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Multiomic Integration Analysis for Monitoring Severe Asthma Treated With Mepolizumab or Omalizumab

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Keywords: asthma | biologicals | biomarkers | metabolomics | proteomics

ABSTRACT

Rationale: Biologics are becoming increasingly important in the management of severe asthma. However, little is known about the systemic immunometabolic consequences of Th2 response blockage.

Objectives: To provide a better immunometabolic understanding of the effects of mepolizumab and omalizumab treatments by identifying potential biomarkers for monitoring.

Methods: In this exploratory longitudinal study severe asthmatic patients were followed for 18 months after initiating mepolizumab ($n = 36$) or Omalizumab ($n = 20$) treatment. Serum samples were collected before, 6, and 18 months after treatment. Targeted omic approaches were performed to analyze inflammatory metabolites ($n = 35$) and proteins ($n = 45$). Multiomic integration was performed individually for each treatment applying supervised analysis Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO) framework. Then, potential biomarkers were confirmed using multivariate ROC analyses and correlated with clinical variables along treatment.

Measurements and Main Results: Mepolizumab and omalizumab were both effective (improved clinical variables) and showed different and specific metabolic and protein profiles in severe asthmatic patients during treatment. Multiomic integration and multivariate ROC analyses identified specific biomarkers, such as arachidonic acid, palmitoleic acid, oleic acid, propionylcarnitine, bilirubin, CCL11, and TNFSF10, which can explain the differences observed with Mepolizumab treatment over 18 months and significantly correlate with clinical improvement. However, no significant biomolecules and no discriminative multivariate ROC curves were found for Omalizumab treatment.

Alma Villaseñor and María M. Escribese contributed equally to this work.

Conclusions: Our results provide a comprehensive insight into the differential effects of mepolizumab and omalizumab on the immunometabolic kinetics of the inflammatory response in severe asthma. We identified a set of biomolecules with potential for monitoring mepolizumab treatment which could be useful for personalized medicine.

1 | Introduction

Asthma pathophysiology is driven by different immune pathways that trigger a chronic inflammatory state, leading to different phenotypes [1]. Notably, approximately 60% of asthmatics are characterized by a Th2-driven response, such as allergic asthma, where Th2 lymphocytes secrete several cytokines (interleukin 4 [IL-4], IL-5, IL-9, and IL-13), resulting in eosinophilia and the production of immunoglobulin E (IgE) by B cells, together with other mediators [1–5].

In recent decades, asthma prevalence and severity have progressively increased, leading some patients to fail at achieving disease control with standard-approved therapies [6] and to develop a severe phenotype. For these patients, the Global Initiative for Asthma (GINA) guidelines recommend add-on biological therapy with monoclonal antibodies (MoAb) targeting key immunomodulators [7, 8]. The first biological drug for asthma treatment approved by the Food and Drug Administration was omalizumab [9], an anti-IgE MoAb used in severe allergic asthmatic patients with high blood levels of IgE [10]. In addition, mepolizumab, a biological which specifically targets IL-5 by preventing its binding to the IL-5-receptor subunit alpha (IL-R5α), was later endorsed for severe uncontrolled eosinophilic asthma [10]. Nowadays, despite the increasing development and use of new biological drugs [11] and their proven efficacy [12, 13], the immunometabolic effects of blocking key immunological routes by these treatments and their time-dependent systemic effects are still not fully understood [14, 15].

Few biomarkers are available for asthma diagnosis and monitoring in daily clinical practice. This includes peripheral or sputum eosinophils, fractional exhaled nitric oxide (FeNO), forced expiratory volume in 1 s (FEV1), and serum IgE [16–18]. Additionally, the clinical history, frequency of exacerbations and/or hospitalizations, Asthma Control Test (ACT), or treatment effectiveness support severe asthma diagnosis [8, 19, 20]. However, it is still crucial to identify new biomarkers to assess asthmatic patients' evolution during treatment, enhancing personalized therapy for severe asthma.

Omic sciences are widely used in the search for new potential biomarkers, allowing us to uncover the multitude of underlying mechanisms behind a complex disease [21]. Previous studies have applied targeted and untargeted proteomics in asthma and allergy, investigating changes in key cytokines and interleukins [22, 23], or untargeted metabolomics, where alterations in several proinflammatory metabolic pathways (e.g., fatty acids (FAs) pathways) are shared by diverse allergic phenotypes [24, 25]. Our group has extensively studied different severe models of respiratory allergy and anaphylaxis by combining untargeted metabolomics, transcriptomics, and proteomics [26–28]. This led to the development of a targeted metabolomic methodology to measure and quantify a set of metabolites associated with allergic inflammation, including the detection of sphingolipids, amino acids, carnitines, and FAs [29]. Thus, the combination of targeted metabolomics and proteomics would shed light on the immunological shifts triggered by biological treatments.

Here, we have performed a multiomic integration analysis using targeted proteomics and metabolomics data from patients treated with mepolizumab or omalizumab and correlated the results with a set of clinical variables used for treatment efficacy evaluation. Our results provide new insights into the effects of blocking the Th2 immune response through different pathways, as well as a new perspective for monitoring biological drug treatment, which could contribute to the better implementation of personalized medicine.

2 | Methods

2.1 | Patients

Adults with severe asthma ($n = 67$) (step 5 of the GINA guidelines [8]) were recruited between April 2018 and July 2019 at the Allergy Service of Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain. The Ethics Committee of this Hospital approved the study protocol (1678/2019), and all patients signed the informed consent.

Patients were classified as severe asthmatics considering they did not control asthma symptoms with the previously prescribed medication and based on different clinical parameters according to the GINA guidelines [8] based on ACT < 20, FEV1 values < 80%, and/or presence of severe exacerbations and/or hospitalizations.

2.2 | Study Design

Severe asthmatic patients were allocated to mepolizumab or omalizumab based on their clinical characteristics. Severe eosinophilic asthmatic patients were treated with mepolizumab, and severe allergic asthmatic patients were treated with omalizumab. Patients were scheduled for a visit at three different time points: before biological treatment (baseline), 6 and 18 months after treatment initiation.

From the total cohort of 67 patients, 3 patients in the omalizumab group dropped out of the study due to lack of response. In the mepolizumab group, 6 patients dropped out of the study, 3 of them due to the presence of nasal polyposis and 3 due to lack of response (showing 2 of them myalgia or recurrent headache as side effects). This leads to $n = 36$ in the mepolizumab group and $n = 20$ in the omalizumab group. A complete and clear scheme of the allocation of the subjects in the study and causes of the dropouts has been clarified in Figure S1. Details on patient exposome and sample collection are in the Supporting Information.

2.3 | Clinical Sampling and Collection

Serum samples were obtained at the three visits and stored at −80°C until further metabolomic and proteomic analyses. In addition, clinical parameters such as number of blood eosinophils, FEV1 values, ACT, and the frequency of hospitalizations and severe exacerbations (increase in cough, shortness of breath, wheezing, and/or progressive lung function decrease) were recorded at the three time points.

2.4 | Metabolomic Analyses

Serum samples (Mepolizumab: $n = 36$; Omalizumab: $n = 18$) were analyzed following a previously published method [29] to quantify 35 metabolites (Table S1) associated with allergic inflammation. In brief, samples were measured using a liquid chromatography system (1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) coupled to a triple quadrupole mass spectrometer with electrospray ionization Agilent Jet Stream source, 6470 Agilent Technologies (Santa Clara, CA, USA). Detailed description of the methodology and information regarding its validation can be found in the Supporting Information on the Methods section.

Raw data is accessible on the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org where it has been assigned Study ID ST002948. The data can be accessed directly via its DOI: 10.21228/M8SB0G.

For the omalizumab group, two patients (O19 and O20) were not included in the analyses due to low sample volume.

2.5 | Proteomic Analyses

Serum proteins were quantified by Proximity Extension Assay (PEA, Olink, Uppsala, Sweden), through Olink Target 48 Cytokine panel (quantify 45 proteins, ref.: 93200, Table S1) in a subset of randomly selected samples from both the mepolizumab ($n = 20$) and omalizumab ($n = 20$) groups. However, two patients from both experimental groups (O13 and O14; Me6 and Me33) were excluded as the values of the incubation controls were outside the median of the plate (±0.3 normalized protein expression [NPX] for both internal controls), resulting in $n = 18$ for each group. Detailed description of the methodology and its validation can be found in the Supporting Information on the Methods section. Raw data can be found in Appendix S1.

2.6 | Statistical Analyses

Baseline differences among the mepolizumab and omalizumab groups were tested using either parametric (*t*-student) or non-parametric (Mann–Whitney *U* or Fisher's exact) tests. Repeated measures ANOVA test was used independently to analyze each treatment along the time using Matlab R2022b (Mathworks, Natick, Massachusetts, USA) and IBM SPSS Statistics V.27.0 (IBM Corp, Armonk, NY, USA).

Multiomic integration was performed using OmicsAnalyst [30] web server through supervised multivariate analysis using Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO) framework 6.26.0 [31] after *Z*-score normalization. The significance level was set at p value < 0.05 and p-adjusted < 0.1. More details on how DIABLO framework operates, and the data used are indicated in the Supporting Information.

Multivariate exploratory receiver operating characteristic (ROC) analysis and paired correlation analyses were performed using MetaboAnalyst 6.0 web server (Wishart Research Group at the University of Alberta, Canada) and range scaling the data. Details of how multivariate ROC curves are generated and how to interpret them are shown in Supporting Information. Briefly, random forest classification and feature ranking methods were used. Furthermore, paired correlation analyses were carried out by Spearman's rank correlation test (ρ). Graphics were generated using GraphPad Prism v10.0.2 (San Diego, CA, USA) and R version 4.2.2 (R Core Team, 2022) with *circlize* package [32].

3 | Results

3.1 | Patients Treated With Mepolizumab or Omalizumab Differ in Their Clinical Profile at Baseline

Patients included in the mepolizumab and omalizumab groups had different clinical characteristics at baseline (Table 1). Patients in the mepolizumab group were significantly older and presented higher body mass index (BMI), levels of eosinophilia and a higher frequency of severe exacerbations compared with those in the omalizumab group (p value < 0.05). Moreover, their pharmacological treatments significantly differed, with a higher proportion of patients in the mepolizumab group being treated with H1-antihistamine plus intranasal corticosteroids (H1-A-InCS), oral corticosteroids (OCS), and/or tiotropium bromide (Tp) (p value < 0.05). Patients in the omalizumab group showed significantly higher IgE levels and were more frequently treated with H1-A, InCS, and/or eye drops (ED) (p value < 0.05). In addition, individual information including comorbidities (e.g., allergic rhinitis and the presence of nasal polyps) and basal IgE levels can be found in Table S2.

These results provide key information regarding the differential clinical characteristics between both groups before treatment.

3.2 | Mepolizumab and Omalizumab Treatments Induce Clinical Improvement

To assess the effect of mepolizumab and omalizumab on the clinical variables at 6 and 18 months, ACT, FEV1, blood eosinophils, and the frequency of severe exacerbations and hospitalizations were evaluated individually (Figure 1 and Table S3). Mepolizumab treatment significantly induced a clinical improvement reflected in the analyzed clinical variables already at 6 months (p value < 0.05), which was maintained up to 18 months (Figure 1). Similar trends, although with less

TABLE 1 | Clinical characteristics at baseline of severe asthmatic patients before being treated with Mepolizumab or Omalizumab.

	Mepolizumab, $n=36$	Omalizumab, $n=20$	p
Gender (%F)	33.33	50.00	0.262
Age (years) (mean ± SD)	53.51 ± 12.39	35.85 ± 15.40	***< 0.001***
BMI (kg/m^2) (mean ± SD)	30.43 ± 5.01	26.14 ± 4.00	***0.004***
IgE (IU/mL) (mean ± SD)	245.90 ± 474.85	392.95 ± 294.96	***0.001***
FEV1 (%)	75 ± 23	74 ± 18	0.822
ACT (mean ± SD)	10.88 ± 5.23	13.18 ± 3.68	0.092
Eosinophils/μL (mean ± SD)	648.33 ± 341.11	506.92 ± 545.10	***0.004***
Severe exacerbations (mean ± SD)	1.40 ± 2.17	0.25 ± 0.55	***0.017***
Hospitalizations (mean ± SD)	2.06 ± 2.27	0.90 ± 0.97	0.057
H1-A-InCS (%)	47.22	0.00	***< 0.001***
H1-A (%)	33.30	95.00	***< 0.001***
InCS (%)	36.10	100.00	***< 0.001***
SABA (%)	88.90	100.00	0.285
LABA (%)	5.60	10.00	0.611
Antileukotriene (%)	63.90	45.00	0.260
ED (%)	0.00	25.00	***0.004***
OCS (%)	47.20	10.00	***0.007***
ICS-MD (%)	0.00	5.00	0.357
ICS-HD (%)	5.60	5.00	1.000
ICS-LD-LABA (%)	0.00	5.00	0.357
ICS-MD-LABA (%)	2.80	10.00	0.288
ICS-HD-LABA (%)	88.90	75.00	0.256
Theophylline (%)	11.10	0.00	0.285
Ipratopium (%)	33.30	10.00	0.063
Tiotropium bromide (%)	44.40	15.00	***0.039***

Note: p values were obtained by Fisher's exact test and unpaired parametric (*t*-student) or nonparametric (Mann–Whitney *U*) tests depending on normality of the data. Significant values (*p* value < 0.05) are highlighted in bold and italics. Theophylline was only administered in low doses to those patients who showed poor symptom control after withdrawal. Currently, GINA does not recommend its use.

Abbreviations: ACT, asthma control test; BMI, body mass index; COPD, chronic obstructive pulmonary disease; ED, eye drops; F, female; FEV1, forced expiratory volume in 1 s; H1-A, H1 antihistamine; HD, high dose; HTN, hypertension; ICS, inhaled corticosteroids; InCS, intranasal corticosteroids; IU, international units; LABA, long-acting inhaled β2 agonist; LD, low dose; MD, medium dose; OCS, oral corticosteroids; SABA, short-acting inhaled β2 agonist.

significant changes, were observed for omalizumab treatment (Figure 1). Thus, these findings show that both biological drugs induced clinical improvement in severe asthmatic patients.

3.3 | Mepolizumab and Omalizumab Treatments Modify the Metabolomic and Proteomic Signatures of Severe Asthmatics

To evaluate the effect of these biological drugs at the metabolic and protein levels over time, omic analyses were performed.

Regarding targeted metabolomics, patients treated with mepolizumab displayed significant changes in 16 out of 32 detected metabolites (50%, *p* value < 0.05) after treatment compared with baseline. Most of them were changed after 6 months and sustained up to 18 months (Figure 2A). Indeed, mepolizumab induced a decrease in FAs (arachidonic acid [AA], lauric acid, oleic acid, and palmitoleic acid), sphingolipids (sphingosine-1-phosphate [S1P] and sphingosine), and arginine, and an increase in carnitines (L-carnitine and propionylcarnitine), most amino acids (such as betaine/valine), and others (pyruvic acid, lactic acid, and bilirubin). The omalizumab group exhibited significant changes in 4 out of the 32 detected metabolites (12%, *p* value < 0.05), mainly at 6 months (Figure 2A). We observed a decrease in S1P levels and an increase in betaine/valine, bilirubin, and pyruvic acid.

In contrast to the metabolomic results, significant protein changes were mainly observed at 18 months compared with

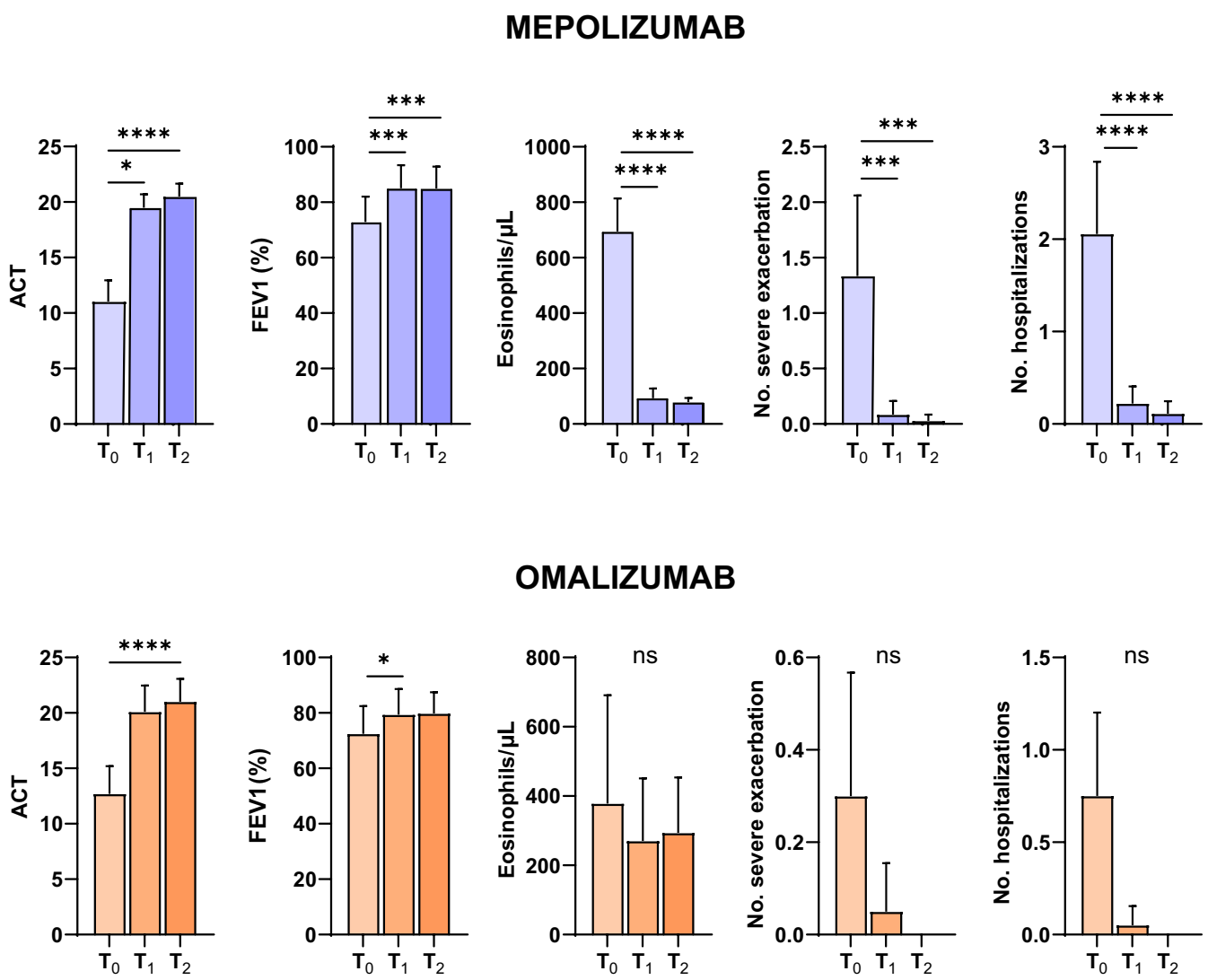

FIGURE 1 | Clinical variables monitoring along biological treatment. Significant differences observed during mepolizumab (purple) ($n = 36$) and omalizumab (orange) ($n = 20$) treatment. T_0 corresponds to baseline, T_1 to 6 months of treatment and T_2 to 18 months of treatment. The values are presented by the mean and the 95% confidence interval. Repeated-measures ANOVA test ****p value < 0.0001, ***p value < 0.001, *p value < 0.05; ns, no significant.

baseline. Mepolizumab induced significant changes in 20 out of 33 detected proteins at 18 months (61%, p value < 0.05). These changes were mostly observed as increases in chemokines (e.g., CCL8 and CCL11), cytokines (e.g., IL-8 and IFN-γ), growth factors (e.g., EGF), TNF family factors (e.g., TNF-α and TNFSF10), and others (e.g., OLR1), and only a decrease in IL-6 and IL-7 (Figure 2B). On the other hand, omalizumab significantly altered 7 out of 33 detected proteins at 18 months (21%, p value < 0.05), including an increase in cytokines (OSM), growth factors (EGF and TGF-α), TNF family factors (TNFSF10), and others (OLR1 and FLT3LG), and a decrease in IL-7 (Figure 2B).

Notably, although mepolizumab and omalizumab drugs display different metabolic and protein responses, there were some metabolites (S1P, betaine/valine, pyruvic acid, and bilirubin) and proteins (TNFSF10, IL-7, EGF, TGF-α, OLR1, and FLT3LG) that were similarly affected by both biologicals. Trajectories for metabolites and proteins that were not significant are shown in Figures S2 and S3.

3.4 | Multiomic Integration Identifies a Set of Potential Biomarkers to Monitor Mepolizumab Treatment in Severe Asthmatics

To understand the regulation of the immune response triggered by mepolizumab and omalizumab and aiming to generate an integrative model, we used the supervised analysis by applying the DIABLO framework to integrate the metabolomic and proteomic datasets, thus resulting in a discriminant multiomic multivariate model.

For mepolizumab, multiomic multivariate analysis showed that baseline differed from 6 and 18 months. This separation can be observed in Figure 3A by component 1 vs. component 2 and

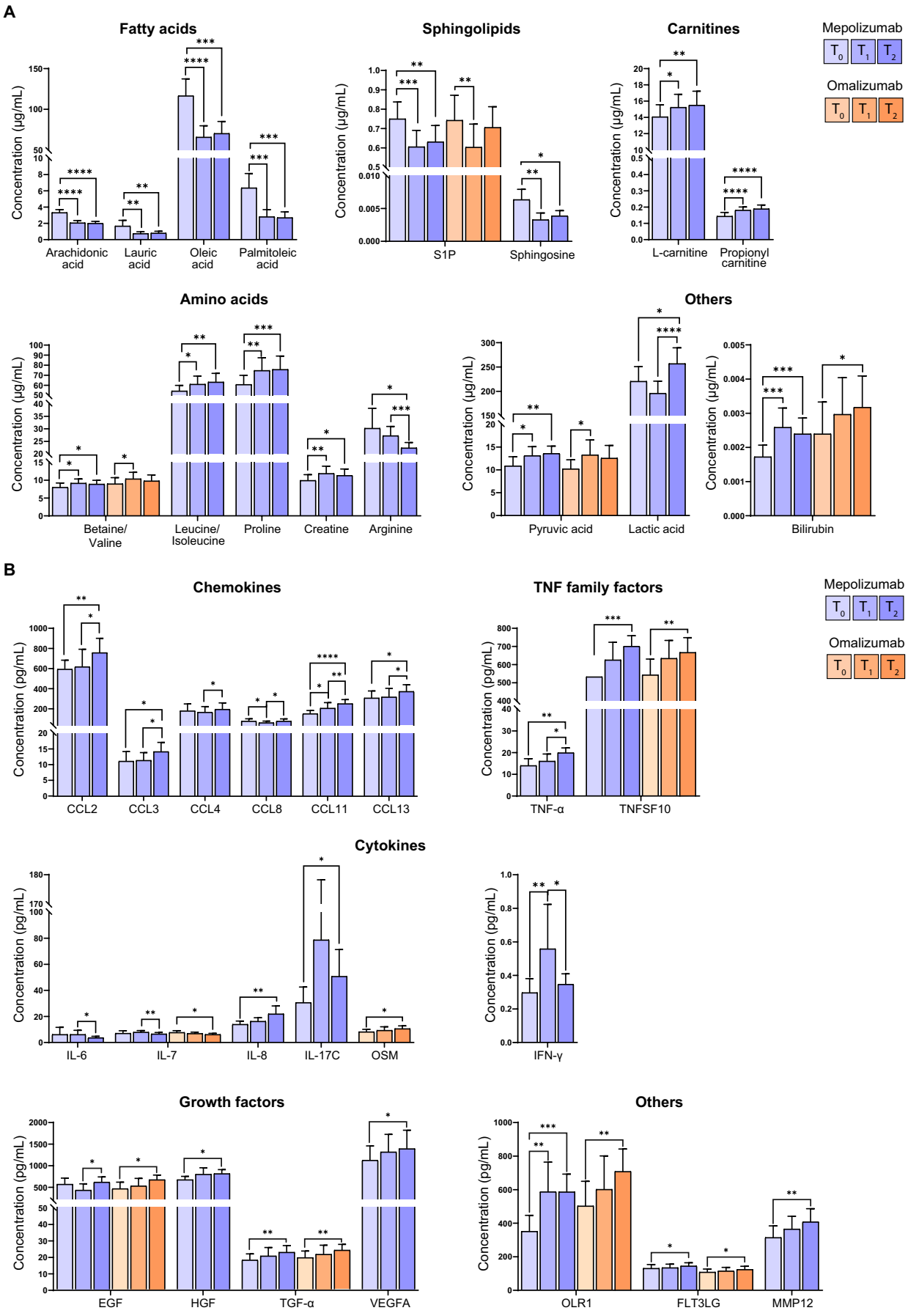

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FIGURE 2 | (A) Metabolomic and (B) proteomic profiles of mepolizumab (purple) ($n = 36$) and omalizumab (orange) ($n = 18$) patients along treatment. T_0 corresponds to baseline, T_1 to 6 months of treatment, and T_2 to 18 months of treatment. The values are presented by the mean and the 95% confidence interval. Repeated-measures ANOVA test ****p value < 0.0001, ***p value < 0.001, **p value < 0.01, *p value < 0.05. CCL, c-c motif chemokine; EGF, proepidermal growth factor; FLT3LG, fms-related tyrosine kinase 3 ligand; HGF, hepatocyte growth factor; IFN-γ, interferon gamma; IL, interleukin; MMP, matrix metalloproteinases; OLR1, oxidized low-density lipoprotein receptor 1; OSM, oncostatin-m; S1P, sphingosine-1-phosphate; TGF, transforming growth factor; TNFSF, tumor necrosis factor superfamily; VEGFA, vascular endothelial growth factor.

component 1 vs. component 3, among the five components obtained. In addition, when looking at component 1 vs. component 3, separated clusters were observed for each of the three time points. Moreover, the total explained variance of each omic layer is represented in Figure 3B. Within the first 3 components, the explained variance was 77.5% (43.8% and 33.7% for metabolomics and proteomics data, respectively). The discriminant analysis resulted in a 3D biplot of the partial least square (PLS) model, which clearly showed that the baseline was separated from the other two time points (Figure 3C, left). Additionally, another perspective of the plot showed a narrower separation between 6 and 18 months, suggesting fewer changes between these time points (Figure 3C, right). Finally, according to the model, the separations observed between the three time points were mainly explained by AA, oleic acid, palmitoleic acid, lactic acid, propionylcarnitine, bilirubin, CCL11, and TNFSF10 (p value < 0.05; p-adjusted < 0.1) (Figure 3C and Table S4).

In the omalizumab group ($n = 16$), the multiomic integration model showed no clear separation between time points with any of the combinations of the five components (Figure 3D). However, according to this model, 93.5% of the variance was explained by the first three components (34.4% and 59.1% for metabolomics and proteomics, respectively) (Figure 3E). This resulted in the 3D biplot PLS model in Figure 3F, which shows no separation between the three time points and highlights that no biomolecule explained any difference in time for omalizumab (p value < 0.05; p-adjusted < 0.1; Table S5), meaning that the differences between the samples may be due to other variables not considered in this model.

Thus, these results point out that AA, oleic acid, palmitoleic acid, lactic acid, propionylcarnitine, bilirubin, CCL11, and TNFSF10 levels are the main changes for mepolizumab treatment over time.

3.5 | Multivariate ROC Analyses Determine a Set of Potential Biomarkers to Classify the Effect of Mepolizumab Treatment

To study whether significant changes in metabolites and proteins over time could be also good classifiers of treatment effect, we performed multivariate ROC analyses between baseline and 6 months, and baseline and 18 months for each biological treatment using the metabolomic and proteomic datasets. We found that the evolution of the omic profiles of patients who received Mepolizumab for 6 months could be correctly predicted from baseline using a multivariate ROC curve composed of 20 potential biomarkers including metabolites and proteins. The outcome of the multivariate ROC curve showed an area under the curve (AUC) = 0.783 with 71.50% accuracy (Figure 4A and Table S6). Of the 20 potential biomarkers, 7 of them (AA, hippuric acid, OLR1, oleic acid, palmitoleic acid, HGF, and bilirubin) presented a selection frequency ≥ 80%. This frequency means how often this is selected in the classification model during crossvalidation (Figure 4B and Table S7).

Furthermore, the obtained multivariate ROC curve for the classification between baseline and 18 months of Mepolizumab treatment also included 20 potential biomarkers with an AUC = 0.871 with a 77.70% of accuracy (Figure 4C and Table S6). In this case, 11 potential biomarkers (AA, palmitoleic acid, oleic acid, propionylcarnitine, TNF-α, lauric acid, CCL11, OLR1, IL-8, adenosine, and TNFSF10) presented a selection frequency ≥ 80% (Figure 4D and Table S8). Interestingly, the potential biomarkers overlap in 70% of coincidence between both multivariate ROC curves at 6 and 18 months, and 85% of the ones that overlapped presented a selection frequency ≥ 80% in at least one comparison. These facts suggest its potential role in the classification of the mepolizumab treatment.

On the contrary, for omalizumab treatment, we did not find any discriminant multivariate ROC curve (Figure S4 and Table S6) as the AUC < 0.75 and accuracy < 60% for both conditions (baseline vs. 6 months or 18 months after treatment).

Notably, there were several biomolecules that were relevant in both the multiomic integration model and in the multivariate ROC curves analyses. Therefore, these results strongly suggest that AA, oleic acid, palmitoleic acid, propionylcarnitine, bilirubin, TNFSF10, and CCL11 could serve as potential biomarkers for monitoring the response to mepolizumab treatment.

3.6 | Potential Biomarkers Significantly Correlate With the Improvement of Clinical Parameters in Severe Asthmatic Patients Treated With Mepolizumab

To investigate the relation of these potential biomarkers with clinical variables, correlation studies were carried out in the Mepolizumab group. Thus, correlations were performed between clinical variables (eosinophilia, FEV1, ACT, frequency of severe exacerbations, and hospitalizations) and those postulated biomarkers obtained from multiomic integration (AA, palmitoleic acid, oleic acid, propionylcarnitine, lactic acid, bilirubin, CCL11, and TNFSF10) and multivariate ROC curve analysis with a selected frequency ≥ 80% (lauric acid, hippuric acid, adenosine, OLR1, HGF, TNF- α, and IL-8). These correlations were evaluated for changes after 6 and 18 months of treatment compared with baseline (Figure 4E,F, Tables S9 and S10, respectively). These results show that all the clinical variables significantly correlated (positively or negatively, p value < 0.05) with

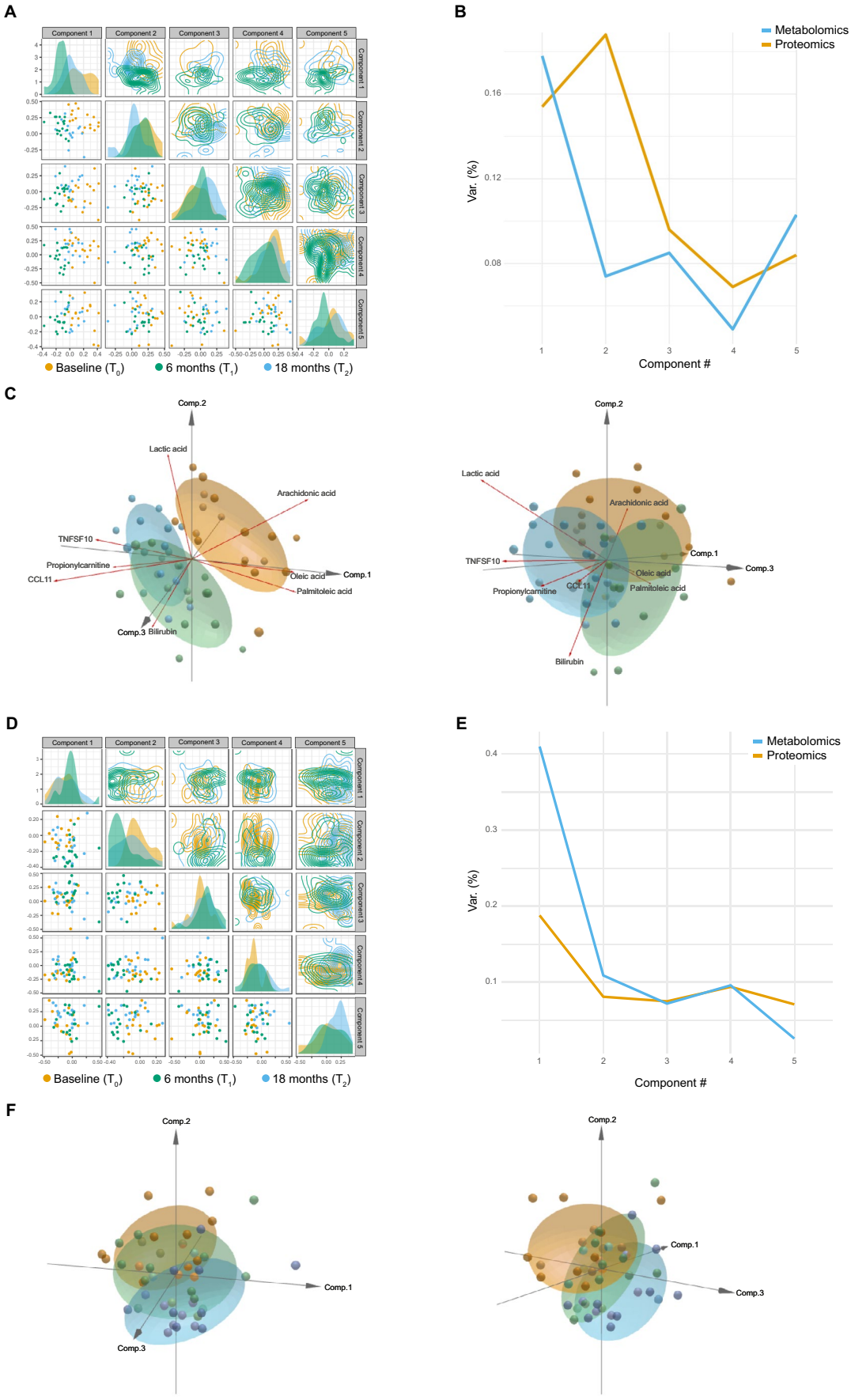

FIGURE 3 | Legend on next page.

FIGURE 3 | Metabolomic and proteomic integration analysis using DIABLO framework. (A) Factor scores plot along different components of the generated models. Samples from the three time points of mepolizumab treatment monitoring ($n = 18$) are represented. (B) Explained variance of each component for each omic layer in the model for mepolizumab group. (C) 3D scatter plot of the samples from the three time points of mepolizumab treatment including the metabolites and proteins that significantly contribute to their differences. (D) Factor scores plot along different components of the generated models. Samples from the three time points of omalizumab treatment monitoring ($n = 16$) are represented. (E) Explained variance of each component for each omic layer in the omalizumab group. (F) 3D scatter plot of the three time points of omalizumab treatment.

either a metabolite or a protein from the above-listed potential biomarkers.

We observed that the number of significant correlations between clinical variables and metabolites and/or proteins increases with longer treatment time. In fact, FEV1 and some biomolecules such as lactic acid, CCL11, TNF-α, and HGF only correlated significantly after 18 months of treatment (Figure 4F).

As previously observed, clinical variables such as eosinophilia, the frequency of severe exacerbations and number of hospitalizations decreased over the course of mepolizumab treatment (Figure 1), correlating positively ($0.33 < \rho < 0.66$) with FAs (AA, lauric acid, oleic acid, and palmitoleic acid) and negatively ($-0.68 < \rho < -0.29$) with propionylcarnitine, bilirubin, CCL11, TNFSF10, and OLR1 (Figure 4E,F, Tables S9 and S10, p value < 0.05). In contrast, FEV1 and ACT showed opposite trajectories increasing throughout treatment (Figure 1), showing negative correlations ($-0.63 < \rho < -0.35$) with FAs (AA, lauric acid, oleic acid, and palmitoleic acid) and positive correlations ($0.23 < \rho < 0.56$) with, e.g., bilirubin, CCL11, and TNFSF10 (Figure 4E,F, Tables S9 and S10, p value < 0.05).

Notably, ACT, eosinophilia, and the frequency of severe exacerbations showed the highest number of correlations, being the strongest correlations mainly after 18 months of mepolizumab treatment.

4 | Discussion

Biological treatments, specifically treatment with MoAb, are an increasingly common therapeutical strategy for chronic inflammatory processes such as asthma, with the capacity to block specific immune pathways [7, 10, 11]. However, the biological effects on the immunometabolic response over time induced by different Th2 response blockers have not been fully reported [33]. In this work, we conducted a multiomic integration analysis combining metabolomics and proteomics, along with correlation analyses between metabolic and protein changes and clinical progression, to identify novel potential biomarkers for monitoring mepolizumab and omalizumab treatment in severe asthma. This provides a deeper understanding of the biological impact of blocking the Th2 immune response at two different steps.

Consistent with other studies [12–14, 19, 34–36] and the GINA guidelines [8], our results demonstrate that the mepolizumab group is older, has a higher eosinophil count and BMI, and lower IgE blood levels at baseline compared with the omalizumab group. This suggests that the immunometabolic features between both groups could be different already at baseline. Furthermore, both treatments exert a positive effect on clinical variables evolution such as eosinophilia, ACT, FEV1, and the frequency of severe exacerbations and hospitalizations. This clinical improvement could also be associated with changes in the metabolic and protein signatures induced by these biologicals.

Our results demonstrate that mepolizumab and omalizumab induce a distinct and specific metabolic and protein profile that suggests a differential regulation of the immune and inflammatory responses at different time points. Indeed, we observed that metabolic changes occur earlier than those observed in the protein profile. As previously published, unlike genes and proteins, metabolites serve as direct signatures of biochemical activity, acting as substrates or products of these processes, making them easier to relate to the observed phenotype [37]. Thus, it is likely that biological treatment first induces changes at the metabolic level [37], and subsequently metabolite–protein and/or protein–protein interactions may occur, which may be related to the induction of transcription and translation of new proteins to regulate the inflammatory response [38].

In addition, some metabolites (S1P, betaine/valine, bilirubin, and pyruvic acid) and proteins (IL-7, EGF, TGF-α, TNFSF10, OLR1, and FLT3LG) appeared altered by both treatments, hinting toward a potential role for all these molecules in the Th2 inflammatory response. In fact, mepolizumab induced further metabolic changes, such as in FAs and carnitines, and in several chemokines that might be specifically linked to IL-5-blockage, a downstream molecule of the Th2 immune response. These changes, combined with the significant improvement in the clinical parameters observed with mepolizumab that were not found in omalizumab, offers new understanding into the mechanisms behind the better clinical efficacy previously reported with mepolizumab [14, 39, 40].

Currently, only a few studies have applied metabolomics [24–26, 41] and/or proteomics [42–44] in asthmatic patients treated with biologicals. Here, by performing multiomic integration analyses, we have generated a unique model with reduced complexity which brings new biological insights into the identification of novel biomarkers for biological treatment monitoring, specifically for mepolizumab treatment.

It should be noted that, although multiomic integration provided a significant model to explain differences related to time after treatment for the mepolizumab group, no significant evidence was found for omalizumab treatment. This, together with their distinct impact over the metabolic and protein profile, may stem from their differing impacts on the Th2 immune response, as we have clinically seen. Therefore, the IgE-blockage, an upstream mediator of the Th2 response, could lead to differences along omalizumab treatment in variables not considered in this study.

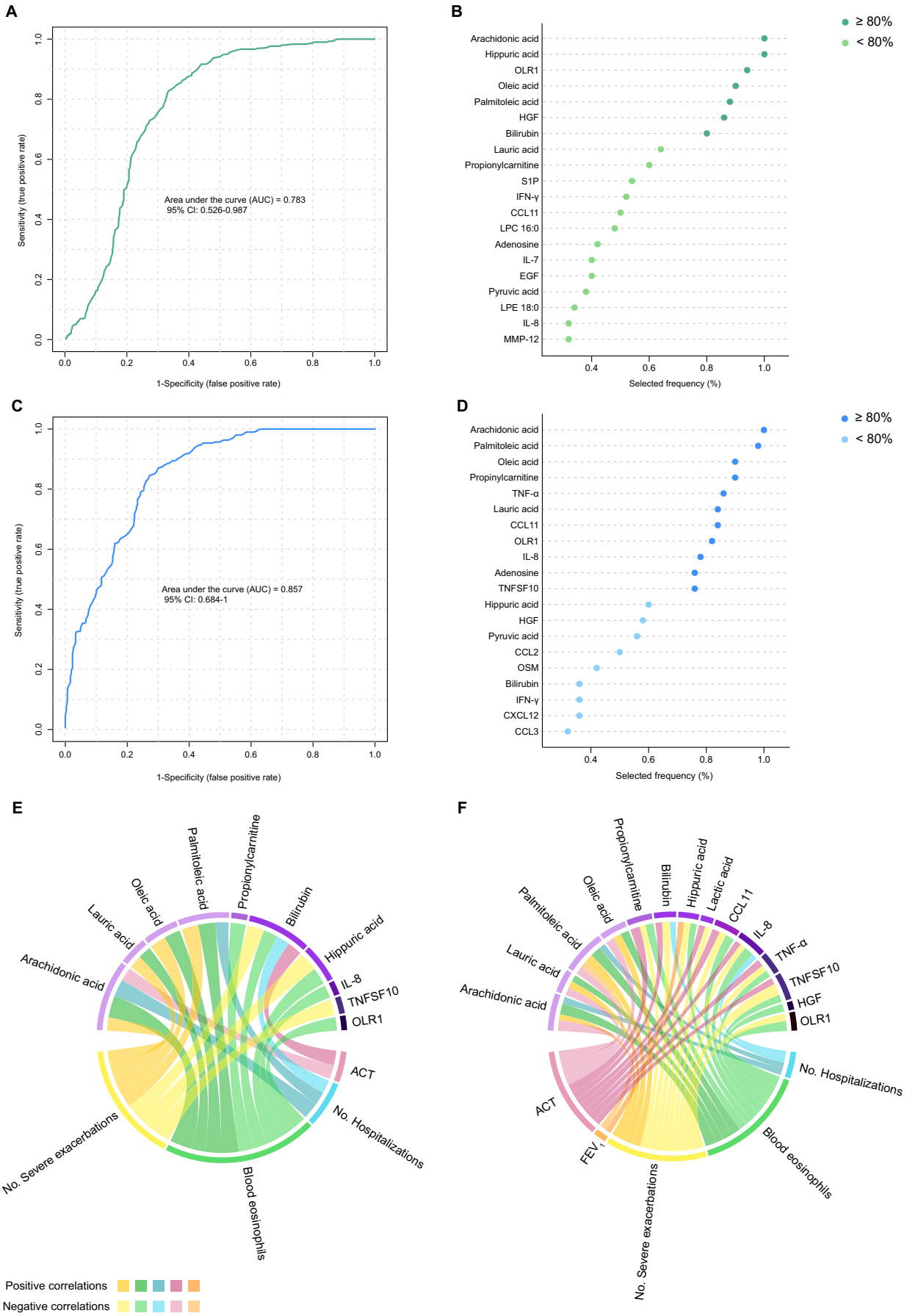

FIGURE 4 | Legend on next page.

FIGURE 4 | Multivariate ROC curves for mepolizumab treatment ($n = 18$) comparing (A) baseline to 6 months and (B) the selected frequency of each biomolecule considered in this model. (C) Multivariate ROC curves for mepolizumab treatment ($n = 18$) comparing baseline to 18 months and (D) the selected frequency of each biomolecule considered in this model. Significant correlations between the selected biomolecules (metabolites and proteins) with the clinical variables (blood eosinophils, levels of FEV1, ACT, and the frequence of severe exacerbations and hospitalizations) (E) after 6 months or (F) 18 months of mepolizumab treatment. Paired Spearman correlation test (p value < 0.05).

Regarding mepolizumab, multiomic integration together with multivariate ROC analyses provide a robust set of biomolecules with the potential of being validated as biomarkers for treatment monitoring. These include AA, palmitoleic acid, oleic acid, propionylcarnitine, bilirubin, CCL11, and TNFSF10. Even more importantly, the changes displayed in these biomolecules along Mepolizumab treatment significantly correlate with clinical improvement. Concretely, this correlation is particularly strong when comparing the increase of ACT and the decrease of blood eosinophils and severe exacerbations' frequency, which are among the most common clinical parameters used for assessing patient evolution [15, 16, 45], demonstrating the potential of these biomolecules to assess treatment effectiveness.

The changes in these potential biomarkers are associated with several molecular mechanisms linked to the immune and/or inflammatory response. Specifically, FAs (AA, palmitoleic acid, and oleic acid) constitute a source of proinflammatory mediators, such as leukotrienes, oxylipins, or prostaglandins (PGD_2) [46–48], being PGD_2 an important eosinophil chemoattractant which is impaired after Mepolizumab administration [49]. Therefore, decreased serum FAs levels correlate positively with blood eosinophils and the frequency of severe exacerbations, and negatively with ACT. Moreover, propionylcarnitine, bilirubin, CCL11, and TNFSF10 increase after Mepolizumab treatment and correlate positively with ACT and negatively with both eosinophilia and the frequency of severe exacerbations. In fact, the correlation between carnitine metabolites and decreased exacerbations has been recently observed [41]. Propionylcarnitine is known to reduce propionyl-CoA bioavailability inducing an anti-inflammatory and antioxidant effect [50]. Bilirubin also exerts an anti-inflammatory effect through the impairment of leukocyte recruitment [51]. TNFSF10 has been previously related to enhancing regulatory T-cell proliferation through its release from dendritic cells in an autoimmune context [52]. On the other hand, CCL11, a crucial eosinophil chemoattractant [53], increases with time of treatment, resembling a shift toward an inflammatory phenotype that could be occurring as a compensatory mechanism due to Mepolizumab blockade of IL-5. In support to this, an increase in CCL11 levels after Mepolizumab treatment has been already described in severe eosinophilic asthmatic patients [42]. However, this change does not seem to have a direct and observable clinical effect as metabolic changes may have [54] when directly blocking IL-5, suggesting that alternative inflammatory mechanisms could be activated to compensate for the IL-5-derived signaling blockade exerted by mepolizumab. Overall, mepolizumab induces initial metabolic changes due to IL-5 blockade which are followed by protein changes which, as in the case of CCL11, could act as a compensatory mechanism for this blockade. All these changes seem to point toward a reduction in the inflammatory profile of severe asthmatic patients.

Despite the potential of this study for personalized medicine, the study has some limitations. Further studies are needed to validate these potential biomarkers in similar cohorts with a larger sample size, and under other biological treatments (such as dupilumab) that block different points of the Th2 immune response, to enhance the robustness of these results. Furthermore, we believe it would be beneficial to include untargeted approaches of metabolomics (including lipidomics) and proteomics to test other metabolites and proteins. In addition, other complementary omic methods such as transcriptomics or epigenomics might be of help to obtain a more complete view of the biological changes during the biological treatment. Additionally, further studies including the nonresponder patients would be interesting to understand the molecular mechanisms behind this lack of response.

Nevertheless, this study provides evidence that both mepolizumab and omalizumab induce a clear distinctly clinical effect that might be related to the blockade step along the Th2 immune response. Furthermore, we observed that both treatments can induce common (e.g., S1P, bilirubin, OLR1, and TNFSF10) and treatment-specific metabolic and protein profiles over time. Finally, we provide an integrative model of metabolomic and proteomic data that has allowed the identification of potential serum biomarkers (AA, palmitoleic acid, oleic acid, propionylcarnitine, bilirubin, CCL11, and TNFSF10) for monitoring treatment with Mepolizumab during the first 18 months.

Author Contributions

Conceptualization: T.C., D.B., A.V., M.M.E. Experimental design: C. Blanco, T.C., D.B., A.V., M.M.E. Sample collection and clinical characterization: H.G.C., J.A.C., V.M., C.C., C.B., T.C. Metabolomic analysis: N.C., D.O. Metabolomic supervision: C. Barbas, A.V. Proteomic analysis: N.C., P.F., M.M.E. Multiomic integration: N.C., A.E.-P. Data analysis and treatment: N.C., A.E.-P., S.A.-D.-P., A.V. Data interpretation: N.C., A.E.-P., M.I.D.-D., E.I., A.V., M.M.E. Manuscript writing: N.C., M.I.D.-D., A.V., M.M.E. All authors contributed to the critical revision of the manuscript and approved the final version of the manuscript.

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Conflicts of Interest

V.M. reports personal fees and/or honoraria from GSK and Astra. C.C. reports personal fees and/or honoraria from Astra, GSK, Sanofi, Gebro, Menarini, and Chiesi and has served Data Safety Monitoring Board and/or Advisory Board at Sanofi, GSK, and Astra. T.C. reports personal fees and/or honoraria from GSK, Sanofi, and AstraZeneca. The other authors declare no conflict of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.