

# Serum Levels of CXCL13 Are Associated With Teriflunomide Response in Patients With Multiple Sclerosis

Nicolás Fissolo, PhD, Agustin Pappolla, MD, Jordi Rio, MD, PhD, Luisa M. Villar, MD, PhD, Santiago Perez-Hoyos, BSc, PhD, Alex Sanchez, PhD, Lucía Gutierrez, BSc, Xavier Montalban, MD, PhD, and Manuel Comabella, MD, PhD

## Correspondence

Dr. Fissolo  
nicolas.fissolo@vhir.org

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## Abstract

### Background and Objectives

To identify biomarkers associated with treatment response in patients with multiple sclerosis (MS) treated with the oral therapies teriflunomide, dimethyl fumarate (DMF), and fingolimod.

### Methods

Serum levels of IL-6, IL-17, TNF- $\alpha$ , granulocyte-macrophage colony-stimulating factor, IL-10, interferon-gamma (IFN- $\gamma$ ), IL-1 $\beta$ , and chemokine ligand 13 (CXCL13) were measured at baseline and 12 months with single molecule array (Simoa) assays in a cohort of patients with MS treated with teriflunomide (N = 19), DMF (N = 22), and fingolimod (N = 25) and classified into “no evidence of disease activity” (NEDA) and EDA patients after 1 year of treatment.

### Results

Serum CXCL13 and TNF- $\alpha$  levels were significantly decreased after treatment with teriflunomide in NEDA compared with EDA patients after 1 year of treatment ( $p = 0.008$  for both cytokines). These findings were validated in an independent cohort of patients with MS treated with teriflunomide (N = 36) and serum CXCL13, and TNF- $\alpha$  levels were again significantly reduced in NEDA patients ( $p < 0.0001$  for CXCL13 and  $p = 0.003$  for TNF- $\alpha$ ). CXCL13, but not TNF- $\alpha$ , showed good performance to classify NEDA and EDA patients according to a cut-off value of 9.64 pg/mL based on the change in CXCL13 levels between baseline and 12 months, with a sensitivity of 75% and specificity of 82% in the original cohort, and sensitivity of 65.4% and specificity of 60% in the validation cohort.

### Discussion

Altogether, these results point to CXCL13 as a treatment response biomarker to teriflunomide in relapsing-remitting patients with MS, and the change in CXCL13 levels during the first year of treatment can be used in clinical practice to identify optimal responders to teriflunomide.

From the Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya, Institut de Recerca Vall d'Hebron (N.F., A.P., J.R., L.G., X.M., M.C.); Departments of Immunology and Neurology (L.M.V.), Multiple Sclerosis Unit, Hospital Ramon y Cajal, (IRYCIS), Madrid; Statistics and Bioinformatics Unit, Vall d'Hebron Institut de Recerca (VHIR) (S.P.-H., A.S.); and Genetics, Microbiology and Statistics Department (A.S.), Universitat de Barcelona, Spain.

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## Glossary

**AUC** = area under the ROC curve; **CXCL13** = chemokine ligand 13; **DMF** = dimethyl fumarate; **EDA** = evidence of disease activity; **EDSS** = Expanded Disability Status Scale; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **IFN- $\gamma$**  = interferon-gamma; **MS** = multiple sclerosis; **NEDA** = no evidence of disease activity; **ROC** = Receiver operating characteristic; **RRMS** = relapsing-remitting MS.

Biomarkers are indicators of normal biological or pathogenic processes that can be objectively measured.<sup>1</sup> Among the different types of biomarkers, treatment response biomarkers are those measured in patients receiving therapies to identify those individuals who are at risk for treatment failure and/or serious adverse drug reactions. Multiple sclerosis (MS) is a complex neurologic disorder characterized by a high degree of heterogeneity regarding several disease aspects including the response to treatment.<sup>2</sup> Although numerous studies have been published aiming to identify biomarkers associated with the response to treatment in patients with MS, very few treatment response biomarkers are used at present in clinical practice to guide treatment decisions.<sup>3,4</sup> This aspect is critical considering the current scenario of MS treatment, with many different options available to treat patients with MS. In this study, we aimed to identify treatment response biomarkers in patients with MS receiving the oral therapies teriflunomide, dimethyl fumarate

(DMF), and fingolimod and classified them according to the presence or absence of disease activity. For this, we measured the serum levels of a panel of inflammatory biomarkers that have been proposed at some point to play a role in MS etiology and included cytokines of the innate immune system (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ), T-cell-related cytokines (IL-17, interferon-gamma [IFN- $\gamma$ ], IL-10, and granulocyte-macrophage colony-stimulating factor [GM-CSF]), and B-cell cytokines (chemokine ligand 13 [CXCL13]).<sup>3,5-7</sup>

## Methods

### Original Cohort

Patients with relapsing-remitting MS (RRMS) treated with teriflunomide, DMF, or fingolimod at the outpatient clinic of the Centre d'Esclerosi Múltiple de Catalunya (Cemcat, Barcelona)

**Table 1** Demographic and Baseline Clinical Characteristics of Patients With MS From the Original Cohort

Baseline characteristics	Teriflunomide			Dimethyl fumarate			Fingolimod		
	NEDA	EDA	<i>p</i> Value	NEDA	EDA	<i>p</i> Value	NEDA	EDA	<i>p</i> Value
<b>N</b>	8	11	—	13	9	—	6	19	—
<b>Age (y)</b>	44.9 (8.8)	43.0 (8.4)	0.3	45.1 (9.9)	44.5 (9.7)	0.3	37.5 (3.5)	37.7 (7.9)	0.9
<b>Female/male (% women)</b>	3/5 (37.5)	10/1 (90.9)	<b>0.01</b>	10/3 (77.0)	7/2 (77.8)	0.9	4/2 (66.7)	14/5 (73.9)	0.7
<b>Duration of disease (y)</b>	12.1 (6.2)	12.2 (6.2)	0.5	10.8 (12.0)	10.6 (11.5)	0.4	7.9 (5.3)	10.1 (6.4)	0.1
<b>Naïve/no naïve (% naïve)<sup>e</sup></b>	1/7 (12.5)	3/8 (27.7)	0.4	8/5 (61.5)	3/6 (33.3)	0.2	1/5 (16.7)	1/18 (5.3)	0.4
<b>EDSS<sup>a</sup></b>	1.9 (1.0–2.0)	2.3 (1.5–2.5)	0.3	2.0 (1.0–2.5)	2.6 (1.5–3.5)	0.3	2.7 (1.9–4.0)	2.7 (2.0–3.5)	0.9
<b>No. of relapses<sup>b</sup></b>	0.6 (0.7)	0.7 (0.7)	0.9	1.0 (0.7)	1.1 (0.8)	0.5	2.1 (1.4)	2.2 (1.5)	0.8
<b>No. of T2 lesions<sup>c,d</sup> [n (%)]</b>									
<b>1</b>	5 (62.5)	1 (9.1)	<b>0.04</b>	5 (38.5)	2 (22.2)	0.08	0 (0)	0 (0)	0.7
<b>2</b>	2 (25.0)	5 (45.4)		8 (61.5)	4 (44.5)		2 (33.3)	5 (26.3)	
<b>3</b>	1 (12.5)	5 (45.4)		0 (0)	3 (33.3)		4 (66.7)	14 (73.7)	
<b>No. of Gd-enhancing lesions<sup>c</sup></b>	0	0.3 (1.0)	0.3	0.7 (1.2)	0.7 (1.4)	0.5	4.4 (4.7)	3.0 (4.2)	0.4

Abbreviations: EDA = evidence of disease activity; EDSS = Expanded Disability Status Scale; NEDA = no evidence of disease activity.

Data are expressed as mean (SD) unless otherwise stated.

<sup>a</sup> Data are expressed as mean (interquartile range).

<sup>b</sup> Refers to the number of relapses in the 2 previous years of blood extraction.

<sup>c</sup> Refers to the number of T2 and gadolinium-enhancing lesions at the time of blood extraction.

<sup>d</sup> T2 lesions were classified into 3 categories according to the number of lesions: 1 = 1–3 lesions, 2 = 4–9 lesions, 3 =  $\geq 10$  lesions.

<sup>e</sup> Before teriflunomide, in the NEDA group, 4 patients were treated with platform therapies (interferon-beta or glatiramer acetate) and 3 patients with dimethyl fumarate; in the EDA group, all 8 patients were treated with platform therapies. Before dimethyl fumarate, in the NEDA group, the 5 patients were treated with platform therapies; in the EDA group, 5 patients were treated with platform therapies and 1 patient with teriflunomide. Before fingolimod, in the NEDA group, all 5 patients were treated with platform therapies; in the EDA group, 12 patients were treated with platform therapies, 3 patients with natalizumab, 2 patients with dimethyl fumarate, and 1 patient with siponimod as part of a clinical trial. *p*-values were obtained after comparisons between NEDA and EDA patients by means of the  $\chi^2$  test (sex, % naïve patients, and number of T2 lesions), Mann-Whitney *U* test (EDSS), and Student *t* test (remaining variables). Significant *p*-values are shown in bold.

**Table 2** Demographic and Baseline Clinical Characteristics of Patients With MS From the Validation Cohort

Baseline characteristics	Teriflunomide		p Value
	NEDA	EDA	
n	26	10	—
Age (y)	42.5 (8.7)	42.1 (7.0)	0.5
Female/male (% women)	21/5 (80.8)	5/5 (50.0)	0.07
Duration of disease (y)	10.7 (9.5)	10.0 (8.2)	0.2
Naïve/no naïve (% naïve) <sup>a</sup>	7/19 (26.9)	4/6 (40.0)	0.4
EDSS <sup>a</sup>	2.1 (1.5–2.5)	2.2 (1.5–2.5)	0.9
No. of relapses <sup>b</sup>	0.7 (0.7)	0.7 (0.8)	0.6
No. of T2 lesions <sup>c,d</sup> [n (%)]			
1	5 (19.2)	1 (10.0)	0.8
2	16 (61.6)	7 (70.0)	
3	5 (19.2)	2 (20.0)	
No. of Gd-enhancing lesions <sup>c</sup>	0.1 (0.2)	0.1 (0.3)	0.1

Abbreviations: EDA = evidence of disease activity; EDSS = Expanded Disability Status Scale; NEDA = no evidence of disease activity. Data are expressed as mean (SD) unless otherwise stated.

<sup>a</sup> Data are expressed as mean (interquartile range).

<sup>b</sup> Refers to the number of relapses in the 2 previous years of blood extraction.

<sup>c</sup> Refers to the number of T2 and gadolinium-enhancing lesions at the time of blood extraction.

<sup>d</sup> T2 lesions were classified into 3 categories according to the number of lesions: 1 = 1–3 lesions, 2 = 4–9 lesions, 3 = ≥10 lesions.

<sup>e</sup> Before teriflunomide, in the NEDA group, 17 patients were treated with platform therapies (interferon-beta or glatiramer acetate) and 2 patients with dimethyl fumarate; in the EDA group, all 6 patients were treated with platform therapies. *p*-values were obtained after comparisons between NEDA and EDA patients by means of the  $\chi^2$  test (sex, % naïve patients, and number of T2 lesions), Mann-Whitney *U* test (EDSS), and Student *t* test (remaining variables).

were included in the study. Patients were classified into responders and nonresponders based on the following criteria after 1 year of treatment: (1) presence or absence of relapses; (2) progression or lack of progression on neurologic disability; (3) presence or absence of radiologic activity on brain MRI. Responders were patients with no evidence of disease activity (NEDA) defined by the absence of relapses, Expanded Disability Status Scale (EDSS) progression, and MRI activity in the first year of treatment. Nonresponders were patients with evidence of disease activity (EDA) defined by the presence during the first year of at least 1 relapse, sustained increase of at least 1 point in the EDSS score, and/or presence of active lesions (either new or enlarging T2 lesions compared with baseline MRI scan or gadolinium-enhancing lesions). A total of 66 patients were included in the study, 19 treated with teriflunomide (8 NEDA and 11 EDA), 22 with DMF (13 NEDA and 9 EDA), and 25 with fingolimod (6 NEDA and 19 EDA). Table 1 summarizes the main baseline demographic and clinical characteristics of patients.

### MRI Lesion Analysis

Total number of T2 lesions was categorized into 3 groups as follows: 1 = 1–9 lesions, 2 = 10–50 lesions, 3 = >50 lesions.

Gadolinium-enhancing (Gd<sup>+</sup>) lesions were counted individually.

### Determination of Serum Cytokine Levels by Single Molecule Array Assays

Peripheral blood was drawn by standard venipuncture and allowed to clot spontaneously for 30 minutes. Serum was obtained by centrifugation and stored frozen at –80°C until used. Levels of cytokines were measured in serum samples using commercially available immunoassay kits (Cytokine 3-Plex B [IL-6, IL-17A, and TNF- $\alpha$ ] cat#101319, IL-10 cat#101643, IFN- $\gamma$  cat#103337, GM-CSF cat#102329, CXCL13 cat#102635, and IL-1 $\beta$  cat#101605, Quanterix, Billerica, MA) run on the fully automated ultrasensitive Simoa HD-1 Analyzer (Quanterix). Samples were run in duplicate in accordance with manufacturers' instructions with appropriate standards and internal controls. Both the mean intra-assay coefficient of variation of duplicates and the mean interassay coefficient of variation were <13% for all assays.

### Validation Cohort

Cytokines associated with the response to treatment were measured in an independent cohort of patients with RRMS treated with teriflunomide at the outpatient clinic of the Hospital Ramón y Cajal (Madrid). Patients were classified into responders and nonresponders after 12 months of treatment according to the same criteria as in the original cohort. A total of 36 patients (26 NEDA and 10 EDA) were included in the study. Demographic and baseline clinical characteristics are summarized in Table 2. Serum levels of TNF- $\alpha$  and CXCL13 were measured using the immunoassay kits cat#103337 and cat#102635 (Quanterix), respectively, on the Simoa HD-1 Analyzer. The intra-assay and interassay coefficients of variation were <10% for both assays.

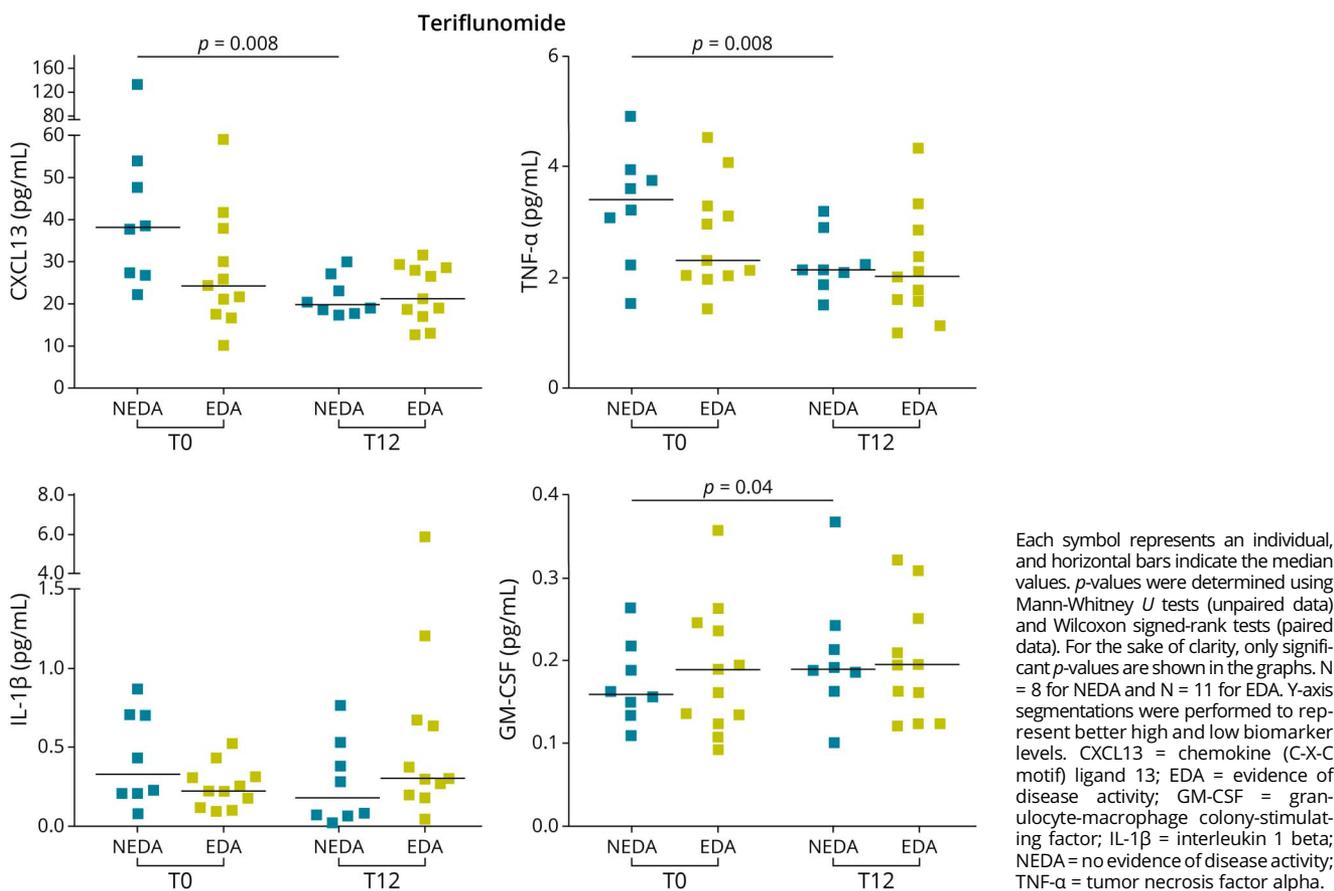
### Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the hospital ethics committee (EPA(AG)57/2013(3834)), and patients gave their informed consent.

### Statistical Analysis

A Mann-Whitney test was used to test for significant differences in cytokine levels between NEDA and EDA at baseline and after 1 year of treatment. Comparisons of cytokine levels before and after treatment with oral therapies in NEDA and EDA patients were assessed by a Wilcoxon signed-rank test. Inasmuch as 3 different oral therapies were analyzed in this study, for the identification of biomarkers associated with the response to treatment, Bonferroni correction was used to correct the alpha level for multiple comparisons between cytokine levels and the response outcome (alpha = 0.01). Correlations between cytokine levels and clinical and radiologic characteristics at baseline were assessed by the Spearman rank correlation coefficient. Receiver operating characteristic (ROC) curve analyses and the Youden Index were used to determine the best cut-off values based on the change of

**Figure 1** Dotplots Showing the Distribution of Serum Cytokine Levels Determined by Simoa in the Original Cohort of Patients With MS at Baseline (T0) and after 12 Months (T12) of Teriflunomide Treatment



serum cytokine levels between baseline and 12 months in the original cohort. Sensitivities, specificities, and likelihood ratios were calculated for the validation cohort.

### Data Availability

The data presented in this study are available on request from the corresponding author.

## Results

### Patients

As shown in Table 1, demographic, clinical, and radiologic variables were comparable between NEDA and EDA patients who will receive DMF or fingolimod treatment (Table 1). For teriflunomide, a significantly higher proportion of female patients and patients with  $\geq 4$  T2 lesions at baseline MRI were observed in EDA compared with NEDA patients (Table 1).

### Serum Cytokine Levels and Clinical and Radiologic Variables at Baseline

Correlations between serum cytokine levels and clinical variables such as disease duration, number of relapses in the previous 2 years before treatment onset, and EDSS at baseline

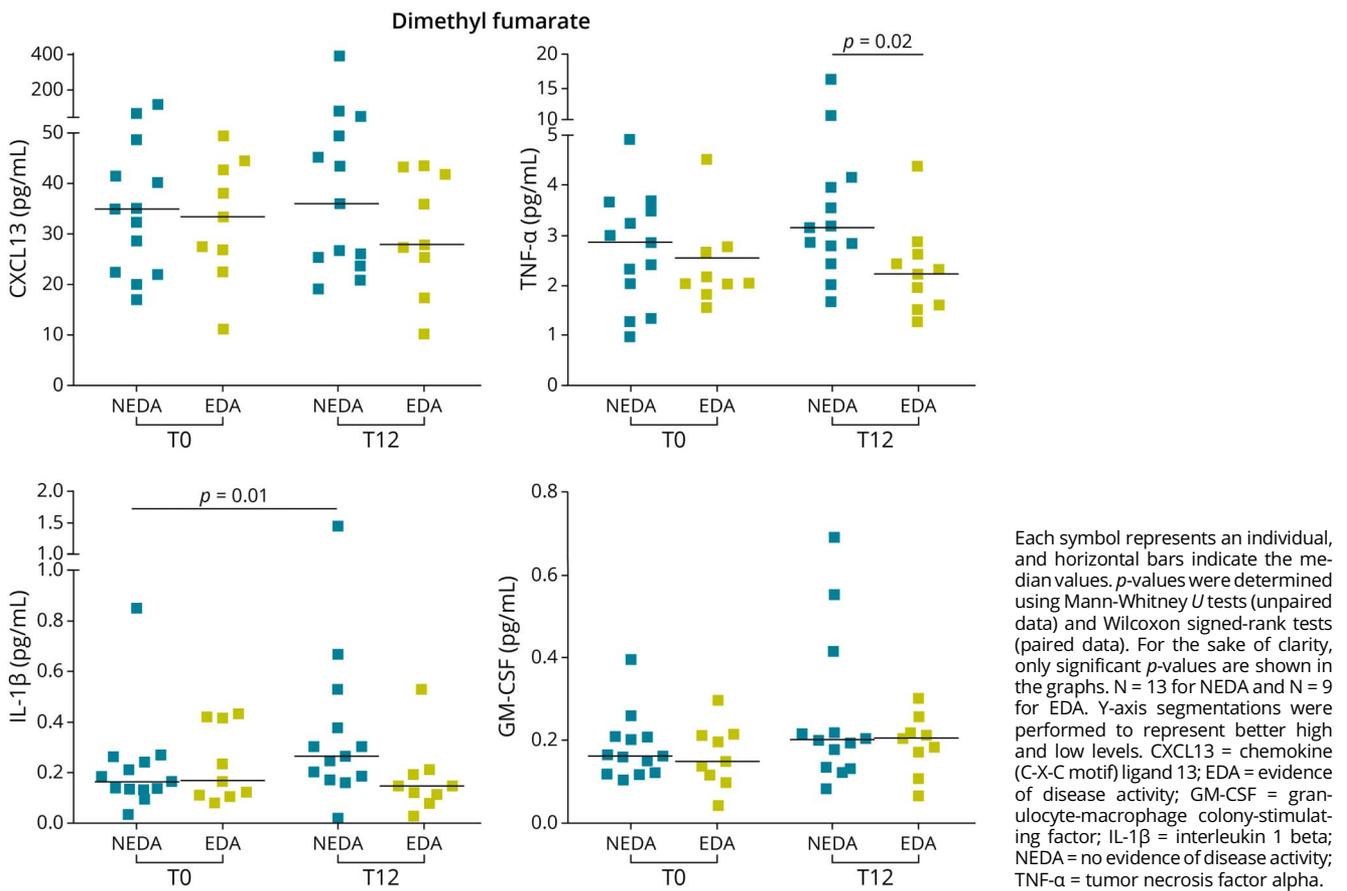
in the whole cohort of patients (N = 66) only revealed a significant correlation between serum IL-10 levels and EDSS ( $\rho = -0.350$ ;  $p = 0.004$ ) (eTable 1, [links.lww.com/NXI/A758](https://links.lww.com/NXI/A758)). There were no significant correlations between baseline serum cytokine levels and radiologic variables such as the number of T2 lesions or the number of contrast-enhancing lesions (eTable 1).

### Serum Cytokine Levels and Response to Oral Therapies

As shown in Figures 1–3 and eFigures 1–3 ([links.lww.com/NXI/A754](https://links.lww.com/NXI/A754), [links.lww.com/NXI/A755](https://links.lww.com/NXI/A755), [links.lww.com/NXI/A756](https://links.lww.com/NXI/A756)), serum cytokine levels at baseline were comparable between NEDA and EDA patients except for IL-6, whose levels were increased in NEDA patients who will receive DMF treatment ( $p = 0.04$ ; eFigure 2).

To identify biomarkers associated with the response to treatment, we focused on significant changes induced by treatment in serum cytokine levels between the baseline and the 12-month-treated time point in NEDA and EDA patients. As shown in Figure 1, treatment with teriflunomide was associated with significant reductions in serum CXCL13 and TNF-α levels in NEDA patients ( $p = 0.008$  for both cytokines) whereas cytokine

**Figure 2** Dotplots Showing the Distribution of Serum Levels Measured by Simoa in the Original Cohort of Patients With MS at Baseline (T0) and After 12 Months (T12) of DMF Treatment



levels remained comparable in EDA patients despite treatment ( $p = 0.21$  and  $p = 0.07$  for CXCL13 and TNF- $\alpha$ , respectively). A trend toward increased GM-CSF levels was also observed in NEDA patients treated with teriflunomide ( $p = 0.04$  in NEDA vs  $p = 0.85$  in EDA patients; Figure 1).

As shown in Figure 2, in patients receiving DMF, a trend toward higher IL-1 $\beta$  levels was observed in NEDA patients after 12 months of treatment compared with EDA patients ( $p = 0.01$  in NEDA patients vs  $p = 0.50$  in EDA patients). Serum levels of IL-6 and TNF- $\alpha$  were elevated in NEDA patients treated with DMF at baseline, and the differences were significant after 12 months of treatment compared with EDA patients ( $p = 0.003$  for IL-6 and  $p = 0.02$  for TNF- $\alpha$ ; eFigure 2, [links.lww.com/NXI/A755](https://links.lww.com/NXI/A755) and Figure 2). However, IL-6 and TNF- $\alpha$  levels were not modified by the effect of DMF treatment inasmuch as cytokine levels remained comparable between the baseline and the treated time point (eFigure 2 and Figure 2).

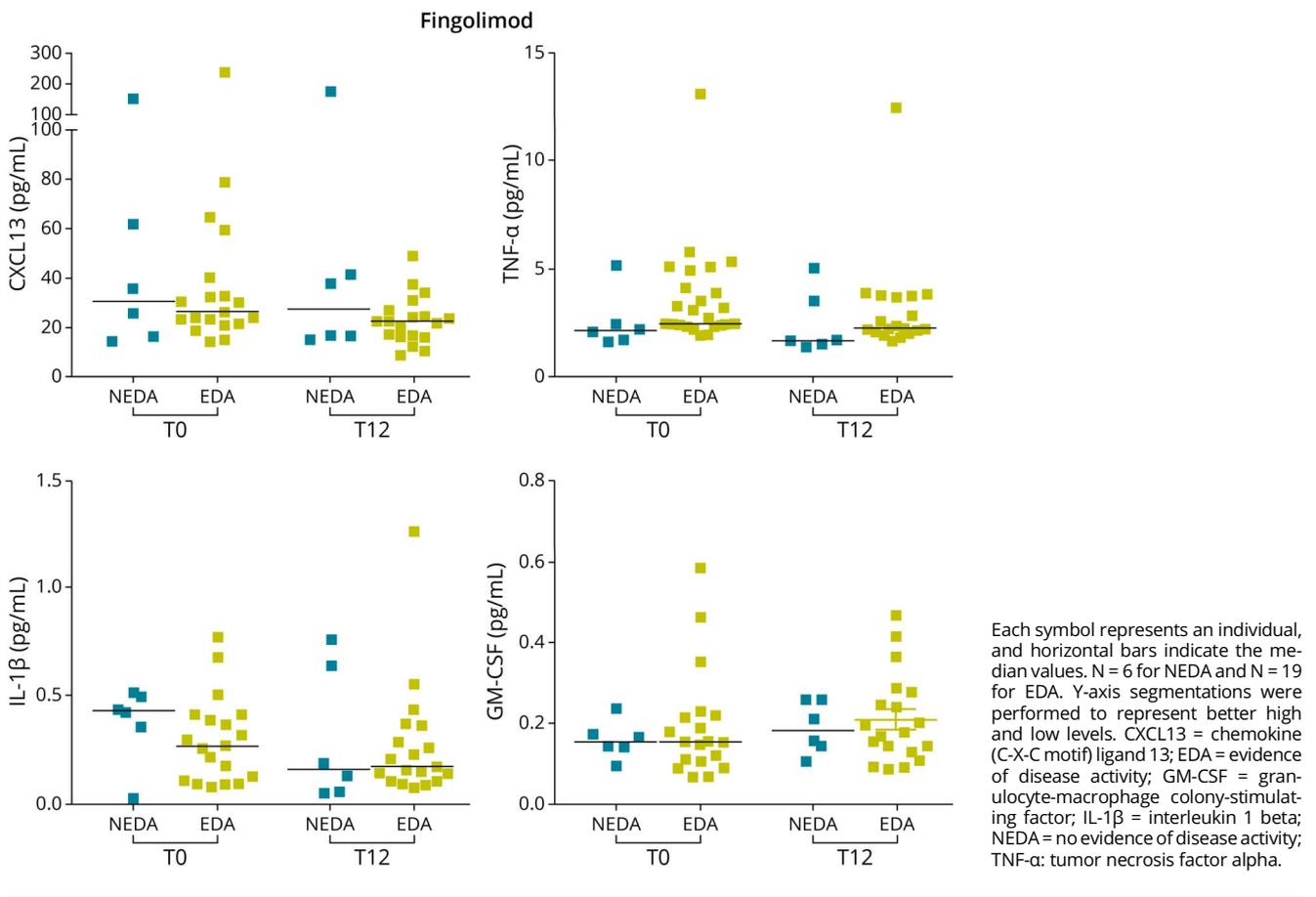
Treatment with fingolimod was not associated with significant changes in serum cytokines levels between the baseline and the treated time point either in NEDA or EDA patients (Figure 3 and eFigure 3, [links.lww.com/NXI/A756](https://links.lww.com/NXI/A756)).

### Validation of Serum TNF- $\alpha$ and CXCL13 Levels as Biomarkers Associated With the Response to Teriflunomide

Based on the results in the original cohort, CXCL13 and TNF- $\alpha$  were selected for validation in an independent cohort of patients with RRMS treated with teriflunomide and classified into NEDA and EDA after 1 year of treatment according to the same criteria as in the original cohort. As shown in Figure 4, both CXCL13 and TNF- $\alpha$  levels were significantly decreased after 12 months of treatment with teriflunomide in NEDA patients whereas no significant changes in cytokine levels were observed in the EDA group ( $p < 0.0001$  in NEDA vs  $p = 0.13$  in EDA for CXCL13;  $p = 0.003$  in NEDA vs  $p = 0.28$  in EDA for TNF- $\alpha$ ).

We finally analyzed the performance of changes in serum levels of CXCL13 and TNF- $\alpha$  between the baseline and the 12-month-treated time point to predict response to teriflunomide. For CXCL13, the area under the ROC curve (AUC) was 77% (95% CI = 54.4%–100%) in the original cohort and 73% (95% CI = 54.1%–91.3%) in the validation cohort (Figure 5A). A change in CXCL13 levels of 9.64 pg/mL between the baseline and 12 months was identified as the best cutoff to classify NEDA and EDA patients in the original

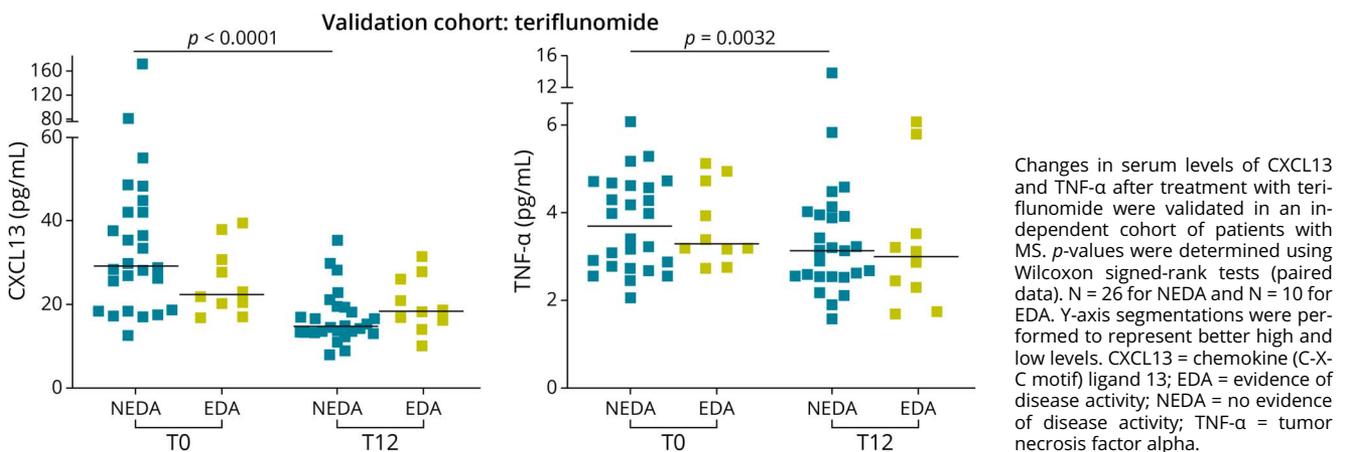
**Figure 3** Dotplots Showing the Distribution of Serum Levels Determined by Simoa in the Original Cohort of Patients With MS at Baseline (T0) and After 12 Months (T12) of Fingolimod Treatment



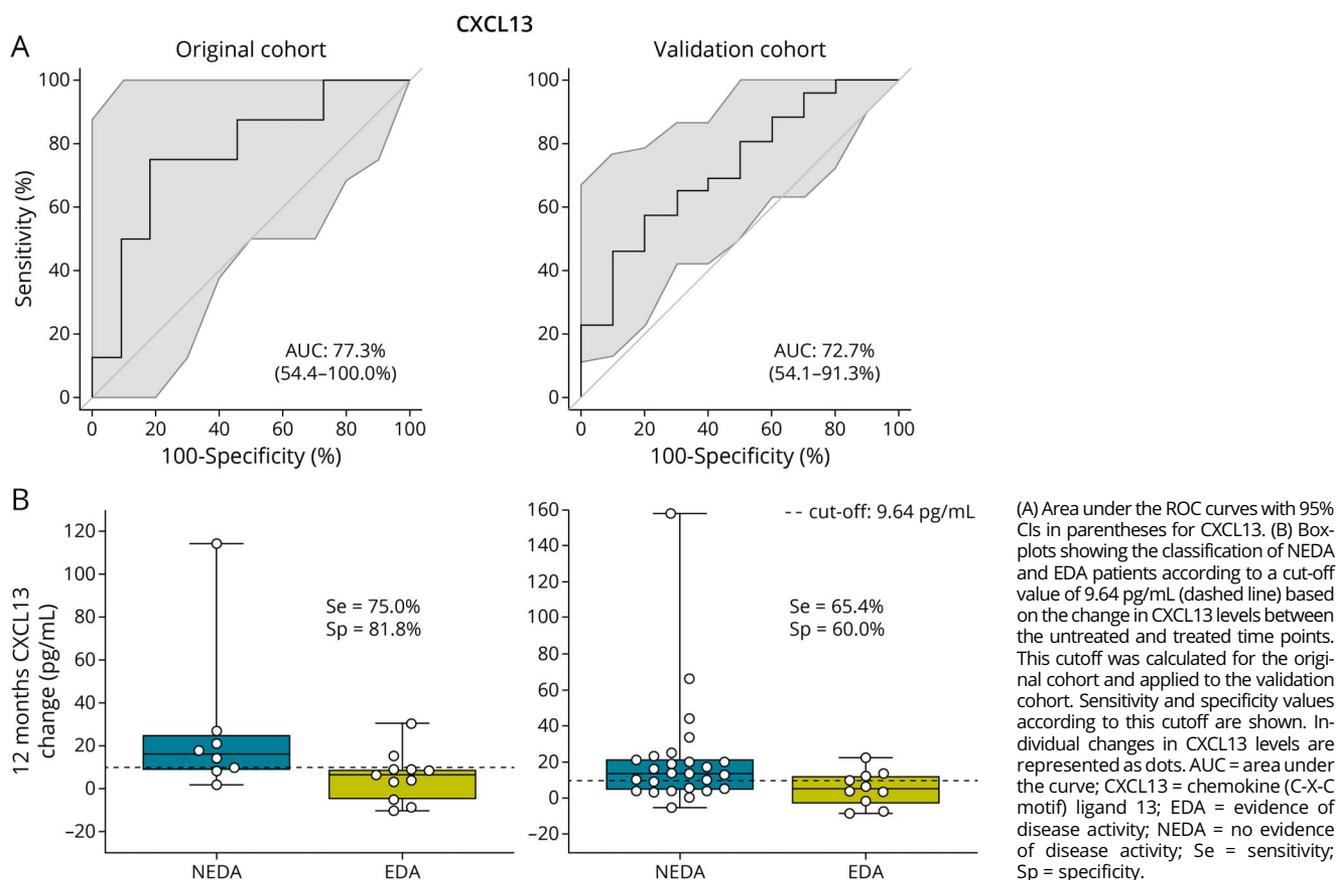
cohort, with a sensitivity and specificity of 75% and 82%, respectively (Figure 5B). When this cutoff was applied to the validation cohort, a sensitivity of 65% and specificity of 60% were achieved (Figure 5B). A similar analysis was performed

for TNF-α, and the AUC were 66% (95% CI = 39.7%–92.1%) in the original cohort and 53% (95% CI = 29.0%–77.1%) in the validation cohort (eFigure 4A, [links.lww.com/NXI/A757](https://links.lww.com/NXI/A757)). A change in TNF-α levels of 0.68 pg/mL between baseline and 12

**Figure 4** Dotplots Showing the Distribution of Serum Levels Measured by Simoa in the Validation Cohort at Baseline (T0) and After 12 Months (T12) of Teriflunomide Treatment



**Figure 5** Performance of Changes in Serum CXCL13 Levels to Discriminate Between NEDA and EDA Patients



months resulted in the best cutoff to discriminate between NEDA and EDA patients in the original cohort, with a sensitivity of 75% and specificity of 63.6% (eFigure 4B). This cutoff was associated with a sensitivity of 38.5% and specificity of 50% in the validation cohort (eFigure 4B).

## Discussion

An important gap in the field of biomarkers in MS is the lack of reliable biomarkers that discriminate between patients who respond to therapies and patients who continue to have relapses, radiologic inflammatory activity, and disease progression despite treatment. NEDA is a concept that depicts the absence of relapses, new MRI T2 lesions, and EDSS progression during follow-up. Although several criteria have been suggested as supportive features to this concept throughout the past years, such as cognitive decline or brain volume loss, for this study, we decided to use the original 3-domain concept for NEDA because it is the most representative of daily clinical practice.<sup>8</sup> Bearing this in mind, we aimed to identify treatment response biomarkers in a cohort of patients with MS receiving the oral therapies teriflunomide, DMF, and fingolimod categorized based on their response to therapies and further validate the data in an independent

cohort of treated patients. In this study, we report that serum CXCL13 and TNF- $\alpha$  levels are decreased after treatment with teriflunomide in patients classified as treatment responders according to the composite outcome measure NEDA. These findings were confirmed in an independent cohort of patients with MS treated with teriflunomide. Furthermore, the change in serum CXCL13 levels between the baseline and 12 months showed good performance to discriminate between teriflunomide responders and nonresponders.

Current disease-modifying therapies achieve their effects primarily by blocking the proinflammatory response in a non-specific manner, inducing changes in the levels of several inflammatory molecules.<sup>9</sup> Among them, cytokines from the innate immune system and T-cell-related and B-cell-related cytokines appear as attractive candidate biomarkers associated with treatment response. In this work, the serum levels of IL-6, IL-17, TNF- $\alpha$ , GM-CSF, IL-10, IFN- $\gamma$ , IL-1 $\beta$ , and CXCL13 were measured by Simoa at baseline and after 12 months of treatment with the above-mentioned oral therapies, to investigate their potential association with the response outcomes.

Interestingly, analysis of serum cytokine levels in the whole untreated cohort revealed a negative correlation between the anti-inflammatory cytokine IL-10 and EDSS. This finding is in

agreement with previous reports indicating a negative relationship between IL-10 levels and EDSS scores.<sup>10</sup> In this regard, B cells of patients with MS were found to exhibit deficient production of IL-10 compared with B cells of healthy controls.<sup>11,12</sup>

At baseline, serum cytokine levels were comparable between responders and nonresponders to the 3 oral therapies except for IL-6, whose levels were increased in patients who will respond to DMF. Taking into account that those levels were not modified by the effect of DMF treatment, and remained significantly higher in treatment responders after 1 year, IL-6 was not considered as a candidate biomarker for validation in an independent cohort of DMF-treated patients.

Comparisons of serum cytokine levels before and after 1 year of treatment revealed that CXCL13 and TNF- $\alpha$  levels were significantly reduced by the effect of treatment in teriflunomide responders and hence were selected for validation. Noteworthy, in the validation cohort, serum CXCL13 and TNF- $\alpha$  levels were again significantly reduced by treatment in teriflunomide responders, while cytokine levels remained unchanged in nonresponders. When we evaluated the performance of the changes in the levels of both CXCL13 and TNF- $\alpha$  to predict treatment response to teriflunomide, only CXCL13 showed good potential to discriminate between responders and nonresponders in both cohorts of patients with MS, indicating that a decrease in CXCL13 levels higher than 9.64 pg/mL by the effect of treatment could be used in clinical practice to identify patients with RRMS having optimal responses to teriflunomide. By contrast, performance of TNF- $\alpha$  to predict teriflunomide treatment response was acceptable in the original cohort but low in the validation cohort, findings that certainly limit its use in clinical practice to identify teriflunomide responders.

CXCL13 is a potent B-cell chemoattractant that plays a role in B-cell recruitment to the CNS during inflammation. Studies of CXCL13 levels showed that concentrations are increased in serum and CSF of patients with active disease, emphasizing that this chemokine might be an important biomarker for MS inflammatory activity.<sup>6,13,14</sup> Interestingly, CSF CXCL13 levels were found to correlate with intrathecal immunoglobulin production and the number of CSF B cells and plasmablasts.<sup>15,16</sup> On the other hand, TNF- $\alpha$  is a potent cytokine that exerts numerous functions in immunity, inflammation, and control of cell proliferation, among others.<sup>17</sup> This cytokine has long been associated with MS pathogenesis,<sup>18</sup> and CSF and serum levels of TNF- $\alpha$  were found to be increased in patients with MS compared with controls and levels correlated with disease severity.<sup>19</sup> Teriflunomide is an oral disease-modifying therapy indicated for the treatment of patients with RRMS, which has shown beneficial effects on both clinical and MRI outcomes in patients with MS.<sup>20,21</sup> The main mechanism of action relies on the inhibition of enzyme dihydroorotate dehydrogenase (DHODH) that catalyzes de novo biosynthesis of pyrimidines, exerting a cytostatic effect on proliferating T and B lymphocytes in the periphery and thus inducing both anti-proliferative and anti-inflammatory properties.<sup>22</sup> Despite this well-documented mechanism of action, it has been reported that teriflunomide may have additional DHODH-independent

mechanisms such as an inhibitory effect on cytokines/chemokines production.<sup>23</sup> In this context, previous studies have shown a lower secretion of TNF- $\alpha$  by T cells after teriflunomide treatment.<sup>24</sup>

B cells are major contributors to MS pathogenesis, a role confirmed with the introduction of B-cell-depleting therapies.<sup>25</sup> Although not specific for B cells, teriflunomide treatment has been shown to reduce the number of effector blood B-cell populations.<sup>26,27</sup> Considering the important role of CXCL13 in the recruitment of B cells into the CNS, the decrease in serum CXCL13 levels in teriflunomide responders could be one of the mechanisms of action by which teriflunomide exerts its beneficial effects. In this context, other MS therapies such as natalizumab could be acting by a similar mechanism.<sup>13</sup>

In conclusion, our approach in serum samples from patients treated with different oral therapies and classified into responders and nonresponders according to NEDA has led to the identification of CXCL13 and TNF- $\alpha$  as biomarkers associated with the response to teriflunomide. In addition, a change in CXCL13 levels above 9.46 pg/mL during the first year of treatment showed good performance to identify teriflunomide treatment responders. Our results support the use of longitudinal measurements of serum CXCL13 levels as a biomarker to monitor treatment response in patients with RRMS receiving teriflunomide.

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## Disclosure

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## Appendix Authors

Name	Location	Contribution
Nicolás Fissolo, PhD	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Design and conceptualized study; acquisition and analysis of the data; drafted the manuscript for intellectual content

## Appendix (continued)

Name	Location	Contribution
<b>Agustín Pappolla, MD</b>	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Acquisition and analysis of the data, and revised the manuscript for intellectual content
<b>Jordi Río, MD, PhD</b>	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Analysis of the data, and revised the manuscript for intellectual content
<b>Luisa M. Villar, MD, PhD</b>	Departments of Immunology and Neurology, Multiple Sclerosis Unit, Hospital Ramon y Cajal, (IRYCIS), Madrid, Spain	Acquisition of the data, and revised the manuscript for intellectual content
<b>Santiago Perez-Hoyos, BSc, PhD</b>	Statistics and Bioinformatics Unit, Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain	Acquisition of the data, and revised the manuscript for intellectual content
<b>Alex Sanchez, PhD</b>	Statistics and Bioinformatics Unit, Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain Genetics, Microbiology and Statistics Department, Universitat de Barcelona, Spain	Acquisition of the data, and revised the manuscript for intellectual content
<b>Lucía Gutiérrez, BSc</b>	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Acquisition of the data, and revised the manuscript for intellectual content
<b>Xavier Montalban, MD, PhD</b>	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Acquisition of the data, and revised the manuscript for intellectual content
<b>Manuel Comabella, MD, PhD</b>	Servei de Neurologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron; Universitat Autònoma de Barcelona, Spain	Design and conceptualized study; analyzed the data; drafted the manuscript for intellectual content

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