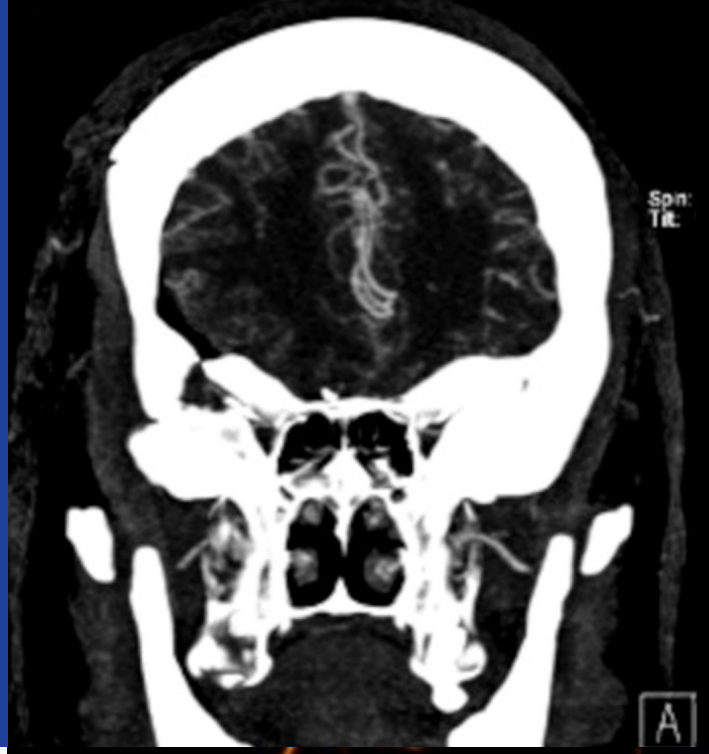
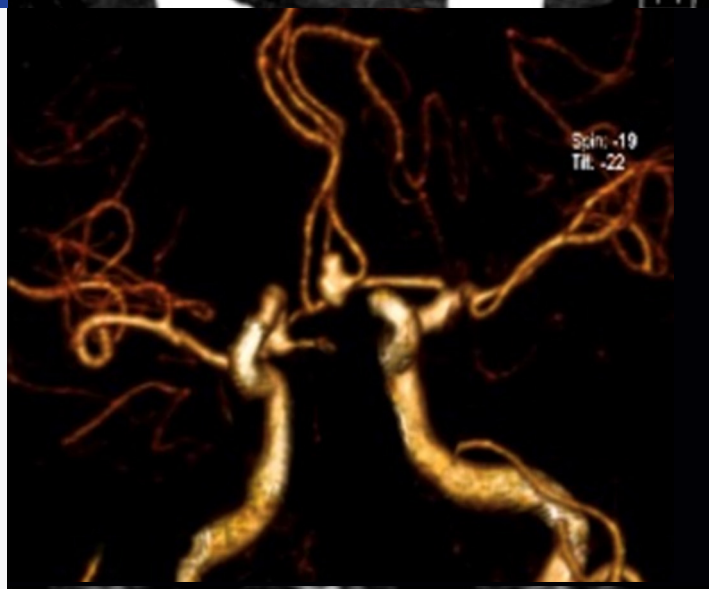
Volumen 28 • número 2 • año 2023

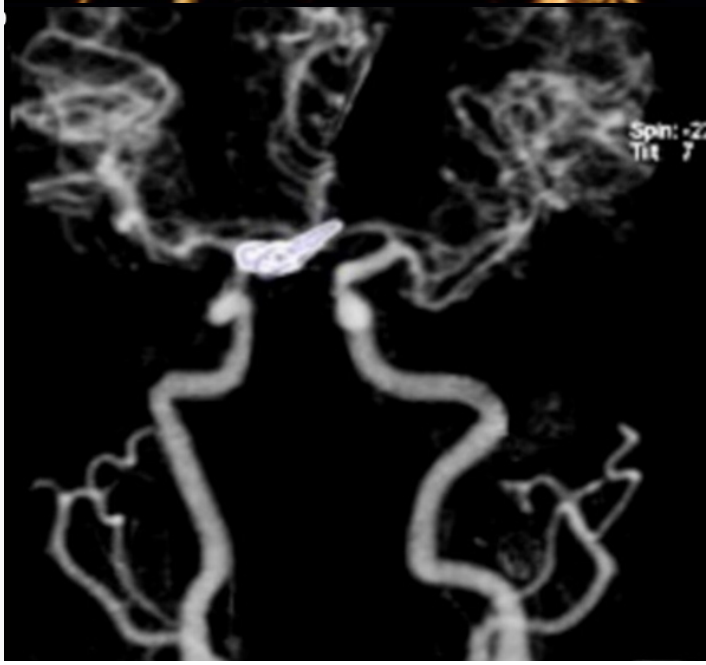
México

ARCHIVOS DE NEUROCIENCIAS

e-ISSN 2954-4122







SALUD
SECRETARÍA DE SALUD

INSTITUTO NACIONAL DE NEUROLOGÍA Y NEUROCIRUGÍA
MANUEL VELASCO SUÁREZ

Directorio

Archivos de Neurociencias del Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, volumen 28, número 2, año 2023; es una publicación trimestral editada por el Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, a través de la Dirección de Enseñanza en Avenida Insurgentes Sur #3877, Colonia La Fama, Alcaldía Tlalpan, C.P. 14269, Ciudad de México. Sitio web: https://archivosdeneurociencias.org; correo electrónico: publicaciones@innn.edu.mx Reserva de Derechos al Uso Exclusivo No. 04-2022-090717242000-203, e-ISSN 2954-4122, otorgados por el Instituto Nacional del Derecho de Autor. La última actualización de este número a cargo del director de Reserva de Derechos Alberto Arenas Badillo, con domicilio en calle Puebla número 143, Col. Roma, Alcaldía Cuauhtémoc, C.P. 06700, Ciudad de México. Editor responsable: Dr. Iván Pérez Neri, correo electrónico: publicaciones@innn.edu.mx Teléfono: (55) 5606-3822, ext. 3009. Fecha de la última actualización de este número 7 de septiembre del 2022. Los conceptos, procedencia y exactitud de los datos en los escritos publicados son responsabilidad exclusiva de sus autores.

Contenido

Arch Neurocien (Mex)| e-ISSN 2954-4122 | Volumen 28, número 2, año 2023

received 9 January 2023 | accepted 9 January 2023 | published 12 January 2023

Doi: 10.31157/an.v28i2.453

APPRECIATION TO REVIEWERS 2022

Peer-review is a critical component of scholarly publications. Some submissions require several review rounds to comply with quality standards. Recommendations from peer-reviewers are very valuable for Editors to make decisions on acceptance or rejection of manuscripts. Archivos de Neurociencias provides recognition to peer-reviewers through Web of Science (formerly Publons), but also offers public acknowledgement for this important contribution.

Peer-reviewers for Archivos de Neurociencias during 2022:

- Abel Santamaría del Angel
- Adriana Casallas Vanegas
- Alejandra Calderón Vallejo
- Alejandro Soto Ospina
- Alfredo Manzano
- Ana Natalia Seubert Ravelo
- Antonio Camiro Zúñiga
- Araceli Díaz Ruiz
- Arnold Rene Thompson Cerna
- Arturo Espíndola Rodríguez
- Bayron Alexander Sandoval Bonilla
- Bruno Antonio Marichal Cancino
- Carlos Seanez
- Carlos Alberto Rodríguez Aceves
- César Adán Almendárez Sánchez
- César Alejandro Martínez de los Santos
- Daniel San Juan Orta
- Denisse Danya Rodríguez Maldonado
- Edgar Gerardo Ordóñez Rubiano
- Elma Paredes Aragón
- Enrique Villarreal García
- Fabiola Eunice Serrano Arias
- Fernanda Flores Alfaro
- Francisco Paz Rodríguez
- Gregorio Macías
- Iván Pérez Neri
- Javier Andrés Galnares Olalde
- Jesús Ramírez Bermúdez
- José Gerardo Garza Marichalar
- Juan Carlos López Hernández
- Juan Manuel Hernández Hernández
- Julio César López Valdés
- Karla María Soler Limón
- Laura Hernández Vanegas
- Lenin Pavón Romero
- Lucía Magis Weinberg
- Luis Alberto Rodríguez Hernández
- Luis Alberto Camputaro
- Marcela Cisneros
- María Alejandra González Patiño
- Maria Anayali Estudillo Guerra
- Mayela Rodríguez Violante
- Miguel Restrepo Martínez
- Miguel Cabanillas Lazo
- Mireya Alcaraz Zubeldia
- Nicola Montemurro
- Omar Cárdenas Saenz
- Oscar Esquivel Zapata
- Pablo Felipe Amaya González
- Pardeep Kumar
- Patricio Zárate
- Ramiro Ruiz García
- Roberto Rodríguez Rivas
- Rodolfo Pedro Molina Martínez
- Rodrigo Uribe Pacheco
- Sabino Luzzi
- Sergio Andrés Castillo Torres
- Silvina Romero
- Vanessa Cano Nigenda
- Verónica Rivas Alonso
- Víctor Gálvez
- Vishal Chavda
- William Florez Perdomo
- Yamil Burguete Fourzali
- Yvonne Flores Medina

Thank you!

Editorial Council

ARCHIVOS DE NEUROCIENCIAS | Letter to Editor

received 29 November 2022 | accepted 29 November 2022 | published 30 November 2022

Doi: 10.31157/an.v28i2.442

BRIEF BIBLIOMETRIC ANALYSIS OF THE PARTICIPATION OF LATIN AMERICAN AUTHORS IN TOP NEUROLOGY JOURNALS DURING 2021: HOW BIG IS THE GAP?

Marrugo-Ortiz Ariel Camilo[1] | Ealo-Cardona Cristina Isabela[1] | Díaz-Vallejo Jhony Alejandro[2] | Cuji-Galarza Wendy Dayanna[3] | Lozada-Martínez Ivan David[4,5]✉

1. School of Medicine, University of Cartagena, Cartagena, Colombia
2. School of Health Sciences, University of Caldas, Manizales, Colombia
3. School of Medical Science, University of Cuenca, Cuenca, Ecuador
4. Colombian Clinical Research Group in Neurocritical Care, Latin American Council of Neurocritical Care, Cartagena, Colombia
5. Prometheus and Biomedicine Applied to Clinical Sciences Research Group, School of Medicine, University of Cartagena, Cartagena, Colombia

Contact

Ivan David Lozada Martínez

✉ ilozadam@unicartagena.edu.co

Conflict of Interest
The authors declare that they have no conflicts of interest to declare relevant to the research, authorship and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship and/or publication of this article.

Dear Editor,

Research is an essential tool for social development. Its outcome must be communicated to the scientific community to acquire applicability. Nowadays, health professionals are encouraged to participate in scientific publications and cultural heterogeneity is a fundamental aspect of the diffusion of different perspectives and realities in their work.[1] In this context, bibliometric analysis of neurology articles is very useful for assessing the quantity and quality of research carried out in low- and middle-income countries, such as Latin American countries, in which there is a particular interest in improving scientific production.[2,3]

In the subject area and category of neurology (clinical), a bibliometric analysis was carried out based on the information available in five leading neurology journals, according to the Scimago Journal & Country Rank (SJR). The aim was to assess the participation of Latin American authors during 2021 in neurology journals with the highest impact factor (according to 2020 metrics). The selected journals were: *The Lancet Neurology* (SJR: 12,776), *JAMA Neurology* (SJR: 5,298), *Brain* (SJR: 5,142), *Annals of Neurology* (SJR: 4,754), and *Neurology* (SJR: 2,910). The typology of publications was organized as follows: original articles, reviews (narrative, systematic, and meta-analysis), and other types (any other type of manuscript, e.g. letters to the editor). Collaborative groups were not considered. Metrics and data on the number of published articles, total number of authors, Latin American authors, and publications according to typology were analyzed.

A total of 2463 articles were considered. 38.4% were original articles (n=946), 3.9% were reviews (n=95) and 57.7% were articles included in the category of "other types" (n=1422) (Figure 1). A total of 19,703 authors participated in the aforementioned publications, 1.3% were Latin American authors (n=265) and only 15.5% of them were main authors (n=41/265). Regarding typology, Latin American authors participated more frequently with articles included in the "other types" category (n=182; 12.8%) and seldom with reviews (n=3; 3.2%). In relation to the total number of publications, Latin American authors represented only 10.8%. *Annals of Neurology* was the journal with the highest participation of Latin American authors, considering the total number of articles published (27.08%) and the total number of authors (2.66%), followed by *Neurology* in both categories (9.97% and 1.42%, respectively). *Neurology* included the highest number of articles with Latinos as main authors (n=21/128; 16.4%); although the journal with the highest proportion of Latinos as main authors in relation to the total number of articles with Latino participation was *The Lancet Neurology* (n=7/10; 70%).

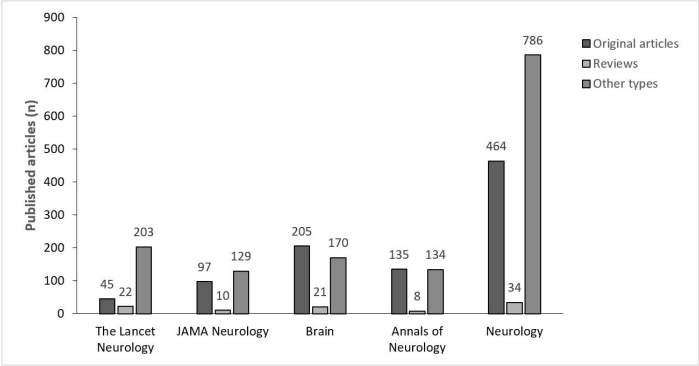


Figure 1. Number of articles published in top neurology journals during 2021, according to text types.

According to the types of articles, *Annals of Neurology* showed the highest Latino participation over the total of original articles (37.04%), *JAMA Neurology* in reviews (10%), and Brain in other types (22.94%). During the analyzed period, out of the total articles published, *Annals of Neurology* was the journal that published the highest proportion of original articles (48.7%), and *The Lancet Neurology* had the highest proportion of reviews (8.1%) and other types (75.2 %).

The results of this bibliometric analysis offer an overview of the high-quality scientific production of Latin America, insofar as top neurology journals – with a significant global impact – published those studies. According to our findings, *Annals of Neurology* is the top journal most attractive and with the greatest coverage for Latin American authors, especially with texts other than originals or reviews. Despite this, Latino authors had a low publication rate in 2021 (10.8%). Only 1 out of 10 articles published in top neurology journals included a Latin American author. This represented 1% of the total number of authors.

This information should be considered as a wake-up call to address the long road ahead in promoting scientific development in low- and middle-income countries. Achieving optimal research -- adequate and applicable to its context --, focused on global challenges in the field of neurology, is essential for such purpose. The gap with high-income countries, evident when analyzing high-quality scientific production, often published in top neurology journals, is still very wide. Therefore, it is necessary to radically modify the way neurology research is conducted in Latin America.

Author contributions

Ariel Camilo Marrugo-Ortiz: Conception and design of the study; analysis and interpretation of the data; drafting of the manuscript; critical revision; approval of the final version.
Cristina Isabela Ealo-Cardona: Analysis and interpretation of the data; drafting of the manuscript; critical revision; approval of the final version.
Jhony Alejandro Díaz-Vallejo: Analysis and interpretation of the data; drafting of the manuscript; critical revision; approval of the final version.
Wendy Dayanna Cuji-Galarza: Analysis and interpretation of the data; drafting of the manuscript; critical revision; approval of the final version.
Ivan David Lozada-Martínez: Analysis and interpretation of the data; drafting of the manuscript; critical revision; approval of the final version.

References

1. Lozada-Martínez ID, Acevedo-Aguilar LM, Mass-Hernández LM, Matta-Rodríguez D, Jiménez-Filigrana JA, Garzón-Gutiérrez KE, et al. Practical guide for the use of medical evidence in scientific publication: Recommendations for the medical student: Narrative review. Ann Med Surg (Lond). 2021; 71:102932. doi: 10.1016/j.amsu.2021.102932
2. Ciocca DR, Delgado G. The reality of scientific research in Latin America; an insider's perspective. Cell Stress Chaperones. 2017; 22(6):847-852. doi: 10.1007/s12192-017-0815-8
3. Pérez-Fontalvo NM, De Arco-Aragón MA, Jimenez-García JDC, Lozada-Martinez ID. Molecular and computational research in low- and middle-income countries: Development is close at hand. J Taibah Univ Med Sci. 2021; 16(6):948-949. doi: 10.1016/j.jtumed.2021.06.010

ARCHIVOS DE NEUROCIENCIAS | Article Original

received 2 September 2022 | accepted 4 January 2023| published 12 January 2023

Doi: 10.31157/an.v28i2.413

NEUTROPHIL-LYMPHOCYTE AND LEUKO-GLYCEMIC RATIOS AS PREDICTIVE MARKERS FOR VENTILATORY SUPPORT IN PATIENTS WITH GUILLAIN-BARRE SYNDROME

Vargas-Cañas Edwin Steven[1] | Hernández-Sánchez Miriam Marcela[2] | Galnares-Olalde Javier Andrés[1] | Jorge de Saráchaga Adib[1] | Bazán-Rodríguez Ana Lissette[1] | May-Mas Raúl Nathanael[1] López-Hernández Juan Carlos[1]

1. Department of Neuromuscular Diseases, Instituto Nacional de Neurología Manuel Velasco Suárez
2. Department of Neurology, Instituto Nacional de Neurología Manuel Velasco Suárez.

Contact

Juan Carlos López Hernández

juanca9684@hotmail.com

Abstract

Background: Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis worldwide. Serum markers such as the neutrophil-lymphocyte (NLR) and leuko-glycemic (LGR) ratios have been studied for the severity and prognosis of non-neurological and neurological disorders.
Methods: Cross-sectional study from a prospective cohort of patients with GBS. A comparison of clinical and paraclinical variables between patients with and without ventilatory support was performed, as well as logistic regression analysis.
Results: 123 patients were included; mean age 45.5±16.5 years, 77 (62.6%) were men and 37 (30%) required ventilatory support. A greater age (51.7±18.2 *vs* 42.9±15.1, $p=0.006$), cranial nerve involvement (75.6% *vs* 40.6%, $p<0.001$), dysautonomia (67.5% *vs* 8.1%, $p<0.001$), median EGRIS score (IQR 2-4) *vs* 5 (IQR 4-6), $p<0.001$], median NLR [6.15 (IQR 4.18-9.23) *vs* 3.1 (IQR 2.21-4.08), $p<0.001$] and median LGR [1.58(IQR 0.99-1.99) *vs* 1.02(IQR 8.85-1.32), $p<0.001$] was observed at admission in patients that needed ventilatory support. The multivariable logistic regression analysis demonstrated that the presence of dysautonomia [OR 30.6 (95% CI 6.9-134), <0.001], a higher score on the EGRIS scale [OR 2.0 (95% CI 1.3-3.1), $p=0.001$], and higher NLR [OR 8.6 (95% CI 2.0-36.7), $p=0.004$] are independent risk factors for invasive mechanical ventilation. LGR and NLR demonstrated high performance for ventilatory support prediction, with 0.70 and 0.81, respectively.
Conclusions: the presence of dysautonomia and increased NLR are independent risk factors for invasive mechanical ventilation in patients with Guillain-Barre syndrome.

Keywords: *Guillain-Barre syndrome, invasive mechanical ventilation, risk factors, neutrophil-lymphocyte ratio, leuko-glycemic ratio*

Introduction

Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis worldwide. Twenty percent of patients become seriously disabled and up to 5% die. About 30% of GBS patients become ventilator dependent, with an increased risk of death, associated complications, and worse clinical outcomes.[1,2]

Scores such as Erasmus GBS Respiratory Insufficiency Score (EGRIS) use clinical data including days from symptom onset and admission, facial or/and bulbar weakness, and Medical Research Council (MRC) sum score to predict respiratory insufficiency within the first week of admission. Additionally, other tools can be used to evaluate pulmonary function (e.g., forced vital capacity and forced expiratory volume in 1s), although special equipment might be required.[3,4]

Several studies have supported the role of molecular mimicry in disease pathogenesis, by examining a causal relationship between *C. jejuni* lipo-oligosaccharides and gangliosides. Lymphocytes and neutrophils are associated with systemic inflammation and production of proinflammatory mediators. GBS, as an autoimmune disease, elicits an upregulation in inflammatory and metabolic pathways, with increased

production of lymphocytes and neutrophils. Serum markers such as the neutrophil-lymphocyte (NLR) and leuko-glycemic (LGR) ratios have been studied for the severity and prognosis of non-neurological disorders such as myocardial infarction, cancer, and autoimmune diseases.[5,6,7] Regarding neurological disorders, little information is available, with some reports in patients with multiple sclerosis and cerebrovascular disease.[8,9] Scarce data has been published on the use of these indices and autoimmune peripheral neuropathies. This inflammatory cascade can appear before GBS clinical symptoms.[10] In this study, we evaluate NLR and LGR in patients with GBS and their relationship as predictive markers for ventilatory support.

Materials and methods

A cross-sectional study from a prospective cohort of patients with GBS was conducted, from January 2018 to February 2021. GBS diagnosis was made by the National Institute of Neurological Disorders and Stroke (NINDS)[11] and all patients had complete blood work on admission to the emergency room (CBC, basic metabolic panel, electrolytes, and liver function tests). Exclusion criteria included patients with diabetes mellitus, previous autoimmune disorders, drug-induced immunosuppression, or HIV infection. Patients with acute bacterial infection (pneumonia, urinary tract infection, etc.) or sepsis on admission, based on the Third International Consensus Definition for Sepsis and Septic Shock, were also excluded.[12] General demographic data was obtained, as well as a history of previous infection, symptom-to-admission (GBS-associated symptoms to ER arrival), assessment of muscle strength based on Medical Research Council (MRC) on admission, GBS disability score (GDS) on admission, cranial nerve involvement, autonomic dysfunction, mechanical ventilation (IMV) and length of stay. Nerve conduction studies were performed on admission and the electrophysiological damage mechanism was classified according to Hadden et al. criteria.[13] Cerebrospinal fluid (CSF) glucose, protein concentration, and cell count were evaluated. Albuminocytological dissociation was defined as elevated CSF proteins (>45mg/dL) with low cell count (≤ 50 cells/μL). For each patient, the leuko-glycemic ratio [glycemia (mg/dl) x leukocytes (106/L) /1,000] and the neutrophil-lymphocyte ratio were calculated by neutrophil count divided by lymphocyte count.

The study was approved by the Ethics Committee and the Institutional Review Board. Signed consent was required for study participation.

Statistical analysis

Demographic data were analyzed with descriptive statistics. Kolmogorov-Smirnov test was used for distribution, and medians with standard deviation or medians with interquartile range were obtained, accordingly. A correlation analysis with the Person test was performed. Comparison between patients with and without ventilatory support was performed with student´s t-test or Mann-Whitney U test based on distribution. The chi-square test was used for categorical variables, and Fisher´s exact test was applied when necessary.

A logistic regression analysis was performed for patients with mechanical support, according to TRIPOD consensus.[14] Variables included age, dysautonomia, EGRIS score, neutrophil-lymphocyte (NLR), and leuko-glycemic (LGR) ratios. Goodness-of-fit was assessed with Hosmer & Lemeshow test, results were reported in odds ratio with 95% confidence intervals, and the performance of the model was assessed through analysis of area under the curve. Both NLR and LGR performances were obtained with an area under the curve analysis, cut-off values, sensitivity, and specificity were established by the Youden index, and a value of $p<0.05$ was considered statistically significant. Data were analyzed using SPSS version 22.

Results

Of 174 patients with GBS, 51 patients were excluded (Figure 1), leaving 123 patients for study analysis. The mean age was 45.5±16.5 years, 77 (62.6%) were men and 37 (30.0%) required ventilatory support. In patients with mechanical ventilation, the mean age was 51.7±18.2 years, and the median time from symptom onset to ventilation was 5 days (IQR 3-5). Moreover, 75.6% had cranial nerve involvement (facial and/or bulbar), a mean MRC sum score of 22.2±16.9, median EGRIS score of 4 (IQR 2-5), median LGR of 1.58 (IQR 0.99-1.99) and median NLR of 6.15 (IQR 4.18-9.23). Baseline demographics patients are shown in Table 1.

The distribution of leukocyte, lymphocyte, neutrophil, and serum glucose counts at admission with respect to the age of the population is shown in Figure 2. In the analysis of correlation between age (years) and the other variables (leukocyte count, lymphocytes, neutrophils, and serum glucose levels) there were no statistically significant results.

When comparing both groups, a greater age (51.7±18.2 *vs* 42.9±15.1, $p=0.006$), cranial nerve involvement (75.6% *vs* 40.6%, $p<0.001$), dysautonomia (67.5% *vs* 8.1%, $p<0.001$),

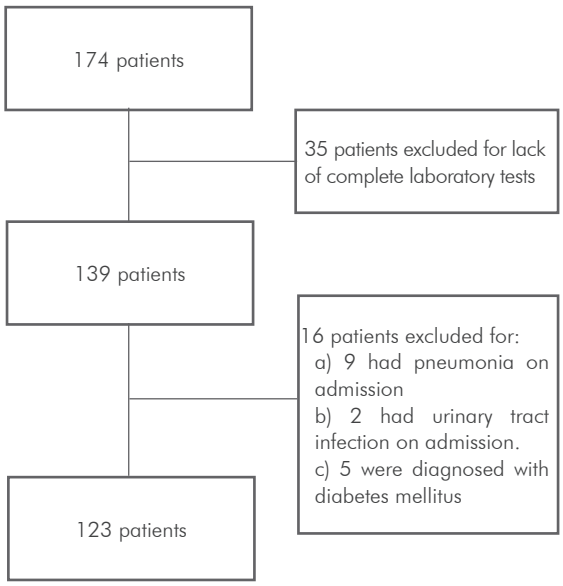


Figure 1. Flow diagram of patient inclusion and exclusion

Table 1. Comparative analysis between GBS patients with invasive ventilation mechanical (IVM) *vs* no-IVM

	Patients requiring ventilatory support n=37	**Patients not requiring ventilatory support n=86**	**P value**
Age – yr, mean	51.7±18.2	42.9±15.1	0.006
Male gender - no. (%)	20(54)	57(66.2)	0.22
Diarrhea, no. (%)	13(35.1)	30(34.8)	>0.99
Cranial nerve involvement – no. (%)	28 (**75.6**)	35 (**40.6**)	<0.001
-Facial nerve-	24(64.8)	34(39.5)	0.011
-Bulbar nerves	25(67.5)	21(24.4)	<0.001
Dysautonomia, no (%)	25(67.5)	7(8.1)	<0.001
GDS score, median (IQR)	4(1-4)	4(2-5)	<0.001
MRC score on admission, mean (SD)	22.2±16.9	35.2 ±16.1	<0.001
EGRIS score, median (IQR)	3(2-4)	5(4-6)	<0.001
Hospital stay (days), median (IQR)	58(31-83)	7(5-10.5)	<0.001
Protein count in LCR (mg/dl), median (IQR)	39(27-100)	446(32-60)	0.83
Leucocyte (103/ml), median, (IQR)	12.51(9.12-16.58)	9.75(8.20-11.72)	0.002
Neutrophil (103/ml), median, (IQR)	8.3 (5.85-11.30)	6.40(5.45-8.10)	<0.001
Lymphocyte (103/ml), median, (IQR)	1.60(1.15-1.90)	2.20(1.60-2.60)	<0.001
Glucose (mg/dl), median (IQR)	122(110-141)	106(95.5-118)	<0.001
Sodium (mEq/dl), median (IQR)	138(135-140)	138(137-140)	0.75
Albumin (g/dl), mean SD	4.28±0.52	4.45±0.46	0.089
NLR, median, (IQR)	6.15(4.18-9.23)	3.1(2.21-4.08)	<0.001
LGR, median, (IQR)	1.58(0.99-1.99)	1.02(8.85-1.32)	<0.001

MRC score (22.2±16.9 *vs* 35.2±16.1, *p<0.001*), and median EGRIS score [3 (IQR 2-4) *vs* 5 (IQR 4-6), *p<0.001*] was observed at admission in patients that needed ventilatory support. No statistically significant differences were encountered neither for demyelinating (38.7% *vs* 42.4%, *p=0.82*) nor axonal subtype (61.2% *vs* 50.6%, *p=0.39*). Both median NLR [6.15 (IQR 4.18-9.23) *vs* 3.1 (IQR 2.21-4.08), *p<0.001*] and median LGR [1.58 (IQR 0.99-1.99) *vs* 1.02 (IQR 8.85-1.32), *p<0.001*] in patients with ventilatory support were significant.

The multivariable logistic regression analysis demonstrated that the presence of dysautonomia [OR 30.6 (95% CI 6.9-134), p=<0.001], a higher score on the EGRIS scale [OR 2.0 (95% CI 1.3-3.1), p=0.001], and higher NLR [OR 8.6 (95% CI 2.0-36.7), p=0.004] are independent risk factors for invasive mechanical ventilation; the model performance is AUC 0.938, 95% CI (0.89-0.98), p= <0.001 (Table 2).

Both LGR and NLR demonstrated high performance for ventilatory support prediction, with 0.70 [95% CI (0.59-0.81), p<0.001] and 0.81 [95% CI (0.72-0.89), p<0.001], respectively. The best cut-off values, according to the Youden index, are 1.12 for LGR (sensitivity 0.70, specificity 0.40) and 3.59 for NLR (sensitivity 0.78, specificity 0.33). The operating characteristic curve analysis is shown in Figure 3.

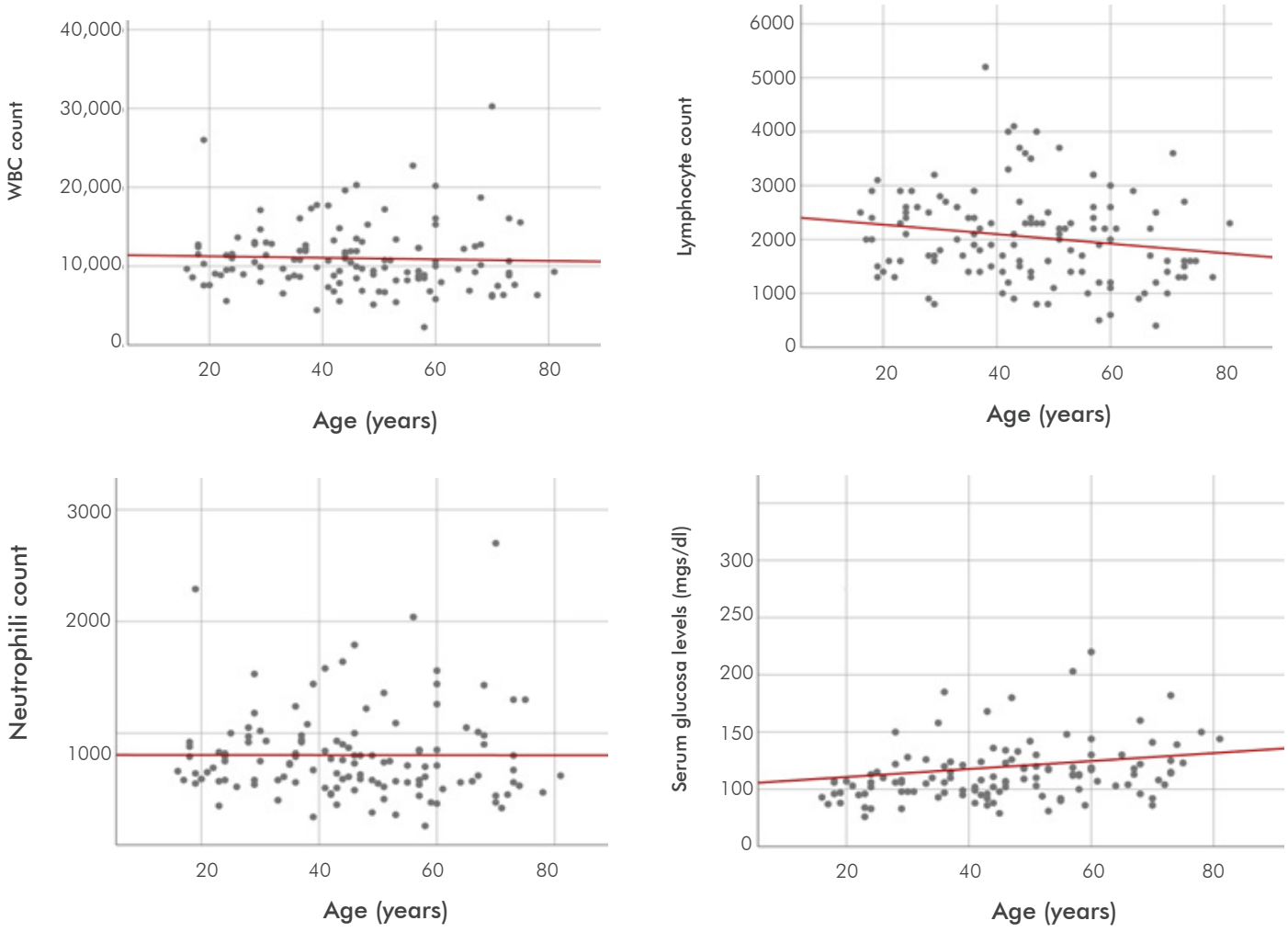


Figure 2. Count of leukocytes, lymphocytes, and serum glucose levels at admission with respect to population age

Table 2. Multivariable analysis for ventilatory support in patients with GBS

	Univariable model					Multivariable model	
	Patients requiring ventilatory support n=37	Patients not requiring ventilatory support n=86	P value	OR (IC 95%)		OR (IC 95%)	P value
Age - yr	51.7±18.2	42.9± 15.1	0.006	1.03(1.0-1.06)		1.06(1.0-1.1)	0.003
Symptom-to-admission -median (days) (IQR)	5(3-5)	6(4-10)	0.003	0.87(0.78-0.98			
Cranial nerve involvement – no. (%).	28(75.6)	35(40.6)	<.001	4.5(1.9-10.7)			
MRC score - mean (SD)	22.2±16.9	35.2 ±16.1	<0.001	0.95(0.92-0.97)			
Dysautonomia, no (%)	25(67.5)	7(8.1)	<0.001	23(8.3-66)		30.6(6.9-134)	<0.001
EGRIS score, median (IQR)	3(2-4)	5(4-6)	<0.001	1.9(1.4-2.4)		2.0(1.3-3.1)	0.001
LGR (%)	1.58(0.99-1.99)	1.02(8.85-1.32)	<.001	3.0(1.6-5.5)		0.7 (0.2-2.5))	0.58
NLR (%)	6.15(4.18-9.23)	3.1(2.21-4.08)	<.001	4.0 (2.0-8.19)		8.6(2.0-36.7)	0.004
Logistic regression description: Overall model fit: chi-square 81.123, df 5, p< 0.0001. Goodness-of-fit test: r2 = 0.483; Hosmer & Lemeshow test, chi-square, 12.47, df 8, p= 0.13. Model performance: AUC 0.938, 95% CI (0.89-0.98), p= <0.001.							

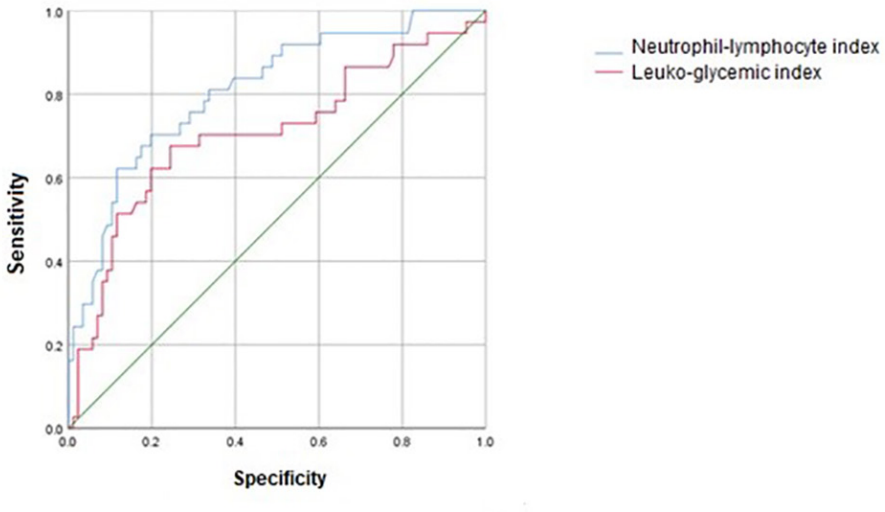


Figure 3. ROC curves for Neutrophil-lymphocyte index and Leuko-glycemic index for ventilatory support prediction

Discussion

Guillain-Barre Syndrome (GBS) is an autoimmune disorder where antibodies are abnormally produced against peripheral nerve gangliosides. Cellular responses potentially play a role in the pathogenesis of GBS and complement-mediated nerve injury. Experimental allergic neuritis models have encountered several pro-inflammatory molecules related to acute nerve injury, such as IFN-γ, IL-1β, tumor necrosis factor (TNF), IL-6, and IL-10. All these inflammatory processes occur in the pre-symptomatic stages of patients with GBS and further portray worse clinical presentations with early ventilatory support requirements.[15]

Classically, old age has been considered a risk factor for ventilatory support and inability to walk independently at 1, 3, and 6 months.[1] Despite decreased immunological responses in the elderly, as described previously by Hagen et al. this subset of patients has increased leukocyte, neutrophil, and lymphocyte counts when compared to younger patients with GBS, and they should be treated as high-risk patients for several complications and worse functional outcomes.[15]

The EGRIS scale, developed in European and North American populations, uses only clinical variables (MRC score, cranial/bulbar weakness, and days between onset and hospital admission) to predict respiratory insufficiency and ventilatory support within the first week of admission.[3] Early involvement of bulbar muscle strength can cause microaspiration, hence the large population of GBS patients with a high EGRIS score with pneumonia upon admission.[16] In these cases, it is difficult to distinguish if the inflammatory process is due to sepsis associated with aspiration pneumonia or GBS inflammatory process. In our study, patients who presented some infectious process (pneumonia) on admission were excluded. We observed that high NLR is a risk factor, independent of the EGRIS scale score, for ventilation requirement; to our knowledge, this data had not been previously reported.

Consequently, we consider that the elevation of NLR in patients with GBS who require VMI is due to the systemic immune response of the disease, as in the case of other neurological autoimmune diseases (multiple sclerosis).[9]

We observed that the presence of dysautonomia is also a risk factor for the requirement of invasive mechanical ventilation, independent of the EGRIS scale score, which has not been previously reported. Cardiovascular dysautonomia is due to injury of the thoracic medullary roots exit that form the paraganglionic chains of the sympathetic autonomic system. The diaphragmatic weakness that occurs in patients with SGB requiring VMI, in part, is attributable to damage of the phrenic nerves in their exit from the cervical roots. Therefore, we theorize that both the presence of dysautonomia and diaphragmatic weakness is due to severe root damage in GBS.[3]

The neutrophil-lymphocyte ratio is an indicator of systemic inflammation and has been described as a marker of severity and short overall survival in subjects with acute respiratory distress syndrome (ARDS). Wang et al. demonstrated an increase in in-hospital, 28-day, and 90-day mortality in patients with ARDS with a NLR of >14: 57.8% *vs* 43.2%, 58.9% *vs* 41.1,% and 58.0% *vs* 42.0%, respectively.[17] Furthermore, inflammatory diseases have higher NLR, as recently described in a meta-analysis of 1550 patients with rheumatoid arthritis.[6] Fewer evidence exists on its utility for neurological disorders. However, a higher NLR (classified by terciles and adjusted for clinical and laboratory values) was associated with unfavorable outcomes in terms of mRS score after acute ischemic stroke.[18] Additionally, higher NLR values have been observed in patients with multiple sclerosis when compared to healthy controls.[9]

Scarce information is available on the usefulness of NLR as an indicator in autoimmune peripheral nerve disorders, such as GBS. One study reported a higher NLR in subjects with acute inflammatory demyelinating polyneuropathy (AIDP), but we did not find any association with electrophysiological subtypes.[19]

Other reports have associated this ratio with severe presentations of GBS, for example, Huang et al. reported a higher level of NLR in subjects with GDS ≥3 and a cut-off value of 2.295 for GBS occurrence and 3.05 for severity.20 Moreover, Pingping N et al. reported several ratios as risk factors for IMV in patients with GBS, particularly NLR, with an OR of 3.319 and a cut-off value of >3.5 for mechanical ventilation.[21] We obtained a similar cut-off value of 3.59 with a sensibility of 78% and a specificity of 33%.

High serum glucose in a patient requiring ventilatory support due to any disease is considered a marker of severity.[22] We observed higher serum glucose levels on admission in GBS patients with ventilatory support compared to those without, as demonstrated in other series.[23] The increase in serum glucose levels might be directly related to the metabolic response to stress in acute inflammatory processes. On the other hand, an interesting fact is the high prevalence of autonomic dysfunction in severe GBS cases and in patients with mechanical support,[23] where cortisol-induced glucose increment can be produced by adrenergic dysregulations.[24]

Although LGR has been extensively studied in acute myocardial infarction and relates to severity,[25] few reports have demonstrated its correlation with severity in acute ischemic stroke.[26] We observed a statistically significant OR in the univariable analysis for LGR but not in the multivariable analysis. To our knowledge, this is the first report of LGR in subjects with GBS. Further studies are needed to confirm the role of these indexes in other circumstances.

Conclusion

The presence of dysautonomia and increased NLR are independent risk factors for invasive mechanical ventilation in patients with Guillain-Barre syndrome.

References

1. López-Hernández JC, Colunga-Lozano LE, Garcia-Trejo S, Gomez-Figueroa E, et al. Electrophysiological subtypes and associated prognosis factors of Mexican adults diagnosed with Guillain-Barré syndrome, a single center experience. J Clin Neurosci. 2020, 80:292-7. doi: 10.1016/j.jocn.2020.04.059
2. van den Berg B, Storm EF, Garssen MJP, Blomkwist-Markens PH, Jacobs BC. Clinical outcome of Guillain-Barré syndrome after prolonged mechanical ventilation. J Neurol Neurosurg Psychiatry. 2018 Sep;89(9):949-54. doi: 10.1136/jnnp-2018-317968
3. López-Hernández JC, Jorge de Saráchaga A, Briseño-Godínez ME, May-Mas RN, Bazán-Rodríguez L, León-Manriquez E, et al. Deltoid muscle strength and autonomic dysfunction as independent risk factors for invasive mechanical ventilation in patients with Guillain-Barré syndrome. Int J Neurosci. 2022; 27:1-13. doi: 10.1080/00207454.2022.2082963
4. Lawn ND, Fletcher DD, Henderson RD, Wolter TD, Wijdicks EF. Anticipating mechanical ventilation in Guillain-Barré syndrome. Arch Neurol. 2001 Jun;58(6):893-8. doi: 10.1001/archneur.58.6.893
5. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014 May 29;106(6):dju124. doi: 10.1093/jnci/dju124
6. Erre GL, Paliogiannis P, Castagna F, Mangoni AA, Carru C, Passiu G, et al. Meta-analysis of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in rheumatoid arthritis. Eur J Clin Invest. 2019; 49(1):e13037. doi: 10.1111/eci.13037
7. Dong CH, Wang ZM, Chen SY. Neutrophil to lymphocyte ratio predict mortality and major adverse cardiac events in acute coronary syndrome: A systematic review and meta-analysis. Clin Biochem. 2018; 52:131-6. doi: 10.1016/j.clinbiochem.2017.11.008
8. Giede-Jeppe A, Bobinger T, Gerner ST, Sembill JA, Sprügel MI, Beuscher VD, et al. Neutrophil-to-lymphocyte ratio is an independent predictor for in-hospital mortality in spontaneous intracerebral hemorrhage. Cerebrovasc Dis. 2017;44(1-2):26-34. doi: 10.1159/000468996
9. Demirci S, Demirci S, Kutluhan S, Koyuncuoglu HR, Yurekli VA. The clinical significance of the neutrophil-to-lymphocyte ratio in multiple sclerosis. Int J Neurosci. 2016; 126(8):700-6. doi: 10.3109/00207454.2015.1050492
10. Martini R, Willison H. Neuroinflammation in the peripheral nerve: Cause, modulator, or bystander in peripheral neuropathies? Glia. 2016 Apr;64(4):475-86. doi: 10.1002/glia.22899
11. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. Ann Neurol. 1990; 27 Suppl:S21-4.
12. M. Singer, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016; 315(8):801-10. doi: 10.1001/jama.2016.0287
13. Hadden RD, Cornblath DR, Hughes RA, Zielasek J, Hartung HP, Toyka KV, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Ann Neurol. 1998; 44(5):780-8.
14. Moons KG, Altman DG, Reitsma JB, Ioannidis JP, Macaskill P, Steyerberg EW, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015;162(1):W1-73. doi: 10.7326/M14-0698
15. Hagen KM, Ousman SS. The neuroimmunology of Guillain-Barré Syndrome and the potential role of an aging immune system. Front Aging Neurosci. 2021 Jan 13;12:613628. doi: 10.3389/fnagi.2020.613628
16. Orlikowski D, Sharshar T, Porcher R, Annane D, Raphael JC, Clair B. Prognosis and risk factors of early onset pneumonia in ventilated patients with Guillain-Barré syndrome. Intensive Care Med. 2006;32(12):1962-9.
17. Wang Y, Ju M, Chen C, Yang D, Hou D, Tang X, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in acute respiratory distress syndrome patients: a retrospective study. J Thorac Dis. 2018;10(1):273-82. doi: 10.21037/jtd.2017.12.131
18. Yu S, Arima H, Bertmar C, Clarke S, Herkes G, Krause M. Neutrophil to lymphocyte ratio and early clinical outcomes in patients with acute ischemic stroke. J Neurol Sci. 2018; 387:115-8. doi: 10.1016/j.jns.2018.02.002
19. Ozdemir HH. Analysis of the albumin level, neutrophil-lymphocyte ratio, and platelet-lymphocyte ratio in Guillain-Barré syndrome. Arq Neuropsiquiatr. 2016 Sep;74(9):718-22. doi: 10.1590/0004-282X20160132
20. Huang Y, Ying Z, Quan W, Xiang W, Xie D, Weng Y, et al. The clinical significance of neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio in Guillain-Barré syndrome. Int J Neurosci. 2018; 128(8):729-35. doi: 10.1080/00207454.2017.1418342
21. Ning P, Yang B, Yang X, Huang H, Shen Q, Zhao Q, et al. Lymphocyte-based ratios for predicting respiratory failure in Guillain-Barré syndrome. J Neuroimmunol. 2021; 353:577504. doi: 10.1016/j.jneuroim.2021.577504
22. Edriss H, Selvan K, Sigler M, Nugent K. Glucose levels in patients with acute respiratory failure requiring mechanical ventilation. J Intensive Care Med. 2017 Dec;32(10):578-84. doi: 10.1177/0885066616636013
23. Wang Y, Li G, Yang S, Gu X, Li X, Liu M, et al. Fasting glucose levels correlate with disease severity of Guillain-Barré syndrome. PLoS One. 2015 Dec 18;10(12):e0145075. doi: 10.1371/journal.pone.0145075
24. Zaeem Z, Siddiqi ZA, Zochodne DW. Autonomic involvement in Guillain-Barré syndrome: an update. Clin Auton Res. 2019; 29(3):289-99. doi: 10.1007/s10286-018-0542-y
25. León-Aliz E, Moreno-Martínez FL, Pérez-Fernández GA, Vega-Fleites LF, Rabassa-López-Calleja MA. Índice leuco-glucémico como marcador pronóstico de la evolución intrahospitalaria en pacientes con infarto agudo de miocardio con elevación del ST [Leuko-glycemic index as an in-hospital prognostic marker in patients with ST-segment elevation myocardial infarction]. Clin Investig Arterioscler. 2014; 26(4):168-75. doi: 10.1016/j.arteri.2014.01.002
26. Hernández-Sánchez MM, Lozano-Nuevo JJ, Suárez-Cuenca JA, Mendoza-Portillo E, Vergara-Orduña FI, Palacios-Pérez E, et al. Índice leuco-glucémico asociado con complicaciones en isquemia cerebral aterotrombótica. Med Int Méx. 2019; 35(1):39-44. doi: 10.24245/mim. v35i1.2180

recibido 2 noviembre 2022 | aceptado 9 febrero 2023 | publicado 13 febrero 2023

Doi: 10.31157/an.v28i2.436

EVALUACIÓN DE UNA BATERÍA DE ESTÍMULOS DE RECONOCIMIENTO EMOCIONAL AUDITIVO

Kreither Johanna[1,2] | Contreras Renata[1] | Cueto José[1]

1. Programa de Investigación Asociativa (PIA) en Ciencias Cognitivas, Centro de Investigación en Ciencias Cognitivas (CICC), Facultad de Psicología, Universidad de Talca, Chile.
2. Centro de Psicología Aplicada (CEPA), Facultad de Psicología, Universidad de Talca, Chile

Correspondencia

Dra. Johanna Kreither
Facultad de Psicología, Universidad de Talca, Campus Lircay. Avenida Lircay s/n, Talca, Chile. CP 3460000.

jkreither@utalca.cl

Resumen

Introducción: el reconocimiento emocional auditivo (REA) es la capacidad de reconocer estados emocionales en otros, poniendo énfasis en las características prosódicas, tales como tono, intensidad y frecuencia. La literatura sobre esta área es escasa y en la actualidad no hay baterías de estímulos de REA validadas en Latinoamérica.

Objetivo: evaluar una batería de estímulos de REA en una muestra de población adulta chilena.

Material y métodos: 140 adultos de entre 18 y 50 años de edad respondieron un formulario online que contenía la batería de estímulos auditivos y preguntas asociadas. Los estímulos fueron elaborados a través de un modulador sintético de acuerdo a los parámetros sugeridos en la literatura. Se solicitó a los participantes que asociaran los estímulos auditivos a estados emocionales.

Resultados: la característica F0M es clave para el reconocimiento de las emociones alegría y enojo, F0SD es importante para la emoción Tristeza y HF500 para la emoción Enojo; se encontraron diferencias significativas entre las medias de respuesta entre participantes que reportaron un trastorno psiquiátrico al momento del estudio y aquellos que no lo reportaron.

Conclusiones: varios de los estímulos fueron evaluados por los/las participantes como representativos de algún estado emocional en alta proporción. Se encontraron similitudes entre los resultados de este estudio y la literatura en cuanto a la importancia de la presencia/ausencia de HF500 en la identificación de las emociones Alegría, Tristeza y Enojo, así como de F0M en el caso de Alegría y F0SD en el de Alegría y Tristeza.

Palabras clave: *emoción, reconocimiento emocional auditivo. prosodia*.

Introducción

El reconocimiento emocional (RE) juega un papel fundamental en la cognición social, pues nos permite predecir el comportamiento de otros en nuestro entorno y, con esto, adaptarnos a las exigencias del contexto social.[1]

El reconocimiento emocional auditivo (REA) es la capacidad de interpretar componentes perceptivos del habla[2] e identificar estados emocionales a través de estas características prosódicas,[3] lo que resulta de gran relevancia puesto que proporciona mayor información para un entendimiento cabal del ambiente social y comunicativo.[4] El estudio respecto a este tipo de reconocimiento es limitado en comparación con el de tipo visual;[5] la mayoría de la investigación desarrollada se ha enfocado en una combinación de estímulos de distinta naturaleza.[6,7,3] La emoción es transmitida por variaciones en el tono de la voz, lo que conduce al proceso de reconocimiento emocional vocal receptivo (también llamado reconocimiento emocional auditivo); la interpretación de esta característica (el tono), permite a los individuos inferir el verdadero estado emocional del hablante, aunque el contenido verbal del discurso sea neutro.[8] La voz, debido a sus características de tono y amplitud, permite expresar emociones más complejas que la expresión facial, pues el hablante tiene la posibilidad de agregar intenciones con mayor eficacia.[4]

Se han identificado patrones relativamente distintivos de señales acústicas según emociones específicas: **1**) tono, el cual tiene los componentes de frecuencia fundamental media (F0M), variabilidad de la frecuencia fundamental (F0SD) y forma del contorno de la frecuencia fundamental (F0contour); **2**) intensidad, que se conforma de intensidad media de la

voz (VoiceintM), variabilidad de la intensidad de la voz (VoiceintSD) y tasa de aumento de la amplitud (ATTACK); **3**) calidad, compuesta por la proporción relativa de energía acústica por encima y por debajo de 500Hz (HF500) y ancho de banda del primer formante (F1BW), y **4**) temporalidad, establecido por la velocidad del habla (ritmo del habla) y cantidad de pausas en el habla (proporción de pausas).[2,8]

Actualmente, en Latinoamérica y en Chile no existen baterías ni estudios de validación de éstas sobre el REA. La literatura sugiere que las características vocales que expresan dichos estados emocionales pueden aproximarse mediante audios no vocales y de ondas sonoras puras, de la misma naturaleza que los utilizados en esta investigación. Así, se vuelve necesario conocer los parámetros exactos de estas características físicas y cómo los cambios en ellas afectan el reconocimiento de ciertas emociones en la población chilena.

Por lo tanto, se buscó evaluar una batería de estímulos de reconocimiento emocional auditivo (REA) generados sintéticamente en una población de adultos chilenos, estableciendo la tasa de respuesta y frecuencia de estímulos de REA según las características físicas asociadas a cada tipo de emoción.

Material y métodos

Muestra

Se trabajó con 140 adultos de nacionalidad chilena (104 mujeres), con edades entre los 18 y 50 años. Del total de participantes, 31 reportaron haber sido diagnosticados con algún trastorno psiquiátrico (esto fue señalado en las respuestas del cuestionario demográfico incluido en la encuesta online, que no consideraban verificación clínica, sólo el reporte de los/las participantes). Los/las participantes leyeron y validaron un consentimiento informado, en el cual se aceptaba la participación voluntaria en la tarea y la utilización de los datos en la investigación.

Diseño y procedimiento

Este proyecto fue revisado y aprobado por el Comité de Ética de la Facultad de Psicología de la Universidad de Talca.

Se desarrolló un cuestionario online a través de la plataforma SurveyMonkey, que incluyó el consentimiento informado, con su correspondiente aceptación o rechazo, un cuestionario demográfico y la secuencia de estímulos auditivos. El cuestionario continuaba solo para aquellos participantes que aceptaban participar en el estudio en la etapa de consentimiento informado.

Las instrucciones para la secuencia de estímulos auditivos se requirió el uso de audífonos y ajustar el volumen al 50%, un estímulo de prueba, y, por último, se presentaron, un estímulo a la vez, los 42 estímulos auditivos aleatorizados en su presentación entre los/las participantes. Cada participante debía reproducir cada sonido y responder a la pregunta "¿qué emoción está demostrando este sonido?", seleccionando una de 5 respuestas (alegría, tristeza, enojo, miedo o sin emoción).

Instrumento y Variables

a. **Cuestionario demográfico**. El cuestionario demográfico incluyó 14 ítems que abarcaban información autoreportada relacionada con la edad, sexo, nivel educacional, diagnósticos psiquiátricos, entre otros.

b. **Batería de estímulos auditivos**. La batería contó con 42 estímulos representativos de 4 emociones básicas (miedo, enojo, alegría y tristeza) y una expresión sin emoción o neutra; el tiempo aproximado de aplicación fue de 15 minutos.

En cuanto a los estímulos de REA, se trataron de archivos de audio, de 500 ms de duración, creados a través de un modulador sintético y de carácter no vocal, puesto que corresponden a un sonido modulado. Fueron creados por la Dra. Johanna Kreither, como parte del proyecto Fondecyt 11180961, mediante el Software Matlab. Los estímulos seguían las características que Juslin y Laukka[9] y Kantrowitz et al.[8] plantean para que el sonido represente una emoción en particular. Adicionalmente, las características operacionales de los estímulos auditivos se detallan a continuación: **1**) F0M: frecuencia fundamental media, su incremento varía la percepción de emociones, siendo sus niveles 125=bajo, 225=medio y 378=alto. Este tono modulado es típicamente percibido como "alegría" (etiqueta "Alegría 378/125"); **2**) F0SD: variabilidad de la frecuencia fundamental, su fluctuación en la altura varía la percepción, siendo sus niveles "bajos" 20, 40 y 60, "medio" 80 y 125 y "alto" 150 y 175. Este tono modulado es típicamente percibido como "tristeza" (etiqueta "Tristeza 125/20"); **3**) HF500: ruido de alta energía que se sobrepone al tono estándar actuando como interferencia sin afectar la frecuencia fundamental ni la variabilidad de la altura. Este sonido es típicamente percibido como "enojo" o "miedo" (etiqueta "Enojo/Miedo HF500"). Dicho parámetro estuvo presente (Si) o ausente (No) en los audios, lo que dividió los estímulos en dos grupos. Estas características se encuentran resumidas en la Tabla 1.

Por último, se realizó un apartado luego de cada estímulo, que refería a la emoción que representaba el ítem según el participante.

Tabla 1. Resumen simplificado de los patrones de señales del sonido, específicos para determinadas emociones básicas

Características	Enojo	Miedo	Felicidad	Tristeza
Tono F0 M	Alto	Medio	Alto	Bajo
F0 SD	Alto	Bajo	Alto	Bajo
F0 cont	Medio	Alto	Alto	Bajo
Intensidad				
Int M	Alta	Baja	Alta	Baja
Int SD	Grande	Grande	Grande	Pequeña
Ataque	Alto	Bajo	Medio	Medio
Calidad				
F1 BW	Bajo	Alto	Alto	Alto
HF 500	Alto	Bajo	Alto	Bajo
Señales Temporales				
Velocidad del sonido	Medio	Alto	Medio	Medio
Proporción de la pausa	Bajo	Medio	Medio	Alto

Nota. Adaptación del resumen aparecido en "Impact of intended emotion intensity on cue utilization and decoding accuracy in vocal expression of emotion", de Juslin y Laukka, 2001.[9]

Plan de Análisis

El análisis estadístico de los datos se realizó mediante los softwares SPSS versión 26 y Excel de Microsoft 365.

a. **Plan de análisis para frecuencias por estímulo**. Con el objetivo de mostrar cuántas respuestas se obtuvieron para cada estímulo auditivo y por cada emoción, se dividieron los estímulos en dos grupos: del 100 al 120, que incluía los estímulos auditivos sin ruido de fondo (HF500), y del 200 al 220, que sí lo presentaban. Luego de esto, se calcularon los porcentajes de frecuencia de las emociones asociadas a cada estímulo.
b. **Plan de análisis para determinar diferencias según reporte de trastorno psiquiátrico**. Para identificar posibles variables intervinientes en el proceso de REA, se compararon las medias de frecuencia respecto a cada emoción según la presencia o ausencia de un trastorno psiquiátrico al momento de la realización del cuestionario. Para ello se llevó a cabo la prueba T de Student para muestras independientes.

Resultados

Resultados de análisis para frecuencias por estímulo. El porcentaje de respuestas de los estímulos del 100 al 120 se presentan en la Tabla 2. Se encontraron estímulos que obtuvieron frecuencias altas (sobre 35%) asociados a Alegría, Tristeza y Sin Emoción.

Tabla 2. Resumen de porcentaje de respuestas por estímulo del 100 al 120

Estímulo	Características		Porcentajes de respuestas (%)				
	F0M/F0SD (Hz)	HF500	Alegría	Enojo	Miedo	Sin Emoción	Tristeza
100	125/20	No	2,9	10,7	20,7	26,4	**39,3**
101	125/40	No	5,0	15,0	20,0	33,6	26,4
102	125/60	No	5,7	17,9	22,9	30,0	23,6
103	125/80	No	7,1	17,1	17,1	34,3	24,3
104	125/125	No	7,1	11,4	29,3	28,6	23,6
105	125/150	No	13,6	9,3	20,7	**38,6**	17,9
106	125/175	No	16,4	16,4	19,3	30,0	17,9
107	225/20	No	5,0	1,4	20,7	15,7	**57,1**
108	225/40	No	13,6	5,0	14,3	21,4	**45,7**
109	225/60	No	12,9	5,7	20,7	21,4	**39,3**
110	225/80	No	15,0	7,1	14,3	34,3	29,3
111	225/125	No	22,1	10,0	16,4	27,9	23,6
112	225/150	No	30,7	10,7	13,6	25,7	19,3
113	225/175	No	30,7	12,1	14,3	30,0	12,9
114	378/20	No	17,9	0,7	14,3	12,1	**55,0**
115	378/40	No	22,9	5,7	17,9	13,6	**40,0**
116	378/60	No	33,6	5,0	14,3	18,6	28,6
117	378/80	No	**39,3**	5,7	12,9	17,1	25,0
118	378/125	No	**45,0**	5,7	17,1	18,6	13,6
119	378/150	No	**56,4**	7,9	7,9	18,6	9,3
120	378/175	No	**62,1**	7,9	10,0	11,4	8,6

Nota. Se han destacado en los porcentajes de respuestas por emoción los valores > 35%. Las características operacionales y moduladas son F0M: frecuencia fundamental, F0SD: altura de la frecuencia y HF500: ruido de alta energía.

Los estímulos del 200 al 220 mostraron porcentajes de respuesta altos (> 35%) para las emociones Enojo y Alegría (Tabla 3).

Resultados de análisis para determinar diferencias según reporte de trastorno psiquiátrico

Se obtuvieron diferencias significativas en la emoción Miedo ($p < 0,01$) entre quienes sí reportaron un trastorno psiquiátrico ($M=11,42$; $SD=5,94$) y aquellos que no lo reportaron ($M=8,52$; $SD=5,15$), al igual que en la emoción Tristeza ($p < 0,05$), entre los participantes que reportaron un trastorno psiquiátrico ($M=10$; $SD=5,61$) y quienes no ($M=7,93$; $SD=4,44$). Algo similar ocurrió en el caso de Sin Emoción: el grupo de quienes reportaron un trastorno psiquiátrico ($M = 5,26$; $SD = 5,26$) y el grupo que no reportó un trastorno psiquiátrico ($M=10,37$; $SD=11,12$) presentaron diferencias significativas ($p < 0,01$).

Tabla 3. Resumen de porcentaje de respuestas por estímulo del 200 al 220

Estímulo	Características		Porcentaje de respuestas (%)				
	F0M/F0SD (Hz)	HF500	Alegría	Enojo	Miedo	Sin Emoción	Tristeza
200	125/20	Sí	2,9	**38,6**	32,9	17,9	**7,9**
201	125/40	Sí	0,7	**45,0**	27,1	21,4	5,7
202	125/60	Sí	2,9	**40,7**	31,4	15,0	10,0
203	125/80	Sí	3,6	**40,7**	30,0	20,0	5,7
204	125/125	Sí	3,6	**38,6**	30,0	17,9	10,0
205	125/150	Sí	7,1	32,1	22,9	**25,7**	12,1
206	125/175	Sí	2,1	**35,7**	30,7	25,7	5,7
207	225/20	Sí	3,6	18,6	32,9	13,6	**31,4**
208	225/40	Sí	4,3	25,7	27,9	20,0	**22,1**
209	225/60	Sí	7,1	34,3	23,6	22,9	**12,1**
210	225/80	Sí	13,6	31,4	22,1	23,6	9,3
211	225/125	Sí	10,7	27,1	25,7	27,1	9,3
212	225/150	Sí	13,6	27,1	25,7	25,0	8,6
213	225/175	Sí	12,1	**35,0**	23,6	25,7	3,6
214	378/20	Sí	8,6	9,3	33,6	15,0	**33,6**
215	378/40	Sí	17,1	13,6	28,6	15,7	**25,0**
216	378/60	Sí	20,7	22,1	21,4	16,4	19,3
217	378/80	Sí	**19,3**	15,7	27,9	23,6	13,6
218	378/125	Sí	**35,0**	24,3	19,3	15,7	5,7
219	378/150	Sí	**37,1**	22,9	23,6	13,6	2,9
220	378/175	Sí	**35,7**	27,9	17,1	14,3	5,0

Nota. Se han destacado en los porcentajes de respuestas por emoción los valores > 35%. Las características operacionales y moduladas son F0M: frecuencia fundamental, F0SD: altura de la frecuencia y HF500: ruido de alta energía.

Discusión

Esta investigación utilizó estímulos sintéticos con variaciones en tono (F0M/F0SD) y calidad (HF500), lo cual permitió evaluar los efectos de la variabilidad de esos tres componentes físicos en el reconocimiento emocional. Esto potencialmente permite, como fue señalado por Kantrowitz et al.,[8] el uso de la misma batería de estímulos en poblaciones interculturales sin las barreras del lenguaje, la adaptación y traducción. Por tanto, fue posible valorar cómo la información de la literatura expuesta y los resultados de estudios anteriores se replicaban en población chilena.

En cuanto a las características físicas de los estímulos utilizados, tono se compone de frecuencia fundamental (F0M) y variabilidad de la frecuencia fundamental (F0SD). Respecto a los resultados de esta investigación, se evidenció que el nivel de la frecuencia fundamental (bajo=125: medio=225; alto=378) tiene un papel clave en el porcentaje de identificación de emociones como Alegría y Enojo; en estímulos con niveles altos de F0M se reconoce Alegría en mayor medida, mientras que en estímulos con niveles bajos de F0M se identifica más Enojo. En relación con la variabilidad de la frecuencia fundamental, se encontró que esta característica determina el porcentaje de precisión para la identificación de Alegría, esto es, hay un mayor porcentaje de identificación de esta emoción cuando aumenta la variabilidad, lo que se aprecia en el aumento en los estímulos 114 al 120 (Ver Tabla 2). En el caso de Tristeza, dicha variabilidad cumple principalmente un rol significativo en su reconocimiento, aunque también juega un papel en la precisión, lo que se evidencia en los estímulos mejor identificados, que tuvieron las características 225/20, 378/20 y 225/40, demostrándose que F0SD es el parámetro que permite el reconocimiento de esta emoción, según los resultados de los estímulos 107, 114 y 108. Con respecto a Alegría, esta característica debe ser mayor a 60 Hz (medio=80 - 125; alto=150 - 175) y su aumento es directamente proporcional con el porcentaje de reconocimiento, por ejemplo, el estímulo con parámetros 378/80 tuvo un menor porcentaje que el estímulo con características 378/175. En contraste, para el reconocimiento de Tristeza, F0SD tiene que encontrarse en niveles bajos (20 - 40 - 60), incluso cuando la característica F0M se encuentre en nivel alto o medio, lo cual amplía los hallazgos de los estudios de Juslin y Laukka[9] y Kantrowitz et al.,[8] quienes proponen que los parámetros para reconocer Tristeza deben ser F0M bajo y F0SD bajo. Cabe mencionar que, si bien el estímulo "estereotípico" de Tristeza (125/20) es identificado en este estudio como tal emoción, el porcentaje de respuesta es de los más bajos, lo que supone que su reconocimiento está más ligado a la característica F0SD que al nivel de F0M.

En cuanto a calidad (HF500), su presencia en los estímulos, según la literatura, está ligada al aumento de la percepción de Enojo y a la disminución con respecto a Tristeza[8], lo que se condice con lo encontrado en este estudio, ya que el primer set de estímulos, que no presentó el ruido HF500, no mostró porcentajes significativos de la percepción de Enojo, a diferencia del segundo set, que sí lo presentó y en el que aumentó la identificación de esta emoción, al tiempo que fueron eliminaron porcentajes significativos en la percepción de Tristeza. En relación con la emoción Alegría, ésta fue identificada transversalmente en ambos sets de estímulos, pero su reconocimiento se redujo en el grupo que contenía el ruido HF500, contrario a lo postulado por Juslin y Laukka.[9]

Era esperable que no se obtuvieran percepciones significativas (>35%) para Miedo, ya que el estudio de Kantrowitz et al.,[8] en el que se basó esta investigación, no trabajó con dicha emoción, por lo que se desconocen los efectos específicos de las características trabajadas en esta batería de estímulos en su reconocimiento. Por su parte, el estudio de Juslin y Laukka[9] sí postula posibles características para que un estímulo pueda identificarse correctamente como Miedo, lo cual no se comprueba en la presente investigación. Lo anterior puede deberse a que los estímulos de la presente se modificaron según 3 aspectos prosódicos, y Miedo podría requerir la modulación F0SD en niveles bajos junto con otros aspectos, como forma del contorno de la frecuencia fundamental (F0cont), que tendría que encontrarse en nivel alto.[2] Dicha característica del tono varía en esta emoción más que en otras, por ejemplo, Tristeza, con la que comparte varios parámetros.

En los estudios que trabajaron con estímulos auditivos para REA con muestras de población clínica (esquizofrenia) y controles,[2,8] se encontraron diferencias significativas en el rendimiento de los grupos según el reconocimiento emocional, sin discriminación por emoción. La presente investigación arrojó diferencias significativas en el porcentaje de respuestas de identificación entre participantes que reportaban un trastorno psiquiátrico y aquellos que no con respecto a las emociones Miedo y Tristeza y la expresión Sin Emoción, lo que coincide parcialmente con la literatura, puesto que las emociones Alegría y Enojo no mostraron diferencias, en contraste con estudios anteriores. Esta diferencia parcial podría deberse a que los parámetros de los estímulos generan distintas respuestas en la población chilena y en la de los estudios de Leitman et al.[2] y Kantrowitz et al.[8]

Cabe mencionar que el presente estudio, al ser, a saber de los autores, el primero que plantea evaluar una batería de estímulos en la población chilena, buscó establecer los parámetros de reconocimiento de emociones por medio de estímulos auditivos en adultos, y evaluarlos de manera exploratoria. Por esta misma razón, se decidió no descartar los resultados de los participantes que reportaron un trastorno psiquiátrico, ya que el porcentaje de prevalencia de éstos es el que se encontraría en la población chilena.

En conclusión, este estudio en población chilena tuvo resultados similares a los de investigaciones previas, específicamente los que se desprenden de los estudios de Juslin y Laukka [9] y Kantrowitz et al.[8] basados en población angloparlante. Por un lado, se confirmó que el parámetro F0M en niveles altos determina la identificación de Alegría, y el parámetro F0SD en niveles bajos la de tristeza, con la salvedad de que, a diferencia de los estudios anteriores, F0M alto y F0SD bajo aún permite el reconocimiento de Tristeza, lo cual muestra que en esta población F0M no cumple un papel determinante en la identificación de dicha emoción. Por otro lado, la presencia o ausencia del sonido de alta energía HF500 se relaciona con la aparición de Enojo y la desaparición de Tristeza, aunque se amplifica para la emoción de Alegría. La literatura postularía que con el sonido de alta energía esta emoción no debería reconocerse tan claramente; en este estudio sólo disminuyó su porcentaje de reconocimiento, que nunca bajó de 35%, lo cual se evidencia en los estímulos 218 a 220.

Este trabajo es relevante dado el número limitado de estudios en reconocimiento emocional auditivo y la inexistencia de investigaciones en español al respecto. Adicionalmente, se encuentra el hallazgo de que, contrario a lo que postulan Kantrowitz et al.[8] respecto al uso de baterías de estímulos no verbales sintetizados y el reconocimiento de las emociones que evocan según distintos contextos culturales, hay menores diferencias en la identificación de ciertas emociones. Esto puede deberse a que dichas emociones estén determinadas por factores culturales, tanto en su expresión como en su interpretación, lo que abre nuevas líneas de investigación en torno a la evaluación de parámetros específicos de los estímulos y sus características físicas (F0M, F0SD y HF500) adaptados a la población participante.

Dentro de las recomendaciones para estudios futuros en poblaciones latinoamericanas, se encuentra contar con un mayor número de participantes en función de la validez y utilidad de los datos, así como con participantes controles y pacientes de algún diagnóstico verificado clínicamente, lo cual debe ser parte de los criterios de inclusión. Además, es necesaria una mayor producción de este tipo de estudios con diversos parámetros prosódicos de los estímulos emocionales auditivos.

Fuentes de Financiamiento

Esta investigación fue financiada por la Agencia Nacional de Investigación y Desarrollo (ANID), y se enmarca en el proyecto Fondecyt Nº 11180961, dirigido por la Dra. Johanna Kreither. Adicionalmente, este trabajo fue apoyado por el Programa de Investigación Asociativa (PIA) en Ciencias Cognitivas (RU-158-2019), Centro de Investigación en Ciencias Cognitivas (CICC), Facultad de Psicología, Universidad de Talca, Chile.

Referencias

1. Celdrán J. La importancia del reconocimiento emocional (RE) en la infancia : estudio, propuesta y validación de una prueba de evaluación de RE dirigida a niños [Tesis doctoral]. Murcia: Universidad de Murcia; 2018. Disponible en: http://hdl.handle.net/10201/55679
2. Leitman D, Laukka P, Juslin P, Saccente E, Butler P, Javitt D. Getting the cue: Sensory contributions to auditory emotion recognition impairments in schizophrenia. Schizophr Bull. 2010; 36(3):545-56. doi: 10.1093/schbul/sbn115
3. Tabernero M, Politis D. Reconocimiento facial de emociones básicas y prosodia emocional en Demencia Frontotemporal variante conductual. Neuropsicología Latinoamericana. 2012;4(4):36-41. Disponible en: https://www.neuropsicolatina.org/index.php/Neuropsicologia_Latinoamericana/article/view/127
4. Morningstar M, Nelson E, Dirks M. Maturation of vocal emotion recognition: Insights from the developmental and neuroimaging literature. Neurosci Biobehav Rev. 2018;90:221-30. doi: 10.1016/j.neubiorev.2018.04.019
5. Chaturvedi R, Kraus M, Keefe RS. A new measure of authentic auditory emotion recognition: Application to patients with schizophrenia. Schizophr Res. 2020;222:450-4. doi: 10.1016/j.schres.2019.11.043
6. Pascual S, Solé B, Castillón J, Jesús A, Tejedor C. Prosodia afectiva y reconocimiento facial y verbal de la emoción en la esquizofrenia. Rev Psiquiatría Fac Med Barna. 2005;32(4):179-83. https://sid-inico.usal.es/idocs/F8/ART13456/prosodia_afectiva.pdf
7. Philip RC, Whalley HC, Stanfield AC, Sprengelmeyer R, Santos IM, Young AW, et al. Deficits in facial, body movement and vocal emotional processing in autism spectrum disorders. Psychol Med. 2010;40(11):1919-29. doi: 10.1017/s0033291709992364
8. Kantrowitz JT, Leitman DI, Lehrfeld JM, Laukka P, Juslin PN, Butler PD, et al. Reduction in tonal discriminations predicts receptive emotion processing deficits in schizophrenia and schizoaffective disorder. Schizophr Bull. 2013;39(1):86-93. doi: 10.1093/schbul/sbr060
9. Juslin PN, Laukka P. Impact of intended emotion intensity on cue utilization and decoding accuracy in vocal expression of emotion. Emotion. 2001;1(4):381-412. doi: 10.1037/1528-3542.1.4.381

ARCHIVOS DE NEUROCIENCIAS | Article original

received 21 August 2022 | accepted 9 October 2022 | published 11 October 202

Doi: 10.31157/an.v28i2.410

THE EFFECTS OF MEMANTINE AND MK801 ON NMDA RECEPTOR SWITCHING 2B AND 2A SUBUNITS IN HIPPOCAMPAL CELL CULTURE

Uribe Ezequiel[1,2] ✉ | Sanchez-Mendoza Eduardo[1]

1. Centro de Biofísica y Neurociencia de la Universidad de Carabobo, Carabobo, Venezuela
2. Departamento de Neurofisiología, Universidad de Carabobo, Carabobo, Venezuela

Contact

Ezquiel Uribe
Apartado postal 3798, El trigal, Valencia, Venezuela.

✉ euribe@uc.edu.ve

Abstract

Background: Schizophrenia (SCZ) is a severe and chronic neurodevelopmental disorder whose onset begins in adolescence or early adulthood. Notwithstanding, brain dysfunction occurs before the disease onset and involves a switch in NMDA receptor subunit composition from GluN2B to GluN2A at early neonatal period. We have recently postulated memantine (MEM) as an effective experimental treatment, due to its effect on modulating NMDA receptor subunit turnover during the postnatal period, as it prevents glutamatergic hypofunction in the maternal deprivation model of SCZ. **Methods**: We evaluated the turnover of pre and postsynaptic glutamatergic synaptic components by using primary mouse hippocampal neurons during the synaptic formation period. **Results**: MK801 stimulation prevented the GluN2B to GluN2A molecular switch at 11 days in vitro (DIV). Vesicular glutamate transporter 2 (VGLUT2) was also reduced at this time point. MEM treatment reverted these effects by normalizing GluN2B, and GluN2A, and over-expressing VGLUT2 expression. **Conclusion**: Our data supports a molecular mechanism by which SCZ may be prevented with MEM treatment through regulation of the glutamatergic synaptic molecular composition.

Keywords: *Schizophrenia, synapses, SNARE, glutamate, development, NMDA receptor*.

Introduction

Schizophrenia (SCZ) is considered a neurodevelopmental disorder,[1] that onsets in adolescence or early adulthood in 80% of cases, without showing clear signs of behavioral dysfunction during childhood.[2] The glutamatergic system has been widely associated with the disease,[3] specifically, the N-Methyl-D-Aspartate Receptor (NMDAR), which undergoes subunit changes along the life of the mammalian brain. The most important of these changes occur in the postpartum period, when the exchange of GluN2B to GluN2A subunits takes place,[4] generating a very low concentration of GluN2B in hippocampal tissue during early adulthood.[5] This change of subunits produces significant variations in synaptic connections between the hippocampus and prefrontal cortex, causing a high risk to develop neuropsychiatric disorders during adulthood.[6] In addition, the inversion of the normal ratio between GluN2A/GluN2B in the hippocampus causes poor synaptic metaplasticity, altering long-term depression and potentiation,[7] a major cause of NMDA receptor hypofunction in schizophrenic patients.[8,9] We have proposed that this, in turn, could cause altered patterns of hippocampal-cortical communication,[10] which may produce the clinical symptoms of the disease in early adult life, during the maturation of the prefrontal cortex.[11]

The GluN2A subunits transcription is a key step in the neonatal brain and is enhanced by the presence of brain-derived neurotrophic factor (BDNF), as well as by the correct activation of GluN2B subunits during the switch.[12] Memantine (MEM) is a low-affinity voltage-dependent uncompetitive antagonist at NMDAR, that has a preference for GluN2B subunits and is able to induce up-regulation of BDNF throughout the modulation of glutamatergic signaling.[13] Previous work from our laboratory showed that MEM treatment in the early postnatal period prevents brain atrophy, and electrophysiological and behavioral abnormalities induced by the maternal deprivation model of SCZ.[14,15] To gain further insight into the molecular mechanisms potentially operating in the development of hippocampal synapses, which is difficult with the maternal deprivation model *in vivo*; we have modeled glutamatergic hypofunction by stimulating hippocampal neurons *in vitro* with the NMDAR no competitive antagonist MK-801. NMDAR antagonists are commonly used to model SCZ as they

produce acute molecular abnormalities consistent with those observed in the disease. We show that GluN2B receptors are downregulated with a parallel decrease in VGLUT2 expression, which is prevented by MEM co-stimulation.

Methods

2.1 Hippocampal cell culture

Brains obtained from E15 mice embryos (*Mus musculus*) were dissected on ice-cold Hank's balanced salt solution (HBBS) buffer. The meninges were removed, and the brains were cut through the midline to expose the hippocampi, which were removed using fine forceps and incubated in HBSS containing 0.25% trypsin (15090–046; Gibco, Schwerte, Germany) and 60 U/mL DNAse-I (D5025; Sigma) for 25 min at 37 °C. Trypsinization was stopped by the addition of 5% fetal bovine serum (FBS; Invitrogen, Waltham, MA, USA) diluted in a Neurobasal medium. The tissue was mechanically dissociated by repeated pipetting. Cells were seeded at a density of 7.5 × 104 cells/mL on coverslips coated with 50 μg/mL polyornithine (P4957; Sigma) and 20 μg/mL laminin-entactin (Corning, New York, NY, USA). Neurons were incubated in a Neurobasal medium containing 2% B27 (Invitrogen), glutamine (20 mM; Invitrogen), and PenStrep antibiotic mix (Invitrogen).

2.2 Drugs administration

MK801 was applied on the 8th DIV, the moment in which synaptic connections are visible at the microscope. A dose ≥ 40 μM of MK801 is cytotoxic *in vitro*, and between 10 - 20 μM ensures the NMDAR hypofunction without cytotoxicity.[16] The medium was washed 24 hours later. On the 10th DIV, MEM, a neuroprotective, was applied (5 μM).[17] Both, MK801 and MEM are noncompetitive antagonists, but act at different places of NMDAR with diverse purposes, for example, MEM interacts with two places, at the magnesium site and with less affinity, the extracellular vestibule of the channel, modulating calcium influx.[18] Besides, MK801 binds within the ion channel vestibule, promoting closure of the ion channel gate, and physically blocking ion permeation.[13]

The study was designed with six groups: control group (CONTROL), Memantine group (MEM), MK801 at 10μM treatment group (MK801 (10μM)); MK801 at 10μM and posterior MEM treatment (MK801 (10μM) + MEM); MK801 at 20μM treatment group (MK801 (20μM)); MK801 at 20μM and posterior MEM treatment (MK801 (20μM) + MEM) (Figure 1). In addition, the time course of GluN2A and GluN2B subunits with no intervention drugs was recorded from DIV 8 to DIV 11 (Figure 2).

DIV 8	DIV 9	DIV 10	DIV 11
X	Wash	X	End
X	Wash	MEM (5µM)	End
Mk801 (10µM)	Wash	X	End
Mk801 (10µM)	Wash	MEM (5µM)	End
Mk801 (20µM)	Wash	X	End
Mk801 (20µM)	Wash	MEM (5µM)	End

Figure 1. Timeline and experimental groups.

2.3 Protein extraction and western Blot

Whole-cell lysates were prepared in a buffer containing 50 mM Tris HCL pH 7.4, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, plus proteinase and phosphatase inhibitors. Samples (20 μg) were run on a 10% SDS-PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% milk in TBS, and incubated overnight at 4°C with primary antibodies against GluN2A (1:500, Life technologies, A-6473), GluN2B (1:500, Life technologies, A-6474), PSD95 (1:10.000, Abcam, ab18258), VGLUT1 (1:2000; Synapticsystem), ab227805), and VGLUT2 (1:1000, Synapticsystem, ab216463); syntaxin-1 (Abcam, ab272736) was used at 1:20.000 as well as B-Actin (1:1000, Santa Cruz Biotechnology H-63, USA) as a loading control. Membranes were washed in TBS, incubated with an HRP-conjugated secondary antibody (1:5000; Santa Cruz Biotechnology) for 1 hour at RT, washed, and incubated with ECL solution (Perkin Elmer) for 1 min. Blots were developed using Amersham ECL Prime Western Blotting Detection Reagent (Life Sciences, Waltham, MA, USA) and scanned using a digital enhanced chemiluminescence (ECL) detection device (Thermo Fisher).

2.4 Statistical Processing

All data points are presented below as means ± SD. Multiple-group values were compared with the non-parametric Kruskal–Wallis (KW) test and the Dunn post-hoc test. To compare the two groups the non-parametric Mann-Whitney test was used. For nonparametric correlations, the Spearman coefficients were calculated. WB percentage was the target protein band signal normalized to the loading control (Ratio of control at Y-axis label). Four (8) WB were made (N=8).

Results

3.1. An NMDA receptor switch is observed between 8 and 10 DIV

We identified an exchange of GluN2B and GluN2A subunits between 8 and 11 DIV (Figure 2A). The levels of GluN2A receptor subunits were undetectable at 8 DIV. GluN2A expression progressively increased (9 DIV: P=0,0003; 10 DIV: P=0,006) until the end of the incubation period at 11 DIV (P=0,0008). Interestingly, GluN2B receptor subunits presented a maximal level of expression at 8 DIV which was progressively lower until 11 DIV.

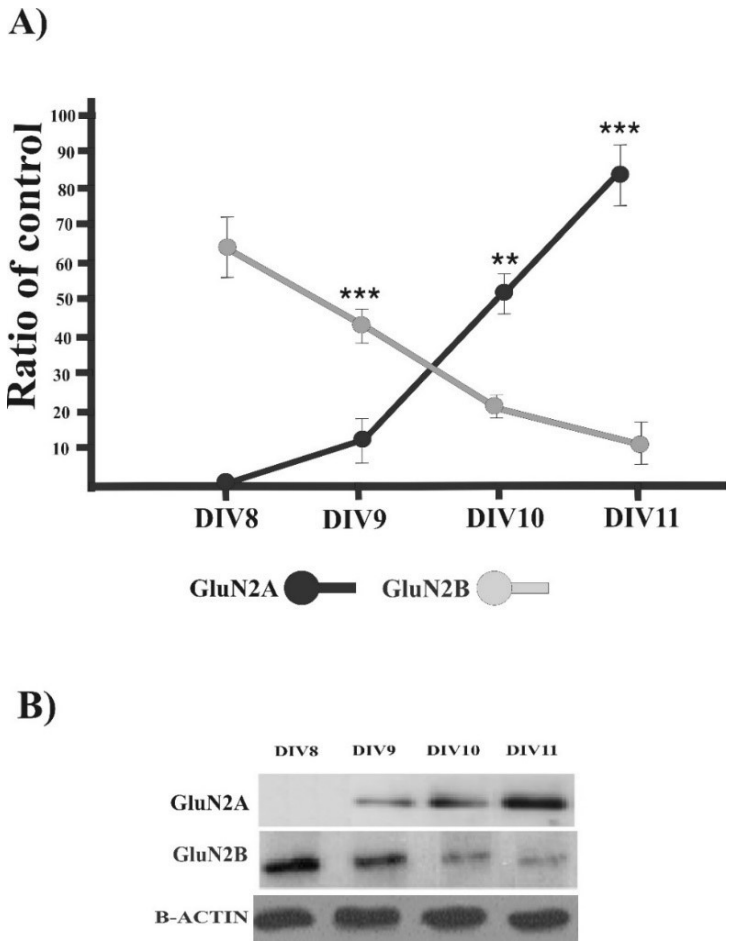


Figure 2. Time course of GluN2A and GluN2B subunits

NMDAR subunits are exchanged in vitro during the early phases of neuronal maturation. **A**) Time course evaluation of GluN2A and GluN2B expression. GluN2A subunits (black) presented a progressive increase while GluN2B subunits (grey) showed a progressive decrease during the incubation period. Significant differences with respect to the initial evaluation condition were identified from 9 to 11 DIV for both NMDAR subunits. **B**) Representative western blots. N=5 experiments analyzed in triplicate. Values are mean +- SDand analyzed by non-parametric Mann Whitney test. **P<0.01 and ***P<0.005 for differences between pairs of groups.

3.2 MEM and MK801 have opposite effects on NMDAR subunit expression *in vitro*

NMDAR subunit composition was then evaluated at 11 DIV after incubation with MK-801 with or without MEM. MK-801 did not show any dose-dependent effect over GluN2A expression. MEM significantly increased the GluN2A expression, although this effect was dose-dependently reduced with MK-801 co-stimulation (Figure 3A). By contrast, MK-801 induced a significant increase in GluN2B expression at 11 DIV which was blocked by MEM co-stimulation (Figure 3B). This suggests an antagonist effect of MK-801 and MEM over GluN2A and GluN2B expression.

3.2.1 GluN2A

MEM increased the GluN2A expression (+34.2%) compared with control (P=0,0021). On the other hand, no differences were found in the expression of GluN2A after MK801 at 10 μM with respect to control (P=0,072); MEM treatment subsequently increased it (+28,9%) (P=0,0042). No differences were found after the application of MK801 at 20 μM with respect to control (P=0,068) nor with the posterior administration of MEM (P=0,091). Moreover, no differences were found in the GluN2A expression after the application of MK801 at 10 (P=0,071) and 20 μM (P=0,089) (Figure 3A).

3.2.2 GluN2B

No differences were found after the administration of MEM compared with control (P=0,27). MK801 at 10 μM increased the GluN2B expression (+47,1%) with respect to control (P=0,0003), and the posterior administration of MEM reduced it (-37,3%) significantly (P=0,0011). MK801 at 20 μM induced an even higher increase (+59,6%) with respect to control (P=0,0001); MEM administration also reduced it (-52,9%) (P=0,0026). Significant differences were found in the GluN2B expression after the application of MK801 at 10 and 20 μM (P=0,003) (Figure 3A).

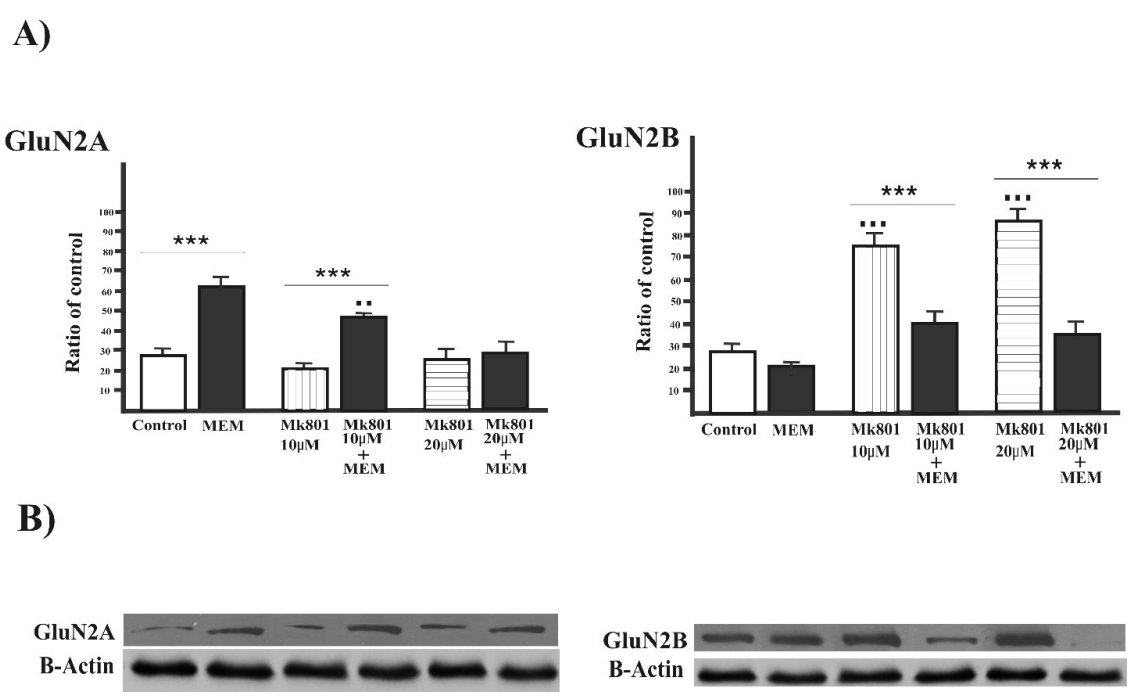


Figure 3. MK-801 and MEM have opposite effects on NMDAR subunit expression *in vitro*

A) MEM induced an increase of GluN2A subunit expression that was dose-dependently reversed by MK-801. MK801 increased GluN2B subunit that was reversed by MEM. **B**) Representative western blots. N=3 experiments. Data are mean values +- SD. ***P<0.001 between conditions. (two squares) P<0.01 and (three squares) P<0.001 for condition vs untreated control (non-parametric Kruskal-Wallis test followed by Dunn post-hoc test).

3.3 MEM promotes the expression of glutamatergic presynaptic components altered by MK801 at 11 DIV

We hypothesized that NMDAR subunit exchange may be accompanied by alterations to other elements of the synaptic machinery. Hence, we evaluated the presynaptic proteins VGLUT1, and VGLUT2, two members of the SNARE complex, synaptotagmin and Syntaxin-1, and the NMDAR interacting component PSD95. VGLUT1 and PSD95 were unaffected by the different treatments (Figure 4). However, MEM caused a significant reduction of VGLUT2 (P=0,02). MEM had no effect on VGLUT2 expression when co-incubated with MK-801 10μM (P=0,5). Moreover, while MK801 20μM significantly reduced VGLUT2 expression compared to the untreated control, co-incubation with both stimuli induced a notable increase of VGLUT2 expression compared to the MK-801 (P=0,0003) and untreated control conditions (P=0,0006) (Figure 4).

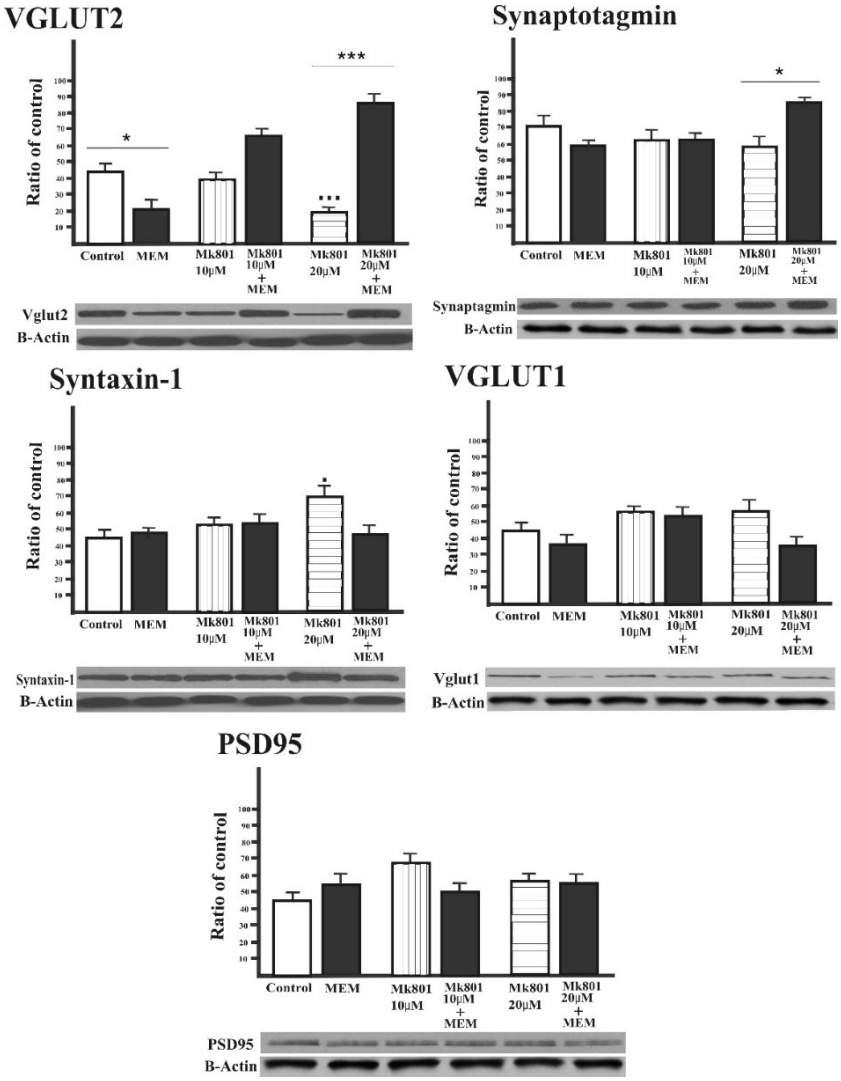


Figure 4. MK-801 treatment alters presynaptic components of the glutamatergic synapse

VLGUT1 and PSD95 were not affected by the different treatments. VGLUT2 expression was significantly reduced compared to the untreated control by MK-801 20μM. MEM reduced VGLUT2 compared to the untreated control but reversed the effect of MK-801 20μM. Syntaxin 1 was significantly increased only when MK-801 was applied. Synaptotagmin was significantly increased only under MK-801 and MEM coincubation. *P<0.05, ***P<0.005 for differences between groups; one black point P<0.05, three black points P<0.005 for differences with Control group (non-parametric Kruskal-Wallis test followed by Dunn post-hoc test).

Also, this protein had an inverse correlation with GluN2A in the MEM group (P= -0,031), and a positive correlation with GluN2A in the MK801 20μM group (P= 0,039) (Table 1). Importantly, Syntyaxin-1 was upregulated only by MK801 20μM (P=0,04), while co-incubation with MEM prevented this effect (P=0,45) (Figure 4). Synaptotagmin was significantly increased by MEM only when co-incubated with MK-801 20μM (P=0,03) (Figure 4), and it had a positive correlation with GluN2B in the control group (P= -0,029) (Table 1). PSD95 had a positive correlation with GluN2A in the MEM group (P=0,048); Synaptotagmin had an inverse correlation with GluN2B in the control group (P= -0,029) (Table 1).

Discussion

The NMDAR GluN2B and GluN2A subunit switch is a key event in the formation of mature synapses, which has been proposed to be disrupted in SCZ.[19] The glutamatergic hypofunction may be causative of long-lasting events affecting cortical connectivity, leading to SCZ-like behavior in rodents.[10] Alteration of normal subunit switch causes abnormalities in synaptic metaplasticity, including long-term depression and potentiation,[7] which have also been identified in the schizophrenic brain.[20] Here we have used in vitro stimulation with MK-801 to model the glutamatergic hypofunction, which is supposed to be the premorbid stage of SCZ.[10] Our results showed that at 11 DIV glutamatergic hypofunction caused an increase in GluN2B subunit expression together with a reduction of the presynaptic marker VGLUT2 and upregulation of Syntaxin-1. These effects induced by MK-801 were reversed by MEM, along with a mild upregulation of Synaptotagmin.

MK-801 has already been shown to modulate the expression of NMDAR subunits in the brain cortex.[21] Both, MK801 and MEM have an affinity for NMDAR containing GluN2B subunits, which are highly expressed during the early stage of brain development.[4,22] There was no alteration in absolute levels of GluN2A and PSD-95 by incubation with MK-801, whose interaction has been otherwise shown to be deficient in the schizophrenic brain.[23] Importantly, the upregulation of GluN2B subunits occurred in parallel to VGLUT2 upregulation while VGLUT1 remained unaltered. The relevance of this finding is substantiated by the observation of VGLUT2 upregulation, but the stability of VGLUT1 levels, observed in post-mortem samples from schizophrenic drug-naive patients.[24] On the other hand, deficits to phosphorylation of Syntaxin 1 have also been associated with schizophrenia.[25] Alterations to VGLUT and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

Table 1. Correlations matrix between NR2A and 2B and the synaptic protein

	NR2A	NR2B
CONTROL		
VGlut1	0,811	0,712
VGlut2	0,192	0,112
PSD95	0,329	0,711
Syntaxin-1	0,772	0,621
Synaptotagmin	0,45	-0,029
MEM		
VGlut1	0,992	0,081
VGlut2	-0,031	0,062
PSD95	0,048	0,099
Syntaxin-1	0,221	0,361
Synaptotagmin	0,401	0,078
MK801 (10MM)		
VGlut1	0,609	0,063
VGlut2	0,701	0,093
PSD95	0,06	0,129
Syntaxin-1	0,218	0,067
Synaptotagmin	0,072	0,213
MK801(10MM) + MEM		
VGlut1	0,228	0,91
VGlut2	0,054	0,188
PSD95	0,251	0,108
Syntaxin-1	0,061	0,083
Synaptotagmin	0,199	0,309
MK801(20MM)		
VGlut1	0,092	0,054
VGlut2	0,039	0,199
PSD95	0,991	0,081
Syntaxin-1	0,872	0,013
Synaptotagmin	0,901	0,072
MK801(20MM) + MEM		
VGlut1	0,085	0,791
VGlut2	0,681	0,078
PSD95	0,141	0,212
Syntaxin-1	0,091	0,064
Synaptotagmin	0,199	0,931

Significant correlations ($P < 0.05$) are expressed with underlined Spearman R correlation coefficient. Inverse correlations are denoted with a negative sign. Control group (CONTROL), Memantine group (MEM), MK801 at 10μM treatment group (MK801 (10μM)); MK801 at 10μM and posterior MEM treatment (MK801 (10μM) + MEM); MK801 at 20μM treatment group (MK801 (20μM)); MK801 at 20μM and posterior MEM treatment (MK801 (20μM) + MEM).

receptor (AMPAR) correlated with abnormal hippocampal neuronal arborization in vivo, which was shown to be actively dependent *in vitro*.[26] Therefore, it could be speculated that the brain atrophy and abnormal electrophysiological activity previously reported on the model of maternal deprivation[15] will be accompanied by decreased GluN2A, increased GluN2B expression, and compensatory effects on VGLUT2 and Syntaxin 1 levels, causing abnormalities in glutamate release, all of which could generate further structural abnormalities within the cortical and hippocampal regions.

The mechanisms by which MK-801 and MEM interact and influence these pre and postsynaptic events are not clear. The NMDAR activation requires the binding of glutamate and glycine together with voltage-dependent relief of magnesium block, resulting in membrane depolarization and calcium influx, which are critical in synaptic transmission and plasticity as well as in cellular mechanisms for learning and memory, elements affected in schizophrenia.[27] Both MK801 and MEM have an affinity for NMDAR containing GluN2B subunits.[5,22] MK801 binds inside the vestibule of the ion channel of the receptor, preventing the flow of calcium and other ions, and blocking the pore in two symmetry-related postures, MEM, on the other hand, seems to block the pore primarily in a single position.[13] Importantly, there are reduced levels of BDNF, GluN2A, and GluN2B sub-units in the hippocampus and the prefrontal cortex in the schizophrenic rats.[28,29] In contrast, MEM was shown to increase BDNF mRNA in SIV-infected macaques[30] and also to reverse the loss of BDNF and TrkB mRNA in the prefrontal cortex.[31] Accordingly, deficiencies of BDNF/TrkB signaling coincided with reduced hippocampal neuron arborization and abnormal VGLUT expression, which reduces the hippocampal neuroplasticity.[26]

Conclusion

Our data suggests that MEM, which has recently been approved for the treatment of dementia,[33] could prevent the emergence of molecular abnormalities of SCZ, regulating pre and post-synaptic elements of the glutamatergic synapse. By understanding the different molecular stages of brain development, it will be possible to prevent the onset of neuropsychiatric disorders in people with a genetic predisposition using adequate treatment.

On the other hand, the present study has several limitations. A simple culture of hippocampal cells excludes the interactions of this group of neurons with the frontal cortex, a brain region highly involved in the development of SCZ. The use of MK801 as a preclinical model of schizophrenia has been questioned,[33] however, it is currently considered useful for reproducing symptoms and brain alterations characteristic of schizophrenia *in vivo*.[34] In this study, it was used as an *in vitro* model to reproduce the NMDAR hypofunction in the hippocampus of the schizophrenic brain, and later, MEM was used to reverse this state. Since both drugs act on the same receptor, this effect could be interpreted as a simple pharmacokinetics consequence in the NMDAR, rather than a solution to the molecular problem. Future research should involve NMDAR subunit knockout to evaluate the effects of MEM.

Acknowledgment

To Hermann Dirk and the Lehrstuhl für Vaskuläre Neurologie, Demenz und Altersforschung, NeuroScienceLab, in Essen, Deutschland, for providing us with the facilities to carry out this research.

Source of financing

This work was supported by the German Academic Exchange Service.

Contributor Roles Taxonomy

Authors contributed equally to this manuscript according to the 14 Contributor Roles.

References

1. Rapoport JL, Addington AM, Frangou S, Psych MR. The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry. 2005; 10(5):434-49. doi: 10.1038/sj.mp.4001642
2. Lehman AF, Lieberman JA, Dixon LB, McGlashan TH, Miller AL, Perkins DO, et al. Practice guideline for the treatment of patients with schizophrenia, second edition. Am J Psychiatry. 2005;161(S2):1-56.
3. Steullet P, Cabungcal JH, Monin A, Dwir D, O'Donnell P, Cuenod M, et al. Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: A "central hub" in schizophrenia pathophysiology? Schizophr Res. 2016;176(1):41-51. doi: 10.1016/j.schres.2014.06.021
4. Liu XB, Murray KD, Jones EG. Switching of NMDA receptor 2A and 2B subunits at thalamic and cortical synapses during early postnatal development. J Neurosci. 2004; 24(40):8885-95. doi: 10.1523/JNEUROSCI.2476-04.2004
5. Liu H, Li Y, Wang Y, Wang X, An X, Wang S, et al. The distinct role of NR2B subunit in the enhancement of visual plasticity in adulthood. Mol Brain. 2015; 8:49. doi: 10.1186/s13041-015-0141-y
6. Monaco SA, Gulchina Y, Gao WJ. NR2B subunit in the prefrontal cortex: A double-edged sword for working memory function and psychiatric disorders. Neurosci Biobehav Rev. 2015; 56: 127-38. doi: 10.1016/j.neubiorev.2015.06.022
7. Philpot BD, Cho KK, Bear MF. Obligatory role of NR2A for metaplasticity in visual cortex. Neuron. 2007; 53(4):495-502. doi: 10.1016/j.neuron.2007.01.027

8. Gonzalez-Burgos G, Lewis DA. NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull. 2012; 38(5):950-7. doi: 10.1093/schbul/sbs010
9. Marballi KK, Gallitano AL. Immediate early genes anchor a biological pathway of proteins required for memory formation, long-term depression and risk for schizophrenia. Front Behav Neurosci. 2018; 12:23. doi: 10.3389/fnbeh.2018.00023
10. Uribe E, Fernández L. Effects of neonatal administration of memantine on hippocampal asymmetry and working memory impairment induced by early maternal deprivation in rats. Neurophysiology. 2019; 51:97-106. doi: 10.1007/s11062-019-09799-4
11. Behrendt RP. Dysregulation of thalamic sensory "transmission" in schizophrenia: neurochemical vulnerability to hallucinations. J Psychopharmacol (Oxford). 2006; 20(3):356-72. doi: 10.1177/0269881105057696
12. Hoffmann H, Gremme T, Hatt H, Gottmann K. Synaptic activity-dependent developmental regulation of NMDA receptor subunit expression in cultured neocortical neurons. J Neurochem. 2000; 75(4):1590-9. doi: 10.1046/j.1471-4159.2000.0751590.x
13. Song X, Jensen MØ, Jogini V, Stein RA, Lee CH, Mchaourab HS, et al. Mechanism of NMDA receptor channel block by MK-801 and memantine. Nature. 2018; 556(7702):515-9. doi: 10.1038/s41586-018-0039-9
14. Uribe E, Sánchez-Mendoza E, Nieves N, Merchor G. Neonatal administration of memantine enhances social cognition in adult rats subjected to early maternal deprivation. Exp neurobiol. 2016; 25(6):328-32. doi: 10.5607/en.2016.25.6.328
15. Uribe E, Fernández L, Pacheco D, Fernandez L, Nayadoleni N, Eblen-Zajjur A. Administration of memantine reverses behavioral, histological, and electrophysiological abnormalities in rats subjected to early maternal deprivation. J Neural Transm (Vienna). 2019; 126(6):759-70. doi: 10.1007/s00702-019-02007-x
16. Hirayama M, Kuriyama M. MK-801 is cytotoxic to microglia in vitro and its cytotoxicity is attenuated by glutamate, other excitotoxic agents and atropine. Possible presence of glutamate receptor and muscarinic receptor on microglia. Brain Res. 2001; 897(1-2):204-206. doi:10.1016/s0006-8993(01)02114-x
17. Emnett CM, Eisenman LN, Taylor AM, Izumi Y, Zorumski CF, Mennerick S. Indistinguishable synaptic pharmacodynamics of the N-methyl-D-aspartate receptor channel blockers memantine and ketamine. Mol Pharmacol. 2013; 84(6):935-47. doi: 10.1124/mol.113.089334
18. Chen HS, Lipton SA. Pharmacological implications of two distinct mechanisms of interaction of memantine with N-methyl-D-aspartate-gated channels. J Pharmacol Exp Ther. 2005; 314(3):961-71. doi: 10.1124/jpet.105.085142
19. Sans N, Petralia RS, Wang YX, Blahos J 2nd, Hell JW, Wenthold RJ. A developmental change in NMDA receptor-associated proteins at hippocampal synapses. J Neurosci. 2000; 20(3):1260-71. doi: 10.1523/JNEUROSCI.20-03-01260.2000
20. Frantseva MV, Fitzgerald PB, Chen R, Möller B, Daigle M, Daskalakis ZJ. Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. Cereb Cortex. 2008;18(5):990-6. doi: 10.1093/cercor/bhm151
21. Xi D, Zhang W, Wang HX, Stradtman GG, Gao WJ. Dizocilpine (MK-801) induces distinct changes of N-methyl-D-aspartic acid receptor subunits in parvalbumin-containing interneurons in young adult rat prefrontal cortex. Int J Neuropsychopharmacol. 2009;12(10):1395-1408. doi: 10.1017/S146114570900042X
22. Ewald RC, Van Keuren-Jensen KR, Aizenman CD, Cline HT. Roles of NR2A and NR2B in the development of dendritic arbor morphology in vivo. J Neurosci. 2008; 28(4):850-61. doi: 10.1523/JNEUROSCI.5078-07.2008
23. Ganguly P, Holland FH, Brenhouse HC. Functional uncoupling NMDAR NR2A subunit from PSD-95 in the prefrontal cortex: effects on behavioral dysfunction and parvalbumin loss after early-life stress [published correction appears in Neuropsychopharmacology. 2016; 41(4):1188]. Neuropsychopharmacology. 2015; 40(12):2666-75. doi: 10.1038/npp.2015.134
24. Schoonover KE, McCollum LA, Roberts RC. Protein markers of neurotransmitter synthesis and release in postmortem schizophrenia substantia nigra. Neuropsychopharmacology. 2017; 42(2):540-50. doi: 10.1038/npp.2016.164
25. Castillo MA, Ghose S, Tamminga CA, Ulery-Reynolds PG. Deficits in syntaxin 1 phosphorylation in schizophrenia prefrontal cortex. Biol Psychiatry. 2010; 67(3):208-16. doi: 10.1016/j.biopsych.2009.07.029
26. 26. Sanchez EH, Camblor S, Martins L, Dzyubenko E, Fleischer M, Carvalho T, et al. Compromised hippocampal neuroplasticity in the interferon-α and toll-like receptor-3 activation-induced mouse depression model. Mol Neurobiol. 2020; 57(7):3171-82. doi: 10.1007/s12035-020-01927-0
27. Bagasrawala I, Memi F, V Radonjic N, Zecevic N. N-methyl d-aspartate receptor expression patterns in the human fetal cerebral cortex. Cereb Cortex. 2017; 27(11):5041-53. doi: 10.1093/cercor/bhw289
28. Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol Psychiatry. 2002; 7(6):609-16. doi: 10.1038/sj.mp.4001036
29. Yu W, Zhu M, Fang H, Zhou J, Ye L, Bian W, et al. Risperidone reverses the downregulation of BDNF in hippocampal neurons and MK801-induced cognitive impairment in rats. Front Behav Neurosci. 2019; 13:163. doi: 10.3389/fnbeh.2019.00163
30. Meisner F, Scheller C, Kneitz S, Sopper S, Neuen-Jacob E, Riederer P, et al. Memantine upregulates BDNF and prevents dopamine deficits in SIV-infected macaques: a novel pharmacological action of memantine. Neuropsychopharmacology. 2008; 33(9):2228-36. doi: 10.1038/sj.npp.1301615
31. Amidfar M, Kim YK, Wiborg O. Effectiveness of memantine on depression-like behavior, memory deficits and brain mRNA levels of BDNF and TrkB in rats subjected to repeated unpredictable stress. Pharmacol Rep. 2018; 70(3):600-6. doi: 10.1016/j.pharep.2017.12.007
32. Folch J, Busquets O, Ettcheto M, Sánchez-López E, Castro-Torres RD, Verdaguer E, et al. Memantine for the treatment of dementia: a review on its current and future applications. J Alzheimers Dis. 2018;62(3):1223-40. doi:10.3233/JAD-170672
33. Eyjolfsson EM, Brenner E, Kondziella D, Sonnewald U. Repeated injection of MK801: an animal model of schizophrenia? Neurochem Int. 2006; 48(6-7):541-6. doi: 10.1016/j.neuint.2005.11.019
34. Daya RP, Bhandari JK, Hui PA, Tian Y, Farncombe T, Mishra RK. Effects of MK-801 treatment across several pre-clinical analyses including a novel assessment of brain metabolic function utilizing PET and CT fused imaging in live rats. Neuropharmacology. 2014; 77:325-33. doi: 10.1016/j.neuropharm.2013.10.001

received 20 March 2022 | accepted 3 June 2022 | published 25 October 2022

Doi: 10.31157/an.v28i2.356

THERAPEUTIC POTENTIAL OF CANNABINOIDS FOR STROKE: SCOPING REVIEW PROTOCOL

Pérez-Neri Iván[1] | Estêvão M Dulce[2] | Mishra Rakesh[3] | Sandoval Hugo[4] | Zárate Manuel[5] | Ríos Camilo[1]

1. Department of Neurochemistry, Manuel Velasco Suárez National Institute of Neurology and Neurosurgery
2. School of Health, University of Algarve
3. Department of Neurosurgery, Institute of Medical Sciences, Banaras Hindu University
4. General Directorate, Luis Guillermo Ibarra Ibarra National Institute of Rehabilitation
5. Autonomous University of Baja California, Mexicali Campus

Contact

Department of Neurochemistry, Manuel Velasco Suárez National Institute of Neurology and Neurosurgery. Insurgentes Sur 3877, Col. La Fama, Alcaldía Tlalpan. Ciudad de México. CP 14269. Mexico City, Mexico.

ipneri03@gmail.com

Abstract

Introduction: Each year, approximately 795,000 people experience a new or recurrent stroke, ischemic or hemorrhagic. The current therapeutical approach for acute ischemic stroke includes thrombolysis and mechanical thrombectomy. However, there are no neuroprotective treatment alternatives to improve its neurological outcome. Some components of the endocannabinoid system are altered after ischemic stroke. Cannabinoids may exert neuroprotective effects, but the use of cannabinoid receptor ligands is a factor to consider due to their psychotropic properties. Regardless of the various studies describing the benefit of administering cannabinoids for experimental stroke, several questions remain unanswered since most information is about non-human species. A previous systematic review detected significant heterogeneity among studies, therefore a scoping review was performed to evaluate the feasibility of an updated systematic review and meta-analysis. This scoping review protocol aims to evaluate the therapeutic potential of modulating the endocannabinoid system for stroke. **Methods**: Published studies (all publication types) will be retrieved from Web of Science, PubMed, Scopus, Ovid, EBSCOhost, and Google Scholar. **Eligibility criteria**: Clinical or preclinical studies reporting endocannabinoid levels or their effects, or reporting administration of cannabinoid modulators (stimulating or inhibiting their synthesis or metabolism, phytocannabinoids, or synthetic cannabinoids) in patients or models of stroke will be considered for inclusion. Studies written in languages different than Spanish or English that could not be properly translated or whose full-text files could not be retrieved will be excluded. **Data charting**: Results will be summarized in tabular form. This protocol complies with PRISMA-P.

Keywords: *Artery occlusion, Endocannabinoid, Ischemia, Phytocannabinoid, Stroke*

Introduction

Overview of *Cannabis spp* use

In recent years, medical research has delved into marijuana use for the possible therapeutic effects derived from its cannabinoid (CB) content. In the United States, a total of 47 states had allowed the medical use of *Cannabis spp* by the end of 2020;[1] nevertheless, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) application are limited for some medical conditions such as end-stage cancer, amyotrophic lateral sclerosis, multiple sclerosis, seizure disorders, Crohn's disease, mitochondrial diseases, Parkinson's disease, and sickle cell disease,[1] among others.

Cannabis spp continues to be the most widely used drug worldwide. The United Nations Office on Drugs and Crime estimates that almost 4 percent of the global population aged 15–64 years consumed *Cannabis spp* at least once in 2019, almost 200 million people.[1] In addition, synthetic cannabinoids (either one of them or their mixture) are also used for recreational purposes.[2]

Stroke

Each year, about 795,000 people experience a new or recurrent stroke. Approximately 610,000 of these are first attacks, and 185,000 are recurrent attacks. Among major stroke types, about 87% are ischemic, 10% are intracranial hemorrhage (ICH), and 3% are subarachnoid hemorrhage (SAH).[3] Stroke is a leading cause of severe long-term disability in the United States. Around 3% of males and 2% of females reported being disabled because of a stroke. Moreover, total direct medical stroke-related costs are projected to increase more than 2-fold between 2015 and 2035, from $36.7 billion to $94.3 billion, with much of those costs arising from people ≥80 years of age.[4]

The current therapeutical approach for acute ischemic stroke includes thrombolysis and mechanical thrombectomy. However, no neuroprotective treatment options currently exist that improve neurological outcomes after ischemic stroke.

In addition, some patients experience a reduced quality of life after stroke, which can be related to some degree of disability, speech disturbances, cognitive impairment, and reduced mood, among other sequelae.[5]

The endocannabinoid system (ECS), integrated by endogenous ligands, cannabinoid receptors, and degrading enzymes, has been proposed as an important pharmacological target in several neurological diseases.[6]

The potential of cannabinoids for stroke therapeutics

The effect of *Cannabis spp* use on stroke incidence is unclear.[7] According to some studies,[8] its consumption is not associated with increased stroke incidence, although these results can be questionable.[9] Neither a relation has been found between its use and negative outcomes in patients with SAH. However, the incidence of some complications may be higher in endovascular-treated *Cannabis spp* users.[10]

Stroke can occur in young *Cannabis spp* users that do not show cardiovascular risk factors.[9] Also, 80% of patients with problematic *Cannabis spp* use may develop post-stroke depression.[11] By contrast, synthetic cannabinoid consumption can cause some neurological symptoms, including somnolence, paresthesia, vertigo, psychomotor retardation, seizures, aggressive behavior, and rhabdomyolysis, but is not associated with stroke.[2]

The relationship between the mechanism of action of *Cannabis spp* and its adverse effects remains unclear. Substantial evidence suggests that chronic *Cannabis spp* consumption, especially during adolescence, is associated with the later development of schizophrenia, and several other psychiatric disorders, including depression, bipolar disorder (mania), anxiety disorders, and antisocial personality disorder.[12] There are limited data regarding the safety of CBs in humans and none in the stroke population.

Some components of the ECS are altered after an ischemic stroke. For example, the expression of cannabinoid CB1 and CB2 receptors is up-regulated in the rat brain after cerebral ischemia, indicating that the ECS may have an important role in the endogenous response to stroke.[13] A THC:CBD formulation is currently being tested in controlled clinical trials to improve spasticity after stroke,[14] that may also be beneficial for post-stroke pain, according to a case report.[15]

Cannabinoids may exert neuroprotective effects,[16] as some studies, mostly preclinical, have informed. It has been reported that CB receptor ligands (endocannabinoids, phytocannabinoids, or synthetic cannabinoids) reduce infarct volume after either transient or permanent ischemia in both rats and mice. However, the effect in non-human primates was non-significant.[13] It has been shown as well that CBD reduced infarct size in an ischemia/reperfusion rodent model.[17] An improved neurological outcome (but not survival) was also observed according to other studies.[13]

Some studies suggest that activation of the CB1 receptor triggers a neuroprotective effect while that of the CB2 receptor is neuromodulatory, although this conclusion might be debated. In addition, the use of CB1 receptor ligands is controversial due to their psychotropic properties.[16] It has also been reported that the deletion of the CB1 receptor increases infarct size, excitotoxicity, and neurological deficits in ischemia models.[18]

Further evidence suggests that CB2 ligands lack some CB1-mediated side effects and may be neuroprotective in models of stroke and other diseases. JWH133, a synthetic CB2 receptor agonist, reduces infarct size, infiltrating neutrophils, myeloperoxidase activity, secretion of inflammatory cytokines, inducible nitric oxide synthase expression, and motor deficits in either transient or permanent ischemia models.[16] This substance decreases glutamate release, preventing excitotoxicity. Also, it reduces brain edema and blood-brain barrier damage in models of hemorrhagic stroke.[16]

Palmitoylethanolamide, an endogenous cannabimimetic, reduces infarct size and neuron loss by diminishing the inflammatory response to anoxia after ischemia-reperfusion in experimental models.[19] In addition, blood levels of this substance correlate with neurological deficits after stroke in humans.[19] Some studies suggest that its administration improves cognition and spasticity in patients with stroke;[19] these effects may be partially mediated by the peroxisome proliferator-activated receptors,[20] which can modulate CB1 receptor activity.[21]

Some synthetic cannabinoids (e.g., HU-211) remain effective when administered several hours after stroke onset.[13] On the other hand, the effect of CB receptor antagonism in stroke is still unclear.[13]

Although many studies describe the benefits of administering cannabinoids for experimental stroke, some questions remain unanswered since most results were observed in non-human species. This scoping review aims to analyze the available evidence of the therapeutic potential of endocannabinoids, phytocannabinoids, or synthetic cannabinoids, as well as their

side effects, possible impact on financial costs and quality of life, in patients with stroke.

The rationale for the study

The neuroprotective potential of cannabinoids for stroke has been recently described in a narrative review,[16] but no systematic approach was applied. In addition, a systematic review and meta-analysis of the effect of cannabinoids in experimental stroke — based on 111 retrieved reports from four databases, excluding human studies — was published in 2015.[13] A systematic review of synthetic cannabinoids was also reported,[2] however, it did not evaluate their role in stroke. A scoping review protocol of current clinical and preclinical evidence for using both natural and synthetic CBs in stroke, utilizing a more comprehensive and updated search strategy, is valuable. A previous systematic review detected significant heterogeneity among studies,[13] therefore a scoping review is necessary to evaluate the feasibility of conducting an updated systematic review and meta-analysis.

Methods

Protocol development

This methodology is based on a preceding protocol,[22] but it is not an update of any previous review. After elaborating on the research question, we used an online tool to define the most appropriate type of review, as previously reported,[23] which was scoping review (https://whatreviewisrightforyou.knowledgetranslation.net/map/results?id=5413&code=GAkWQRoevx).

The International Prospective Register of Systematic Reviews (PROSPERO), the Clinical Online Network of Evidence for Care and Therapeutics (JBI COnNECT+), and the Open Science Framework (OSF) were consulted to identify ongoing protocols for systematic or scoping reviews related to our main research question (July 17th, 2021) but no relevant records were found.

This protocol was drafted by the research team and revised as necessary. Supporting materials (checklists and forms) are available through the OSF (https://osf.io/8erk4/?view_only=142c7bd4f3b84f51bef04eab1baaab58) as previously reported 24 (registration date Nov. 16th, 2021; last updated Feb. 17th, 2022).

Our research team is composed of specialists with different profiles: clinical, preclinical, and socio-medical. This protocol complies with the JBI Manual for Evidence Synthesis,[25] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020[26]), complemented with the PRISMA extensions for abstracts (PRISMA-A[27]), protocols (PRISMA-P[28]), search strategies (PRISMA-S[29]), and scoping reviews (PRISMA-Scr[30]). Those guidelines were applied as much as feasible in this scoping review protocol.

Objectives

The primary objective of this study is to evaluate the therapeutic potential of modulating the ECS for stroke. The secondary objectives are as follows:

- To evaluate the therapeutic potential of either endocannabinoids, phytocannabinoids, or synthetic cannabinoids for stroke.
- To describe possible interactions between either endocannabinoids, phytocannabinoids, or synthetic cannabinoids with conventional treatments for stroke.
- To describe possible side-effects of either phytocannabinoids or synthetic cannabinoids.
- To estimate the possible financial cost of cannabinoid-based treatment for patients with stroke.
- To estimate the possible impact of either endocannabinoids, phytocannabinoids, or synthetic cannabinoids, on quality of life in patients with stroke.

Research questions[31] for this review are described in Table 1.

Search strategy

The search strategy was created by a trained investigator and was peer-reviewed using the *PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement.*[32] Published studies (all publication types) will be retrieved from Web of Science (Clarivate), Medline (PubMed), Scopus, Ovid, and EBSCOhost (Academic Search Ultimate), from the database inception to the present. Additionally, the first 100 results from Google Scholar (https://scholar.google.com/), sorted by relevance without citations, will be retrieved[24] using Publish or Perish.[33]

Databases to be consulted, their providers, and dates of coverage are listed in Appendix A (https://osf.io/8erk4/?view_only=142c7bd4f3b84f51bef04eab1baaab58). Authors will be contacted if necessary. Collections from the authors of the present manuscript will also be considered. No additional sources will be consulted. No limits or filters will be applied. Search algorithms were elaborated using an online tool and are publicly available (https://app.2dsearch.com/new-query/612a734d758bc70004e35990). These algorithms were adjusted when necessary for each database, during the line-by-line analysis described in Appendix B. (https://osf.io/8erk4/?view_only=142c7bd4f3b84f51bef04eab1baaab58).

Table 1. Research questions for this systematic scoping review.

Question type	Framework	Description
Main research question	CoCoPop Framework (Condition, Context, Population)	What is the therapeutic potential (Co) of modulating the endocannabinoid system (Co) in patients with stroke (Pop)?
Secondary research question 1	CoCoPop Framework (Condition, Context, Population)	What is the therapeutic potential (Co) of endocannabinoids, phytocannabinoids, or synthetic cannabinoids (Co) in patients with stroke (Pop)?
Secondary research question 2	CoCoPop Framework (Condition, Context, Population)	Is there any interaction between (Co) endocannabinoids, phytocannabinoids, or synthetic cannabinoids with conventional treatment (Co) for stroke patients (Pop)?
Secondary research question 3	CoCoPop Framework (Condition, Context, Population)	What are the possible side-effects (Co) of phytocannabinoids or synthetic cannabinoids (Co) in patients with stroke (Pop)?
Secondary research question 4	CoCoPop Framework (Condition, Context, Population)	What could be the financial cost (Co) of cannabinoid-based treatment (Co) in patients with stroke (Pop)?
Secondary research question 5	CoCoPop Framework (Condition, Context, Population)	What is the effect (Co) of endocannabinoids, phytocannabinoids, or synthetic cannabinoids on quality-of-life measures (Co) in patients with stroke (Pop)?

Articles written in languages other than English and Spanish will be included if adequately translated using Google Translate[34] and/or DeepL, or if appropriate translations are found.[35] Gray literature will be consulted through the Conference Proceedings Citation Index-Science (Web of Science Core Collection) and OpenDissertations (EBSCOhost).

Study selection

Retrieved references will be de-duplicated using Rayyan QCRI's default algorithm, complemented with Zotero and Endnote. Duplicates will be confirmed manually and will be eliminated.[36] Two independent researchers will assess all references for eligibility using Sysrev according to predefined criteria. A third researcher will resolve discrepancies. Inter-rater reliability will be calculated using the Sysrev concordance tool.[37] Two screening stages will be performed: Title/Abstract, and Full-text; each stage will be pilot-tested with a random sample of 25-50 studies.[25,38]

Selected studies will be retrieved using the Retraction Watch database (http://retractiondatabase.org/) to identify retracted studies, which will be eliminated. After twelve months, the search will be rerun to identify recent studies for possible inclusion. Search strategy results will be described in a PRISMA flow diagram.

Eligibility criteria

Inclusion criteria

- Clinical or preclinical studies reporting endocannabinoid levels in any biological sample , assessed by any imaging or biochemical method, in stroke patients or experimental models.
- Clinical or preclinical studies reporting the administration of endocannabinoid modulators (stimulating or inhibiting their synthesis or metabolism, any pharmacological treatment) in stroke patients or experimental models.
- Clinical or preclinical studies reporting the effect of either phytocannabinoid or synthetic cannabinoids (any pharmacological treatment) in stroke patients or experimental models.
- Clinical or preclinical studies showing an effect of stroke on endocannabinoid levels in the blood and/or the brain of patients or experimental animals.
- As previously reported,[39] no specific diagnostic criteria for stroke will be required if the studies describe their population as presenting the condition.
- Analysis will not be limited to a clinical setting. All quantitative, qualitative, or mixed-method studies will be considered.

Exclusion criteria

- Studies written in languages other than Spanish or English that could not be appropriately translated using Google Translate and/or DeepL.
- Studies whose full-text files could not be retrieved.

Eligibility criteria may be adjusted during the screening process, as previously reported.[24] Adjustments will be applied to all studies and reported accordingly.

Data charting

Charting variables include age [years (humans), bodyweight or months (experimental animals)], gender (male/female), cannabinoid class (phyto-, endo-, synthetic), dose, duration of treatment, study type (clinical study, experimental model or theoretical study), species analyzed and their respective strains and/or genetic modifications (cell culture, rodents, non-human primates), type of stroke (ischemic, hemorrhagic, other), disease stage, therapeutic effect (survival, neurological

deficit, infarct size), pathophysiological mechanisms (oxidative stress, cell death, excitotoxicity), interaction with conventional treatment (present, absent), cannabinoids' side-effects, patients' comorbidities, quality-of-life measures. Only original research studies are eligible for these charting methods. Therapeutic effects are the main objective of this review. No data will be extracted from the figures.

Data will be reported in the units of their original report; no conversions will be applied. Unclear information will not be considered. Two independent researchers will extract data using Sysrev; a third researcher will resolve discrepancies. Inter-rater reliability will be calculated using the Sysrev concordance tool.[37] This process will be pilot-tested with a random 25-50 studies sample.[25,38]

Data synthesis

All studies are eligible for narrative synthesis. Results will be summarized in tables. Clinical and preclinical studies will be analyzed separately but may be discussed together. Preclinical studies will be discussed by study type (cell culture, rodent models, non-human primates).No statistical synthesis will be applied.

Strengths and limitations

This scoping review will provide an integrative perspective of the therapeutic potential of cannabinoids for stroke based on both clinical and preclinical studies. Also, possible side effects of this treatment were included to determine an objective recommendation for its use. Finally, the costs of current treatments for this disease will be included — when possible — to evaluate its possible general application.

In contrast to other protocols,[24] our research question complies with a systematic framework that also supports our search strategy, which was peer-reviewed. An effort will be made to include articles written in any language. The multidisciplinary research group provides complementary perspectives. This protocol complies with several guidelines, not only for scoping reviews (PRISMA-Scr, JBI Manual for Evidence Synthesis) but also for systematic reviews (PRISMA 2020, PRISMA-P, PRISMA-S, PRISMA-A).

Only a narrative analysis of the evidence will be presented. No risk-of-bias analysis or certainty of evidence assessment will be considered. The heterogeneity of the included studies allows an exhaustive analysis of the research topic. However, this could be a limitation since it might preclude performing a systematic review of intervention or meta-analysis.

Authors' contributions

I.P.N. provided methodological expertise, contributed to the design of the protocol's methodology (including search strategy), coordinated the co-author's contributions, corrected and approved the final draft, will implement protocol amendments if necessary, and is the guarantor of the review. R.M. and M.D.E. contributed with topic expertise, the design of the protocol's methodology (including search strategy), and corrected and approved the final draft. H.S. provided methodological expertise, contributed to the design of the protocol's methodology (including search strategy peer-review), and corrected and approved the final draft. M.Z. provided topic expertise, contributed to the design of the protocol's methodology, and reviewed and approved the final draft. C.R. provided topic expertise, contributed to supervising the review team, and approved the protocol's methodology and the final draft.

Conflicts of interest

I.P.N. is an Editor for *Archivos de Neurociencias*.

Funding

This protocol did not receive funding from any academic or governmental entity.

Acknowledgments

The authors want to thank Ana Paulina Murillo López for contributing to protocol development.

References

1. United Nations Office on Drugs and Crime. World Drug Report 2021. United Nations publication, Sales No. E.21.XI.8; 2021. doi: 10.18356/9789210058032
2. Tournebize J, Gibaja V, Kahn JP. Acute effects of synthetic cannabinoids: Update 2015. Subst Abus. 2017;38(3):344-66.
3. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart disease and stroke statistics-2021 update: A report from the American Heart Association. Circulation. 2021;143(8):e254-e743.doi:10.1161/CIR.0000000000000950
4. Nelson S, Whitsel L, Khavjou O, Phelps D, Leib A. Projections of cardiovascular disease prevalence and costs: 2015–2035. RTI International; 2016.

5. Stewart C, Subbarayan S, Paton P, Gemmell E, Abraha I, Myint PK, et al. Non-pharmacological interventions for the improvement of post-stroke quality of life amongst older stroke survivors: a systematic review of systematic reviews. Eur Geriatr Med. 2019;10(3):359-86. doi: 10.1007/s41999-019-00180-6
6. Zarruk JG, Fernández-López D, García-Yébenes I, García-Gutiérrez MS, Vivancos J, Nombela F, et al. Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. Stroke. 2012;43(1):211-9. doi: 10.1161/STROKEAHA.111.631044
7. Jouanjus E, Raymond V, Lapeyre-Mestre M, Wolff V. What is the current knowledge about the cardiovascular risk for users of cannabis-based products? A systematic review. Curr Atheroscler Rep. 2017;19(6):26. doi: 10.1007/s11883-017-0663-0
8. Yoo SGK, Seth M, Vaduganathan M, Ruwende C, Karve M, Shah I, et al. Marijuana use and in-hospital outcomes after percutaneous coronary intervention in Michigan, United States. JACC Cardiovasc Interv. 2021;14(16):1757-67. doi: 10.1016/j.jcin.2021.06.036
9. Gómez Ochoa SA. Stroke and cannabis use in patients with no cardiovascular risk factors: a systematic review of case reports. Neurologia (Engl Ed). 2021;36(3):222-8. doi: 10.1016/j.nrl.2017.09.016
10. Chiu RG, Fuentes AM, Patil SN, Chiu R, McGuire LS, Mehta AI. Cannabis abuse and perioperative complications after treatment of intracranial aneurysms: A nationwide analysis. World Neurosurg. 2022 Feb;158:e184-e195. doi: 10.1016/j.wneu.2021.10.156
11. Rabat Y, Sibon I, Berthoz S. Implication of problematic substance use in poststroke depression: An hospital-based study. Sci Rep. 2021;11(1):13324. doi: 10.1038/s41598-021-92639-5
12. Gorelick DA, Hermann R. Cannabis use and disorder: Epidemiology, comorbidity, health consequences, and medico-legal status. UpToDate. 2020; 20172-150:1-36.
13. England TJ, Hind WH, Rasid NA, O'Sullivan SE. Cannabinoids in experimental stroke: A systematic review and meta-analysis. J Cereb Blood Flow Metab. 2015;35(3):348-58. doi: 10.1038/jcbfm.2014.218
14. Marinelli L, Balestrino M, Mori L, Puce L, Rosa GM, Giorello L, et al. A randomised controlled cross-over double-blind pilot study protocol on THC: CBD oromucosal spray efficacy as an add-on therapy for post-stroke spasticity. BMJ open. 2017;7(9):e016843. DOI: 10.1136/bmjopen-2017-016843
15. Moser U. Tetrahydrocannabinol and cannabidiol as an oromucosal spray in a 1: 1 ratio: a therapeutic option for patients with central post-stroke pain syndrome. BMJ Case Reports CP. 2021;14(7):e243072. doi: 10.1136/bcr-2021-243072
16. Hashiesh HM, Jha NK, Sharma C, Gupta PK, Jha SK, Patil CR, et al. Pharmacological potential of JWH133, a cannabinoid type 2 receptor agonist in neurodegenerative, neurodevelopmental and neuropsychiatric diseases. Eur J Pharmacol. 2021;909:174398.
17. Sultan SR, Millar SA, England TJ, O'Sullivan SE. A systematic review and meta-analysis of the haemodynamic effects of cannabidiol. Front Pharmacol. 2017;8:81. doi: 10.3389/fphar.2017.00081
18. Wang LN, Xing MD, Qu WT, Wang CB, Liu ZQ, Han J, et al. Impaired vessel relaxation response and increased infarct size in smooth muscle cannabinoid receptor 1 knockout mice. Microvasc Res. 2022;139:104263. doi: 10.1016/j.mvr.2021.104263
19. Davis MP, Behm B, Mehta Z, Fernandez C. The Potential Benefits of Palmitoylethanolamide in Palliation: A Qualitative Systematic Review. Am J Hosp Palliat Care. 2019;36(12):1134-54. doi: 10.1177/1049909119850807
20. Annunziata C, Pirozzi C, Lama A, Senzacqua M, Comella F, Bordin A, et al. Palmitoylethanolamide promotes white-to-beige conversion and metabolic reprogramming of adipocytes: Contribution of PPAR-α. Pharmaceutics. 2022;14(2):338. doi: 10.3390/pharmaceutics14020338
21. Azar S, Udi S, Drori A, Hadar R, Nemirovski A, Vemuri KV, et al. Reversal of diet-induced hepatic steatosis by peripheral CB1 receptor blockade in mice is p53/miRNA-22/SIRT1/PPARα dependent. Mol Metab. 2020;42:101087.
22. Pérez-Neri I, Diéguez-Campaa CE, Sandoval H, Chávez VA, Castro-Martínez E, Ríos C. Therapeutic potential of dehydroepiandrosterone for Parkinson's disease: scoping review protocol. Arch Neurocien. 2022;27(2):39-46. doi: 10.31157/an.v27i2.331
23. Rosca EC, Tudor R, Cornea A, Simu M. Parkinson's disease in Romania: A scoping review protocol. Brain Sci. 2021;11(2):251.
24. Polhemus AM, Bergquist R, Bosch de Basea M, Brittain G, Buttery SC, Chynkiamis N, et al. Walking-related digital mobility outcomes as clinical trial endpoint measures: protocol for a scoping review. BMJ Open. 2020;10(7):e038704. doi: 10.1136/bmjopen-2020-038704.
25. Aromataris E, Munn Z, editors. JBI Manual for Evidence Synthesis. JBI; 2020.
26. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: Updated guidance and exemplars for reporting systematic reviews. BMJ. 2021;372:n160. doi: 10.1136/bmj.n160
27. Beller EM, Glasziou PP, Altman DG, Hopewell S, Bastian H, Chalmers I, et al. PRISMA for Abstracts: reporting systematic reviews in journal and conference abstracts. PLoS Med. 2013;10(4):e1001419. doi: 10.1371/journal.pmed.1001419
28. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015;350:g7647. doi: 10.1136/bmj.g7647
29. Rethlefsen ML, Kirtley S, Waffenschmidt S, Ayala AP, Moher D, Page MJ, et al. PRISMA-S: an extension to the PRISMA Statement for Reporting Literature Searches in Systematic Reviews. Syst Rev.

2021;10(1):39. doi: 10.1186/s13643-020-01542-z.

30. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med. 2018;169(7):467-73. doi: 10.7326/M18-0850
31. Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. Int J Evid Based Healthc. 2015;13(3):147-53. doi: 10.1097/XEB.0000000000000054
32. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. J Clin Epidemiol. 2016;75:40-6. doi: 10.1016/j.jclinepi.2016.01.021
33. Bramer WM, de Jonge GB. Improving efficiency and confidence in systematic literature searching. Workshop given at International Congress of Medical Librarianship (ICML) & European Association for Health Information and Libraries (EAHIL). Dublin; 2017:1-16.
34. Willcox MDP, Walsh K, Nichols JJ, Morgan PB, Jones LW. The ocular surface, coronaviruses and COVID -19. Clin Exp Optom. 2020;103(4):418-44. doi: 10.1111/cxo.13088
35. Pérez-Neri I, González-Aguilar A, Sandoval H, Pineda C, Ríos C. Therapeutic potential of ultrasound neuromodulation in decreasing neuropathic pain: Clinical and experimental evidence. Curr Neuropharmacol. 2021;19(3):334-48. doi: 10.2174/1570159X18666200720175253
36. McKeown S, Mir ZM. Considerations for conducting systematic reviews: evaluating the performance of different methods for de-duplicating references. Syst Rev. 2021;10(1):38. doi: 10.1186/s13643-021-01583-y
37. Bozada T, Borden J, Workman J, Del Cid M, Malinowski J, Luechtefeld T. Sysrev: A FAIR platform for data curation and systematic evidence review. Front Artif Intell. 2021;4:685298. doi: 10.3389/frai.2021.685298
38. Garritty C, Gartlehner G, Nussbaumer-Streit B, King VJ, Hamel C, Kamel C, et al. Cochrane Rapid Reviews Methods Group offers evidence-informed guidance to conduct rapid reviews. J Clin Epidemiol. 2021;130:13-22. doi: 10.1016/j.jclinepi.2020.10.007
39. Usher R, Stapleton T. Approaches for assessing decision-making capacity in older adults: a scoping review protocol. JBI Evidence Synthesis. 2020;18(4):832-40. doi: 10.11124/JBISRIR-D-19-00068

received 10 November 2022 | accepted 26 February 2023 | published 28 February 2023

Doi: 10.31157/an.v28i2.432

COMPARATIVE EFFICACY OF FIRST AND SECOND GENERATION LONG-ACTING INJECTABLE ANTIPSYCHOTIC IN SCHIZOPHRENIC PATIENTS: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

Saucedo-Uribe Erasmo[1,2,3] ✉ | González-Mallozzi Pedro Jehu[1,2] | Medrano-Garza Raúl Ricardo[1,2] | Díaz González-Colmenero Fernando[1,3] | Carranza-Navarro Farid[1,2] | Castillo-Morales Patricia Lizeth[1,3] | Leyva-Camacho Paloma C.[1,3] | Herrera-Montemayor Yessica[1,2] | Vidal-Tijerina Mauricio[1,2] | Enríquez-Navarro Moisés Karika[1,2] | Medrano Samantha B.[1,3] | Fernández-Zambrano Stefan Mauricio[1] | Mancías- Guerra Claudia Magdalena[2] | Saucedo-Mancías Claudia Lizeth[2] | Sánchez-Ramírez Manuel Ramiro[2]

1. Department of Psychiatry, Faculty of Medicine, Universidad Autonoma de Nuevo Leon (UANL). Mexico.
2. Advanced Neuroscience Center Universidad Autonoma de Nuevo Leon (UANL). Mexico.
3. INVEST Medicina UANL-KER Unit Mayo Clinic (KER Unit Mexico), Universidad Autonoma de Nuevo Leon, México.

Correspondence

Erasmo Saucedo Uribe
Faculty of Medicine, Universidad Autónoma de Nuevo Leon (UANL), Av. Francisco I. Madero and Av. Gonzalitos, Col. Mitras Centro, Monterrey, Nuevo León, México. C.P 64460

✉ drerasmosaucedouribe@gmail.com

Abstract

Introduction: Long-acting injectable antipsychotics (LAIA) can influence the course of treatment and have the potential to improve treatment adherence. This systematic review and network meta-analysis aimed to evaluate the efficacy of second-generation LAIAs (SG-LAIAs) and first-generation LAIAs (FG-LAIAs) in the treatment of schizophrenia. **Methods**: The present study adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and is registered in Prospero (ID CRD42019128700). A comprehensive search was conducted in Medline, Embase, Web of Science, and Scopus databases. The search encompassed the period from June 17, 2020, with an update from June 2020 until September 14, 2021. **Results**: The standardized mean differences (SMDs) for four antipsychotics (80%) demonstrated significant reductions in PANSS scores compared to the placebo. The SMDs ranged from -0.72 (95% CrI -0.99 to -0.46) for haloperidol to -0.45 (-0.54 to -0.37) for paliperidone. Eight studies provided usable results for both negative and positive symptoms, involving a comparison of four antipsychotics. The SMDs for three antipsychotics (75%) significantly reduced negative symptoms compared to placebo, ranging from -0.40 (95% CrI -0.53 to -0.26) for aripiprazole to -0.32 (-0.44 to -0.19) for risperidone. The SMDs for the three drugs (100%) that significantly reduced positive symptoms compared to placebo ranged from -0.50 (95% CrI -0.63 to -0.37) for aripiprazole to -0.19 (-0.57 to 0.20) for zuclopenthixol. **Discussion**: Our findings suggest that all long-acting injectable antipsychotics, except for zuclopenthixol, exhibit comparable efficacy in symptom reduction. **Conclusions**: The majority of LAIAs demonstrate similar effectiveness in reducing overall symptoms, and the differences between individual LAIAs are not statistically significant.

Keywords: *Schizophrenia, antipsychotics, long-acting injectable antipsychotics*

Introduction

Schizophrenia, a severe chronic disorder, affects more than 21 million people worldwide[1,2] It has been recognized as a crucial mental health concern within the Grand Challenges in Global Mental Health Initiative.[3–6] The diagnosis of schizophrenia profoundly impacts life expectancy, with mortality rates increasing 2-3 times among younger individuals.[7]

Long-acting injectable antipsychotics (LAIA) have the potential to lead the course for schizophrenia treatment and to increase adherence, as well as reduce healthcare costs in the long term. Additionally, LAIAs have been associated with a lower risk of all-cause mortality, relapses, and hospitalizations compared to other antipsychotics.[8–11]

However, the question of LAIA efficacy in the treatment of schizophrenia remains uncertain. Previous systematic reviews comparing second-generation (SG) and first-generation (FG) LAIAs have primarily focused on mortality risk[12] or discontinuation rates.[13] Only one meta-analysis conducted by our research group[14] has specifically addressed efficacy and tolerability; nevertheless, results are considered preliminary due to the lack of evidence.

Considering the critical role of depot antipsychotics for long-term symptom stability, we performed a systematic review and network meta-analysis of randomized clinical trials (RCT) of patients with diagnosis of schizophrenia who are at least 18 years old with a minimum >12 weeks of treatment, in which SGA-LAIs were compared to FG-LAIs or placebo, to evaluate efficacy through clinimetry (PANSS global score, PANSS Positive subscale, and PANSS Negative subscale) and clinical criteria.

Methods

Search strategy and selection criteria

This network meta-analysis adheres to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (the PRISMA checklist is available in the supplementary material). The protocol for this study was registered in PROSPERO under the registration number CRD42019128700. An experienced librarian (EG), in collaboration with the lead researcher, developed and implemented the search strategy across multiple databases, including Medline, Embase, Web of Science, and Scopus, from database inception to June 17, 2020, with an update from June 2020 to September 14, 2021. Additionally, references from eligible studies and reviews were also screened for eligibility (search can be found in supplementary material Table 1).

To be included in this systematic review and network meta-analysis, studies were required to be randomized controlled trials (RCTs) investigating depot antipsychotics, both typical (first generation) and atypical (second generation), comparing them with each other, or placebo, and that also meet the following criteria: A) Inclusion of patients aged 18 years or older with a confirmed diagnosis of schizophrenia based on recognized diagnostic criteria, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Classification of Diseases 10th Edition (ICD-10); B) Evaluation of depot antipsychotics efficacy measured by various scales, including the Positive and Negative Symptoms Scale (PANSS), the Clinical Global Impression (CGI), the Brief Psychiatric Rating Scale (BPRS), as well as assessments of the quality of life, treatment adherence, suicide risk, aggressiveness, relapse, and rehospitalization; and C) Minimum treatment duration of ≥12 weeks. Studies were eligible for inclusion if they compared one LAIA treatment with another or with a placebo. Studies were excluded if they were nonrandomized clinical trials, involved patients with treatment-resistant schizophrenia or with a diagnosis other than schizophrenia, treatment durations of less than 12 weeks, or comparisons with oral antipsychotics or other psychotropic medications; studies not measuring efficacy were also not considered.

Three pairs of investigators (ESU, AFG, FCM, PLCM, RM, and PJGM) independently selected the studies, reviewed the primary reports and supplementary materials, extracted the relevant information from the included trials, and assessed the risk of bias. Before each screening phase, pilots were conducted to ensure satisfactory inter-rater reliability, with a Fleiss' kappa value exceeding 0.70.[15] Any discrepancies were resolved through consensus and arbitration by a panel of investigators within the review team (FCN and PLCM).

Outcomes of interest and data extraction

A web-based extraction form was developed and evaluated by all reviewers before data extraction. General information from the included studies (author names, publication year, country of origin, funding sources, and study design) was extracted. The primary efficacy outcome of interest was symptoms, assessed by changes in the Positive and Negative Syndrome Scale (PANSS) total score. The PANSS score was chosen as the primary measure due to its utility in defining symptom severity.[16,17]

Secondary efficacy outcomes encompassed changes in the PANSS Positive and Negative subscales.

Data analysis

Effect sizes for each treatment comparison reported in the included studies were estimated with odds ratios (OR). Subsequently, frequentist network meta-analysis models were constructed to examine the primary and secondary efficacy outcomes, with placebo serving as the reference group in all models.

Pairwise meta-analytical techniques were employed to estimate effect sizes based on the mean changes in the PANSS score reported in each study. If the degree of statistical heterogeneity was considerable (i.e., an I2 statistic >50%), both random and fixed effects models were explored. In cases of a significant Q test for heterogeneity, the random effects results were utilized.

In the network meta-analysis models, both random effects and fixed effects models were considered. The assumption of transitivity was assessed using network graphs containing at least one closed loop; inconsistency within the models was evaluated through the Q statistics and the netsplit techniques (i.e., comparing the difference between indirect and direct estimates in closed loops within the network graph). If a significant level of inconsistency was detected, the results from the random effects model were reported. Treatment comparisons without direct estimates did not allow for the assessment of inconsistency. Treatment ranking was conducted using the P-score technique, and the results were presented in a forest plot that depicted the pooled effect sizes of each treatment estimated using the network meta-analysis.

A significance level of $p < 0.10$ was considered indicative of statistical significance for all analyses of heterogeneity and inconsistency. For all other analyses, a threshold of $p < 0.05$ was considered. Data analysis was performed using the R software (version 4.1.2), in conjunction with RStudio (version 2022.02.03+492) and the following packages: "meta", "netmeta", and "dmetar".

Risk of bias

The risk of bias in the included studies was assessed following the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions.[18] Two independent reviewers (RM, SM), working in duplicate, evaluated the risk of bias for each individual RCT using the Cochrane risk of bias tool 2.0 (RoB2.0). This tool encompasses six domains, which include bias arising from the randomization process, deviations from the intended intervention, missing outcome data, mismeasurement of outcomes, and selection of reported results. According to the tool, the overall risk of bias for each study was classified as low, moderate (referred to as "with some concerns" in the tool), or high.[19] In the event of any discrepancies between the reviewers, resolution was achieved through consensus or, if necessary, by consulting a third reviewer.

GRADE assessment

The certainty of the evidence for outcomes with significant clinical significance, such as PANSS, positive symptoms, and negative symptoms was assessed and categorized using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE).[19] To determine the certainty of treatment effect estimates from the network meta-analysis, it was necessary to evaluate the level of evidence for both direct and indirect comparisons, as well as the best estimates derived from both direct and indirect evidence, including the network meta-analysis (combining direct and indirect evidence).[20] The quality of evidence was classified as high, moderate, low, or very low, reflecting the certainty of the evidence for the meta-analysis.

Results

The initial search included a total of 6,525 citations, from which 5,658 unique reports were identified. After screening the titles and abstracts, 215 full-text articles were retrieved, resulting in 17 studies with a total of 7,139 participants.

An additional search update was conducted from June 2020 to September 14, 2021, which identified 184 citations; only 26 articles were selected for full-text assessment, however, no articles met the inclusion criteria. The PRISMA flowchart depicting the study selection process, including reasons for exclusion, can be found in the Supplementary Material.

Among the 17 included studies, the treatment groups consisted of 7 studies using risperidone, 11 using paliperidone, 2 using aripiprazole, 1 using haloperidol, 1 using zuclopenthixol, and 10 placebo groups; with the most common comparison being between paliperidone and risperidone (5 studies).

Network Plot

Figure 1 shows the network plots, where nodes and edges represent the different LAIAs treatments, comparisons, and placebo. Overall, a well-connected network was observed. The examined comparisons focused on PANSS score and its

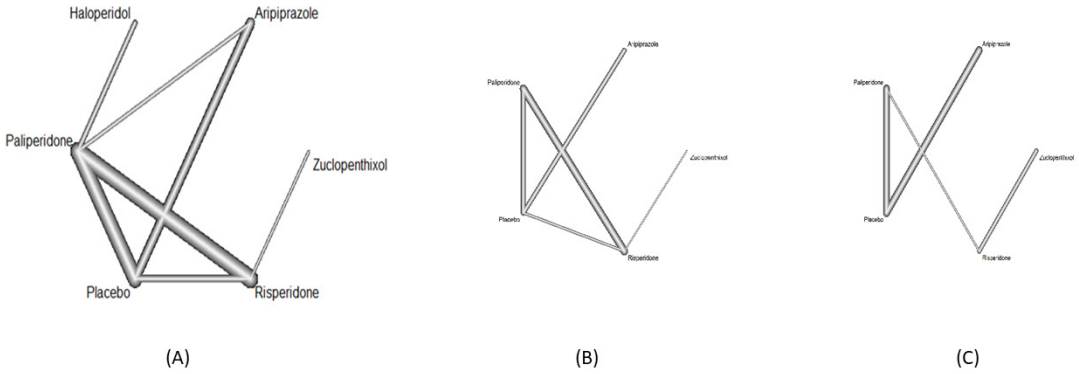


Figure 1. Network plot illustrating the meta-analysis results. A) PANSS total score, B) Negative symptoms score, C) Positive symptoms score.

Positive, and Negative symptoms subscales as reference as the primary efficacy outcome; secondary outcomes as results can be appreciated between various LAIA treatments and placebo. The most frequently examined comparisons in terms of PANSS score were between paliperidone *vs*. risperidone, as well as paliperidone vs. placebo. On the other hand, there were fewer direct comparisons between zuclopenthixol or haloperidol compared to other treatments.

Efficacy

Out of the 17 included studies, 15 reported usable results for PANSS score involving the comparison of five antipsychotics. The remaining two studies were excluded from subsequent analyses due to inconsistency with the rest of the LAIA treatments or placebo studies. The SMDs for the four antipsychotics (80%) that significantly reduced PANSS score compared with placebo ranged from –0.72 (95% CrI –0.99 to –0.46) for haloperidol to –0.45 (–0.54 to –0.37) for paliperidone, as shown in Figure 2. In hierarchical order, haloperidol, aripiprazole, risperidone, and paliperidone demonstrated significantly greater reduction in PANSS score compared to other drugs, contrary to the belief that newer antipsychotics are more effective than older ones. However, it is worth mentioning that there was only one study comparing haloperidol with paliperidone using the overall PANSS scale, without specifically assessing the efficacy of haloperidol in reducing negative and positive symptoms, limiting the results. Further details of the direct and indirect comparisons are presented in Figure 3.

Eight out of 11 studies assessed for negative symptoms reported usable results (four antipsychotics compared). The most common comparisons were between paliperidone and placebo (4 studies), as well as paliperidone and risperidone (3 studies). The SMDs for three antipsychotics (75%) that significantly reduced negative symptoms compared to placebo ranged between –0.40 (95% CrI –0.53 to –0.26) for aripiprazole to –0.32 (–0.44 to –0.19) for risperidone as depicted in Figure 2. In hierarchical order, aripiprazole, paliperidone, and risperidone demonstrated a significant reduction in negative symptoms compared to other drugs. Among the antipsychotics examined for negative symptoms, zuclopenthixol was the only one that did not show improvement in the negative symptoms subscale when compared to risperidone. Additional information on the direct and indirect comparisons is presented in Figure 3.

For the reduction of positive symptoms, 8 out of 11 studies provided usable results (involving the comparison of four antipsychotics). Similar to the negative symptoms section, the most common comparisons were between paliperidone and risperidone (3 studies) and paliperidone and placebo (4 studies). The SMDs for the three drugs (100%) that significantly reduced positive symptoms compared to placebo ranged from –0.50 (95% CrI –0.63 to –0.37) for aripiprazole to –0.19 (–0.57 to 0.20) for zuclopenthixol, as shown in Figure 2. In hierarchical order, aripiprazole, paliperidone, and risperidone demonstrated a significant reduction in positive symptoms compared to other drugs. Further details of the direct and indirect comparisons can be found in Figure 3.

In terms of the primary outcome, typical antipsychotic haloperidol ranked first in reducing PANSS scores, which is considered a crucial and comprehensive measure of efficacy. As for the secondary outcomes, aripiprazole exhibited the most

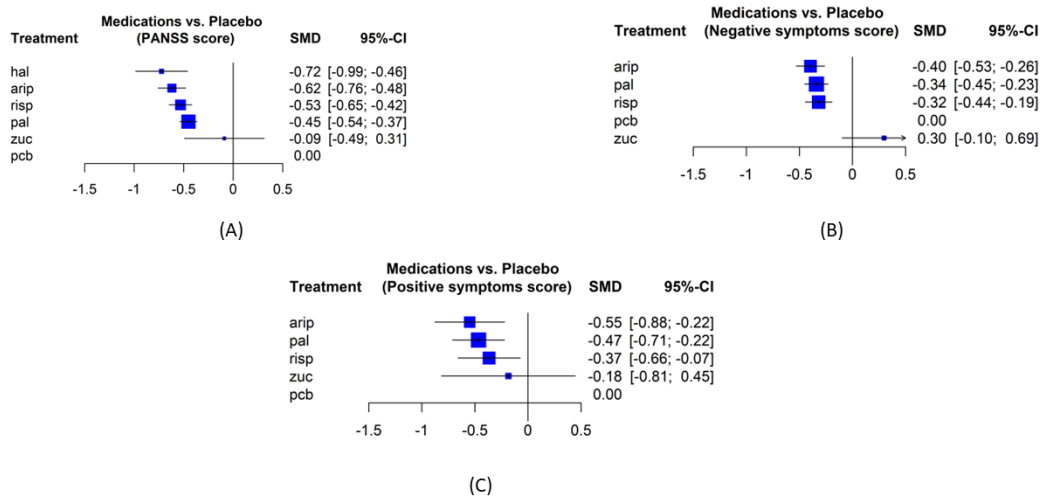


Figure 2. Treatment ranking based on the network meta-analysis of all trials. A) PANSS total score, B) Negative symptoms score, C) Positive symptoms score.

Note: Placebo serves as the reference group in both efficacy plots. SMD: standardized mean difference, OR: overall risk, Arip: ariperidone, Hal: haloperidol, Pal: paliperidone, Pcb: placebo, Risp: risperidone, Zuc: zuperidone, Flph: flupenthixol.

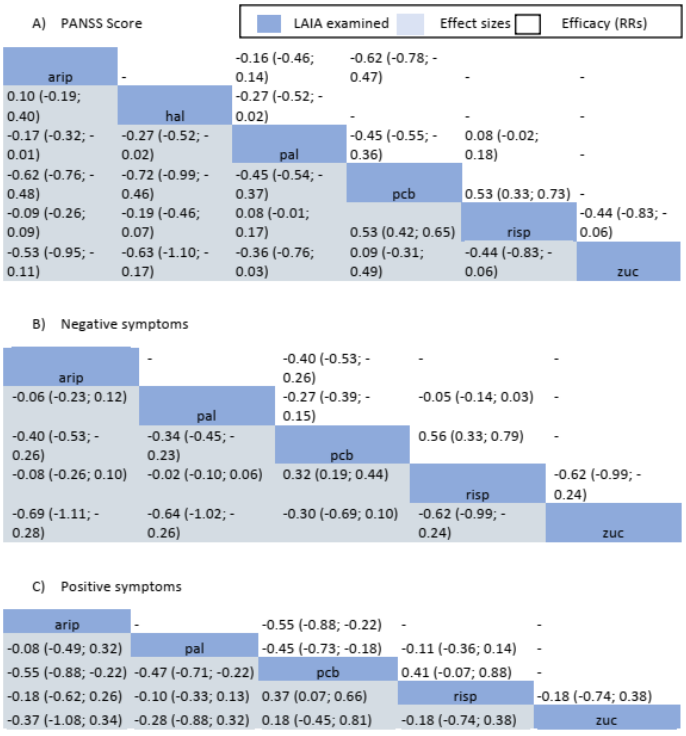


Figure 3. Direct and indirect comparisons in the network meta-analysis of all efficacy trials.

Note: The diagonal represents the various long-active injectable antipsychotics examined in the study. On the left side of the diagonal, effect sizes are presented as standardized mean difference (SMD) with corresponding 95% confidence intervals (CIs) and 95% prediction intervals, with each cell indicating values for a specific comparison between the LAIAs. On the right side of the diagonal, efficacy values are presented as relative risk (RR) with 95% CIs and 95% prediction intervals. Statistically significant data are shown in bold.

significant reductions in positive and negative symptoms and ranked second, following haloperidol, regarding PANSS score reduction. These findings indicate that one typical and one atypical antipsychotic emerged as leading treatments in terms of efficacy outcomes. These results are considered because the internal consistency of the network meta-analysis of these outcomes could be evaluated, and statistically significant differences were observed.

Discussion

In this network meta-analysis involving 17 studies and 7139 participants, we evaluated the comparative efficacy of eight different long-acting injectable antipsychotics and placebo in the treatment of schizophrenia. This study builds upon previous findings from pairwise meta-analyses that compared first versus second-generation LAIAs and examined various outcomes assessed by clinician-administered rating scales, such as positive and negative symptoms.

Our results indicate that, with the exception of zuclopenthixol, all LAIAs were more effective than placebo in reducing overall PANSS scores. The SMD ranged from –0.72 for haloperidol decanoate to –0.45 for paliperidone palmitate. However, these findings also suggest that the differences between individual LAIAs are not statistically significant. In our previous meta-analysis, we found that first and second-generation LAIAs had similar efficacy in reducing general psychopathology, although only three studies were included. The overlapping confidence intervals in this network meta-analysis further support the notion that most LAIAs have similar effectiveness in reducing overall symptoms, including haloperidol decanoate. Although node splitting assessment revealed no inconsistencies, only one study included haloperidol LAIA, which may limit the generalizability of these findings.

For positive and negative symptoms, the available data primarily originated from studies involving newer LAIAs such as paliperidone palmitate, aripiprazole, lauroxil, and risperidone microspheres, with all LAIAs exhibiting a similar effect in reducing these symptom dimensions. Even though this statement may hold true for the positive dimension, the evaluation of negative symptoms in the clinical trials included was based on the PANSS negative symptoms subscale, which does not differentiate between primary and secondary negative symptoms.[21] Therefore, it remains unclear whether the improvements observed in these core symptoms with LAIAs are directly related to their actions on the primary biochemical deficits in schizophrenia or if they are mediated through other mechanisms.

Regarding zuclopenthixol LAIA, it is worth noting that the presence of comorbid substance use disorder in the studied population may alter the homogeneity of the sample, potentially explaining the observed differences with other LAIAs.

Results obtained from the previous analyses suggest that older, less expensive LAIAs such as haloperidol decanoate exhibit comparable efficacy to second-generation LAIAs (aripiprazole, lauroxil, paliperidone palmitate, risperidone microspheres). However, only aripiprazole lauroxil (OR 0.2), risperidone microspheres (0.26), and paliperidone palmitate (0.39) demonstrated a significantly lower odds ratio (OR) for psychotic exacerbation. While clinician-administered rating scale improvements were commonly used as primary outcome measures in the included RCTs, relapse, and exacerbations are more frequently employed in clinical practice. Discrepancies in the criteria used across studies often prioritize the focus on rating scales, which may explain some differences observed between the PANSS mean changes and other outcomes.

Although the main focus of this manuscript is the efficacy of LAI´s antipsychotics, our protocol also encompasses data on safety and tolerability. Due to the heterogeneous nature of these studies (inconsistency among studies and evaluation methods), a separate analysis and discussion of the information pertaining to efficacy were necessary.

We conducted a search for various safety variables, including treatment-emergent adverse events (TEAEs), serious treatment-emergent adverse events (sTEAEs), extrapyramidal symptoms (EPS), use of antiparkinsonian drugs, clinically significant weight gain, suicide ideation, and attempts, pain at the injection site, discontinuation due to any cause, discontinuation due to adverse effects, and discontinuation due to lack of efficacy. However, given the limited literature available on most of these variables, we were only able to provide a critical review of the literature for the following variables.

Nonetheless, we can discuss some of the results included in our protocol, considering the significant relevance of the safety aspect of antipsychotics, particularly given the prevalence of metabolic alterations and neurologic adverse events associated with this class of drugs.

Regarding TEAEs and sTEAEs, aripiprazole and risperidone demonstrated favorable outcomes when compared to placebo, respectively. However, aripiprazole exhibited a non-protective effect against sTEAEs.

Only one study reported two deaths, with the majority of studies reporting a low incidence of mortality. Among LAIs, risperidone demonstrated the lowest mortality rate compared to placebo.

The all-cause discontinuation rate of LAIs indicated a protective effect for all treatments, except for haloperidol decanoate. Aripiprazole exhibited the highest rate of treatment continuation compared to placebo. Regarding discontinuation due to adverse events, aripiprazole had the lowest rate of treatment abandonment attributed to adverse events. Please refer to Figure 4 for detailed results.

In a meta-analysis conducted by our group,[14] we aimed to assess safety aspects related to the use of LAIs. Despite the numerous variables we attempted to evaluate, measures were often reported using different methods, making it difficult to draw conclusions regarding these variables. However, indirect and direct comparisons allowed for some insights to be gained. Regarding extrapyramidal symptoms and tardive dyskinesia, SG-LAIs were more likely to be associated with these adverse events compared to placebo, although this did not impact treatment discontinuation. The metabolic profile of LAIs was evaluated in only one study that conducted a metabolic assessment including measures of glucose, HbA1c%, and lipid profile over a 24-month period.[22]

This study compared haloperidol decanoate and paliperidone palmitate, with no differences observed within this comparison. These findings are of great interest, as SGA-LAIs are associated with metabolic parameters increase. However, SGA-LAIs were associated with weight gain and increased body mass index during long-term use.

Another study comparing paliperidone palmitate at different dosages (50mg, 100mg, and 150mg) versus placebo over a three-month period in acutely ill patients with schizophrenia evaluated safety outcomes. The overall incidence of TEAEs did not differ significantly between the groups. The frequency of extrapyramidal symptoms and glucose increase as TEAEs was low. However, clinically significant weight gain was more frequently observed in the paliperidone palmitate group (12% for 50 mg, 10% for 100 mg, 4% for 150 mg) compared to the placebo group (2%).[23]

A study comparing paliperidone palmitate (at dosages of 50mg, 100mg, and 150mg) and risperidone LAI (at dosages of 25mg, 37.5mg, and 50mg) over a 13-week period, assessing safety through TEAEs, clinical laboratory findings, EPS, electrocardiogram findings, and physical examination findings, found no significant differences between the two groups, with no new findings compared to previous studies.[24]

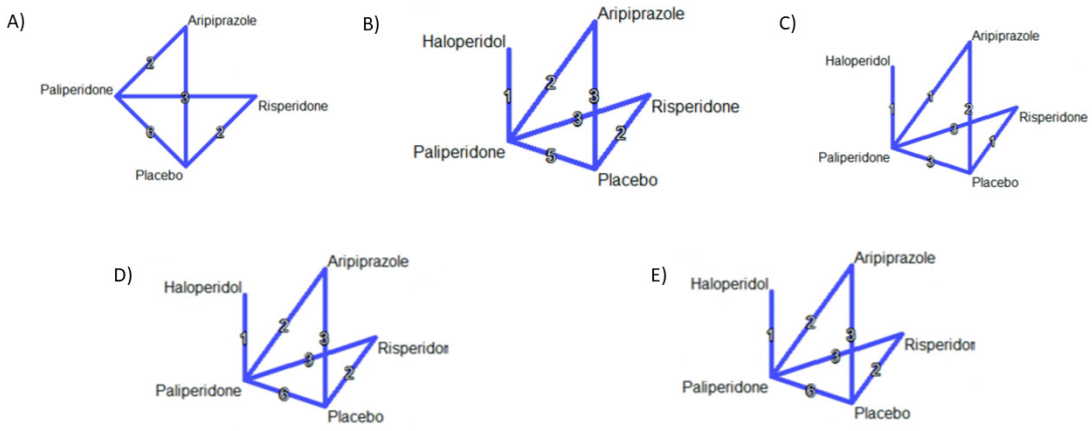


Figure 4. Safety outcomes. A) Treatment Emergent Adverse Events (TEAEs), B) Serious Treatment Emergent Adverse Events (STEAEs), C) Deaths, D) All-cause discontinuation, E) Discontinuation due to adverse events.

Strengths and limitations

To the best of our knowledge, this network meta-analysis represents the first attempt to compare the efficacy of first and second-generation long-acting injectable antipsychotics (LAIAs) and placebo in the treatment of schizophrenia. Previous meta-analyses in this area have primarily focused on mortality risk or discontinuation rates, without considering evidence regarding efficacy.

Overall, our analysis revealed no evidence of network inconsistency, while the risk of bias ranged from low to moderate across the included studies. The quality of evidence varied from very low to high. Detailed results pertaining to the risk of bias and the GRADE assessment can be found in the supplementary material.

When interpreting our findings, several limitations should be taken into account. We were unable to examine all variables that previous studies have identified as potentially influencing the efficacy of LAIAs, such as discontinuation rates, mortality, or adverse events. Our focus was specifically on assessing efficacy based on PANSS scores within the included trials, aiming for precision. However, we have identified other variables that may be worthwhile to explore in future research. Lastly, it should be noted that unpublished studies were not included in our analysis.

Conclusions

Clinical practice guidelines recommend individualized antipsychotic selection based on side effect profiles. The distinct pharmacokinetics of oral and depot antipsychotics pose challenges in managing adverse events, necessitating careful consideration before drug selection. In terms of PANSS scores, all LAIAs demonstrated similar performance compared to the placebo.

References

1. Brown A, Lau F. Chapter 2 - A review of the epidemiology of schizophrenia. Handb Behav Neurosci. 2016;23:17-30.
2. World Health Organization. Schizophrenia. https://www.who.int/news-room/fact-sheets/detail/schizophrenia
3. Collins PY, Patel V, Joestl SS, March D, Insel TR, Daar AS, et al. Grand challenges in global mental health. Nature. 2011, 475:7-10.
4. Karadag F, Sengul CB, Enli Y, Karakulah K, Alacam H, Kaptanoglu B et al. Relationship between serum bilirubin levels and metabolic syndrome in patients with schizophrenia spectrum disorders. Clin Psychopharmacol Neurosci. 2017;15(2):153-62.
5. Kim YK, Choi J, Park SC. A novel bio-psychosocial-behavioral treatment model in schizophrenia. Int J Mol Sci. 2017;18(4):734.
6. Tripathi A, Kar SK, Shukla R. Cognitive deficits in schizophrenia: Understanding the biological correlates and remediation strategies. Clin Psychopharmacol Neurosci. 2018;16(1):7-17.
7. Chesney E, Goodwin G, Fazel S. Risks of all-cause and suicide mortality in mental disorders: a meta-review. World Psychiatry. 2014;13(2):153-60.
8. Barkhof E, Meijer CJ, de Sonneville LMJ, Linszen DH, de Haan L. Interventions to improve adherence to antipsychotic medication in patients with schizophrenia-A review of the past decade. Eur Psychiatry [Internet]. 2012;27(1):9-18. doi: 10.1016/j.eurpsy.2011.02.005
9. Kishimoto T, Nitta M, Borenstein M, Kane JM, Correll CU. Long-acting injectable versus oral antipsychotics in schizophrenia: A systematic review and meta-analysis of mirror-image studies. J Clin Psychiatry. 2013;74(10):957-65.
10. Leucht C, Heres S, Kane J, Kissling W, Davis J, Leucht S. Oral versus depot antipsychotic drugs for schizophrenia--a critical systematic review and meta-analysis of randomised long-term trials. Schizophr Res. 2011; 127(1-3):83-92.
11. Park SC, Choi MY, Choi J, Park E, Tchoe HJ, Suh JK, et al. Comparative efficacy and safety of long-acting injectable and oral second-generation antipsychotics for the treatment of schizophrenia: A systematic review and meta-Analysis. Clin Psychopharmacol Neurosci. 2018; 16(4):361-75.
12. Kishi T, Matsunaga S, Iwata N. Mortality risk associated with long-acting injectable antipsychotics: A systematic review and meta-analyses of randomized controlled trials. Schizophr Bull. 2016; 42(6):1438-45.
13. Gentile S. Discontinuation rates during long-term, second-generation antipsychotic long-acting injection treatment: A systematic review. Psychiatry Clin Neurosci. 2019;73(5):216-30.
14. Saucedo E, Carranza F, Fernanda A, Medrano G, Iscely K, Cervantes G, et al. Preliminary efficacy and tolerability profiles of first versus second-generation long-acting injectable antipsychotics in schizophrenia : A systematic review and meta-analysis. J Psychiatr Res. 2020; 129:222-33.
15. Falotico R, Quatto P. Fleiss' kappa statistic without paradoxes. Qual Quant. 2015; 49:463-70.
16. Leucht S, Davis JM, Engel RR, Kissling W, Kane JM. Definitions of response and remission in schizophrenia: Recommendations for their use and their presentation. Acta Psychiatr Scand Suppl. 2009; (438):7-14.
17. Schennach-Wolff R, Seemüller FH, Mayr A, Maier W, Klingberg S, Heuser I, et al. An early improvement threshold to predict response and remission in first-episode schizophrenia. Br J Psychiatry. 2010;196(6):460-6.
18. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al., editors. Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane; 2019.

www.training.cochrane.org/handbook

19. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924-6. doi: 10.1136/bmj.39489.470347.AD.
20. Puhan MA, Murad MH, Li T, Singh JA, Kessels AG, Guyatt GH, et al. A GRADE Working Group approach for rating the quality of treatment effect estimates from network meta-analysis. BMJ. 2014;349:g5630:1-10. doi: 10.1136/bmj.g5630
21. Marder SR, Kirkpatrick B. Defining and measuring negative symptoms of schizophrenia in clinical trials. Eur Neuropsychopharmacol [Internet. 2014;24(5):737-43. doi: 10.1016/j.euroneuro.2013.10.016
22. McEvoy JP, Byerly M, Hamer RM, Dominik R, Swartz MS, Rosenheck RA, et al. Effectiveness of paliperidone palmitate vs haloperidol decanoate for maintenance treatment of schizophrenia: A randomized clinical trial. JAMA - J Am Med Assoc. 2014; 311(19):1978-86.
23. Gopal S, Hough DW, Xu H, Lull JM, Gassmann-Mayer C, Remmerie BM, et al. Efficacy and safety of paliperidone palmitate in adult patients with acutely symptomatic schizophrenia: A randomized, double-blind, placebo-controlled, dose-response study. Int Clin Psychopharmacol. 2010;25(5):247-56.
24. Pandina G, Lane R, Gopal S, Gassmann-Mayer C, Hough D, Remmerie B, et al. A double-blind study of paliperidone palmitate and risperidone long-acting injectable in adults with schizophrenia. Prog Neuro-Psychopharmacology Biol Psychiatry. 2011; 35(1):218-26. doi: 10.1016/j.pnpbp.2010.11.008

ARCHIVOS DE NEUROCIENCIAS | Case Report
received 9 October 2022 | accepted 27 January 2023 | published 29 January 2023
Doi: 10.31157/an.v28i2.421

MEDIAN ARTERY OF THE CORPUS CALLOSUM IN THE CONTEXT OF ANTERIOR COMMUNICATING ARTERY ANEURYSM RUPTURE: THE RELEVANCE OF PERIANEURYSMAL ANATOMY

Degollado-García Javier[1] | Marian-Magaña Ricardo[1] | Hernández-Hernández Alan[1]
Villanueva-Castro Eliezer[1] | Ballesteros-Herrera Daniel[1] | Gómez-Amador Juan Luis[1]

1. Department of Neurosurgery, Manuel Velasco Suárez National Institute of Neurology and Neurosurgery

Correspondence

Juan Luis Gómez Amador
Department of Neurosurgery, Manuel Velasco Suárez National Institute of Neurology and Neurosurgery. Av. Insurgentes Sur 3877, Col. La Fama, Tlalpan, Mexico City, Mexico. C.P. 14269.

jlga@neurocirugia-innn.com

Abstract

It is important to understand the patient's vascular anatomy before treating cerebral aneurysms. The middle artery of the corpus callosum is one of the least common variations of the anterior communicating artery (AComA) complex. We describe the case of a 59-year-old woman who suffered a subarachnoid hemorrhage due to an AComA complex aneurysm that had ruptured. Fluorescein injection during the aneurysm clipping procedure revealed a partial obstruction of the middle artery, requiring clip repositioning. The vascular variations that patients may exhibit must be considered in aneurysm clipping surgery.

Keywords: *AComA aneurysm, clipping, vascular abnormalities, subarachnoid hemorrhage, fluoresceine.*

Background

The anterior communicating artery (AComA) has been identified around 41-48 days of gestation during embryological development.[1] The AComA develops from a multi-channeled vascular network that coalesces at the time of birth and unites the anterior cerebral arteries (ACA) in the lamina terminalis cistern,[2,3] completing the anterior circle of Willis.[4] In the embryo, it can reach a maximum length of 18 mm.[5] The average AComA diameter is 1.2 mm when the difference in diameter between the right and left precommunicating (A1) segments of the anterior cerebral artery (ACA) is 0.5 mm or less, and 2.5 mm if the difference is more than 0.5 mm.[1,6] A variety of vascular abnormalities of the anterior communicating artery complex include AComA aplasia, duplication, A1 hypoplasia or aplasia, azygos, and median artery of the corpus callosum (MACC).[2,3,4,7,8,9]

The median artery of the corpus callosum is found in 2% of cases and dissections.[2,10,11] It runs parallel to and behind the normal pericallosal artery,[12] and gives branches to the corpus callosum, splenium, and paracentral lobules of both sides.[6,7] Its diameter ranges between 0.4 and 3.1 mm, with an average of 0.9 mm.9 There are two anatomic variants of this vessel, the classical and hemispheric, the first one terminating along the body of the corpus callosum in the midline and the second one serving as a second pericallosal with medial cortical branches.[9] It is well known that hemodynamic abnormalities create stress in artery bifurcations, resulting in aneurysm formation.[6]

Considering all vascular abnormalities of the AComA complex, we present a case of an AComA aneurysm with a median artery of the corpus callosum.

Case presentation

A 59-year-old woman with a previous history of smoking and alcohol consumption, poorly controlled chronic arterial hypertension, and associated obesity, with a body mass index (BMI) of 40 kg/m2, arrived at the emergency room due to cephalalgic syndrome with secondary characteristics consisting of frontotemporal bilateral intense severity associated with loss of consciousness and posterior recovery. The patient presented transient motor aphasia with full recovery after one hour. Physical examination revealed morbid obesity with no other findings. Neurologic examination showed meningeal signs with a positive jolt accentuation maneuver and nuchal rigidity. Simple computerized tomography (CT) scan showed a subarachnoid hemorrhage and a left Sylvian clot corresponding to Fisher I, Hunt-Hess II, and World Federation of Neurological Surgeons (WFNS) I. CT angiography revealed a superior projecting anterior communicating aneurysm and a median callosal artery (Figure 1).

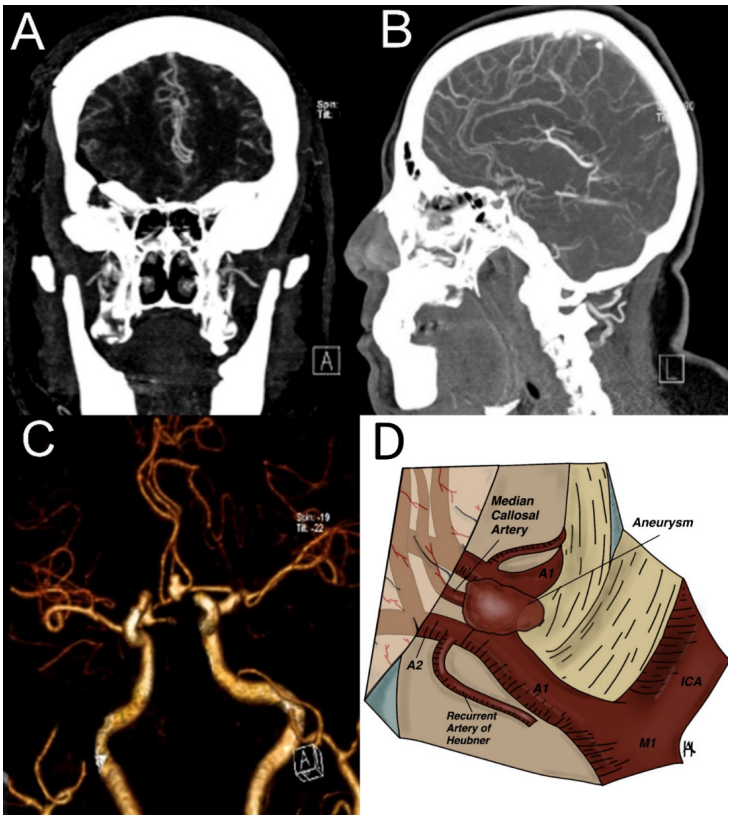


Figure 1. Preoperative aneurism. A, B. Preoperative CT angiography in coronal and sagittal projections with evidence of an AComA aneurysm with a superior projecting dome and a median callosal artery. **C.** 3D reconstruction showing the vascular preoperative relations of the aneurysmal dome. **D.** Anatomy diagram of the anterior communicating complex with the aneurysm. CT: computerized tomography; AComA: anterior communicating artery; 3D: three-dimensional; A1: precommunicating segment; A2: infracallosal segment; Ml: sphenoidal segment; ICA: internal carotid artery.

Urgent surgery with standard pterional approach was performed, followed by a sub-frontal corridor and posterior depletion of the carotid and optic-carotid cisterns. Optical nerves, chiasm, carotid artery, and ACA were identified. Opening of the lamina terminalis was performed to achieve brain relaxation. A1 was identified and followed toward the AComA. An anterior-inferior bi-lobulated projecting aneurysm was identified. Dissection of the aneurysm dome was done with bipolar forceps and aspirator. We identified both the infracallosal (A2) arteries; a third artery arising from the AComA was also identified. After the correct identification of all the AComA complex arteries, definitive clipping was performed with a 9 mm straight clip. Fluorescein was administered in a 4 mg/kg dose to confirm parent vessel patency and total aneurysm occlusion. When exposing both A2, a median callosal artery was detected partially occluded by the clip, which made it necessary to relocate it. Aneurysm residual was found in the inferior portion, and a second definitive 5 mm straight clip was used with no residual after clipping. Dural watertight reconstruction, bone repositioning, and aponeurotic repair were performed in the usual manner (Figure 2).

A postoperative CT- angiography (Figure 2) confirmed total occlusion of the aneurysm neck with no residual. The patient evolved satisfactorily with no vasospasm during follow-up and was discharged after six days.

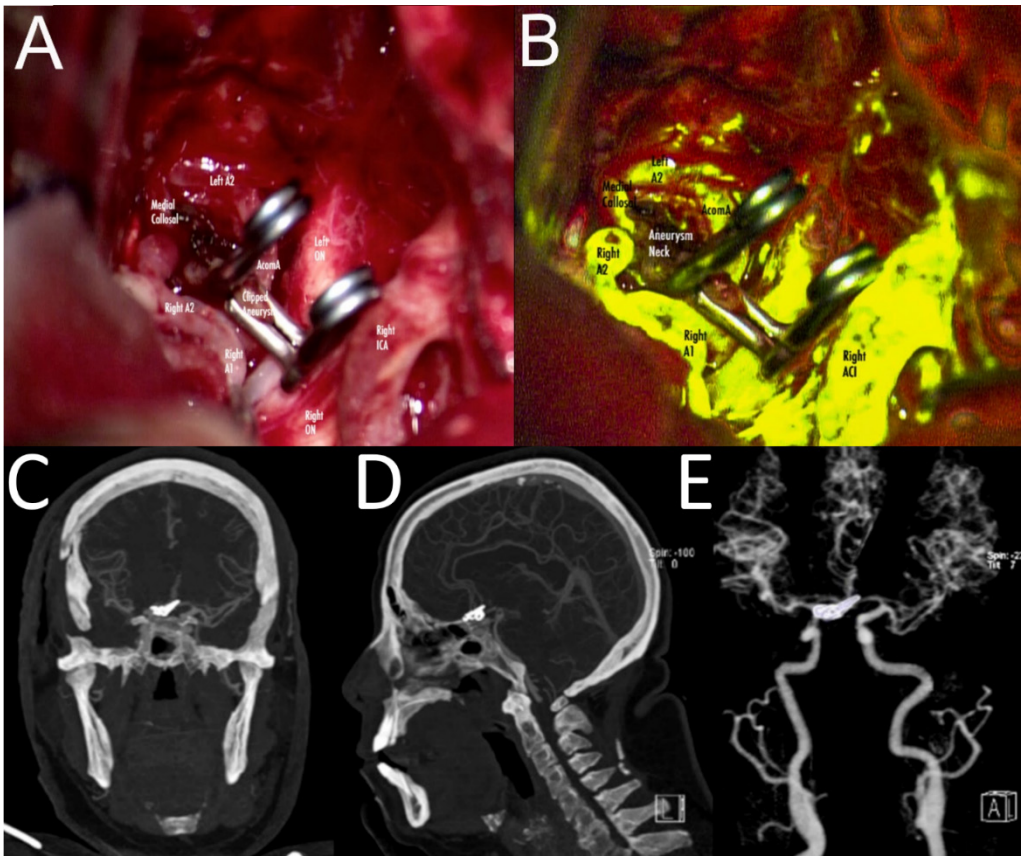


Figure 2. T**ransoperative photograph and postoperative CT angiography**. **A**. Transoperative photograph showing the AComA complex and the final clip display with no residual. **B**. Intraoperative fluorescein angiography control with complete patency of the AComA complex and no residual filling of the aneurismal dome. **C, D**. CT angiography in coronal and sagittal projections displaying clipped AComA aneurysm with a superior projecting dome without residual, and a patent AComA complex with median callosal artery. E. 3D reconstruction of CT angiography showing clip configuration. A1: precommunicating segment; A2: infracallosal segment; MI: sphenoidal segment; ICA: internal carotid artery; ON: optic nerve; AcomA: anterior communicating artery. CT: computerized tomography; 3D: three-dimensional.

Discussion and conclusion

Since the exposure obtained in the laboratory is different from that obtained in the operating room, adequate knowledge of the anterior communicating artery complex and its variants is required; this also becomes relevant when considering that the most common aneurysm site on the anterior cerebral artery is at the level of the AComA, which represents the most frequent intracranial aneurysm.[6]

As described by Yasargil and simplified by Lawton, AComA aneurysm dissection identifies 14 arteries: ipsi- and contralateral A1 segments; ipsi-and contralateral A2 segments; AComA; ipsi-and contralateral recurrent arteries of Heubner; ipsi-and contralateral orbitofrontal arteries; ipsi-and contralateral frontopolar arteries, and the collection of AComA hypothalamic perforators, the proximal origin of the callosomarginal arteries and a third A2 segment.[2,3,4]

Multiple surgical approaches have been used to treat anterior communicating artery aneurysms as the operative field is mainly dependent on the selected approach and the amount of dissection made for exposure.

All possible variants must be identified during the preoperative angiography because this will allow the surgeon to select the best approach and operative plan. This also saves time in the operating room since differentiating the median artery of the corpus callosum and an early branching pattern of the A2 may be challenging during surgery. In addition, MACC can be difficult to identify because it runs parallel to and behind the normal pericallosal artery, and it is easy to damage during surgery.[2] As Ogawa et al[13] have mentioned, there are two types of patterns: type A is when the aneurysm is at the trifurcation of the MACC, the branching point of the AComA, and the ipsilateral A1 or A2; type A2 is when the aneurysm is formed at the junction of the AComA and the ipsilateral A.

In this case, previous acknowledgment of the MACC variant allowed extending the AComA dissection until the complete identification of the aneurysm; both A2 and the MACC had a Type A pattern. It is important to highlight that even if a vascular anomaly has not been identified in the preoperative studies, all these variations should be considered, including a MACC, to avoid a postoperative stroke.

Preoperative diagnosis of this anatomic variation is mandatory to avoid occlusion of this artery. Despite not being identified in the preoperative image, it should always be considered that this variation may be present and intentionally discard this possibility to avoid ischemic complications secondary to the occlusion of these abnormal arteries.

Funding

The authors declare that no funding was received for this article.

Declaration of no interests

The authors declare that they have no conflict of interest to disclose.

Author contributions

Conception and design: Javier Degollado-García, Ricardo Marian-Magaña, Alan Hernández-Hernández, Eliezer Villanueva-Castro, Daniel Ballesteros-Herrera, Juan Luis Gómez-Amador. Acquisition of data: Javier Degollado-García, Ricardo Marian-Magaña, Alan Hernández-Hernández, Eliezer Villanueva-Castro, Daniel Ballesteros-Herrera, Juan Luis Gómez-Amador. Drafting the article: Javier Degollado-García, Ricardo Marian-Magaña, Alan Hernández-Hernández, Eliezer Villanueva-Castro, Daniel Ballesteros-Herrera, Juan Luis Gómez-Amador. Critically revising the article: Javier Degollado-García, Ricardo Marian-Magaña, Alan Hernández-Hernández, Eliezer Villanueva-Castro, Daniel Ballesteros-Herrera, Juan Luis Gómez-Amador.

References

1. Altafulla JJ, Simonds EA, Dupont G, Lachkar S, Litvack Z, Iwanaga J, et al. A median artery of the corpus callosum. Cureus. 2018;10(9):e3355. doi:10.7759/cureus.3355
2. Yasargil MG, Fox JL, Ray MW. The operative approach to aneurysms of the anterior communicating artery. In: Krayenbühl H, editor. Advances and Technical Standards in Neurosurgery. Vienna: Springer; 1975. p. 113-70.
3. Yasargil MG, Fox JL. The microsurgical approach to intracranial aneurysms. Surg Neurol. 1975;3(1):7-14.
4. Lawton MT. Anterior communicating artery aneurysms. In: Lawton MT, editor. Seven aneurysms tenets and techniques for clipping. Stuttgart: Thieme; 2011. p. 94-120.
5. Kardile PB, Ughade JM, Pandit SV, Ughade MN. Anatomical variations of anterior communicating artery. J Clin Diagn Res. 2013;7(12):2661-4. doi: 10.7860/JCDR/2013/6664.3725
6. Rhoton AL Jr. The supratentorial arteries. Neurosurgery. 2002;51(4 Suppl):S53-S120.
7. Marinković S, Milisavljević M, Marinković Z. Branches of the anterior communicating artery. Microsurgical anatomy. Acta

Neurochir (Wien). 1990;106(1-2):78-85. doi: 10.1007/BF01809337

8. Martínez F, Spagnuolo E, Calvo-Rubal A, Laza S, Sgarbi N, Soria-Vargas VR, et al. Variants of the anterior circle of Willis. Anatomic and angiografic correlation and its implications in the surgery of intracranial aneurysms. (Acigos anterior cerebral artery, median artery of the corpus callosum and accessory middle cerebral artery). Neurocirugia (Astur). 2004;15(6):578-89. doi: 10.1016/s1130-1473(04)70449-2
9. Türe U, Yaşargil MG, Krisht AF. The arteries of the corpus callosum: a microsurgical anatomic study. Neurosurgery. 1996;39(6):1075-85. doi: 10.1097/00006123-199612000-00001
10. Kwak R, Niizuma H, Hatanaka M, Suzuki J. Anterior communicating artery aneurysms with associated anomalies. J Neurosurg. 1980;52(2):162-4. doi: 10.3171/jns.1980.52.2.0162
11. Nathal E, Yasui N, Sampei T, Suzuki A. Intraoperative anatomical studies in patients with aneurysms of the anterior communicating artery complex. J Neurosurg. 1992;76(4):629-34. doi: 10.3171/jns.1992.76.4.0629
12. Okahara M, Kiyosue H, Mori H, Tanoue S, Sainou M, Nagatomi H. Anatomic variations of the cerebral arteries and their embryology: a pictorial review. Eur Radiol. 2002;12(10):2548-61. doi: 10.1007/s00330-001-1286-x
13. Ogawa A, Suzuki M, Sakurai Y, Yoshimoto T. Vascular anomalies associated with aneurysms of the anterior communicating artery: microsurgical observations. J Neurosurg. 1990;72(5):706-9. doi: 10.3171/jns.1990.72.5.0706

recibido 21 noviembre 2022 | acceptado 30 diciembre 2022| publicado 12 enero 2023

Doi: 10.31157/an.v28i2.443

SIGNO DE ROMBERG: CONCEPCIÓN HISTÓRICA

Violante-Villanueva Arturo[1] | Lopez-Hernández Juan Carlos[1]

1. Departamento de Urgencias Neurológicas, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suarez, Ciudad de México, México

Correspondencia

Juan Carlos López Hernández
Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suarez, Av. Insurgentes Sur 3877, Col. La Fama, Tlalpan, CP 14269, Ciudad de México.

juanca9684@hotmail.com

Abstract

El signo de Romberg es uno de los signos clásicos y de mayor ayuda en la exploración neurológica. Fue propuesto por el alemán Mortiz Heinrich Romberg en 1851, al describir la pérdida del control postural que experimentaban los pacientes con tabes dorsal después de cerrar los ojos o en la oscuridad.

En la literatura se reporta diferentes descripciones del signo de Romberg, y actualmente no existe un consenso. En las primeras descripciones, dicho signo fue relacionado con el daño a la vía propioceptiva. Asimismo, no solo se emplea en la neurología sino también en otras áreas médicas, por lo que resulta de gran utilidad.

Palabras clave: *conceptos historicos, descipcion, revisión, signo de Romberg*

Introducción

El signo de Romberg es una prueba que nos permite valorar alteraciones en la sensibilidad propioceptiva de los pacientes al observarse pérdida del control postural en ausencia de estímulo visual. De forma general, la prueba de Romberg se realiza solicitando al paciente que se pare con los pies juntos, primero con los ojos abiertos y después se le pide que los cierre. Se dice que el signo está presente cuando se observa pérdida de la estabilidad o incluso caída en el momento en que el paciente cierra los ojos.[1,2]

El signo de Romberg es uno de los hallazgos clínicos más antiguos que se han descrito. Inicialmente fue descrito como signo patognomónico de tabes dorsal en la neurosífilis, sin embargo, actualmente este signo persiste como parte integral de la exploración neurológica porque constituye una herramienta útil para valorar la propiocepción.[1,3]

Desde su descripción a mediados del siglo XIX por Mortiz Heinrich Romberg, el signo de Romberg ha tenido diversas modificaciones y adaptaciones e incluso hoy en día no existe un consenso sobre las variantes en ejecución e interpretación y, sobre todo, su traducción clínica, lo que ha producido discrepancias acerca de la validez e importancia semiológica de esta prueba en la práctica neurológica y en otras áreas afines, como la otorrinolaringología.[4,5,6]

Desarrollo histórico de la descripción del signo

Quizás la primera descripción del signo de Romberg como hoy lo conocemos se deba atribuir al neurólogo inglés Marshal Hall, quien en 1836, en su obra *Lectures on the Nervous System an its Diseases*, describió la pérdida de control postural en la oscuridad en pacientes con compromiso importante de la propiocepción: "Este día he visto un paciente con un ligero grado de debilidad. El camina de forma segura y normal mientras tiene los ojos fijos sobre el suelo, pero trastabilla si trata de caminar en la oscuridad (…) Sus propias palabras fueron; mis piernas se sienten adormecidas, no le podría decir donde se encuentran mis pies en la oscuridad, no puedo mantenerme en equilibrio."[7]

Por su parte, en Alemania, Mortiz Heinrich Romberg describió en la segunda edición de su obra *Lehrbook der Nervenkrankheiten des Menschen* (1851) la pérdida del control postural que experimentaban los pacientes con tabes dorsal después de cerrar los ojos o en la oscuridad. Basado en la descripción de Marshall Hall, Romberg ideó una prueba para demostrar el fenómeno, lo que después dio lugar al multicitado signo neurológico. En la obra antes mencionada, Romberg señala: "Si al paciente se le dice que cierre sus ojos mientras está en posición de bipedestación, inmediatamente comienza a moverse de lado a lado y a oscilar incluso al grado de caer".

En otro apartado, Romberg describe la forma en que, al caminar, los pacientes con pérdida de la propiocepción colocan la planta del pie con gran fuerza, con la mirada fija en sus extremidades.[8]

Paralelamente a los trabajos de Romberg, el médico alemán Bernardus Brach describió manifestaciones similares. En 1850, Brach describió lo siguiente: "Es bien sabido que las personas con tabes dorsal tienen una marcha inusual (…) el paciente con tabes dorsal levanta las piernas con las rodillas extendidas y con dificultad. Cuando da pasos golpea el piso con sus pies con fuerza (…) No siente el movimiento que hace con sus piernas, no tiene sensación en sus extremidades. Su marcha es muy propensa a caer y tiene que usar su cuerpo y brazos para mantener el balance. En las pruebas para sensibilidad de temperatura, presión y dolor, el paciente responde como cualquier persona sana. Entonces no podemos afirmar que no tiene sensibilidad".[5] Brach notó que estos pacientes no presentaban debilidad. De hecho, como anécdota curiosa, refiere que, en 1838, un paciente de 36 años caminó durante 4 horas para ir a verlo. Días antes, dicho paciente le había descrito a Brach sus síntomas en una carta: "Debo usar mis ojos para guiarme. En la oscuridad no tengo equilibrio, incluso en lugares que me son familiares, y ciertamente tiendo a caer. Cuando camino, debo concentrarme totalmente en la tarea que están realizando mis pies (…) Doy pasos muy fuertes que inclusive las plantas de mis pies presentan lesiones y se inflaman después de caminar distancias cortas."[5]

Adopción del signo

Durante el siglo XIX, la identificación de la pérdida del control postural con los ojos cerrados o en la oscuridad fue una contribución atribuida con mayor frecuencia a Moritz Romberg, aunque algunos autores daban crédito tanto a Romberg como a Bernardus Brach, o incluso se presentaba la descripción del signo sin autoría. Era notoria la discrepancia entre los neurólogos más destacados sobre la atribución de este hallazgo clínico. Por citar algunos ejemplos, el médico estadounidense William Osler,[9] el notable neurólogo Frances Jean Martin Charcot[8] y el destacado neurólogo británico Willam Gowers,[5] en sus diferentes publicaciones denominaban al fenómeno descrito como signo de Romberg.[2,5,9,10] Sin embargo, otros neurólogos importantes de la época, como Charles K. Mills o Charles Loomis[9], se referían al hallazgo como signo de Brach-Romberg.[5] En contraste, autores como Duchenne de Bologne, Alexander Hammond y Charles Radcliffe[8,9] citaban el fenómeno sin acreditarlo a nadie.[5,10,11] A pesar de las diversas descripciones y atribuciones a lo largo del siglo XIX, fue Romberg el primero en evaluar dicho fenómeno en el examen neurológico, lo que continúa siendo parte integral de la exploración física en neurología.

En 1871, William Hammond presentó sus observaciones sobre pacientes con tabes dorsal, en las que señaló que el signo era independiente de algún grado variable de paresia y estableció la utilidad de éste para distinguir la tabes dorsal de una afección de tipo cerebeloso.[10] Por su parte, Jean Martin Charcot, tal vez el neurólogo más importante de la época, refería al signo en las clases que impartía en La Salpêtrière, hospital de París, en las que convergían estudiantes de diferentes partes del mundo. Esta audiencia internacional contribuyó a la difusión del fenómeno clínico. Charcot, al igual que Hammond, consideraba el signo como característico de la tabes dorsal, sin embargo, describió que pacientes con enfermedad de Friedreich, neuropatía alcohólica y algunos pacientes con histeria también lo presentaban.[5]

Posteriormente, en 1888, William Gowers, en su obra *A Manual of Diseases Of the Nervous System*, estableció claramente las bases anatomofisiológicas del signo de Romberg y agregó la instrucción de que el paciente debía asumir una postura en la que la base de sustentanción se redujera, es decir, debía pararse con los pies juntos. A diferencia de cuando el paciente únicamente cerraba los ojos, Gowers consideraba que de esta forma la sensación de posición de los pies se ponía aún más a prueba, y que exigía al máximo la propiocepción del paciente, lo que posibilitaba valorar si existía una alteración.[3]

Recuento anatómico de la propiocepción

De forma general se acepta que existen tres modalidades de sensibilidad: superficial o exteroceptiva, que incluye dolor, tacto y temperatura; profunda, también conocida como propiocepción, que refiere a las variantes de vibración, sentido de posición articular y dolor profundo, y de asociación o combinada, que utiliza vías de asociación cortical para integrar la sensación y el reconocimiento del medio externo.

Los diversos axones de neuronas de un mismo tipo de receptores forman un haz (tracto) para crear una vía sensorial, en el caso de la sensibilidad propioceptiva, la información viaja a través de la vía del lemnisco medial, también denominada de los cordones posteriores.[12,13,14]

El sistema lemniscal (cordón posterior) conduce tacto, sensaciones articulares, discriminación entre dos puntos y sentido de vibración. Los receptores, de Pacini, Golgi y muy especialmente el huso neuromuscular, se localizan en músculos, tendones y articulaciones. El impulso se transmite a

través de unas fibras muy mielinizadas del ganglio dorsal de las astas posteriores y asciende por los cordones posteriores hasta el bulbo, donde se produce una segunda sinapsis en los núcleos gracilis y cuneatus, luego cruza la línea media para colocarse del lado contralateral, y asciende por el lemnisco medio al tálamo y después a la corteza parietal en el área somatosensitiva 3, 1 y 2 de Brodman.[12,13,14]

La propiocepción es el tipo de sensación que informa al organismo de la posición de los músculos y constituye la capacidad de sentir la posición relativa de partes corporales contiguas. De igual modo, regula la dirección y rango de movimiento, permite reacciones y respuestas automáticas, interviene en el desarrollo del esquema corporal, y en la relación de éste con el espacio, y sustenta la acción motora planificada. Otras funciones en las que actúa con más autonomía son el control del equilibrio y la coordinación de ambos lados del cuerpo. Cabe destacar que varias de estas funciones, como el equilibrio, el sentido de posición y el movimiento, son un constructo conformado no sólo por la propiocepción y las estructuras neuroanatómicas, ya que también intervienen el cerebelo, el sistema vestibular, la visión, entre otros.[12,13,14]

Como ya se ha mencionado, en los orígenes de la descripción del signo de Romberg, este se atribuía exclusivamente al tabes dorsal, que se trata de un proceso observado en la neurosífilis, caracterizado por lesiones desmielinizantes a lo largo de la vía de los cordones posteriores, lo que se traduce en alteraciones de las modalidades de sensibilidad propioceptiva, entre ellas, la aparición del signo de Romberg. Sin embargo, una vez que se documentaron las bases neuroanatómicas del signo, éste no fue más un signo patognomónico del tabes dorsal. Cualquier condición neurológica que afecte la vía de los cordones posteriores en su trayecto desde el receptor hasta la corteza somatosensorial en el lóbulo parietal podría manifestarse con signo de Romberg, por ejemplo, esclerosis múltiple, traumatismo, enfermedad Charcot-Marie-Tooth, incluso deficiencia de vitamina B12. Asimismo, se debe puntualizar que también se ha documentado la presencia de signo de Romberg en afecciones no relacionadas directamente con la vía priopioceptiva, como daño vestibular agudo (neuritis vestibular aguda) y en afecciones cerebelosas.[12,15,16]

Técnica y variantes del signo

Desde su descripción original, y hasta los últimos años, han existido variaciones en la forma de realizar y evaluar el signo de Romberg, surgidas de la necesidad de dar mayor sensibilidad o especificidad al signo al evaluar la función propioceptiva. La descripción original indica: el paciente al estar de pie y cerrar los ojos comienza a tambalearse, o al estar en obscuridad la marcha se vuelve insegura. Como puede observarse, esta descripción no señala características sobre la base de sustentación o la posición de las manos, aunque con el tiempo se agregaron especificaciones al respecto. Por ejemplo, se ha recomendado que la base de sustentación sea una área pequeña, esto es, que se solicite al paciente que mantenga los pies juntos y en una superficie firme.[1,9]

También se han propuesto variantes con respecto a la posición de las manos; algunos autores señalan que las manos deben estar a los lados del cuerpo, otros, que deben estar extendidas hacia el frente, o cruzadas sobre el tórax. Sin embargo, no hay datos que indiquen que la posición de las manos afecte la sensibilidad de la prueba. De tal manera que, actualmente, la mayoría de las descripciones del la prueba expresan: al paciente, en bipedestación, con los pies juntos y con los brazos extendidos al frente, se le pide que cierre los ojos y mantenga esa posición.[1,5]

El signo de Romberg tandem constituye otra variación; en ella el paciente se coloca de pie en posición tamden, es decir, con un pie frente al otro de tal forma que la punta de un pie toque el talón del pie delantero. La interpretación del signo es similar al Romberg tradicional.[5]

Romberg caminando, otra variante de la prueba, consiste en que el paciente camine cinco metros con los ojos abiertos y posteriormente con los ojos cerrados; el signo se registra como positivo si el paciente se balancea, cae o no puede realizar la prueba cuando tiene los ojos cerrados. En un estudio que comparó esta variante con el Romberg tradicional, la primera resultó ser más útil para detectar anormalidades propioceptivas en pacientes con mielopatía crónica.[9]

Ya que la prueba de Romberg, como se comentó anteriormente, no es utilizada exclusivamente para detectar lesiones propioceptivas, también se han descrito pruebas modificadas. Por ejemplo, en un estudio la prueba fue designada exclusivamente para evaluar la función vestibular: el participante debía mantener el equilibrio sobre una base de hule espuma —empleada para confundir la información propioceptiva— con los ojos cerrados.[4]

A continuación se expone brevemente la experiencia de los autores con respecto al signo de Romberg troncal. La prueba consistía en pedirle al paciente, sentado sobre la mesa de exploración, que extendiera sus brazos hacia

delante llevando la cabeza hacia atrás, todo esto con los ojos abiertos. Una vez que el paciente estaba en dicha posición se le pedía que cerrara los ojos. En caso de ser positivo el signo, el paciente comenzaba a presentar inestabilidad troncal con balanceo del mismo. Este hallazgo de documentó en cuatro pacientes con esclerosis múltiple con lesión espinal a nivel de la vía de los cordones posteriores.

Interpretación de la prueba

El test de Romberg es difícil de interpretar ya que existen múltiples variaciones en los criterios para considerar positivo el signo. En general se acepta que el signo es positivo cuando el paciente al cerrar los ojos comienza a balancearse, pierde la postura o cae. Esto sería un indicador de lesión de las vías propioceptivas, puesto que, al eliminarse la información visual, el paciente depende únicamente de la información propioceptiva para mantener la posición. Sin embargo, factores como la experiencia del examinador y el grado de lesión pueden generar falsos positivos o negativos.[1,2] De igual modo, existe variación según el grado de balanceo que debe presentarse para que el signo se considere positivo. Muchos médicos no consideran el balanceo de cadera, e insisten en observar el balanceo de tobillos antes de confirmar el signo. Otros especialistas prefieren que el paciente esté descalzo durante la prueba y confirman el signo sólo si éste da un paso correctivo a un lado, o si casi se produce una caída.[12] Por otra parte, muchos pacientes de edad avanzada se balancean ligeramente con los ojos cerrados, así que esto puede resultar poco significativo. No obstante, el balanceo mínimo puede desparecer pidiendo al paciente que se mantenga totalmente inmóvil.[17] En suma, dicha falta de unificación de criterios puede alterar la sensibilidad de la prueba y restar confiabilidad al diagnóstico.

Otros usos de la prueba

Ya que el signo de Romberg aparece no sólo en el caso de lesiones en las vías propioceptivas, sino también en lesiones cerebelosas y vestibulares, su uso no se restringe al campo de la neurología, e incluso ha sido empleado como prueba de control postural para indagar en la relación entre defectos secundarios de éste y esguinces de tobillo.[15]

Asimismo, el signo ha sido utilizado como indicador de riesgo de caída en personas ancianas, resultando un predictor confiable de caídas dentro de un lapso de dos años, por lo que ha sido sugerido como herramienta útil de prevención. Al respecto, una investigación que evaluó la sensibilidad y especificidad de los test de movibilidad, mostró que un conjunto de pruebas clínicas —que incluían radio de balanceo con prueba de Romberg, máximo apoyo anterior y posterior y arco de movimiento, lateral y medial, al sentarse y levantarse, así como grado de balanceo al sentarse y levantarse— presentaban una sensibilidad de 80% y especificidad de 74% para predecir caídas (*fall status*) o riesgo de caídas en personas ancianas.[17]

El test, en su variante de Romberg modificado, descrita anteriormente, también se ha utilizado en la detección de déficit de equilibrio y afecciones vestibulares. Se ha argumentado que esta variante evalúa específicamente la función vestibular, sin embargo, se desconoce la validez de esta afirmación, ya que, como se ha explicado antes, la base fisiopatológica del signo de Romberg refiere a la valoración de las vías propioceptivas.

Conclusión

Aunque la literatura ofrece diferentes modificaciones del signo de Romberg, éste continúa siendo uno de los signos clínicos de mayor utilidad para el médico neurólogo en la valoración de alteraciones en la vía propioceptiva.

Contribución de los autores

VVA: redacción, revisión y edición; LHJC: redacción, revisión y edición.

Referencias

1. Campbell, WW. DeJong's The Neurologic Examination. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
2. Lanska D. The Romberg sign and early instruments for measuring postural sway. Semin Neurol. 2002;22(4):409-18. doi: 10.1055/s-2002-36763
3. Gowers WR. Locomotor Ataxy (tabes dorsalis: posterior sclerosis). En: A manual of diseases of the nervous system. American edition. Philadelphia: P. Blakiston, Son & Co.; 1888. p. 285-323.
4. Agrawal Y, Carey JP, Della Santina CC, Schubert MC, Minor LB. Disorders of balance and vestibular function in US adults. Arch Intern Med. 2009;169(10):938-44. doi: 10.1001/archinternmed.2009.66
5. Lanska D, Goetz C., Romberg´s sign: Development, adoption and adaptation in the 19th century. Neurology 2000;55:1201-1206. doi: 10.1212/wnl.55.8.1201
6. Wilkins R, Brody I. Neurological Classics. Philadelphia: American Association of Neurological Surgeons; 1997.
7. Hall M. Lectures on the nervous system and its diseases. London: Sherwood, Gilbert, & Piper; 1836.
8. Poore V. Selections from the clinical works of Dr. Duchenne. London: The new Sydenham society; 1883.

9. Findaly GF, Balain B, Trivedi JM, Jaffray DC. Does walking change the Romberg sign? Eur Spine J. 2009;18(10):1528-31. doi: 10.1007/s00586-009-1008-7
10. Hammond WA. Sclerosis of the posterior columns of the spinal cord (locomotor ataxia). En: A treatise on diseases of the nervous system. New York: D. Appleton & Co.; 1871. p. 484-516.
11. Pearce JM. Romberg and his sign. Eur Neurol. 2005; 53(4):210-3. doi: 10.1159/000086732
12. Afifi A, Bergman R. Neuroanatomía funcional: texto y atlas. 2ª ed. Ciudad de México: McGrawHill; 2006.
13. Crossman A, Neary R. Neuroanatomía: texto y atlas a color. 3ª ed. Barcelona: Elsevier; 2007.
14. Waxman S. Clinical Neuroanatomy. 26ª ed. EUA: McGrawHill; 2009.
15. McKeon P, Hertel J. Systematic review of postural control and lateral ankle instability, part II: is balance training clinically effective? J Athl Train. 2008; 43(3):305-15. doi: 10.4085/1062-6050-43.3.305
16. Notermans NC, van Dijk GW, van der Graaf Y, van Gijn J, Wokke JH. Measuring ataxia: quatification based on the estándar neurological examination. J Neurol Neurosurg Psychiatry. 1994; 57(1):22-6. doi: 10.1136/jnnp.57.1.22
17. Ortuño M, Martín E, Barona R. Posturografía estática frente a pruebas clínicas en ancianos con vestibulopatía. Acta Otorrinolaringol Esp. 2008;59(7):334-40. doi:10.1016/S0001-6519(08)75552-3