTS, JCO, SMG, RMMN, FI, VGQ and AOD collected data. MTS, GOV and SGF designed the experimental procedures and performed the ELISA. SPP, MTS and AOD analyzed data and drafted the manuscript. SPP, MTS, VGQ and AOD critically revised the manuscript. All authors read and approved the final manuscript.

**Funding** This work has received funding from the Carlos III Health Institute, Madrid, Spain (Grant ISCIII-FISS PI15/01285) and private funds of Marqués de Valdecilla Research Institute (IDIVAL), Santander, Spain.

**Data availability** All relevant data are within the article. Raw data can be obtained by contacting the corresponding author (agustin.oterino@gmail.com).

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.
References

1. Geppetti P, Capone JG, Trevisani M, Nicoletti P, Zagli G, Tola MR (2005) CGRP and migraine: neurogenic inflammation revisited. J Headache Pain 6:61–70
2. Ho TW, Edvinsson L, Goadsby PJ (2010) CGRP and its receptors provide new insights into migraine pathophysiology. Nat Rev Neurol 6:573–582
3. Matias-Guiu J, Porta-Etessam J, Mateos V, Díaz-Insa S, Lopez-Gil A, Fernández C et al (2011) One-year prevalence of migraine in Spain: a nationwide population-based survey. Cephalalgia marzo de 31:463–470
4. Natoli JL, Manack A, Dean B, Butler Q, Turkel CC, Stovner L et al (2010) Global prevalence of chronic migraine: a systematic review. Cephalalgia 30:590–609
5. Durham P, Papapetropoulos S (2013) Biomarkers associated with migraine and their potential role in migraine management. Headache 53:1262–1277
6. Buse DC, Manack A, Serrano D, Turkel C, Lipton RB (2010) Sociodemographic and comorbidity profiles of chronic migraine and episodic migraine sufferers. J Neurol Neurosurg Psychiatry 81:428–432
7. Lee MJ, Lee S-Y, Cho S, Kang E-S, Chung C-S (2018) Feasibility of serum CGRP measurement as a biomarker of chronic migraine: a critical reappraisal. J Headache Pain 19:53
8. Gallai V, Sarchielli P, Floridi A, Franceschini M, Codini M, Glioti G et al (1995) Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. Cephalalgia 15:384–390
9. Rodriguez-Osorio X, Sobrino T, Brea D, Martínez F, Castillo J, Leira R (2012) Endothelial progenitor cells: a new key for endothelial dysfunction in migraine. Neurology 79:474–479
10. Fusayasu E, Kowa H, Takeshima T, Nakaso K, Nakashima K (2007) Increased plasma substance P and CGRP levels, and high ACE activity in migraineurs during headache-free periods. Pain 128:209–214
11. Goadsby PJ, Edvinsson L, Ekman R (1990) Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol 28:183–187
12. Ashina M, Bendtsen L, Jensen R, Schifter S, Olesen J (2000) Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. Pain 86:133–138
13. Sarchielli P, Pini LA, Zanchin G, Alberti A, Maggioni F, Rossi C et al (2006) Clinical-biochemical correlates of migraine attacks in rizatriptan responders and non-responders. Cephalalgia 26:257–265
14. Tvedskov JF, Lipka K, Ashina M, Iversen HK, Schifter S, Olesen J (2005) No increase of calcitonin gene-related peptide in jugular blood during migraine. Ann Neurol 58:561–568
15. Edvinsson L, Ekman R, Goadsby PJ (2010) Measurement of vasoactive neuropeptides in biological materials: problems and pitfalls from 30 years of experience and novel future approaches. Cephalalgia 30:761–766
16. Cernuda-Morollón E, Ramón C, Martínez-Cambor P, Serrano-Pertierra E, Larrosa D, Pascual J (2015) OnabotulinumtoxinA decreases interictal CGRP plasma levels in patients with chronic migraine. Pain 156:820–824
17. Dominguez C, Viettes-Prado A, Pérez-Mato M, Sobrino T, Rodriguez-Osorio X, López A et al (2018) CGRP and PTX3 as predictors of efficacy of onabotulinumtoxin type a in chronic migraine: an observational study. Headache 58:78–87
18. Cernuda-Morollón E, Larrosa D, Ramón C, Vega J, Martínez-Cambor P, Pascual J (2013) Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. Neurology 81:1191–1196
19. Cernuda-Morollón E, Martínez-Cambor P, Ramón C, Larrosa D, Serrano-Pertierra E, Pascual J (2014) CGRP and VIP levels as predictors of efficacy of Onabotulinumtoxin type A in chronic migraine. Headache 54:987–995
20. Cernuda-Morollón E, Martínez-Cambor P, Alvarez R, Larrosa D, Ramón C, Pascual J (2015) Increased VIP levels in peripheral blood outside migraine attacks as a potential biomarker of cranial parasympathetic activation in chronic migraine. Cephalalgia 35:310–316
21. Cernuda-Morollón E, Riesco N, Martínez-Cambor P, Serrano-Pertierra E, García-Cabo C, Pascual J (2016) No change in interictal PACAP levels in peripheral blood in women with chronic migraine. Headache 56:1448–1454
22. Rubio-Beltrán E, Correnti E, Deen M, Kamk K, Kelderman T, Papetti L et al (2018) PACAP38 and PAC1 receptor blockade: a new target for headache? J Headache Pain 19:64
23. Tuka B, Helyes Z, Markovics A, Bagoly T, Szocsálnyi J, Szabó N et al (2013) Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. Cephalalgia 33:1085–1095
24. Zagami AS, Edvinsson L, Goadsby PJ (2014) Pituitary adenylate cyclase activating polypeptide and migraine. Ann Clin Transl Neurol 1:1036–1040
25. Han X, Dong Z, Hou L, Wan D, Chen M, Tang W et al (2015) Interictal plasma pituitary adenylate cyclase-activating polypeptide levels are decreased in migraineurs but remain unchanged in patients with tension-type headache. Clin Chim Acta 450:151–154
26. Schytz HW, Birk S, Wienecke T, Kruuse C, Olesen J, Ashina M (2009) PACAP38 induces migraine-like attacks in patients with migraine without aura. Brain 132:16–25
27. Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J (2002) CGRP may play a causative role in migraine. Cephalalgia 22:54–61
28. Edvinsson L, Linde M (2010) New drugs in migraine treatment and prophylaxis: telcagepant and topiramate. Lancet 376:645–655
29. Marcus R, Goadsby PJ, Dodick D, Stock D, Manos G, Fischer TZ (2014) BMS-927711 for the acute treatment of migraine: a double-blind, randomized, placebo controlled, dose-ranging trial. Cephalalgia 34:114–125
30. Voss T, Lipton RB, Dodick DW, Dupre N, Ge YJ, Bachman R et al (2016) A phase IIb randomized, double-blind, placebo-controlled trial of ubrogepant for the acute treatment of migraine. Headache 56:1393–1404
31. Aschermann W, Mücke D, Wienecke T, Hansen JM, Fahrenkrug J, Olesen J et al (2013) PACAP-38-like immunoreactivity in the plasma of young headache patients: a novel potential biomarker for headache disorders? Cephalalgia 33:629–808

Ethical approval The Ethics Committee of Cantabria of Cantabria, Spain, approved this study (2015.155) and each patient provided written informed consent.
36. Ramón C, Cernuda-Morollón E, Pascual J (2017) Calcitonin gene-related peptide in peripheral blood as a biomarker for migraine. Curr Opin Neurol 30:281–286
37. Riesco N, Cernuda-Morollón E, Martínez-Camblor P, Pérez-Alvarez AI, Verano L, García-Cabo C et al (2017) Relationship between serum levels of VIP, but not of CGRP, and cranial autonomic parasympathetic symptoms: a study in chronic migraine patients. Cephalalgia 37:823–827
38. Kelman L (2006) Migraine changes with age: IMPACT on migraine classification. Headache 46:1161–1171
39. Valdemarsson S, Edvinsson L, Hedner P, Ekman R (1990) Hormonal influence on calcitonin gene-related peptide in man: effects of sex difference and contraceptive pills. Scand J Clin Lab Invest 50:385–388

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.