LETTER TO EDITOR

FANSY POSTCOV: A composite clinical immunological predictive index for post-COVID-19 syndrome unveils distinctive features in a cohort study of mild to critical patients

Dear Editor,

The post-coronavirus disease 2019 (COVID-19) syndrome encompasses the persistence of symptoms for more than 12 weeks after infection onset.[1] It causes multiorgan damage and disability, has an unclear pathogenesis and lacks biomarkers.[1] Our study provides evidence of the clinical and immunological signatures of post-COVID-19 syndrome to identify at-risk patients who may benefit from therapy in a timely manner.

In this observational cohort study, 103 patients with a positive polymerase chan reaction (PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and who received treatment between August 2020 and February 2021 at the Unidad Temporal, a reference hospital for COVID-19 in Mexico, were followed up until May 2021 (mean follow-up, 107.82 days). The primary outcome was the presence of post-COVID-19 syndrome 12 weeks after infection onset as diagnosed in patients with fatigue and at least two of the multiorgan symptoms previously described.[1] Symptoms were assessed using a standardised questionnaire, which was developed after a multidisciplinary group reached a consensus as shown in the Supplementary Material. The systems addressed were respiratory, cardiovascular, neurological, gastrointestinal, musculoskeletal, psychological/psychiatric, ear, nose and throat and dermatological.[1] All subjects provided written informed consent, and the study was approved by the institutional ethical review committee (Reference: 3341).

On the day the patients sought medical attention or were admitted to the hospital (baseline), and at 1 and 3 months later, a venous blood sample was drawn to characterise T- and B-cell subsets, cytokines, chemokines, serum anticellular antibodies, anti-SARS-Cov-2 immunoglobulin G (IgG) antibodies, neutrophil extracellular traps (NETs) and tripartite motif (TRIM)63 as detailed in the Supplementary Material.

Post-COVID-19 syndrome was diagnosed in 46.6% of patients ($n = 48$) and was more frequent in women ($n = 31$, 64.6%). Table S1 shows the clinical features of patients with COVID-19. The clinical and immunological features of patients at baseline according to the development of post-COVID-19 syndrome are shown in Tables S2–S5. Patients classified with post-COVID-19 syndrome had an increased serum level of interleukin (IL)-1β ($p = .01$), elevated proportion of CD24$^+$CD38$^{lo/-}$ B cells ($p = .01$), enhanced expression of CD57 in CD8$^+$ T cells ($p = .05$), decreased proportion of naïve CD4$^+$ T cells ($p = .01$) and lower Th17 cells ($p = .04$). Other biological features at baseline were not associated with post-COVID-19 syndrome (Table S6). Univariate analysis for the prediction of post-COVID-19 syndrome is shown in Table S7. After multivariate analysis, we constructed a composite predictive index for post-COVID-19 syndrome, named **FANSY** POSTCOV as an acronym of the following explanatory variables: **F**emale, percentage of CD24$^+$CD38$^{-/lo}$ (**A**typical memory B cells), **N**aïve CD4$^+$ T cells, expression of the **S**enescence marker CD57 in CD8$^+$ T cells and the number of **SY**mptoms at COVID-19 diagnosis (Figure 1). The cut-off point and score of each explanatory variable are shown in Table 1. Using a cut-off point of ≥ 7, the FANSY POSTCOV index showed a sensitivity of 0.89 (95% confidence interval (CI) 0.77–0.96), specificity of 0.43 (95% CI 0.30–0.57), positive predictive value of 0.58 (95% CI 0.43–0.81), negative predictive value of 0.82 (95% CI 0.65–0.89), positive likelihood ratio of 1.58 (95% CI 1.23–2.04) and a negative likelihood ratio of 0.23 (95% CI 0.09–0.57). The optimism-corrected predictive parameters are shown in Table 2. The receiver operator curve and calibration plot for the FANSY POSTCOV index are shown in Figure 2.

The serum cytokines, chemokines, anti-SARS-CoV-2 IgG antibodies, NETs and TRIM63 recorded 3 months after COVID-19 symptom onset or hospital discharge are shown in Table S8. Patients with the post-COVID-19 syndrome

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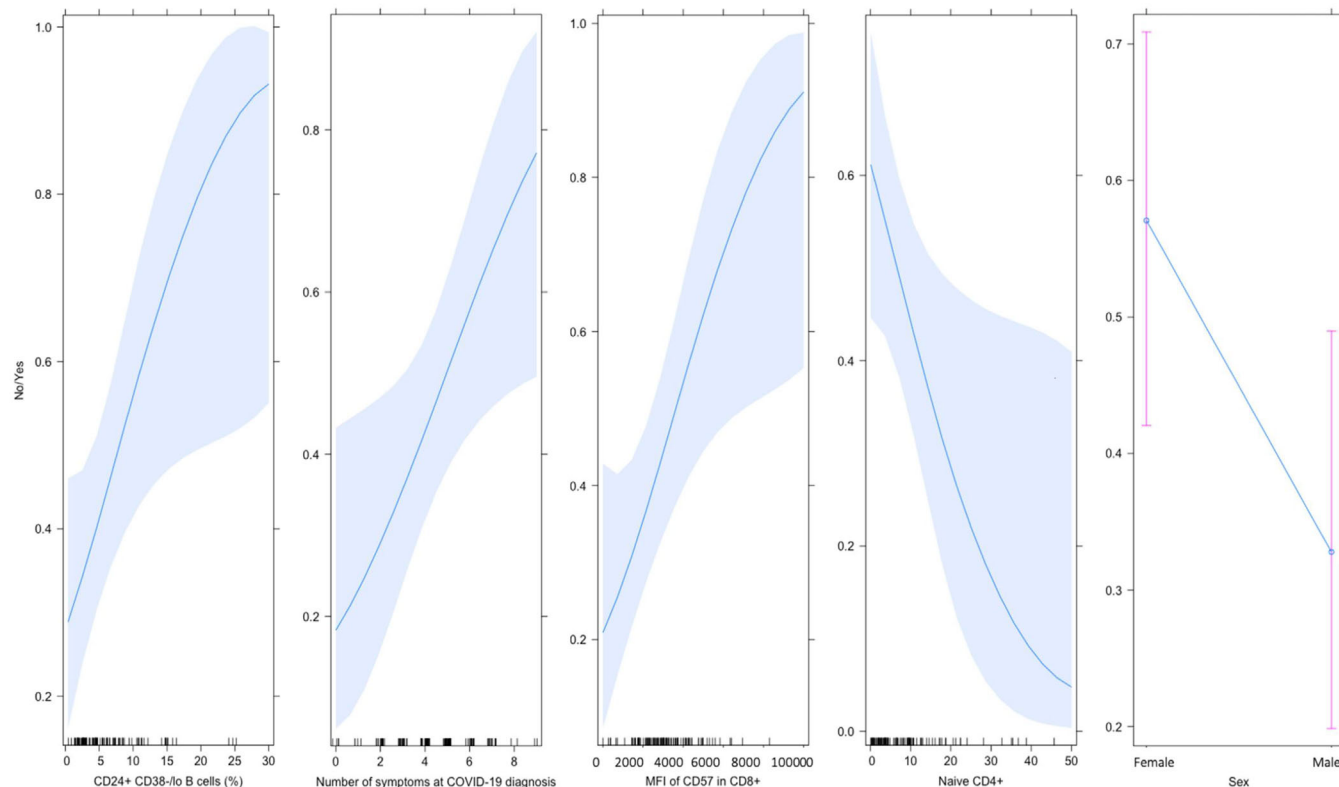
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FIGURE 1 Graphical representation of the effect of the variables included in the FANSY POSTCOV (**F**emale, percentage of CD24$^+$CD38$^{-/lo}$ (**A**typical memory B cells), **N**aïve CD4$^+$ T cells, expression of the **S**enescence marker CD57 in CD8$^+$ T cells and the number of **SY**mptoms at coronavirus disease 2019 (COVID-19) diagnosis) predictive index for the diagnosis of post-COVID-19 syndrome

TABLE 1 Post-COVID-19 syndrome clinical-immunological predictive index (FANSY POSTCOV)

	OR	95% CI	*p*-value	Cut-off point	Score
Sex	2.73	1.12 to 7.09	0.03	Female	5
Number of symptoms at COVID-19 diagnosis	1.35	1.06 to 1.77	0.012	>3.5	4
CD24$^+$CD38$^{lo/-}$ B cells	1.13	1.02 to 1.25	0.03	>2.805	3
Naive CD4$^+$ T cells	0.93	0.87 to 0.99	0.009	<9.58	2
MFI of CD57 in CD8$^+$ T cells	1.00	1.00 to 1.00	0.04	>22,785.5	1

Note: All features were evaluated at baseline (at hospital admission).

Abbreviations: COVID-19, coronavirus disease 2019; CI, confidence interval; FANSY POSTCOV, **F**emale, percentage of CD24$^+$CD38$^{-/lo}$ (**A**typical memory B cells), **N**aïve CD4$^+$ T cells, expression of the **S**enescence marker CD57 in CD8$^+$ T cells and the number of **SY**mptoms at COVID-19 diagnosis; MFI, mean fluorescence intensity; OR, odds ratio.

TABLE 2 Optimism-corrected predictive parameters of the FANSY POSTCOV composite index

	Original index	Training	Test	Optimism index-corrected	Optimism
AUC	0.720	0.719	0.720	0.721	−0.001
C-statistic	0.720	0.719	0.720	0.721	−0.033
Somers' D	0.440	0.438	0.440	0.443	−0.003
E$_{max}$	0.000	0.000	0.013	0.013	0.013
Brier score	0.213	0.210	0.216	0.220	−0.007

Abbreviations: AUC, area under the curve; FANSY POSTCOV, **F**emale, percentage of CD24$^+$CD38$^{-/lo}$ (**A**typical memory B cells), **N**aïve CD4$^+$ T cells, expression of the **S**enescence marker CD57 in CD8$^+$ T cells and the number of **SY**mptoms at COVID-19 diagnosis; MFI, mean fluorescence intensity; OR, odds ratio.
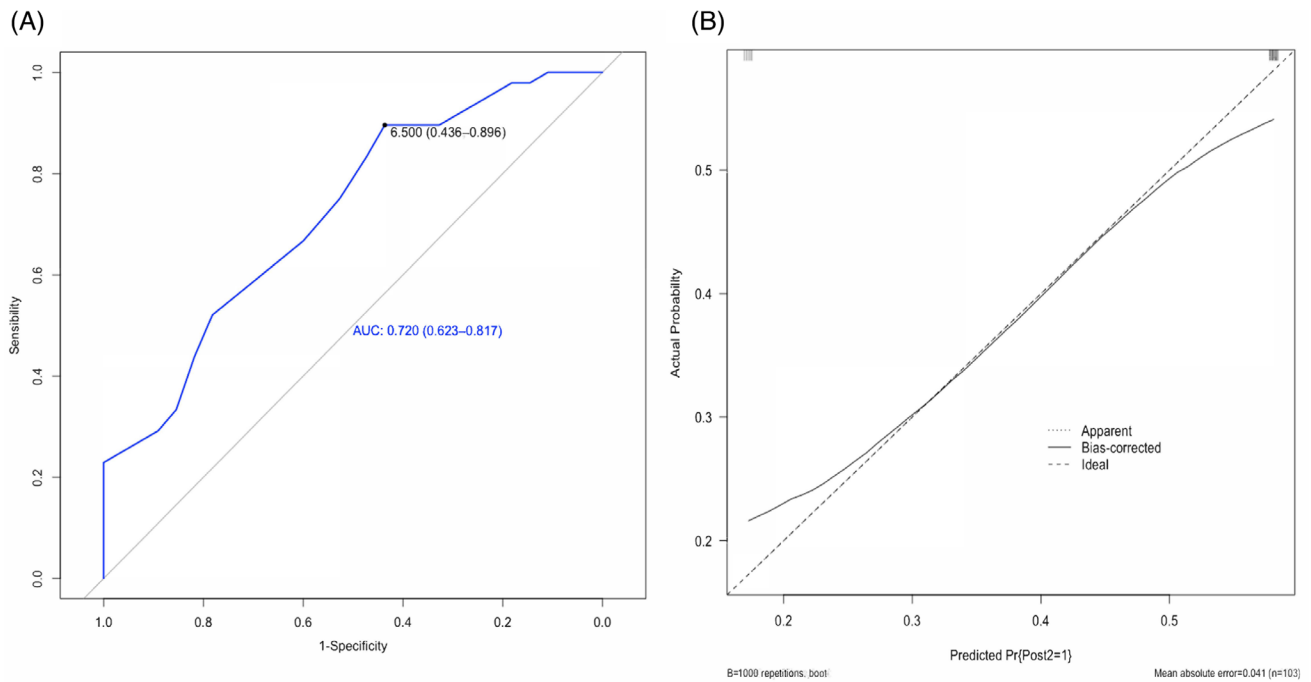
FIGURE 2 Receiver operator curve and calibration plot for the FANSY POSTCOV index are shown in (A) and (B), respectively; AUC = area under the curve

had higher serum levels of anti-SARS-CoV2 IgG antibodies (p = .01), granulocyte-macrophage colony-stimulating factor (GM-CSF; p = .02) and vascular endothelial growth factor (VEGF; p = .009).

Univariate analysis of the biomarkers assessed at post-COVID-19 syndrome diagnosis is shown in Table S9. After multivariate analysis, the only variable to remain significant was serum VEGF. The serum VEGF cut-off point associated with the post-COVID-19 syndrome was > 71.71 pg/ml This cut-off point showed a sensitivity of 0.62 (95% CI 0.48–0.74), specificity of 0.71 (95% CI 0.58–0.81) and positive likelihood ratio of 2.18.

According to previous studies, women and patients with a higher number of symptoms and elevated levels of anti-SARS-CoV-2 antibodies were more prone to post-COVID-19 syndrome.[2] A greater number of symptoms may reflect organ damage, one of the features of post-COVID-19 syndrome.[3] We found that patients with long-lasting COVID-19 symptoms showed an exhausted anergic phenotype, with a high proportion of CD24+CD38^{lo/−} B cells and elevated expression of CD57 in CD8+ T cells, which may promote viral persistence or reactivation.[3] The CD24+CD38^{−/lo} B cell population includes mature naïve and atypical memory B cells, which have been shown to have a dampened proliferative response upon antigenic stimulation.[4] The naïve CD4+ lymphopenia and the decreased absolute number of Th17 reflect a global defect in the T helper compartment with a reduced viral clearance capacity as was shown in patients with post-COVID-19 neurologic symptoms.[5] A low proportion of naïve CD4+ T cells is also associated with a good prognosis in SARS-CoV-2 infection,[6] and therefore our data may also reflect the survival status of patients with COVID-19. It has been suggested that post-COVID-19 syndrome shares clinical and immunological features with chronic fatigue syndrome and fibromyalgia,[7] including increased levels of IL-1β, lower expression of activation markers in T cells and a dysregulated B-cell compartment.[8] Our study highlights the serum GM-CSF as a key driver of COVID-19 severity[9] and VEGF as a probable marker of chronic hypoxia after SARS-CoV-2-induced lung damage.[10]

In conclusion, the FANSY POSTCOV index is the first tool to include both clinical and immunological features to predict the development of post-COVID-19 syndrome. Our study is also the first to reveal VEGF as a biomarker of this syndrome. We believe that the FANSY POSTCOV will be particularly useful for clinicians to provide closer follow-up and treatment. Since all our patients are Latin American, additional studies are needed to validate our findings and to elucidate the potential inducers of VEGF in patients with post-COVID-19 syndrome. Furthermore, this tool may only be applicable in reference care centres with the capability to measure the included parameters.

CONFLICT OF INTEREST

Jiram Torres-Ruiz[1,2]
Julieta Lomelín-Gascón[3]
Jaquelin Lira-Luna[1]
Alfredo Pérez-Fragoso[1]
Roberto Tapia-Conyer[3,4]
Miroslava Nuñez-Aguirre[1]
Beatriz Alcalá-Carmona[1]
Abdiel Absalón-Aguilar[1]
José Luis Maravillas-Montero[5]
Nancy Raquel Mejía-Domínguez[5]
Carlos Núñez-Álvarez[1]
Luis Llorente[1]
Sandra Romero-Ramírez[5]
Victor Andrés Sosa-Hernández[5]
Rodrigo Cervantes-Díaz[5]
Guillermo Juárez-Vega[5]
David Meza-Sánchez[5]
Marina Rull-Gabayet[1]
Luis Alberto Martínez-Juárez[3,6]
Linda Morales-Juárez[3,7]
Lizeth Naomi López-López[3]
José Adrián Negrete-Trujillo[3]
Jorge Abelardo Falcón-Lezama[3]
Rafael Ricardo Valdez-Vázquez[7]
Héctor Gallardo-Rincón[3,8]
Diana Gómez-Martín[1,5]

[1] Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

[2] Emergency Medicine Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

[3] Departments of Operative and Global Solutions, Fundación Carlos Slim, Mexico City, Mexico
Email: julieta.lomelin@gmail.com;
luis.martinez@lshtm.ac.uk

[4] Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

[5] Red de Apoyo a la Investigación, Universidad Nacional Autónoma de México e Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

[6] Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK

[7] Temporary COVID-19 Hospital, Mexico City, Mexico

[8] Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, Mexico

Correspondence

Diana Gómez-Martín, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Belisario Domínguez Sección XVI, Tlalpan, 14080, Mexico City, Mexico.
Email: diana.gomezm@incmnsz.mx
Héctor Gallardo-Rincón, Fundación Carlos Slim and Universidad de Guadalajura CUCS, Lago Zurich 245, Presa Falcón, Building Floor 29, Miguel Hidalgo, 11529, Mexico City, Mexico.
Email: gallardodr@yahoo.com

Jiram Torres-Ruiz and Julieta Lomelin-Gascon contributed equally to this work.

ORCID

Jiram Torres-Ruiz https://orcid.org/0000-0002-7882-1488
Julieta Lomelín-Gascón https://orcid.org/0000-0001-7665-5351
Jaquelin Lira-Luna https://orcid.org/0000-0001-7601-3826
Alfredo Pérez-Fragoso https://orcid.org/0000-0003-2978-4200
Roberto Tapia-Conyer https://orcid.org/0000-0001-9425-7322
Miroslava Nuñez-Aguirre https://orcid.org/0000-0002-9154-7938
Beatriz Alcalá-Carmona https://orcid.org/0000-0002-0985-5609
Abdiel Absalón-Aguilar https://orcid.org/0000-0003-3967-8516

José Luis Maravillas-Montero https://orcid.org/0000-0003-3755-662X

Nancy Raquel Mejía-Domínguez https://orcid.org/0000-0002-5788-4586

Carlos Núñez-Álvarez https://orcid.org/0000-0002-5051-0389

Luis Llorente https://orcid.org/0000-0002-3464-3747

Sandra Romero-Ramírez https://orcid.org/0000-0002-9751-087X

Victor Andrés Sosa-Hernández https://orcid.org/0000-0003-4203-4228

Rodrigo Cervantes-Díaz https://orcid.org/0000-0001-7245-7315

Guillermo Juárez-Vega https://orcid.org/0000-0002-0633-0567

David Meza-Sánchez https://orcid.org/0000-0003-0392-296X

Marina Rull-Gabayet https://orcid.org/0000-0003-4409-7548

Luis Alberto Martínez-Juárez https://orcid.org/0000-0001-7550-7867

Linda Morales-Juárez https://orcid.org/0000-0002-1028-2465

Lizeth Naomi López-López https://orcid.org/0000-0003-2312-7015

José Adrián Negrete-Trujillo https://orcid.org/0000-0003-3072-6120

Jorge Abelardo Falcón-Lezama https://orcid.org/0000-0001-9081-4064

Rafael Ricardo Valdez-Vázquez https://orcid.org/0000-0002-5026-9433

Héctor Gallardo-Rincón https://orcid.org/0000-0002-0811-4606

Diana Gómez-Martín https://orcid.org/0000-0002-1807-0045

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