Editorial

Nervous system and COVID-19
Bertha Torres-Oliva, Karina Vélez-Jiménez, Idelfonso Rodríguez-Leyva, and Lorena Guerrero-Torres

Original Articles

Preventive treatment in migraine. Used drugs and related variables. Results of the European work and migraine survey
Ma. Teófila Vicente-Herrero, Ma. Victoria Ramírez-Iñiguez de la Torre, Elena Ruiz-de la Torre, and Luis Reinoso-Barbero

Executive dysfunction in middle-aged hypertensive adults
Edwin J. Palma-Díaz, Damaris F. Estrella-Castillo, Rita E. Zapata-Vázquez, Edgar García-Santamaría, and Héctor A. Rubio Zapata

Clinical considerations on the introduction of ocrelizumab in Mexico

Cranial electrical stimulation for the treatment of insomnia, anxiety, and depression symptoms in adults
Jesús A. Moo-Estrella, and María F. Pérez-Pichardo

Review Article

Synaptes and neural communication in neuropathological conditions
Alejandro Sánchez, Dora L. Flores, Eugenio Leyva, and Carlos Castro
A new disease has been reported recently: COVID-19, caused by a novel virus which was named SARS-CoV-2 coronavirus. COVID-19 started in Wuhan, China, in late December 2019 at a seafood market. Due to its high rate contagion, 59 suspected patients were transferred to a designated hospital in Wuhan, China. The predominant symptoms were fever, dry cough, myalgia, fatigue, and, less frequently, headache, hemoptysis, and diarrhea. More than half of the patients developed dyspnea during the 2nd week of the onset of symptoms and all presented pneumonia with abnormal chest CT, as well as lymphopenia. A significant elevation in inflammatory markers was found in patients admitted to the intensive care unit (ICU). In 41 of the 59 cases, nCoV 2019 infection was confirmed by next-generation sequencing or real-time RT-PCR methods.

In this case series, a third of the patients had underlying diseases such as diabetes, hypertension, and cardiovascular disease. Furthermore, it was more common in males (73%), with a median age of 49 years. Other studies have confirmed that age and comorbidities are associated with higher rates of hospitalization for COVID-19.

Regarding the neurological manifestations of COVID-19 infection, in the retrospective study published by Mao et al., the presence of neurological symptoms was sought intentionally in 214 hospitalized patients with confirmed SARS-CoV-2 disease. They were classified into three categories: (1) signs and symptoms of affection to the central nervous system (CNS) such as headache, dizziness, alteration of consciousness, ataxia, acute cerebrovascular disease, and epilepsy, (2) signs and symptoms of affection to the cranial and peripheral nerves, such as taste impairment, smell impairment, vision impairment, or neuralgia, and (3) skeletal and muscular injury manifestations. Neurological manifestations were found in 78 (36.4%) of patients. CNS symptoms were the main form of neurological injury, present in 53 (24.8%) of the cases. The most common symptoms were dizziness (16%) and headache (13%). Cranial nerve involvement was infrequent, with hypogeusia in 12 (5.6%), hyposmia in 11 (5.1%), and vision problems in 3 (2.3%). There were 23 (10.7%) patients with muscle injury.

This study also showed differences between the characteristics of patients with severe infection (41%) and nonsevere infection (58.9%). Patients with severe infection were significantly older (58.2 ± 15.0 vs. 48.9 ± 14.7; p < 0.001), had more comorbidities (47.7% vs. 32.5%; p < 0.05), especially hypertension (36.4% vs. 15.1%; p < 0.01), and had fewer typical symptoms compared to patients with nonsevere infection. Furthermore, neurological disease was more common in patients with severe infection compared with nonsevere infection (45.5% vs. 30.2%; p < 0.02), particularly acute cerebrovascular disease (5.7% vs. 0.8%; p < 0.001), altered consciousness (14.8% vs. 2.4%; p < 0.001), and skeletal muscle damage (19.3% vs. 4.8%; p < 0.001).

Although neurological manifestations of COVID-19 seemed uncommon, the self-reported olfactory and taste disorders (OTD) questionnaire carried out by Giacomelli.
et al. in 59 hospitalized patients with COVID-19 in Milan, Italy, revealed the necessity to expand the study of these manifestations in nonhospitalized infected patients with COVID-19. These investigators reported an alteration in taste or smell in 20 (33.9%) of patients and the presence of both symptoms in 11 (18.6%) of the patients. Furthermore, 20.3% of the patients experienced the symptoms before hospital admission and 13.5% after hospital admission. Changes in taste were frequently (91%) identified before hospitalization. Moreover, OTDs were more common in women and younger people.

In a recent report of 58 patients hospitalized for SARS-CoV-2 acute respiratory distress syndrome, neurological abnormalities were detected in 49 (84%) of them; 40 (69%) presented agitation and confusion, and 39 (67%) a corticospinal tract injury. Magnetic resonance image of the brain performed in 13 patients revealed meningeal reinforcement in 8 (62%), cerebrovascular disease in 3 (23%), and perfusion abnormalities in 11 (100%) patients who underwent this sequence. In the cerebrospinal fluid (CSF) examination of seven ties in 11 (100%) patients who underwent this sequence. revealed meningeal reinforcement in 8 (62%), cerebrovascular disease in 3 (23%), and perfusion abnormalities in 11 (100%) patients who underwent this sequence. In the cerebrospinal fluid (CSF) examination of seven patients who underwent lumbar puncture, the presence of oligoclonal bands was found in 2 (29%), high proteins and IgG in 1 (14%), and low albumin in 4 (57%). All CSF samples were negative for SARS-CoV-2.

Other manifestations caused by SARS-CoV-2 have emerged. As reported by Italian researchers, five patients with COVID-19 among their 1000 to 12,000 patients presented a typical Guillain-Barré syndrome (GBS), with an interval from 5 to 10 days between the onset of COVID-19 symptoms and the first Guillain-Barré symptom. Three of these patients had ageusia or anosmia 5-7 days before the start of GBS. The CSF analysis reported an average protein level in two of them and a normal leukocyte count in all patients. Antiganglioside antibodies were tested in three patients and found normal. All RT-PCR of the CSF was negative for SARS-CoV-2. The electrophysiological studies reported an axonal variant in three patients and a demyelination variant in two patients. Gadolinium-based MRI showed an enhancement of the caudal nerve roots in two patients, enhancement of the facial nerve in one, and a demyelination variant in two patients. Gadolinium-based MRI showed an enhancement of the caudal nerve roots in two patients, enhancement of the facial nerve in one, and a demyelination variant in two patients. Angiotensin-converting enzyme 2 (ACE-2) receptors have recently been identified as the site of entry into the cell by the SARS-CoV-2 virus. These receptors are present in multiple organs such as the lung, the nervous system, and skeletal muscle, such that SARS-CoV-2 can cause neurological symptoms by direct or indirect mechanisms. These receptors have been detected in glial cells and neurons, making it a potential target for SARS-CoV-2. The pathogenic mechanism for invading the central nervous system of SARS-CoV-2 is suspected to be hematogenous or the retrograde neuronal pathway.

The proximity of the cribriform plate to the olfactory bulb could enable the SARS-CoV-2 virus to reach and inflict cerebral damage. Therefore, changes in smell (hyposmia) could appear as an early symptom in the uncomplicated stage of COVID-19. Similarly, ACE-2 receptors are used by the SARS-CoV2 virus to penetrate the epithelial cells of the mucosa of the oral cavity and on the tongue. These findings could explain the pathogenic mechanism in the alterations of taste and odor in SARS-CoV-2 infection.

On March 11, 2020, the World Health Organization (WHO) declared the COVID-19 as a pandemic due to the exponential increase in cases outside of China, the number of countries affected, and the high mortality. To date, 2,319,066 confirmed cases had been reported in 213 countries, with 157,970 deaths worldwide.

Humanity faces the most significant challenge in 100 years: the appearance of this new COVID-19 caused by a novel virus capable of affecting all the organs and systems, especially the lungs. Increasing evidence shows that SARS-CoV-2 may also invade the CNS and cause neurological manifestations. Damage to the nervous system can come in different forms and severity. Sudden loss of smell and taste as a symptom of infection should be further studied, as it could be a screening tool that contributes to early diagnosis and timely isolation. Innovating and learning about this disease is one of the critical areas to prevent infections, save lives, and hinder its effects.

References
Preventive treatment in migraine. Used drugs and related variables. Results of the European work and migraine survey

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Abstract

Background: Migraine is a chronic debilitating and costly illness, the etiology of which is not yet fully known. Treatment is based on the control of acute attacks and the prophylactic management of chronic forms. Objective: The objective of this study is to find out the migraine preventive treatments which are used by patients in different countries in Europe, as well as observing the differences according to their social and demographic conditions. Methods: A cross-sectional observational study performed by means of an anonymous web-based survey of 3342 patients from Spain, Italy, France, Portugal, Ireland, United Kingdom, Germany, and other European Union (EU) countries. Study variables: Age, gender, country, type of town/city, level of studies completed, and rural or urban area have been dismissed. The different uses of preventive treatments are defined as: i always take preventive treatments, I take seasonal preventive treatments, I do not take preventive treatments, I do not know what a preventive treatment is. Results: The regular use of preventive treatments increases with age, their use is greater in patients over the age of 40 years (p < 0.0001), and they are most commonly used in Spain, Germany, United Kingdom, Italy, and in the rest of the countries in the EU (p < 0.0001). Out of all of the countries included in this survey, Spain has the highest use of seasonal preventive medication (p < 0.0001). The lowest use of preventive treatments is in patients under the age of 40 years (p = 0.002) and in female patients (p = 0.028). The highest percentages of patients who do not use preventive treatments (p < 0.0001) are from Spain, Germany, and the rest of the countries in the EU. Young patients under the age of 40 years (p < 0.0001), patients in Spain, Germany, and the rest of the countries in the EU that were not included in the initial design (p < 0.0001) have the greatest lack of knowledge with regard to preventive treatments. Conclusions: The use of preventive pharmacological therapies in migraine remains low despite the fact that these therapies are scientifically backed. It is important to further develop the training of physicians and reinforce patient information, assessing patient preferences to improve their adherence to treatment.

Key words: Migraine. Preventive treatment. Public health.
Tratamiento preventivo en migraña. Fármacos usados y variables relacionadas. Resultados de la encuesta europea sobre trabajo y migraña

Resumen

Antecedentes: La migraña es un trastorno crónico incapacitante y costoso, cuya etiología aún no se conoce completamente; el tratamiento se basa en el control de los ataques agudos y el manejo profiláctico de las formas crónicas. Objetivo: El objetivo de este trabajo es descubrir el uso de tratamientos preventivos en pacientes con migraña de países europeos y las diferencias observadas según sus condiciones sociales y demográficas. Método: Estudio observacional transversal mediante encuesta web anónima a 3342 pacientes de España, Italia, Francia, Portugal, Irlanda, Reino Unido, Alemania y otros países de la Unión Europea (UE). Variables de estudio: edad, sexo, país, tipo de ubicación, nivel de estudios y área rural o urbana. Las opciones de uso de los tratamientos preventivos recopilados son: tratamientos preventivos siempre, tratamientos preventivos en temporadas, «no tomo tratamiento preventivo» y «no sé qué es un tratamiento preventivo». Resultados: El uso de tratamientos preventivos es superior en los mayores de 40 años (p < 0.0001), con la mayor utilización en España, Alemania, Reino Unido, Italia y el resto de los países de la UE no incluidos en el diseño inicial (p < 0.0001). España es el país con mayor uso de preventivos en temporadas (estacional) (p < 0.0001). El uso más bajo de tratamientos preventivos ocurre en personas menores de 40 años (p = 0.002) y en mujeres (p = 0.028). España, Alemania y el resto de los países de la UE tienen el mayor porcentaje de pacientes sin tratamiento preventivo (p < 0.0001). La mayor falta de conocimiento sobre los preventivos ocurre en los mayores de 40 años de edad (p < 0.0001), en España, Alemania y el resto de los países de la UE no incluidos en el diseño inicial (p < 0.0001). Conclusiones: El uso de terapias farmacológicas preventivas en la migraña sigue siendo bajo a pesar de contar con respaldo científico. Es importante reforzar la capacitación del médico y la información al paciente, evaluando las preferencias del paciente para mejorar su adherencia al tratamiento.


Introduction

Migraine is a debilitating and costly chronic illness, the etiology of which is not yet fully known; however, it is understood that it is partly attributable to genetically determined factors that play a relevant role. It is estimated that migraines affect 18% of women and 6% of men1.

Treatment is based on the control of acute attacks and the prophylactic management of chronic forms. This includes the use of different categories of medication, although it has been demonstrated that not all subjects have the same clinical response to these forms of medication. The general picture is further exacerbated by the need for the frequent use of polytherapy to treat comorbidities, which may interfere with the pharmacologic action of migraine medications, including both symptomatic and preventative treatments. The main objective of personalized medicine is to set optimal therapies in the light of the functional biochemical active substance and of the comorbidities of each individual patient, to obtain the best clinical response. There are now novel therapeutic perspectives that have provided options for managing this pathology; nonetheless, the pharmacologic interactions and their metabolic destiny must always be studied by the application of pharmacogenomics2.

In the last decade, migraine research has identified novel pharmacologic targets and therapies that represent great progress3. However, preventive treatments continue to be underused, and this is due to significant factors, including adherence to treatment and patient preferences. Adherence to therapy, though a key factor for successful treatment, is low among patients with chronic conditions such as migraines. Dose frequency plays a major role in adherence, as is having flexible dosing options which allow for greater and better acceptance and adherence to treatment among adults with migraine4.

The objective of this study is to find out whether preventive treatments are used by patients with migraine in different countries in Europe, as well as observing the differences according to their social and demographic conditions, as by doing so it will be possible to contemplate more effective and targeted actions based on the results obtained.

Methods

A cross-sectional observational study performed by means of an anonymous web-based multiple-choice questionnaire with 32 questions, not validated, located on the European Migraine and Headache Alliance (EMHA)’s website, and scientifically backed by the Spanish Association of Specialists in Occupational
Medicine (AEEMT). 3352 patients participated from Spain, Italy, France, Portugal, Ireland, United Kingdom, Germany, and other European Union (EU) countries which were not included in the initial study design and who responded to it. The inclusion criteria were that the patients must have been previously diagnosed with migraines, be working at the time of the questionnaire, or have been working in the previous year, and the patients had to participate voluntarily. The data were collected from September 2018 to January 2019.

Based on the initial description, the responses corresponding to the management of the migraines were analyzed according to sociodemographic variables: age up to 20 years, between 21 and 40, between 41 and 60, more than 61; gender: man, and woman; place of residence: Spain, Italy, France, Portugal, Ireland, United Kingdom, Germany, and other country in the EU; type of town/city where they live: up to 500 inhabitants, 501-10,000 inhabitants, 10,001-250,000 inhabitants, 250,001-1 million inhabitants, and more than a million inhabitants; level of studies completed: elementary, intermediate, and higher; and environment in which they live: rural (town) and urban (capital).

The options for preventive treatment were defined by question 12 of the survey: i always take preventive treatment, I take seasonal preventive treatment, I always take several preventive treatments, I take several seasonal preventive treatments, I do not take preventive treatments, I do not know what a preventive treatment is.

Bivariate analysis was performed for each of the proposed options, as well as in relation to the different sociodemographic parameters.

Contingency tables were presented, which showed the absolute frequency (n) and the percentage (%) for each cross tab. Depending on the nature of the variables in the survey (categorical variables), the Chi-squared test or Fisher’s exact test was used to analyze the possible relationship between the characteristics of the migraine and the sociodemographic variables.

The data for each of the possible answers were analyzed separately.

Results

The sociodemographic characteristics of the population who responded to the survey are shown in table 1 and indicate a heterogeneous distribution by country, and the highest percentage of responses was received from Spain and Germany. About 85.13% of the individuals who filled out the survey were in the middle age block and were predominantly women (90%). The majority of the participants responded that they lived in an urban environment (68.63%), in medium-large sized cities (35% in towns/cities with more than 250,000 inhabitants and 72.5% in towns/cities with more than 10,000 inhabitants), that they were qualified workers (69% with higher studies and 27% with intermediate studies), and that they received moderate support from their environment during the migraine attacks (44.06%).

The overall results for the use of the different preventive treatment options for migraines and their perceptual relationship with the different sociodemographic variables that have been studied are shown in table 2.

By differentiating between each of the preventive options in relation with the studied variables and by only taking the statistically significant results into account, we see that.
The use of preventive treatments increases with age in all cases (Table 3), and these treatments are most commonly used in patients over the age of 40 years. Preventive treatments are most commonly used in Spain, Germany, the United Kingdom, Italy, and the rest of the EU countries ($p < 0.0001$). Out of all of the countries, Spain is the country with the highest use of seasonal preventive medication (Table 4) ($p < 0.0001$). The use of preventive treatments is lowest in patients under the age of 40 years ($p = 0.002$) and in female patients ($p = 0.028$). Spain, Germany, and the rest of the countries in the EU have the highest percentage of patients who do not take preventive treatments ($p < 0.0001$) (Table 5). Patients under the age of 40 years ($p < 0.0001$) in Spain, Germany, and the rest of the countries in the EU that were not included in the initial design but who responded to the survey ($p < 0.0001$) (Table 6) have the greatest lack of knowledge regarding preventive treatment.

### Discussion

To be able to evaluate the rates and predictive factors of adequate care for patients with migraines, three

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preventive treatment of the crisis</th>
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<tbody>
<tr>
<td></td>
<td>One preventive treatment always, n (%)</td>
</tr>
<tr>
<td>Age (years old)</td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>53 (5.58)</td>
</tr>
<tr>
<td>Between 21 and 40</td>
<td>374 (39.41)</td>
</tr>
<tr>
<td>Between 41 and 60</td>
<td>496 (52.27)</td>
</tr>
<tr>
<td>&gt; 61</td>
<td>26 (2.74)</td>
</tr>
<tr>
<td>Total</td>
<td>949 (100)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>88 (9.26)</td>
</tr>
<tr>
<td>Woman</td>
<td>862 (90.74)</td>
</tr>
<tr>
<td>Total</td>
<td>950 (100)</td>
</tr>
<tr>
<td>Country</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>178 (18.76)</td>
</tr>
<tr>
<td>Italy</td>
<td>106 (11.17)</td>
</tr>
<tr>
<td>France</td>
<td>31 (3.27)</td>
</tr>
<tr>
<td>Portugal</td>
<td>41 (4.22)</td>
</tr>
<tr>
<td>Ireland</td>
<td>70 (7.38)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>142 (14.96)</td>
</tr>
<tr>
<td>Germany</td>
<td>175 (18.44)</td>
</tr>
<tr>
<td>Other EU country</td>
<td>206 (21.71)</td>
</tr>
<tr>
<td>Total</td>
<td>944 (100)</td>
</tr>
<tr>
<td>Characteristics of the town/city</td>
<td></td>
</tr>
<tr>
<td>&lt; 500 inhabitants</td>
<td>46 (4.87)</td>
</tr>
<tr>
<td>500-1,000 inhabitants</td>
<td>227 (24.05)</td>
</tr>
<tr>
<td>&gt; 1,000-250,000 inhabitants</td>
<td>355 (37.61)</td>
</tr>
<tr>
<td>&gt; 250,000-1 million inhabitants</td>
<td>124 (13.14)</td>
</tr>
<tr>
<td>&gt; 1 million inhabitants</td>
<td>192 (20.34)</td>
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<tr>
<td>Total</td>
<td>944 (100)</td>
</tr>
<tr>
<td>Level of studies completed</td>
<td></td>
</tr>
<tr>
<td>Elementary</td>
<td>34 (3.58)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>252 (26.5)</td>
</tr>
<tr>
<td>Higher</td>
<td>665 (69.93)</td>
</tr>
<tr>
<td>Total</td>
<td>951 (100)</td>
</tr>
<tr>
<td>Environment in which they live</td>
<td></td>
</tr>
<tr>
<td>Rural (Town)</td>
<td>317 (33.37)</td>
</tr>
<tr>
<td>Urban (Capital)</td>
<td>633 (66.63)</td>
</tr>
<tr>
<td>Total</td>
<td>950 (100)</td>
</tr>
</tbody>
</table>
essential steps are required: medical consultation, accurate diagnosis, and the minimal necessary pharmacologic treatment (with acute and preventive treatments). Socioeconomic, demographic, and headache-specific variables have a joint influence.

The Chronic Migraine Epidemiology and Outcomes study showed that more than 5% of people with chronic migraines have to traverse these barriers (consultation, diagnosis, and treatment), which represents an unmet need for improving care in this population group. The predictive factors in this professional consultation and treatment indicate that the consultation increases with age, more in women, and likewise the need for public health efforts to improve the results obtained in patients with migraine through interventions and educational efforts aimed at improving the consultation rates, diagnostic accuracy, and adherence to minimal symptomatic and/or preventive pharmacologic treatment were indicated5.

The results from our survey demonstrated that age does not significantly modify the use of preventive treatments in migraine; nonetheless, statistically significant results were observed in terms of the lack of knowledge about or the non-use of these treatments; likewise, it was observed that younger patients know less about these preventive treatments (especially those patients aged under 20 years) and that it is patients of adult age (between 41 and 60 years) who make the greatest use of said treatments.

Studies show that primary headaches, especially migraines, and tension-type headaches are some of the most frequent conditions at a young age. In the case of these young patients, even before pharmacological treatment, an appropriate lifestyle must be adopted, avoiding triggers, given that the specific and effective pharmacologic treatments for migraines and tension-type headaches are never lacking in side effects, nonetheless, in specific cases their recommended use is scientifically backed, both for specific medications for treating the crisis and the prophylactic pharmacologic therapies when the situation so requires6.

Most headaches in young patients can be classified according to the International Classification of Headache Disorders criteria. Migraine is the most frequent diagnosis, and it is commonly associated with a negative impact on the quality of life; however, the majority of young patients receive little preventive treatment before being referred to specialized clinics7.

The approach to headaches in young patients is complex; nonetheless, it is one of the most common conditions affecting children, adolescents, and young people in industrialized countries. Although effective pharmacologic treatments without secondary effects are still lacking, over the last few years, several options (Ginkgolide B) have proven to be an effective and well-tolerated preventive treatment for reducing precipitating factors of young migraine.

### Table 3. Preventive treatment of migraine crises and statistically significant variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preventive treatment: preventive treatment always</th>
<th>No, n (%)</th>
<th>Yes, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>&lt; 20</td>
<td>341 (14.25)</td>
<td>53 (5.58)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Between 21 and 40</td>
<td>1062 (44.38)</td>
<td>374 (38.41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between 41 and 60</td>
<td>913 (38.15)</td>
<td>496 (52.27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 61</td>
<td>77 (3.22)</td>
<td>26 (52.27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2393 (100)</td>
<td>949 (100)</td>
<td></td>
</tr>
</tbody>
</table>

*Only variables with p < 0.05 are included. Gender, level of education, characteristics of the town/city, and area of residence have been dismissed as p > 0.05.

### Table 4. Preventive treatment in the season of migraine crises and statistically significant variables*

<table>
<thead>
<tr>
<th>Variable country</th>
<th>Preventive treatment: seasonal preventive treatment</th>
<th>No, n (%)</th>
<th>Yes, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>855 (30.56)</td>
<td>184 (34.07)</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>229 (8.18)</td>
<td>50 (9.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>79 (2.82)</td>
<td>8 (1.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>110 (3.93)</td>
<td>22 (4.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>184 (6.58)</td>
<td>38 (7.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>257 (9.19)</td>
<td>42 (7.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>576 (20.59)</td>
<td>128 (23.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other EU country</td>
<td>508 (18.16)</td>
<td>68 (12.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2798 (100)</td>
<td>540 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only variables with p < 0.05 are included. Age, gender, level of studies completed, characteristics of the town/city, and area of residence have been dismissed as p > 0.05.

EU: European Union.
migraine attack frequency and in attenuating the use of symptomatic medication for primary headaches in this group of young patients\(^6\).

In our survey, no significant results were observed among men and women in the use of preventive therapy, although there seems to be a greater lack of awareness among men. In contrast, the Migraine in America Symptoms and Treatment study carried out in 2016 in the United States evaluated gender differences in sociodemographics and headache features, consultation and diagnosis patterns, and patterns of acute and preventive treatment for migraine. The results showed that men (14.5\%) were more likely than women (10.4\%) to take daily oral preventive medication (p < 0.001), but that in both, acute prescription and preventive migraine treatments are underused\(^6\).

In our survey, significant differences in the use of preventive treatments were observed depending on the country. Patients in Spain and Germany demonstrated the greatest lack of knowledge with regard to preventive treatment, and these treatments are least commonly used in Spain and Portugal. Italy, the United Kingdom, and other countries in the EU which were not included in the initial design but who responded to the questionnaire make the greatest use of one or several preventive treatments always. There was no relationship between the size of town/city in terms of the number of inhabitants and the use of preventive treatments. Studies carried out in Spain among neurologists are in line with the majority of the internationally established guidelines where first choice preventive drugs are concerned. This is recorded in the survey which was recently carried out by the Spanish Society of Neurology\(^10\); however, the My Migraine Voice survey published in 2018 demonstrated that in a large proportion of patients with more than four migraine attacks per month, and for whom at least one preventive migraine treatment had failed which had led to resistance to use, future treatments could address existing unmet needs, allowing these individuals with migraine to be able to maximize their contribution to society\(^11\).

There are no statistically significant differences in our survey between the cultural level and the use of preventive treatments; however, there are differences in the knowledge of the same, as it is lower in patients with elementary or intermediate levels of studies than in those with higher levels of studies.

### Table 5. Preventive treatment of migraine crises and statistically significant variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preventive treatment: without preventive treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>219 (10.57)</td>
</tr>
<tr>
<td>Between 21 and 40</td>
<td>875 (42.25)</td>
</tr>
<tr>
<td>Between 41 and 60</td>
<td>918 (44.33)</td>
</tr>
<tr>
<td>&gt; 61</td>
<td>59 (2.85)</td>
</tr>
<tr>
<td>Total</td>
<td>2071 (100)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>189 (9.11)</td>
</tr>
<tr>
<td>Woman</td>
<td>1886 (90.89)</td>
</tr>
<tr>
<td>Total</td>
<td>2075 (100)</td>
</tr>
<tr>
<td>Country</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>556 (26.86)</td>
</tr>
<tr>
<td>Italy</td>
<td>198 (9.57)</td>
</tr>
<tr>
<td>France</td>
<td>53 (2.56)</td>
</tr>
<tr>
<td>Portugal</td>
<td>80 (3.86)</td>
</tr>
<tr>
<td>Ireland</td>
<td>156 (7.54)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>219 (10.58)</td>
</tr>
<tr>
<td>Germany</td>
<td>452 (21.84)</td>
</tr>
<tr>
<td>Other EU country</td>
<td>356 (17.2)</td>
</tr>
<tr>
<td>Total</td>
<td>2070 (100)</td>
</tr>
</tbody>
</table>

*Only variables with p < 0.05 are included. Level of studies completed, characteristics of the town/city and area of residence have been dismissed as p > 0.05.

### Table 6. Preventive treatment of migraine crises and statistically significant variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preventive studies: I do not know what preventive treatment is</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>274 (9.16)</td>
</tr>
<tr>
<td>Between 21 and 40</td>
<td>1264 (42.94)</td>
</tr>
<tr>
<td>Between 41 and 60</td>
<td>1333 (44.58)</td>
</tr>
<tr>
<td>&gt; 61</td>
<td>99 (3.31)</td>
</tr>
<tr>
<td>Total</td>
<td>2990 (100)</td>
</tr>
</tbody>
</table>

*Only variables with p < 0.05 are included. Gender, characteristics of the town/city, and area of residence have been dismissed as the p > 0.05.

EU: European Union.
With regard to the environment in which they live, our results do not show any relationship between this and the use or knowledge of these preventive treatments; however, prior systematic reviews of the global prevalence of migraine at the community level (302 studies which included 6,216,995 participants) showed that migraine affects one in ten people worldwide, with higher prevalence among women, among young people, and among urban residents in comparison with those living in a rural environments (11.2% among urban residents and 8.4% among rural residents)\(^2\).

The results of our survey confirm the scarce use and knowledge of preventive treatments in migraine as confirmed by prior studies despite the scientific evidence that supports their use adjusted to international criteria. The American Academy of Neurology and the Canadian Headache Society have published evidence-based guidelines for preventive pharmacologic treatments for migraines that provide valuable guidance for clinicians; however, these pharmacologic therapies continue to be underused in clinical practice. The primary objective of these guidelines is to assist the practitioner in choosing an appropriate prophylactic medication for a person with migraine, based on current evidence in the medical literature and expert consensus. These guidelines are focused on patients with episodic migraine (headache on ≤ 14 days a month) and there is good evidence from randomized controlled trials for the use of a number of different prophylactic medications in patients with migraines.

Medication choice for an individual patient requires careful consideration of patient clinical features\(^1\). The principles of preventive treatment are important to improve compliance, minimize side effects, and improve patient outcomes. The choice of treatment should be based on the presence of comorbid and coexistent illness, patient preference, reproductive potential, and best available evidence\(^14\). The route of administration and preventive treatment-related adverse events has an impact on patient preference and their adherence to treatment\(^5,16\). Current treatment options for migraine prophylaxis are associated with poor tolerability and low adhesion and persistence, with an irregular course, frequent gaps, and discontinued prophylaxis by the end of the 1st year\(^17\). Persistence to oral preventive treatments is poor at 6 months and declines further by 12 months. Switching between treatments is common, but persistence worsens as patients cycle through various preventive treatments\(^18\).

Scientific evidence supports the fact that preventive treatment is an important part of migraine therapy. When prescribing medications, physicians should understand patient’s preferences and select drugs that most closely meet their needs. Understanding the factors that influence these preferences increases physicians’ ability to select appropriate migraine therapy. The results of patient surveys indicate that patients rated efficacy as the most important aspect of preventive therapy of migraine\(^19\). In addition to the functional impact of migraine, the decision to start prophylaxis is based on a complex of considerations from the patient’s perspective (e.g., perceived burden of migraine, expected benefits or disadvantages, interaction with relatives, colleagues, and physician), therefore, when advising migraine patients about prophylaxis, their opinions should be taken into account. Patients need to be open to advice and information and intervention have to be offered at an appropriate moment in the course of migraine\(^20\).

The biases of this study include the use of a non-validated survey, the subjectivity of the responses, the greater participation by women, the non-uniform distribution of participants by countries, with greater participation from Spain and Germany, and the inclusion of respondent patients from countries that were not contemplated in the initial design.

The sample size and the comparative study by European countries are considered the strengths of this study, as well as the social and demographic variables that have been incorporated.

**Conclusions**

The use of preventive treatments increases with age, and the use of these treatments is greater in patients over the age of 40 years. The greatest lack of knowledge was observed among patients under the age of 40 years.

No relationship has been observed between the use of preventive treatments in migraine and the size of their place of residence or whether they live in a rural or urban area.

Knowledge of preventive treatments is lower in individuals with elementary or intermediate studies than in those with higher studies.

The greatest use of regular preventive treatments is in Spain, Germany, the United Kingdom, and Italy.

Spain and Germany have the greatest percentage of patients who do not take treatment or who do not know about preventive treatments.
The use of preventive pharmacologic therapies in migraine remains low despite the fact that it is scientifically backed. It is important to further develop the training of physicians and reinforce patient information, assessing patient preferences to improve their adherence to treatment.

Acknowledgments

We are grateful to the European Migraine and Headache Alliance (EMHA) patients for their voluntary collaboration in this survey, to the Spanish Association of Specialists in Occupational Medicine for its scientific support and backing, and to Silvia Lladosa for the statistical study of the data.

Funding

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Conflicts of interest

There are no conflicts of interest in this work.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

References

Executive dysfunction in middle-aged hypertensive adults

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Abstract

Objective: The objective was to compare the executive functions between hypertensive and non-hypertensive middle-aged Mexican adults. Methods: An observational and analytic study was designed. Participants were men and women residents of Southeastern Mexico, aged between 40 and 60 years, with at least 5 years of hypertension diagnosis. The control group was people without hypertension. All participants completed a digit symbol substitution test (DSST), clinical and epidemiological data. Statistical analysis unpaired Student's t-test, p < 0.05. Results: DSST score in control men was 37.78 ± 11.94, control women: 42.96 ± 11.19, hypertensive men: 16.81 ± 9.82, and hypertensive women: 26.88 ± 12.04. Significant differences were found between hypertensive and non-hypertensive groups. Men had worse scores than women. No difference between normotensive men and women. Inverse correlation was found between DSST score and age, values of systolic and diastolic blood tension in the hypertension group. Conclusion: Hypertension decreases the executive function in middle-aged people, mainly in men. This dysfunction could be an early indicator of brain deterioration.

Key words: Hypertension. Executive cognitive function. Mature age. Brain dysfunction.

Disfunción ejecutiva en adultos hipertensos de edad madura

Resumen

Objetivo: comparar la función ejecutiva entre adultos mexicanos de mediana edad, hipertensos y no hipertensos. Métodos: se diseñó un estudio observacional y analítico. Los participantes eran hombres y mujeres residentes del sureste de México, con edades comprendidas entre 40 y 60 años y al menos cinco años de diagnóstico de hipertensión. Los controles fueron personas sin hipertensión. Todos los participantes completaron la prueba de Sustitución de Símbolo y Dígitos (DSST), datos clínicos y epidemiológicos. Análisis estadístico t-Student no pareada, p < 0.05. Resultados: la puntuación DSST en los hombres control fue de 37.78 ± 11.94 y las mujeres control: 42.96 ± 11.19, los hombres hipertensos: 16.81 ± 9.82 y las mujeres hipertensas: 26.88 ± 12.04. Se encontraron diferencias significativas entre el grupo hipertenso y no hipertenso. Los hombres tuvieron peores puntuaciones que las mujeres. No hay diferencia entre hombres y mujeres normotenso. Se encontró correlación inversa entre la puntuación DSST y la edad, los valores de tensión arterial sistólica y diastólica en el grupo con hipertensión.
Introduction

According to the National Health Survey (ENSANUT MC) 2016, 25.5% of Mexican adults have hypertension\(^1\,\text{,}^2\). Hypertension causes functional and structural alterations in blood vessels, especially affecting arteries of medium and small caliber, which predominate in some organs like brain\(^3\). Several studies have established a causal relationship between hypertension and brain diseases such as stroke, vascular dementia, and recently Alzheimer’s disease\(^4\). Due to factors that are not yet completely clear, vascular damage induced by hypertension seems to be more aggressive in the frontal lobe, which could lead to executive dysfunction\(^5\).

Executive functions refer to a collection of cognitive abilities that enable and drive adaptive, goal-oriented behavior. These include the ability to generate thought and think flexibly, to update and manipulate information mentally, to inhibit what is irrelevant to current goals, to self-monitor, and to plan and adjust behavior as appropriate to the present context. Executive dysfunction impairs efficient performing of daily activities and increases the risk of morbidity and mortality by accidents inside and outside the home, increases dependence on caregivers, limits productive activities, and reduces quality of life\(^6\,\text{,}^7\). Chronicity and aging can worsen brain damage caused by hypertension\(^8\). Executive dysfunction may be one of the first manifestations of brain damage\(^8\), especially in patients who do not achieve control despite consuming pharmacological treatment\(^9\).

In most cases, brain damage is irreversible\(^8\), so it is an important early diagnosis. Young and middle-aged hypertensive people usually have no symptoms of a cognitive impairment, however, there are standardized tools to evaluate the executive function\(^10\). The aim of this study was to compare the executive cognitive function in middle-aged hypertensive and no hypertensive Mexican adults and related with some epidemiological data.

Methods

It is an observational and analytical study. Patients were recruited at the Unit of Social Insertion (UIS) of University of Yucatán, Mexico. This institution provides medical care to approximately 1179 users annually. The sample size was non-probabilistic and included all patients with and without hypertension, men and women from 41 to 60 years, with and without diagnosis of hypertension, and with minimum secondary schooling who attended the external consultation from October 2018 to December 2018. All selected patients with hypertension had at least 5 years of diagnosis to ensure chronicity period. To avoid biases on interpretation of cognitive function, results were excluded patients with a history of cerebral vascular event, known brain diseases, with obesity (body mass index \([\text{BMI}] > 30\) ), dyslipidemias, and hypo or hyperthyroidism. Motor, visual or auditory dysfunction, or under neurological or psychiatric treatment were not included in the study. Ninety-two people agreed to participate and met the selection criteria.

We eliminated patients who at the time of the evaluation had mild cognitive deterioration and/or depressive symptomatology. Seven people were eliminated due to symptoms of depression and/or cognitive impairment. Forty-one hypertensive and 44 normotensive patients were included in final analysis.

Procedures

A physician in a clinic room evaluated patients, during the morning (8-10 am). A brief clinical history was complete emphasizing aspects related to hypertension, time of hypertension evolution, type of treatment it carries, drugs, doses, and achievement of therapeutic goal. All participants in fasting were weighed and measured, without shoes, trousers or skirts, and shirt or blouse. The patients were weighed and measured with Detecto® brand stadiometer and scale; with these values, the BMI was determined, according to the following formula: weight (Kg)/Size (M\(^2\)).

Blood pressure (BP) was determined with a Check-ATeK® Baumanometer calibrated according to the official Mexican standard NOM-009-SCFI-1993 with the technique and specifications indicated by NOM-030-SSA2-2017\(^12\). It was considerate as therapeutic goal if patient at time of measurement systolic BP (SBP) < 140 mmHg and diastolic < 90 mmHg. If in the past 3 months, average arterial BP would not

**Palabras clave:** Hipertensión. Función cognitiva ejecutiva. Edad madura. Disfunción cerebral.

**Conclusión:** la hipertensión disminuye la función ejecutiva en personas de mediana edad, principalmente en hombres. Esta disfunción podría ser un indicador temprano de deterioro cerebral.
have exceeded reference values (taken from the clinical record), patients were considerate controlled.

**Questionnaires**

1. Digit symbol substitution test (DSST) evaluates the working memory, organization of perceived stimuli, visomotor coordination, and selective attention, which are executive cognitive functions. DSST was validated in Europe and the United States, mainly in the older adult population. Due to its iconographic nature, no linguistic translation is required, and the test has been used and validated in multiple contexts, regions, and languages, including Spanish and Mexican population. All participants were explained how to respond and used as an example the first 10 boxes with their respective symbols to ensure that patient understood how to perform the test. Participant had to match numbers with their respective symbol in order and without skipping any box, as fast as possible and without any kind of external help. Test had a total duration of 90 s (in triplicate). The number of binomials number-symbol paired correctly constituted score of the participant in the DSST. Blank space between two completed items does not invalidate the test; however, two or more consecutive blank spaces point to the end of the test. Paired symbols after two or more blank spaces are not considered in total score. DSST has no cutting points, score constitutes a continuous variable and has no individual value; it takes utility at population level when different groups are compared and is also useful when applied in the same individual overtime. Score reflects the speed of information processing as an executive function, and in comparison with other cognitive tests, DSST performance is strongly correlated with the volume of the prefrontal cortex.

2. Mini-mental state examination (MMSE): this is a widely validated tool, values in < 10 min cognitive state examining functions such as the ability to record, attention, calculus, memory, language, ability to follow simple instructions, and guidance. MMSE is used primarily to detect patients with mild cognitive impairment and other more severe forms of cognitive deterioration. Cutting point < 25 was an elimination criterion.

3. Beck-II depression inventory (BDI-II): BDI-II requires 5-10 min to be completed and it explores data of major depression in the past 2 weeks, consistent with DSM-5 criteria. Depression has significant repercussions on global cognitive function and can affect test results such as DSST, so score > 19 (of a maximum of 63) was an elimination criterion.

The procedures were the same for the control group (not hypertensive), only the interrogation on arterial hypertension was excluded from the study.

**Ethical considerations**

The study was carried out in accordance with the provisions of the General Law on Health in the field of research, Mexican Secretariat of Health 1987. Ethical principles of the Helsinki World Medical Assembly and The International Code of Medical Ethics, as well as the provisions and Guidelines of the National Bioethics Commission (Conbioetica) 2016, were attended too. The project was evaluated and approved by the Ethics and Research Committee of the UUIS of the Autonomous University of Yucatán.

**Statistical analysis**

It was carried out with the statistical program GraphPad Prism 7. The normality of the data was determined with the Shapiro-Wilk test. We compared the values of the scores of DSST with values of BP, age, duration of hypertension, using Student’s t-test for unrelated samples. For variables: sex, therapeutic status, and Chi-square test were used. Linear correlation was performed with hypertension length, age, SBP, and diastolic blood pressure (DBP) related to the DSST score. The statistical significance was 95%, p < 0.05.

**Results**

The results presented below correspond to 85 adults. Table 1 describes its main characteristics, grouped as a control group (not hypertensive) and hypertension group.

We found similar distribution of sex, age and BMI in both groups.

There were no differences in BP between men and women, neither in the hypertension group nor in the control group. DSST values in the hypertension group were lower than the control group. Hypertensive men performed less than women did (p = 0.007). In the control group, there was no sex difference (p = 0.15).

Diagnostic average duration in the hypertension group was 11.9 ± 5 years, on men was 11.31 ± 5.5 years and woman 12.28 ± 4.7 (p = 0.55). Most hypertensive patients (82.9%) were receiving some form of pharmacological treatment, 17% of patients had abandoned
Table 1. Characteristics and ejective function of adults with and without hypertension

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypertension (n = 41)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>61%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>39%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.70 ± 6.70</td>
<td>52.50 ± 5.30</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.38 ± 1.98</td>
<td>27.07 ± 2.16</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>Systolic</td>
<td>115 ± 9.76</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>74.32 ± 6.05</td>
</tr>
<tr>
<td>DSST</td>
<td>Total group</td>
<td>40.84 ± 11.66</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>42.96 ± 11.95</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>37.78 ± 11.99</td>
</tr>
</tbody>
</table>

Table 2. Comparison of controlled and uncontrolled hypertensive patients

<table>
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<th>Characteristic</th>
<th>Subgroup</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Controlled (41.4%)</td>
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</tr>
<tr>
<td></td>
<td>Uncontrolled (58.6%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>47.4%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>50%</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>Systolic</td>
<td>116.50 ± 10.57</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>75.88 ± 7.12</td>
</tr>
<tr>
<td>Pharmacotherapy</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>DSST</td>
<td>Total group</td>
<td>27.12 ± 12.23</td>
</tr>
</tbody>
</table>

*Chi-square.

Discussion

This study showed that hypertension in middle-aged Mexican adults is associated with lower scores in the DSST, a validated test with a high sensitivity to detect variations in executive function. Hypertension is a well-known risk factor for numerous adverse cardiovascular outcomes. Given their impact in brain vessels vasodilatory capacity, it could decrease biological efficacy to execute some cognitive functions. Although there is a normal increase in the BP while aging due to calcification and atherosclerosis, hypertension in younger subjects suggests a possible genetic predisposition negatively modulated by environmental and lifestyle factors such as obesity and elevated salt intake and others.

Mean duration of hypertension in participants in our study was higher than that reported by the National Health Survey, which states that most hypertensive individuals in Mexico had 4-10 years with the diagnosis. This could be explained by our selection criteria only allowed people with at least 5 years with hypertension. About 83% of hypertensive participants in our study had pharmacological treatment, similar with 79.3% reported in other studies for Mexican hypertensive patients. There is evidence that lack of pharmacological treatment in hypertensive people increases their risk of ischemic heart disease, heart failure, stroke, and kidney diseases.

Although the treatment of hypertension is cheap and simple compared to other chronic diseases, their asymptomatic nature can cause patients to refuse to undergo lifelong treatment or reduce their adherence. Remarkably, only 50% of patients with treatment had achieved therapeutic goals. Mexican National Health Survey reported a hypertensive control proportion of 45.6%. Most hypertensive patients in our study consume antihypertensive drugs in monotherapy, and though this approach facilitates compliance, it is well documented that few patients achieve adequate...
hypertensive control without two or more drugs. We observed an important number of patients receiving beta-blockers, which, according to recent guidelines should not be used as first choice antihypertensive drugs because they promote the development of dyslipidemias, impair glucose tolerance and hinder weight reduction. It is important to analyze individual patient conditions to offer the best treatment in each case and achieve therapeutic goals.

Our findings show that hypertensive patients have a worse performance in the DSST compared with normotensive people. Cognitive impairment is a gradual process and having hypertension could accelerate this process. Controversial results of the effect of hypertension on cognitive function have been found. In our study, SBP was responsible for 16% of the variance in DSST scores in the hypertensive patients.

Some studies have found a better executive performance in men; however, we found that men hypertensive obtained lower scores in DSST compared with woman hypertensive or normotensive people. There is evidence that in women, prenatal exposition to different hormonal concentrations promotes the overdevelopment of specific neuronal pathways and the neurotrophicic effects of estrogens are well described. The patients in this study were around 50 years old, so the women were in the menopausal period; consequently, it is likely that they maintained some degree of neurotrophic estrogenic stimulation and better cerebral blood flow compared to man.

The mechanisms that regulate arterial BP are similar in men and women; however, there are physiological differences at the molecular, cellular, and tissue levels between the sexes that contribute to differences in disease onset, susceptibility, prevalence, and treatment responses. The sympathetic nervous system, the renin-angiotensin-aldosterone system, and the immune system are differentially activated in males and females. Sex hormones such as estrogens or testosterone as well as sex chromosome complement likely contribute to sex differences in BP and cardiovascular disease. At the cellular level, differences in cell senescence pathways may contribute to increased longevity in women and may limit brain damage caused by hypertension. Therefore, this may be an explanation because the women in our study were less affected in their executive function. In addition, many lifestyles and environmental factors such as smoking, alcohol consumption, and diet, they are usually different in men and women, as well as their possible effect on BP and brain function, were not evaluated in the present study.

SBP and DBP were higher than those reported in the previous studies in Mexico, and higher BP readings were correlated with lower DSST scores. Uncontrolled hypertension increases vascular stiffness which rises pulse pressure. Increased BP is a risk factor for white matter lesions and subclinical hemorrhages that can cause cognitive alterations. In concordance with other authors, we could not find differences in executive function between controlled or uncontrolled hypertensive patients, maybe due to the small sample size. Several studies have reported that an elevated BP during middle age predicts cognitive impairment 20-30 years later and SBP control since middle age reduces this risk.

Many studies have reported intense prefrontal activation during DSST resolution using functional MRI and electroencephalography. These areas are particularly vulnerable to subclinical ischemia because they depend on distal blood supply. Vasomotor dysfunction characteristic of hypertension impairs their capacity for compensatory redistribution of blood flow in response to cognitive challenge. DSST is a powerful tool to explore the executive cognitive domain associated with brain regions most affected by hypertensive vasculopathy. On the hypertensive group, we found an inverse correlation between age and DSST performance, which is consistent with the previous reports. Motor dexterity decline through aging may contribute to this consistent finding and hypertension could accelerate this process. Although we guarantee a minimum level of education in our inclusion criteria, we did not specifically explore the influence of the educational level on DSST performance. However, the previous reports state that there is no relationship between education and DSST performance probably due to their iconicographic nature, making it useful in poor educated populations like ours. As opposed to most studies revised, we could not observe a significant correlation between DSST scores and duration of disease. The duration of hypertension is relevant because there is evidence that their neurodegenerative effects are accumulative.

Effective hypertension management requires a substantial amount of self-planning and adherence to pharmacological and non-pharmacological treatment. Thus, we propose that executive dysfunction may worsen self-care on hypertensive patients. Assessing executive function since middle age with easy administrated tests.
like the DSST in primary health-care settings could promote early interventions that preserve the functional independence of hypertensive patients.

Conclusion
In conclusion, mature adults with hypertension had less efficiency in the executive function test. Men showed worse test performance compared to women. In this population the control of hypertension and the duration of the disease did not affect the performance of the executive function.

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The present investigation has not received specific aid from public sector agencies, commercial sector, or non-profit entities.

Conflicts of interest
The authors declare no conflicts of interest.

Ethical disclosures
Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

References


Clinical considerations on the introduction of ocrelizumab in Mexico

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Abstract

Multiple sclerosis (MS) is the leading cause of neurological disability among young adults. The disease-modifying treatments (DMTs) have been a breakthrough in the care of this patients, becoming a treatable disease. Today, we face a broad spectrum of treatment possibilities, which should be used rationally to provide the maximum benefit for the patients. In the context of the introduction of ocrelizumab as a treatment option in the Mexican MS DMT portfolio, a group of neurologists was convened to analyze the potential transition among DMT from their experience, through a desk research and expert opinion. As a result, here we describe the different considerations suggested for switching from different DMT to ocrelizumab that includes profiling studies, washout periods, and follow-up considerations. We concluded that the switch from other DMT previously used to ocrelizumab could be convenient and safe, as long as there is an adequate selection and profiling of the patients.

**Introduction**

Multiple sclerosis (MS) is the main neurological cause of disability in young adults around the world. The diagnosis of MS has increased substantially in the past few decades, with a prevalence of 1.6/100,000 habitants in 1972\(^1\),\(^2\). According to the previous studies, it has been estimated that there are at least 15,000 people in Mexico who suffer from MS, with a prevalence of 7.5-30/100,000 habitants\(^2\),\(^3\).

Treatment aimed at modifying the natural history of MS has progressed considerably. The first disease-modifying treatment (DMT) approved was interferon beta-1b in 1993\(^1\), since then, we have had major changes in the understanding of the disease and now much more is known about environmental risk factors and genetic susceptibility, and the specific pathogenesis of MS may be explained in more detail. That is why there is now a wide range of treatment options available that should be used rationally to better benefit patients\(^3\).

This work was carried out in the context of the introduction of ocrelizumab (Ocrevus\(^\text{®}\)) to the Mexican market. Ocrelizumab is an IgG1 humanized monoclonal antibody that depletes B lymphocytes that express the CD20+ surface protein in their membrane. This limits immunological events linked to autoimmune conditions, specifically, MS\(^4\). Having a new DMT available, make it possible to debate over its use, which is why Roche has brought together a group of Mexican neurologists to examine the therapeutic transition from different points of view, based on their experience. The opinions given herein are the responsibility of the physicians who gave them and are independent from the unrestricted support given.

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**Materials and methods**

This analysis was carried out in the second half of 2017. Eleven neurologists considered as opinion leaders in MS, highly experienced in DMT and its mechanism of action (MoA), who understood the implications of changing treatment and were asked to give their point of view.

The group was made up of 11 neurologists who worked at some of the major public and private health institutions and hospitals in Mexico (INNN, IMSS, Hospital Español, Hospital Ángeles Lomas, INCNSZ, ISSSTE, ISSEMyM, etc.)

The work was carried out in two hands-on sessions, each lasting 2 days. Points to be considered included: (a) defining the guidelines for the proper use of ocrelizumab; (b) establishing the medical reasons for why a switch in treatment could be considered and its implications; (c) suggested paraclinical studies according to the treatment from which they are switching; (d) suggested washout period to migrate each DMT to ocrelizumab; and (e) suggested paraclinical control studies.

The work has been carried out for academic purposes, design as a non-experimental and documentary research that involved open discussion in teams and reflections as a group.

The work was divided into two sessions: in the first, the group was divided into teams to discuss in depth of rationale behind each subject and reach a consensus; in the second, all proposals were discussed extensively followed by an open discussion on what was learned and final comments and consensus.

**Results**

Placing ourselves in the MS treatment algorithm context, in Mexico, we have beta-1b interferon, beta-1a interferon, glatiramer acetate, teriflunomide, dimethyl fumarate, fingolimod, natalizumab, and alemtuzumab. Ocrelizumab is now one of the many drugs available.

Treatment guidelines around the world say that the choice of DMT depends on the characteristics of patients, comorbidities, activity/severity of the disease, safety profile, access to treatment, and other aspects\(^5\),\(^7\).
**Table 1. Recommendations to switch from an oral disease-modifying treatment to ocrelizumab**

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Teriflunomide&lt;sup&gt;5-11&lt;/sup&gt;</th>
<th>Dimethyl fumarate&lt;sup&gt;12-15&lt;/sup&gt;</th>
<th>Fingolimod&lt;sup&gt;14,16-20&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoA to consider before switching</td>
<td>Inhibits the mitochondrial</td>
<td>Reduces oxidative stress and</td>
<td>As it is a functional antagonist of the S1P</td>
</tr>
<tr>
<td></td>
<td>DHO-DH enzyme selectively</td>
<td>inhibits pro-inflammatory</td>
<td>receptor, it blocks the capability of</td>
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<td></td>
<td>and reversibly; reduces rapid</td>
<td>cytokines, causing lymphopenia</td>
<td>lymphocytes to exit the lymph</td>
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<tr>
<td></td>
<td>replication of lymphocytes;</td>
<td></td>
<td>nodes, causing lymphopenia.</td>
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<tr>
<td></td>
<td>blocks proliferation of the</td>
<td></td>
<td>Specifically, the MoA</td>
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<tr>
<td></td>
<td>activated T and B lymphocytes</td>
<td></td>
<td>to be considered on circulating</td>
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<td></td>
<td>Enterohepatic recycling</td>
<td></td>
<td>B cells is the potential decrease</td>
</tr>
<tr>
<td></td>
<td>The accelerated elimination</td>
<td></td>
<td>in activated B memory cells (CD38)</td>
</tr>
<tr>
<td></td>
<td>process may be used, if</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>necessary.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible switching scenarios</td>
<td>No response to treatment</td>
<td>No response to treatment</td>
<td>No response to treatment</td>
</tr>
<tr>
<td></td>
<td>(clinical activity and/or in</td>
<td>(clinical activity and/or in MRI)</td>
<td>(clinical activity and/or in MRI)</td>
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<td></td>
<td>MRI) after 6 months of</td>
<td>after 6 months of continuous use</td>
<td>after 6 months of continuous use</td>
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<td></td>
<td>continuous use and having</td>
<td>and having checked adherence to</td>
<td>and having checked adherence to</td>
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<td></td>
<td>checked adherence to treatment</td>
<td>treatment</td>
<td>treatment</td>
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<td></td>
<td>Patients who do not adhere to</td>
<td>Patients who do not adhere to</td>
<td>Patients who do not adhere to</td>
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<tr>
<td></td>
<td>or are intolerant to treatment</td>
<td>treatment because of dosage</td>
<td>treatment because of dosage</td>
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<tr>
<td></td>
<td>Inherent adverse effects of</td>
<td>Adverse events of DMF that makes</td>
<td>Adverse events of fingolimod that</td>
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<td></td>
<td>teriflunomide</td>
<td>it difficult to continue treatment</td>
<td>makes it difficult to continue</td>
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<td></td>
<td>Choice of patient because of</td>
<td>Choice of patient in dosage due</td>
<td>treatment Choice of patient in</td>
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<td></td>
<td>convenience of dose</td>
<td>to convenience</td>
<td>dosage due to convenience</td>
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<td>Additional screening</td>
<td>Standard screening previously</td>
<td>Standard screening previously</td>
<td>Standard screening previously</td>
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<td></td>
<td>described for ocrelizumab,</td>
<td>described for ocrelizumab,</td>
<td>described for ocrelizumab,</td>
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<td>including pregnancy test</td>
<td>including pregnancy test</td>
<td>including pregnancy test</td>
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<td></td>
<td>If needed serum level of</td>
<td></td>
<td>Rule out chicken pox</td>
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<td></td>
<td>teriflunomide</td>
<td></td>
<td>Rule out skin cancer and breast</td>
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<tr>
<td>Washout time</td>
<td>Immediate if all screening</td>
<td>It is recommended to wait for total</td>
<td>It is recommended to check</td>
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<tr>
<td></td>
<td>paraclinics are normal</td>
<td>lymphocyte recovery and, ideally,</td>
<td>recovery of total lymphocyte count</td>
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<td></td>
<td>If there is an alteration to</td>
<td>measure sub-populations of</td>
<td>(at least 800 cell/ml) in blood</td>
</tr>
<tr>
<td></td>
<td>the liver function</td>
<td>lymphocytes by flow cytometry,</td>
<td>count and, ideally, measure</td>
</tr>
<tr>
<td></td>
<td>If lymphocytes are below</td>
<td>and check status of liver function</td>
<td>sub-populations of lymphocytes</td>
</tr>
<tr>
<td></td>
<td>normal limits, consider</td>
<td>tests. If the parameters are</td>
<td>by flow cytometry</td>
</tr>
<tr>
<td></td>
<td>accelerated elimination</td>
<td>normal, you may switch immediately</td>
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<td></td>
<td>Consider induced washout</td>
<td>A washout time of 6-12 weeks is</td>
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<td></td>
<td>with activated carbon or</td>
<td>recommended depending on recovery</td>
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<tr>
<td></td>
<td>cholestyramine</td>
<td>of lymphocytes and liver function</td>
<td></td>
</tr>
<tr>
<td>Monitoring when switching to ocrelizumab</td>
<td>Monitor disease’s activity</td>
<td>Monitor disease’s activity (EDSS</td>
<td>Monitor disease’s activity (EDSS</td>
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<tr>
<td></td>
<td>(EDSS and MRI) every 6 months</td>
<td>(EDSS and MRI) every 6 months</td>
<td>and MRI) every 6 months</td>
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<tr>
<td></td>
<td>Lymphocyte count</td>
<td>Lymphocyte count</td>
<td>Lymphocyte count</td>
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<td></td>
<td>Monitor liver function</td>
<td>Convenience of whether to carry</td>
<td>Monitor cancer</td>
</tr>
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<td></td>
<td>Convenience of whether to</td>
<td>out anti-JVC antibodies or not is</td>
<td>Convenience of whether to carry</td>
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<tr>
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<td>carry out anti-JVC antibodies</td>
<td>still in debate</td>
<td>out anti-JVC antibodies or not is</td>
</tr>
<tr>
<td></td>
<td>or not is still in debate</td>
<td></td>
<td>still in debate</td>
</tr>
</tbody>
</table>


The following aspects were analyzed as part of the work to consider switching from other DMT to ocrelizumab:

a. The MoA to be considered and its clinical implications
b. The most relevant safety and efficacy considerations of each DMT for which a switch may be needed
c. Elimination time of the previous DMT from which the switch is made and, therefore, washout time, if necessary. Paraclinical studies before the switch is made
d. Paraclinical follow-up studies to monitor safety
Table 2. Recommendations to switch from a monoclonal antibody group disease-modifying treatment to ocrelizumab

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Natalizumab&lt;sup&gt;16,20&lt;/sup&gt;</th>
<th>Alemtuzumab&lt;sup&gt;20-24&lt;/sup&gt;</th>
<th>Rituximab&lt;sup&gt;16,25&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoA to be considered before switching</td>
<td>Humanized α4-integrin antagonist mAb, inhibiting migration of lymphocytes through the blood–brain barrier. Its MoA should be considered when switching treatment due to the risk of IRIS</td>
<td>Anti-CD52 mAb that depletes T and B lymphocytes. The effect of alemtuzumab on B cells may be transitory and there may be an early rebound, so anti-CD20 would be a suitable option</td>
<td>Anti-CD20 mAb</td>
</tr>
<tr>
<td>Possible switching scenarios</td>
<td>No response to treatment (clinical activity and/or in MRI) after 6 months of continuous use and having checked adherence to treatment. Risk of PML in patients with &gt; 24 infusions of natalizumab and/or a high JCV index. Patients who do not adhere to treatment. Adverse effects of natalizumab that makes it difficult to continue treatment. Choice of patient because of convenience of dose</td>
<td>Disease activity (clinical and/or radiological) after the 2nd year of treatment. Reconstitution syndrome measured by B lymphocytes; it is recommended to check sub-populations of lymphocytes by flow cytometry. Adverse effects that make it difficult to continue with infusions (incomplete cycles). Choice of patient. Patients in transitional/progressive disease. Approved as therapy for active secondary progressive and PPMS in adults by the FDA. Consider the local labeling in Mexico is approved for RMS and PPMS.</td>
<td>Adverse reactions (infusion related). Off-label use may limit insurance approval</td>
</tr>
<tr>
<td>Additional screening</td>
<td>Anti-JCV antibody titers recommended, particularly in patients who switch due to the risk of PML. MRI no &gt; 3 weeks, with FLAIR/T2, DWI sequence to discard PML. Monitoring MRI every 3 months after the 1st year to assess risk of PML. Rule out syphilis and chicken pox.</td>
<td>Lymphocyte count, considering flow cytometry to measure cell sub-populations, bearing in mind that immunosuppression in these patients is greater. Tests to rule out cancer (mammography, papanicolaou, APE, SOH, skin cancer). Tests to rule out other autoimmune conditions. Consider prophylaxis with acyclovir, TMP-SMX.</td>
<td>If profiling carried out previously for rituximab, continue with routine monitoring</td>
</tr>
<tr>
<td>Washout time</td>
<td>4-12 weeks, discarding lesions suggestive of PML by MRI. Risk of IRIS should be taken into account.</td>
<td>At least 6 months after the last infusion.</td>
<td>Unnecessary, should continue with application scheme established (every 6 months)</td>
</tr>
<tr>
<td>Evidence</td>
<td>Literature reports improved efficacy results in patients who switched from natalizumab to anti-CD20 treatment versus oral DMT.</td>
<td>Cases have been reported in literature of patients not responding to alemtuzumab who benefited from switching to anti-CD20 therapy. Anti-CD20 therapy has been used in cases of early B lymphocyte reconstitution rebound.</td>
<td>Still no evidence</td>
</tr>
</tbody>
</table>

In every case, the suggested paraclinical studies for patients to start treatment with ocrelizumab include the following:
- Blood count
- Blood chemistry
- Liver function tests
- Hepatitis testing – surface antigen and anti-core antibodies of the virus (AgHBVs and anti-HBVC).
- Rule out tuberculosis – recommend for the population exposed.
- Rule out HIV – recommend for the population exposed.
- Magnetic resonance imaging (MRI) – it is recommended to have a baseline MRI of no more than 3 weeks, ruling out any suspected progressive multifocal leukoencephalopathy. The following sequences must be taken into consideration: T1, T1 with gadolinium, T2, and fluid-attenuated inversion recovery, according to the international standard recommended by the MAGNIMS group. The frequency and make-up of each follow-up is determined by the needs of the individual patient.

If switching from other DMT, specific recommended studies may be added based on what is known about the MoA, as shown in tables 1 and 2. Therapies were divided into two large DMT groups: (a) oral DMT and (b) monoclonal antibodies (mAb). The tables below summarize the considerations made in the discussion groups.

We should point out that there is currently not enough evidence to draw final conclusions, so in this study, we will look at recommendations based on evidence available of the MoA and the recommendations to switch from each DMT. The vast experience of clinical neurologists in using innovative DMT for MS was taken into account.

Conclusions

Some 25 years after the introduction of the first interferon for treating MS, we have witnessed how DMT has evolved, aiming to adjust the pathological processes of this disease that we understand better than before. We are well aware that there is no single treatment algorithm and decisions should be made based on the knowledge of the MoA and the experience gained with these therapies.

When discussing DMT, we may classify its development in three eras: (i) from 1993 to 2003, when the first interferons were introduced and drugs were developed to better understand the immune physiopathological process of MS; (ii) the second was from 2003 to 2009 with the advent of more efficacious DMT, such as natalizumab, the first monoclonal antibody, and fingolimod, the first oral DMT; and (iii) the third from 2009 to date, in which not only were biological therapies developed but also small molecules, such as dimethyl fumarate. The range of MoA from the DMT has been expanded during this time, the results are encouraging.

Bearing this in mind, it is of particular interest to reflect on the experience and opinions of clinical neurologists about the potential switching from other DMT to those recently approved, such as ocrelizumab.

The group concludes that the switch of current DMT to ocrelizumab may be convenient and safe, as long as the patients are selected and evaluated correctly. We should bear in mind that the patients should be monitored closely during the first 24 h after the switch.

Real-life evidence is needed by means of several cases and evidence of safety in the medium and long term.

Ethical declaration

This work was carried out with the unrestricted support of Roche de México, including transport, travel expenses, and the fees of each person attending meetings.

Acknowledgments

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Conflicts of interest

Dr. Ordoñez reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Novartis, personal fees and non-financial support from Stendhal, personal fees and non-financial support from Teva, personal fees and non-financial support from Sanofi, personal fees and non-financial support from Merck, personal fees and non-financial support from Roche, personal fees and non-financial support from Biogen, and personal fees and non-financial support from Synthon, outside the submitted work; Dr. Velazquez Quintana reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study; Dr. Skromne Eisenberg reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Merck, personal...
fees and non-financial support from Sanofi, personal fees and non-financial support from Stendhal, personal fees and non-financial support from Teva, and personal fees and non-financial support from Biogen, outside the submitted work; Dr. Treviño Frenk reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Teva, personal fees and non-financial support from Novartis, personal fees and non-financial support from Stendhal, personal fees and non-financial support from Merck, and personal fees and non-financial support from Sanofi, outside the submitted work; Dr. Rivas Alonso reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Biogen, personal fees and non-financial support from Merck, personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Biogen, personal fees and non-financial support from Merck, personal fees and non-financial support from Teva, personal fees and non-financial support from Stendhal, and grants from Probiomedic, outside the submitted work; and Dr. Gonzalez Amezquita reports personal fees and non-financial support from Roche Servicios de Mexico, during the conduct of the study, personal fees and non-financial support from Bayer, personal fees and non-financial support from Merck, personal fees and non-financial support from Sanofi, personal fees and non-financial support from Stendhal, personal fees and non-financial support from UCB, personal fees and non-financial support from Probiomedic, personal fees and non-financial support from Armstron, personal fees and non-financial support from Allegran, and personal fees and non-financial support from Merz, outside the submitted work.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

References

Cranial electrical stimulation for the treatment of insomnia, anxiety, and depression symptoms in adults

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Abstract

Background: Clinical advantages of the cranial electrical stimulation (CES) are not yet clear. Objective: The objective of the study was to know the effects as a result of the CES intervention for the treatment of insomnia symptoms, anxiety, and depression. Method: Twenty-four individuals agreed to participate in the study, all of them with initial insomnia diagnosis (ISDC-2), with an average age of 32.10 (± 14.24) years old (58% women), distributed in control (n = 11) and experimental group (n = 13). The intruments used were Beck’s depression inventory, State-Trait Anxiety Inventory, the Insomnia Severity Index, and the Insomnia Symptoms Questionnaire. A CES device (Fisher Wallace-100) was given to each participant for its use at nights and mornings (20 min each session) for 10 days. Results: Insomnia symptoms decreased significantly in both the control and experimental groups (p < 0.01), but only in the experimental group there was a significant reduction in the severity of insomnia (p < 0.05). According to the effect size (Cohen’s d), the experimental group had a larger effect in the insomnia severity and a moderated effect for anxiety and depression. The placebo group had a small effect in anxiety and a medium in insomnia and depression. Conclusions: The CES effect is superior to placebo to reduce the insomnia severity, but it is no different from placebo for anxiety and depression symptoms.

Key words: Anxiety. Cranial electrical stimulation. Depression. Insomnia.
su uso en la noche y en la mañana (20 minutos cada sesión), durante 10 días, empleado durante 20 minutos cada noche antes de dormir y 20 minutos al despertar. Los cuestionarios fueron aplicados antes y después del tratamiento. **Resultados:** Los síntomas de insomnio disminuyeron significativamente tanto en el grupo control como el experimental (p < 0.01), pero solo en el grupo experimental hubo una reducción significativa de la gravedad del insomnio (p < 0.05). Con base en el tamaño del efecto (d de Cohen), el grupo experimental tuvo un efecto mayor en la reducción de la gravedad del insomnio y un efecto moderado para la ansiedad y la depresión. En el grupo de placebo (control) la magnitud del efecto fue leve para la ansiedad y moderada tanto para el insomnio como para la depresión. **Conclusiones:** El efecto EEC fue superior al placebo para reducir la gravedad en el tratamiento del insomnio, pero no fue diferente al placebo y tuvo efectos similares en la disminución de los síntomas de ansiedad y depresión.

**Palabras clave:** Ansiedad. Estimulación eléctrica craneal. Depresión. Insomnio.

**Introduction**

Anxiety, depression, and insomnia are considered to be among the alterations that generate disturbance in people who suffer them\(^1,2\). It is estimated that these changes may be present even in one-third of the world’s population\(^3,5\), and in Mexico, they can reach similar numbers\(^5-8\). These disorders are associated with the pathophysiology of the brain. Insomnia and anxiety include an increase of emotional, cognitive, somatic, and cortical excitability\(^9,10\) while depression is associated with the decrease of cortical activity in prefrontal areas and the anterior cingulate gyrus\(^11\). Many interventions focus on drug treatments\(^12-14\), but they often have certain limitations, such as cost, side effects, and a decrease of its efficiency and the quality of patient’s lifestyles\(^15-17\). The non-pharmacological approaches for the treatment of these disorders are diverse and its effectiveness is still in research\(^18,19\). Non-invasive brain stimulation techniques are used as an alternative to pharmacological treatment and they have shown a notable upturn in recent decades\(^20\). Cranial electrical stimulation (CES) is a non-invasive neurophysiological technique that works through low-intensity pulses of electric current of ≤ 4 milliamperes (mA) in frequencies from 0.5 to 15,000 Hz, through two electrodes placed on the scalp\(^21\). The CES is safe\(^22\) and it has been employed on different disorders such as major depression, anxiety, post-traumatic stress disorder, and insomnia\(^23\). Brain stimulation with alternating current (AC) such as CES has important approaches in the treatment of anxiety, depression, and insomnia\(^24\), however, it requires greater attention on its mechanism of action, especially in clinical research.

The mechanism of the CES on clinical symptoms has been explained at different levels on the nervous system. On the one hand, it is believed that the CES affects subcortical structures such as the reticular activating system, the thalamus, hypothalamus, and limbic system\(^16,21\). Brain imaging studies suggest a cortical deactivation in the prefrontal and parietal midline of the brain after 20 min of CES\(^25\). In addition, it is proposed that the effects of CES include both cortical and subcortical areas producing changes similar to the use of anti-anxiety medications\(^26\). At the electrophysiological level, it is observed an increase in alpha activity and a decrease in delta and beta activity\(^27\). At a biochemical level, it is suggested an increase in endorphins, adrenocorticotropic hormone, serotonin, melatonin, norepinephrine, cholinesterase, and reduction of cortisol\(^21\).

Given that the electrodes are placed over the scalp, this technique is considered a form of peripheral nerve stimulation. Although the positive effects of the CES have been observed in different levels of the nervous system, its efficacy on clinical symptoms is not yet conclusive. However, the use of the CES in the clinical practice has increased steadily in recent decades, so it is required the application of empirical studies which can be distinguished from the placebo effect.

**Method**

**Participants**

Twenty-six cases that covered the insomnia symptoms based on the International Classification of Sleep Disorders (ICSD) were considered initially\(^2\). The inclusion criteria were to be over 18 years old and the exclusion criteria were having antecedents of epilepsy, receive treatment for insomnia or drug use with effects on the central nervous system or metallic implants and/or pacemakers. Of the 26 cases, two were eliminated from the control group, for failing to comply with the age of majority and the second report of phosphenes as a side effect in the first session of the treatment of CES (Fig. 1 shows the eligibility diagram). The final sample included 24 subjects with an average age of 32.10 (± 14.24) years old (58% women), with a diagnosis of insomnia. Participants were randomized between the control
(n = 11) and experimental groups (n = 13) in the order, in which they arrived at the sleep laboratory. No differences in sex and age were observed between the groups.

**Instruments**

The study consisted of four self-administered questionnaires completed by participants to provide information on insomnia, anxiety, and depression symptoms. The first instrument is the insomnia symptoms questionnaire, based on the ICSD-3, which consists of 43 dichotomous questions, grouped into five factors: (1) general symptoms of insomnia, (2) psychophysiological insomnia, (3) stress-related insomnia, (4) inadequate sleep hygiene, and (5) idiopathic insomnia. These same instruments included questions related to habits and sleep schedules. The second questionnaire was the insomnia severity index, which consists of seven questions with a format of five options of response (from 0 to 4), where a high score means more severity of insomnia, considering the following cutoff points: no clinically significant insomnia (0 to 7), subthreshold insomnia (8 to 14), moderate (15 to 21), and severe (22 to 28).

The State-Trait Anxiety Inventory was administered for the evaluation of anxiety. This questionnaire includes 20 questions with four answer options (1 = not at all; 2 = somewhat; 3 = moderately so; and 4 = very much so), whereas the cutoff points are high (scores with four -44), and low (< 30). The inventory of depression of Beck (Beck’s depression inventory) was used for the measurement of depression symptoms. The cutoff points are minimum (0-13), mild (14-19), moderate (20-28), and severe (29-63).

The technique was used for treatment, neuropsychological CES using the “Fisher Wallace (FW) stimulator” device FW 100 model. This device includes two electrodes which are located bilaterally in the temples of the scalp, which sends small pulses of AC with a square wave in three frequencies: 15 Hz, 500 Hz, and 15,000 Hz, with variation of the temples of the scalp, which is study was 2 mA.

**Procedure**

The questionnaires were applied before and after the treatment, following the ethical guidelines according to the Official Mexican Norm NOM004-SSA3-201 in research for health. A consent letter was given for voluntary participation with freedom to withdraw at any time during the study, including information about the purpose of the study, the justification, risks and benefits and confidentiality on the individual’s data in written form.
device was delivered to each subject for home use for 10 days. Previously held a training session in the sleep laboratory (Faculty of Psychology, Universidad Autónoma de Yucatán) indicating the use of the device for 20 min every night before bedtime and 20 min in the morning. For the control group, it was indicated that the therapeutic level was zero (placebo). It is worth mentioning that a brochure on sleep hygiene was provided for the two groups. During the study, participants did not receive additional treatment. At the end of the protocol, control was provided to participants in the group the device 10 days more in active mode (level 2).

The statistical test was used for data analysis Student’s t-test for related samples whereas alpha minimum of 0.05. Similarly, the effect size was analyzed to assess the relevance of the effects of intervention among groups within the practical context of research. For the effect size, following cutoff points were considered: no effect aI0.20, small = 0.21-0.49, medium effect = 0.50-0.79, and large effect =0.80.

**Results**

A difference was found in relation to the sleep habits (p < 0.01) in the experimental group, which reported one more hour of sleep after the intervention (Table 1). There was a significant reduction of four symptoms of insomnia in both groups. In addition, the psychophysiological insomnia symptoms decreased in the control group (p = 0.026) and severity of insomnia in the experimental group (p = 0.002).

In the report of anxiety symptoms, there were no differences between groups, but in depression symptoms, there was a reduction of four points in the control group (p = 0.018) and five points in the experimental (p = 0.016), as shown in table 2.

Based on the analysis of the effect size (Cohen’s d), both CES and placebo had a positive effect in reducing the symptoms, but at different levels. For the insomnia index the effect was strong in the CES group (d = 0.88) and moderated in the placebo group (d = 0.52). For symptoms of anxiety, the effect was modest with the CES (d = 0.54) and moderated with placebo (d = 0.35). In both groups the effect size of depression was moderated (d = 0.69, and d = 0.58, respectively).

**Discussion**

It is suggested that the CES can have positive effects over the symptoms of anxiety, insomnia, and depression due to its influence on the limbic system and the autonomic nervous system, interconnected with the hypothalamic-pituitary-adrenal-immune axis. However, our results, after low-voltage CES, suggest a variety on the effects on the symptoms of anxiety, depression, and insomnia. In this sense, the increase of an hour of sleep and decrease the severity of insomnia as a result of the CES are observed, but psychophysiological insomnia symptoms reduced as the result of a placebo effect. Symptoms of anxiety and depression decreased in CES group, but they were not superior to placebo group.

The increase in the time dedicated to sleep during the week supports the hypothesis that the CES changed the perception of sleep. However, there are no changes in bedtime schedules and get up to match this increase. Reporting sleeping an extra hour as the effect of the CES was consistent with the results previously obtained. It is noteworthy that even with an increase of an hour of sleep (from 5 to 6 h), they are below recommended hours of sleep time. Possibly, these results can increase by extending the number of sessions of CES. In addition, it is proposed to consider that the application of the CES should be accompanied by strategies such as hygiene of sleep and therapy cognitive behavior to improve the effectiveness of the CES and maintain longer the positive effects on sleep.

In relation with insomnia, our results are similar to the results of a meta-analysis study that suggests that the CES is effective for the treatment of insomnia severity. However, we recommend to obtain objective sleep measurements to establish the physiological mechanism associated with clinical changes. Our results indicate that some types of insomnia such as the psychophysiological may improve as a result of the placebo effect. Psychophysiological insomnia is characterized by concerns over not being able to sleep, occurring particularly at the time of going to bed. Furthermore, it is possible that placebo shifted the focus of attention: the concern to the concentration in the sense of the device, providing relaxation to fall asleep.

In relation to anxiety, some authors have suggested that the CES technique should not be used in the treatment of their symptoms unless that is used for its value as placebo. In our study, the effect of CES improved the symptoms of anxiety, as it has been reported in the previous studies, but the effect was similar to the placebo’s when analyzed the results considering the statistical significance, no statistical differences were observed. Similar results in different studies are discussed based on statistical significance, however, analysis with an alternative statistic as the d Cohen may
Table 1. Pre-post intragroup comparison of the sleep schedule and insomnia

<table>
<thead>
<tr>
<th>Hours</th>
<th>CES</th>
<th></th>
<th>Placebo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>t</td>
<td>CI (95%) LI-LS</td>
<td>Cohen's d</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lay down on weekdays</td>
<td>23.11 ± 1.19</td>
<td>0.79</td>
<td>0.45</td>
<td>-1219.67-2604.28</td>
</tr>
<tr>
<td>Lay down on weekends</td>
<td>24.23 ± 1.53</td>
<td>1.1</td>
<td>0.29</td>
<td>-2013.43-6167.28</td>
</tr>
<tr>
<td>Wake up on weekdays</td>
<td>6.59 ± 1.57</td>
<td>0.81</td>
<td>0.43</td>
<td>-972.82-2126.6</td>
</tr>
<tr>
<td>Wake up on weekends</td>
<td>9.11 ± 2.07</td>
<td>1.07</td>
<td>0.3</td>
<td>-1861.84-5461.84</td>
</tr>
<tr>
<td>Sleep on weekdays</td>
<td>5.08 ± 0.90</td>
<td>3.17</td>
<td>0.009</td>
<td>-1.48-0.267</td>
</tr>
<tr>
<td>Sleep on weekends</td>
<td>6.69 ± 1.79</td>
<td>0.81</td>
<td>0.435</td>
<td>-4.26-1.95</td>
</tr>
<tr>
<td>Insomnia symptoms</td>
<td>9.53 ± 1.56</td>
<td>3.85</td>
<td>0.002</td>
<td>1.57-5.65</td>
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<tr>
<td>Inadequate sleep hygiene</td>
<td>2.83 ± 2.03</td>
<td>1.89</td>
<td>0.085</td>
<td>-0.137-1.8</td>
</tr>
<tr>
<td>Psychophysiological insomnia</td>
<td>3.3 ± 0.947</td>
<td>2.15</td>
<td>1.77</td>
<td>2.087</td>
</tr>
<tr>
<td>Stress-related insomnia</td>
<td>1.53 ± 1.19</td>
<td>1.04</td>
<td>0.316</td>
<td>-0.416-1.18</td>
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<tr>
<td>Insomnia severity index</td>
<td>18.3 ± 4.21</td>
<td>3.85</td>
<td>0.002</td>
<td>2.17-7.82</td>
</tr>
</tbody>
</table>

Conclusions

CES is a safe and efficient treatment for insomnia. The analysis of the effect suggests that this treatment can be clinically encouraging for anxiety, but its effect on depression is not superior to placebo.

Declaration of conflicts of interest

The authors reported no potential conflicts of interest.

Financing

No financial support was received for this scientific report.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

References


Table 2. Pre-post intragroup comparison of anxiety and depression symptoms

<table>
<thead>
<tr>
<th>Hours</th>
<th>CES</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>t</td>
<td>p</td>
<td>CI (95%)</td>
<td>Cohen’s d</td>
<td>Mean ± SD</td>
<td>t</td>
<td>p</td>
<td>CI (95%)</td>
<td>Cohen’s d</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td>LI-LS</td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td>LI-LS</td>
<td></td>
</tr>
<tr>
<td>STAI</td>
<td>47.46 ± 12.60</td>
<td>39.69 ± 15.51</td>
<td>2.15</td>
<td>0.052</td>
<td>−1.11-15.95</td>
<td>0.54</td>
<td>44.09 ± 10.87</td>
<td>39.63 ± 13.95</td>
<td>1.39</td>
<td>0.193</td>
</tr>
<tr>
<td>BDI</td>
<td>13.16 ± 6.52</td>
<td>8.08 ± 7.99</td>
<td>2.84</td>
<td>0.016</td>
<td>1.14-9.02</td>
<td>0.69</td>
<td>14.90 ± 5.59</td>
<td>10.63 ± 6.4</td>
<td>2.81</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Synapses and neural communication in neuropahtological conditions

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Abstract

The central nervous system (CNS) is an extraordinary and complex communication network that receives voluminous amounts of information simultaneously but is vulnerable to disease and injury. This research emphasizes on the importance of gap junctions in neuropathological conditions and neurodegenerative diseases. Namely, we explore the degree of participation of chemical and electrical synapses within an updated panorama of integrative molecular techniques used for their study. The number of electrical and chemical synapses that coexist in the human brain is unknown, yet they are present in almost all types of cells. Frequently, electrical and chemical synapses are found separately, yet sometimes mixed electrical-chemical synapses are found coupled or together. Unfortunately, synaptic dysfunction has adverse effects on organs and vital structures. In this review, we discuss the most common diseases derived from synaptic dysfunction, as well as their occurrence in Mexico. Similarly, we discuss current treatments for diseases involving human connexins. Studies on the CNS are important to understand how this system works and explain current action mechanisms used as treatments of neuropathologies.

Key words: Gap junctions. Nervous system. Neuropathologies.
Introduction

The nervous system has five forms of communication: mechanical signaling, electrical signaling, short-range signaling, local signaling, and long-range signaling. Mechanical signaling involves adhesion and recognition, whereas electrical signaling is triggered by electrical synapses and gap junctions (GJs). Short-range signaling occurs thanks to chemical synapses and neurotransmitters, local signaling is triggered by hemichannels, and finally, long-range signaling involves neurohormones.1,2

In most animal cells, except isolated moving cells, intracellular channels are formed between the cytoplasm of adjacent cells. These channels ensure the exchange of ions, metabolites, and other messenger molecules, but they also mediate electrical coupling among connected cells3,4. Similarly, specific intracellular channels are formed by multi-protein complexes, commonly known as GJs. Pathologies affecting GJs have adverse effects on vital organs, senses, and bones.

There are three families of unrelated proteins that are involved in electric synapses connexins, pannexins, and innexins (Fig. 1). The family of connexins comprises 20 members which are found only in chordates4, and some of them are expressed in the central nervous system (CNS)5. On the other hand, pannexins are found in vertebrates, and innexins exist only in invertebrates. A GJ channel is formed by two hemichannels, each from one of two neighboring cells, leaving a narrow 2-4 nm gap between them. Furthermore, some hemichannels only serve as a backdoor to important metabolites. Studies on the functional role of innexins in the nervous system of invertebrates generate results that are applicable to all animal species, particularly mammals. Therefore, in this review, we emphasize on the importance of invertebrate animal models, namely, Hirudo medicinalis, to study synaptic function and thus respond to complex questions in neuroscience. Similarly, we present a panorama of neurological disorders, in which synaptic dysfunction is a characteristic. Then, we compare this information to that reported in the literature about human connexins and current treatments of human neurological diseases.

Hirudo development of the nervous system

It is well-known that molecular interactions influence the development of virtually all types of cells, including nerve cells, during embryogenesis6,7. GJs are considered as important mediators of cell communication8. In fact, research has demonstrated that GJs play a key role in the development and preservation of nerve cells; GJ inhibition causes progenitor cells to cease proliferation, thus leading to cell differentiation, or even death9. Cells can affect one another and promote growth. Electrical synapses work both during the development of the nervous system and in adulthood. If an innexin is ectopically expressed, it creates new connections with adjacent neurons, thus forming new communication channels. However, if some innexins are blocked, new channels cannot be formed (Fig 2).

H. medicinalis is a well-known species of the genus Hirudo and the family of Hirudinidae; however, it is usually confused with another leech species, Hirudo verbana, mainly due to their physical resemblance and similar behavior (Fig 1). Due to the size of its neurons and the structure of its nervous system, H. medicinalis is a popular model in biology. It is used to study neuron-to-neuron communication, specifically GJs. The nervous system of H. medicinalis comprises 32 ganglia, which is considered as motor brains and contains 400 neurons of approximately 10-50 μ in diameter. These characteristics make H. medicinalis neurons more accessible to neuroscience studies. Furthermore, ganglia six and seven contain the male and female sexual organs of this species, respectively.

Important cells of the nervous system

GJs take part in electrical coupling and contribute to synapse regeneration after neuronal injury. There is an average of ten glial cells or neuroglia per neuron, which makes them the most abundant cell types in the CNS. Glial cells comprise macroglia (astrocytes and oligodendrocytes), microglia, and ependymal cells. Astrocytes are associated with the majority of neurological disorders and diseases, such as brain ischemia, Alzheimer’s disease (AD), and epilepsy. Similarly, GJs can be related to the malignant degree and metastasis of
brain tumors, yet the functional role of most of these proteins is still unknown, particularly in the case of diseases of the nervous system\(^9\). Oligodendrocytes form the myelin that surrounds and protects axons, whereas microglia are the first and major form of active immune defense in the CNS. Astrocytes also exchange ions and metabolites, such as inositol 1,4,5-triphosphate, and adenosine triphosphate (ATP) through GJs. Similarly, oligodendrocytes maintain myelin using GJs. Furthermore, studies on brain ischemia and epilepsy using mice as biological models have managed to block GJs activity, thus addressing the possibility of neural protection to prevent brain ischemia or metabolic stress. Finally, it has also been demonstrated that macrophages can influence GJ expression in astrocytes, whereas activated microglia communicate using GJs\(^9\).

**Role of GJs in neuropathological conditions**

**Diseases introduction**

Knowing the functional role of GJs in conditions such as brain ischemia, neurodegenerative diseases, epilepsy, heart arrhythmia, cataracts, and tumors could allow experts to determine and understand the real molecular mechanism of GJ proteins network during disease...
processes, a practical separation of diseases based on the type of synapse involved, to simplify their study. Consequently, it could also help design treatments to successfully eliminate these conditions. Connexins and pannexins are key proteins to the bone and skeletal muscle development, maintenance, and regeneration. Research has found that connexin channels are present in the bone among osteoblasts, osteoclasts, and osteocytes. Similarly, connexins are important for bone growth and adaptation.

The permeability of GJ channels is increased by multiple factors, including low pH levels, high cytosolic free calcium levels, and voltage gradient across the GJ. In vertebrate species, embryonic tissues are easily dissociated by treating them with low concentrations of a proteolytic enzyme, such as trypsin, along with a divalent-cation chelator, such as ethylenediaminetetraacetic acid, to disrupt the protein-protein interactions that hold the cells together.

The connection between cancer development and GJs was first reported by Loewenstein and Kanno when they studied liver cancer using electrophysiology techniques in 1966. Twenty-four years later, it was already established that GJ mutations were linked to multiple human genetic diseases. Nowadays, connexins are known to be related to the development of conditions such as hearing loss, skin disorders, congenital cataracts and other vision disorders, heart arrhythmia, demyelinating diseases, and oculodentodigital dysplasia.

Although the exact percentage of electrical, chemical, or combined synapse participation is currently unknown, a simplification would help unravel the workings of opening neuronal communication channels during different conditions and would allow progress in these areas. At the molecular level, cellular communication plays a decisive role in the development or treatment of these diseases, and of course, the channels through which these signal molecules travel (Fig 3).

**Brain ischemia**

Cerebrovascular ischemia, or brain ischemia, occurs when there is insufficient blood flow to the brain. It leads to limited oxygen supply and can cause the death of brain cells. The side of the ischemic lesion has the same physiopathological mechanism and local consequences with different clinical manifestations, its severity, and its duration, yet sufferers can risk death. When a person presents symptoms of brain ischemia, they must receive emergency treatment. Even a temporary deficit in oxygen supply can impair the brain, when the brain is damaged as a result of ischemia, the consequences are severe (i.e., physical or mental disability or death). In fact, cerebrovascular diseases such as brain ischemia are a leading cause of death in the USA. Cerebral vascular disease is ranked as the second leading cause of death (9.7%) in Mexico.
The functional role of astrocytes and GJs in brain ischemia is still unclear, yet the two are known to fulfill a potentially neuroprotective function that involves ischemic tolerance and remodeling of neuronal networks by phagocytosis. Experiments using cell culture techniques have demonstrated that blocking GJs in astrocytes make the cells more vulnerable to glutamate cytotoxicity. Likewise, using hippocampal slice cultures, it was found that blocking GJs cause neuronal damage in ischemic conditions, such as oxygen and glucose deprivation. GJs channels are known to remain open during these conditions.

The most promising strategy for evaluating the functional role of connexins involves blocking them using interfering RNA, drugs such as carbenoxolone (CBX), and mimetic peptides, including Gap 19 and Gap 16. Yet any state of overregulation can lead to microglial activation and neuroinflammation. This strategy can help experts identify which connexins are implicated in apoptosis and inflammation. Consequently, it would be possible for scientists to develop new treatment options for brain ischemia. To date, Connexin 43 (Cx43) is the most promising connexin. Cx43 expression is sensitive to neuronal injury and can be detected as early as 2 h post permanent middle cerebral artery occlusion (pMCAO). These findings underscore Cx43 GJ as a potential early target for therapeutic intervention in ischemic stroke. Cx43 is potentially a useful early marker to delineate and investigate the ischemia progression.

**Epilepsy**

Leading causes of epilepsy include infectious diseases and abnormal brain development. Epilepsy is a neurological disorder that develops as a result of abnormal brain function, neural communication anomalies, or imbalanced neurotransmitters – which are responsible for neurotransmission (i.e., communication between neurons). Hence, epilepsy occurs when neurons occasionally send abnormal signals in the brain.

After a head injury, brain stroke, or any other accident, the brain might try to self-repair; however, this process can accidentally lead to abnormal brain connections, and thus epilepsy. During the epileptic crisis, many neurons send signals at the same time – as many as 500 times/s. This event causes changes in a person’s behavior, movements, feelings, and levels of consciousness. Nowadays, epilepsy is referred to as a spectrum of disorders with a myriad of causes, severities, and effects. Some people may experience convulsions or lose consciousness, whereas others may simply stop what they are doing, have a short lapse of awareness, and stare into space for a moment without knowing what is happening around them.

In general, a person is considered to have epilepsy after two unprovoked seizures; a “seizure” is a paroxysmal alteration of neurologic function caused by the excessive, hypersynchronous discharge of neurons in the brain, separated by at least 24 h. Provoked seizures are caused by known precipitating factors, such as high fever, nervous system infections, acute traumatic brain injury, or fluctuations in blood sugar, or electrolyte levels. To date, nearly 2.3 million adults and 450,000 children and adolescents in the US suffer from epilepsy. In Mexico, there are about 2 million people with epilepsy, according to the Ministry of Health. Anyone can have epilepsy, men and women, regardless of their race, ethnicity, or age.

There are also severe cases of epilepsy, such as Dravet syndrome. This is a rare but lifelong dysfunction of the brain characterized by medically refractory seizures and causing serious learning disabilities. Current medications can control epilepsy in 60% of sufferers, whereas the remaining 40% experience what is commonly known as drug-resistant epilepsy. Nowadays, treatment options for epilepsy include about 20 different types of drugs, special diets, and surgical techniques, and medical specialty like neurosurgery. Common tests for epilepsy diagnosis include imaging and monitoring techniques (e.g., electroencephalography and magnetoencephalography), patient medical history, blood tests, behavioral tests, and neurological exams. Cx43 is related to cryptogenic epilepsy that is defined as a group of focal or generalized epilepsy, which are believed to be symptomatic of a histopathological or cellular occult alteration, but not of a genetic nature.

Research has found that Cx43 in GJs and hemichannels adversely affects astrocyte function, thereby causing epileptic seizures, which in turn induce Cx43 expression. On the other hand, changes in Connexin 36 (Cx36) expression cause cell death and neural communication anomalies, thus resulting in epilepsy. Furthermore, GJs in astrocytes play a crucial role in ionic regulation; particularly, they can change the concentration of potassium ions. Changes in the strength of GJ coupling can cause neuronal hyperexcitability, and consequently, spontaneous epileptic activity, which ultimately leads to astrocyte uncoupling. Finally, there is evidence that pannexin 1 (Panx1) contributes to the maintenance of epileptic seizures by releasing ATP.
potentiates seizure manifestation due to low levels of adenosine kinase, whereas the absence of Panx1 in neurons reduces seizure manifestation.

**GJ blockers to treat epilepsy**

In a recent research Scemes et al., they studied 13 GJ blockers as treatment options for epilepsy: CBX, quinine, mefloquine, quinidine, anandamide, oleamide, heptanol, octanol, meclofenamic acid, niflumic acid, flufenamic acid, glycyrrhetic acid, and retinoic acid. In the *in vitro* experiments, all these compounds demonstrated to have anticonvulsant effects in brain slices. The blockers modified the behavioral parameters related to seizures induced by 4-aminopyridine, pentyleneetetrazol, pilocarpine, penicillin, and maximal electroshock. Similar research works suggest that GJ blockers are a future alternative for the treatment of epilepsy. Nevertheless, most of these compounds have been discontinued as treatments due to their side effects, thus implying that further research must be conducted to identify the action mechanisms for neurological disorders such as epilepsy (Table 1).

Multiple studies on connexins have been conducted with the aim of understanding and reporting the devastating effects of connexin dysfunctions (Table 2). Connexins are important, since they form synapses. Moreover, GJs are present in all biological cells, except spermatozoids, and some types of blood cells.

**Neurodegenerative diseases**

**AD**

AD is the most common degenerative disease, in México, approximately 800,000 people have AD. Clinically, AD is caused by cerebral atrophy of the frontal cortex, along with neurofibrillary tangle and large numbers of senile plaques. The ApoE4 allele is usually seen as a risk factor for AD, yet health experts also look for other indicators. That is, not everybody carrying the ApoE4 gene variant will go onto develop AD as they grow old, although the probabilities are significantly higher. Moreover, some experts state that knowing that a person carries the ApoE4 allele does not benefit at all since none of the current treatment options cures the disease. ApoE gene polymorphic form ApoE4 (ε4) allele is the most commonly associated genetic risk factor linked with the late onset of the AD. In response to injury or neuroinflammation, ApoE4 undergoes neuron-specific proteolysis. Even if it is not clear the relationship between this allele and Cx43, it will be interesting to focus on Cx43 function under this condition. Thus far, the relationship between AD and GJs remains unclear, yet glial cells are thought to contribute to the aggressive propagation of this disease by inhibiting cell communication and promoting neuroinflammatory responses. Moreover, scientific evidence suggests a relationship between neurotoxicity of Aβ42 deposits in AD and up-regulation of Cx43 as a result of released neurotoxic molecules that cause oxidative stress.

That said, researchers have found that Cx43 prevents, to some extent, the brain from deteriorating; however, Cx43 overexpression identified in experiments on AD-like pathologies seems to severely affect intercellular communication, thus relating Cx43 overexpression with neuroinflammation.

Cx43 also seems to be involved in glutamate excitotoxicity induced by manganese exposure, thus promoting neurodegenerative diseases, such as AD and Parkinson's disease (PD). Moreover, in the relationship between GJ activity in glial cells and glutamate excitotoxicity, the latter is linked to excessive NMDAR activity and neurodegeneration. That said, two promising treatments of AD include blocking specific GJs for Cx43 and the Metabolic Enhancement for Neurodegeneration (MEND) approach. MEND is a 36-point therapeutic personalized program including aspects such as medication, comprehensive dietary changes, vitamins, brain stimulation, and exercise, to name but a few. There is a revision that mentions the importance of GJ in AD, their positive and negative role in neurons and other cell types, expressing that there is a need for further investigation in a GJ oriented treatment for AD.

**PD**

PD is characterized by the loss of dopaminergic neurons in the part of the brain known as the substantia nigra (SN). There are no exact numbers of Parkinson's patients in Mexico. However, the National Institute of Neurology and Neurosurgery estimates a prevalence of 50 new cases per 100,000 inhabitants/year. Clinical symptoms include shaking, rigidity or tremor, and motor disorders such as bradykinesia which means slowness of movement, and it is one of the cardinal symptoms of Parkinson's (i.e., difficulty with walking). The 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridine (MPTP) chemical model is used to represent the conditions of PD, and it causes permanent symptoms. MPTP is extensively modeled in mice and is characterized by overexpression of Cx43 in both the striatum and the astrocytic
hemichannels in the SN, thus contributing to the death of dopaminergic neurons\textsuperscript{34}. In addition, MPTP animal models have shown accelerated neuron loss in the absence of Cx30 as a result of a disruption in astrocyte energy metabolism\textsuperscript{35}.

A breakthrough in research on PD is the study of the inferior olivary nucleus (ION), which is a complex structure forming a bulge in the ventral surface of the medulla oblongata. The ION receives a wide range of sensory and motor afferents, and it is the source of the climbing fibers ascending to the cerebellum. Similarly, the ION is thought to play a key role in the generation of tremor in PD. Nowadays, olivary neurons are coupled by means of GJs, yet further research is necessary to determine their functional role. In other publications they mention the importance of focusing attention on the GJs for PD, for example, they say that GJ blocker treatment combined with inhibition of K+ channels might be a good approach to correct motor dysfunction in PD patients\textsuperscript{36} and that the use of GJ blockers in PD appears to be a promising treatment, it has been reported to improve motor function in hemiparkinsonian rats since GJ activity elicits beta oscillations in the basal ganglia nuclei, leading to akinesia in PD\textsuperscript{37}.

### Brain tumors (glioma)

A glioma is only one type of CNS tumor that originates in the glial cells of the brain or the spine. Gliomas are the most common type of brain tumors – they are space-occupying lesions causing intracranial pressure, vascular occlusion, and cerebral edema. In Mexico, an average of 30,000 new cases of brain cancer is detected annually, according to data provided by the Mexican Council of Neurological Surgery. The literature reports that in cases of severe gliomas, Cx43 is present in low concentrations; however, it is also known that the structure of Cx43 itself encourages glioma proliferation\textsuperscript{38}. In addition, Cx43 suppresses tumor growth, regardless of GJ channels. Namely, it is believed that Cx43 C-terminus directly inhibits tumor metastasis, possibly due to the correlation between Cx43 expression and protein kinase B (Akt)/extracellular signal-regulated kinase\textsuperscript{39}. In other words, Cx43 acts as an inhibitory regulator of the activation of growth factor receptors, usually related to treatments for glioblastomas (GBM).

The study of brain tumor cells entails a deep study of the cell cycle. For instance, preliminary data have shown that Cx43 helps regulate the cell cycle during the S phase by interacting with a protein kinase\textsuperscript{40}. However, Cx43 is also known to promote chemoresistance during GBM treatment by reducing the apoptotic effect of

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### Table 1. GJ blockers and their side effects

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Connexin</th>
<th>Side effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbenoxolone</td>
<td>Non-selective</td>
<td>Hydroelectrolytic disorders</td>
<td>58</td>
</tr>
<tr>
<td>Quinine</td>
<td>C x 36, C x 45, C x 50</td>
<td>Serious adverse events tinnitus, deafness, dizziness, vomiting</td>
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<tr>
<td>Mefloquine</td>
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<td>Neurotoxic side effects following large doses</td>
<td>12</td>
</tr>
<tr>
<td>Anandamide</td>
<td>C x 32, C x 43</td>
<td>Impact on calcium channels Toxicity following large doses</td>
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<tr>
<td>Heptanol</td>
<td>C x 32, C x 43, C x 45</td>
<td>Unstated</td>
<td></td>
</tr>
<tr>
<td>Octanol</td>
<td>C x 43, C x 46, C x 50</td>
<td>Unstated</td>
<td></td>
</tr>
<tr>
<td>Meclofenamic acid</td>
<td>C x 36, C x 43, C x 50</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>Niflumic acid</td>
<td>C x 43, C x 45, C x 50</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>C x 26, C x 32, C x 40, C x 43, C x 46, C x 50</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>Glycyrrhetinic acid</td>
<td>Non-selective</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>C x 38</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>Mimetic peptides</td>
<td>Specific</td>
<td>Reduce connexin function, temporarily</td>
<td>10</td>
</tr>
<tr>
<td>siRNA</td>
<td>Specific</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

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temozolomide. From this perspective, researchers have been able to identify cancer cell lineages with these characteristics. In fact, identifying cells with aberrant Cx43 expression allow experts to develop new treatment options for brain tumors and block GJs to prevent chemoresistance.

New approaches have been developed to identify tumor genesis response mechanisms. For instance,

<table>
<thead>
<tr>
<th>Connexin (H. sapiens)</th>
<th>Tissue or site of expression</th>
<th>Mutation-related pathologies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C x 26 (GJB2)</td>
<td>Ependymal cells, pinealocytes, breasts, cochlea, placenta, hepatocytes, pancreas, kidneys, intestine, and epidermis</td>
<td>Sensorineural hearing loss, palmoplantar hyperkeratosis</td>
<td>22, 62, 63, 64</td>
</tr>
<tr>
<td>C x 29/ C x 30.2-31.3* (GJC3)</td>
<td>Heart, skeletal muscle, liver, myelinating Schwann cells, Bergmann glia, and oligodendrocytes</td>
<td>Hearing impairment with pathological changes in cochlea</td>
<td>4, 62, 63, 64</td>
</tr>
<tr>
<td>C x 30 (GJB6)</td>
<td>Epidermis, cochlea, astrocytes, and hippocampal pyramidal neurons</td>
<td>Nonsyndromic hearing loss, hidrotic ectodermal dysplasia</td>
<td>11, 22, 64, 65</td>
</tr>
<tr>
<td>C x 31.9 (GJD3)</td>
<td>Smooth muscle cells expressed in the heart</td>
<td>Arrhythmia</td>
<td>25, 31, 58, 67</td>
</tr>
<tr>
<td>C x 30.3 (GJB4)</td>
<td>Epidermis and kidneys</td>
<td>Erythrokeratoderma variabilis (EKV)</td>
<td>29, 64, 66</td>
</tr>
<tr>
<td>C x 31 (GJB3)</td>
<td>Cochlea, auditory nerves, placenta, and epidermis</td>
<td>Hearing impairment, erythrokeratoderma variabilis (EKV)</td>
<td>4, 22, 31, 36, 58, 63, 64</td>
</tr>
<tr>
<td>C x 31.1 (GJB5)</td>
<td>Middle and outer layers of the corneal epithelium and epidermis</td>
<td>Expressed upregulation in corneas affected with Stevens-Johnson syndrome (SJS)</td>
<td>63, 68</td>
</tr>
<tr>
<td>C x 32 (GJB1)</td>
<td>Highly expressed in liver, pancreas, and kidneys. Expression in the nervous system is reduced to oligodendrocytes.</td>
<td>Hereditary peripheral neuropathy, Charcot-Marie-Tooth disorder (CMTX)</td>
<td>4, 5, 31, 58, 64, 65</td>
</tr>
<tr>
<td>C x 36 (GJA9/GJD2)</td>
<td>Neurons, microglia, and pancreatic B-cells</td>
<td>Involved in gliomas, astrocytomas, and glioblastomas</td>
<td>14, 22, 65, 69</td>
</tr>
<tr>
<td>C x 37 (GJA4)</td>
<td>Endothelial cells, granulosa cells, lungs, and epidermis</td>
<td>Atherosclerosis</td>
<td>4, 13, 29, 58, 66</td>
</tr>
<tr>
<td>C x 40.1 (GJD4)</td>
<td>Pancreas, kidneys, skeletal muscle, liver, placenta, myoblasts, and heart</td>
<td>Muscular dystrophy</td>
<td>3, 4, 31, 58</td>
</tr>
<tr>
<td>C x 40 (GJA5)</td>
<td>Cardiomyocytes, endothelial cells, and lungs</td>
<td>Arrhythmia</td>
<td>4, 31, 66</td>
</tr>
<tr>
<td>C x 43 (GJA1)</td>
<td>Ubiquitous, highly expressed in astrocytes, retinal cells, and basal layers of the corneal epithelium and anterior stroma</td>
<td>Oculudentodigital dysplasia (ODDD), syndactyly type III, vaso atrial heterotaxy, astrocytomas</td>
<td>6, 40, 57, 58, 64, 68, 70</td>
</tr>
<tr>
<td>C x 45 (GJA7/GJC1)</td>
<td>Endothelial cells, neurons, smooth muscle, myoblasts, neurons of the cerebral cortex, claustrum, and olfactory bulb glomeruli</td>
<td>Muscular dystrophy</td>
<td>3, 4, 31, 58</td>
</tr>
<tr>
<td>C x 46 (GJA3)</td>
<td>Highly expressed in heart, placenta, testis, chondrocytes, and ocular lens</td>
<td>Autosomal dominant zonular pulverulent cataract-3 (CZP3) upregulated in breast cancer cells</td>
<td>7, 12, 22, 69</td>
</tr>
<tr>
<td>C x 47 (GJA12/GJC2)</td>
<td>Oligodendrocytes along the surface of internodal myelin</td>
<td>Linked to neuropathies such as Pelizaeus-Merzbacher-like disease 1</td>
<td>22, 69</td>
</tr>
<tr>
<td>C x 50 (GJA8)</td>
<td>Ocular lens</td>
<td>Zonular pulverulent cataract-3 (CZP3)</td>
<td>21, 22</td>
</tr>
</tbody>
</table>
laser scanning microscopy (confocal laser scanning microscopy) and simple molecule localization microscopy are employed for the spatial localization of Cx43 in tumor genesis and the formation of metastasis\textsuperscript{44}. Moreover, the literature reports epigenetic approaches for Cx26, Cx30, and Cx43\textsuperscript{45}. In fact, cell homeostasis can help suppress gliomas, yet it is also theorized that tumor cells affect neighbor cells through GJs\textsuperscript{16,47}, whereas increased GJ regulation decreases the proliferation of glioma cells\textsuperscript{48}.

**Autism**

Autism spectrum disorder (ASD) is a developmental disorder whose causes are usually unknown. It is influenced by multiple factors across race, ethnicity, and socioeconomic status aspects. According to the literature, in the USA, over 100 million families are affected by ASD. In 2017, around 1\% of all children in Mexico, about 400,000 have autism. Recent evidence suggests that alterations of gut microbial-associated epitopes, including GJ alpha 1, can adversely affect the function of Cx43\textsuperscript{49}. Such results associate gastrointestinal problems in ASD with GJs and neuronal loss. In addition, research has found an increase in astrocytic Cx43 expression in a part of the brain’s frontal cortex (i.e. Brodmann area 9) of subjects with autism, thus suggesting abnormal glial-neuronal communication in brains of ASD sufferers. Finally, studies on brain connectivity relying on electrophysiological techniques have used *H. medicinalis* as a key model to reduce the expression of specific connexins. In humans, this could help find a way to reduce overconnectivity in the brains of subjects with autism\textsuperscript{50}.

New studies have emerged to diagnose neuropathologies with different novel approaches. Histochemistry was used to proper identify neurons, neuritic processes and axons, myelin sheaths, neuroglial cells, and connective tissue in the nervous system\textsuperscript{50}. New strategies have been developed to predict ASD. One approach can be using genetic information contained in the Autism Genetic Resource Exchange; this database was used to predict the diagnostic of ASD in combination with an instrument of behavioral evaluation\textsuperscript{51}. The other approach is the use of neuroimaging information which can be useful in the evaluation of psychiatric disorders. For ASD computational techniques were used to process magnetic resonance images of subjects with ASD and control groups\textsuperscript{52,53}. There is no relationship between neuroimaging and GJs. Neuroimaging can be used as alternative information to try to evaluate the condition of a patient. Magnetic resonance procedures can be helpful to see the brain activity of patients with ASD. Moreover, computational techniques can be useful to process that information.

Symptom onset, genetic, and neuropathological data were examined from patients with Lewy body proteins to determine the relationship between those proteins and AD\textsuperscript{54}. In addition, postmortem magnetic resonance was used to examine serial coronal sections, horizontal sections of brainstem, and cerebellum to find neuropathological lesions\textsuperscript{54,55}.

**Discussion**

The study of both the nervous system and neuropathologies through integrative molecular neuroscience requires not only fully-equipped laboratories and key biological models – such as *H. medicinalis* – but also extensive training on techniques such as injection of individual neurons, nanoballistics, and confocal microscopy, not to mention neuroimmunology and advanced histological techniques (Fig. 3).

Data show that connexin mimetic peptides can be used as GJ function blockers\textsuperscript{56,57} to disrupt innexin functions and to analyze the specific function and action of each member of the innexin family in *H. medicinalis*. In this sense, results from research on the nervous system of *H. medicinalis* can be extrapolated to understand the functional role of GJs in the nervous systems of other species, including vertebrates.

**Conclusion**

According to the World Health Organization (OMS), disability is a complex phenomenon that reflects a close and borderline relationship between the characteristics of the human being and the characteristics of the environment where he lives. It is a broad term that contains and encompasses deficiencies, limitations to perform certain activities, and restrictions on participation. According to the National Human Rights Commission (CNDH) in Mexico, disabilities are divided into motor, sensory, cognitive-intellectual, and psychosocial disabilities. If we analyze these disabilities at the cellular-molecular level, we can see a clear lack of cellular communication or defective communication between the neurons of the central and/or peripheral nervous system, a disconnection between different regions of the body.

The CNS plays a central role in the control of our bodily functions, yet failures in this system can cause multiple
diseases and disabilities. Any CNS problem diagnosis is devastating. Studying GJs is important when trying to understand neuron-to-neuron communication. In fact, knowing and understanding the functional role of GJs in certain neuropathologies could allow experts to develop new treatment options to tackle such pathologies from the root cause. In this sense, *H. medicinalis* is a well-known model used in *in vivo* experiments on neurons, innexins, and neurotransmitters.

Our work demonstrates that cell communication through GJs and electrical/chemical synapses is closely related to neurological conditions. However, evidence suggests that turning off connexins causes collateral damage or side effects that do not benefit humans. Additional and deeper *in vivo* studies are needed for a medical breakthrough and to find treatment options to cure neurological diseases.

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**Conflicts of interest**

None.

**Ethical disclosures**

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

**References**


