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Plasma Galectin-3 and urine proteomics predict FEV_1 improvement in omalizumab-treated patients with severe allergic asthma: Results from the PROXIMA sub-study

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ABSTRACT

Background: Patients with severe allergic asthma (SAA) when treated with omalizumab may exhibit different extent of response. Identifying biomarkers that can predict the extent of treatment effectiveness in patients can be useful in personalizing omalizumab treatment.

Methods: Patients from the longitudinal phase of the PROXIMA study were selected for this ancillary study. After 12 months of omalizumab treatment, patients were categorized according to their response to treatment as: "clinical responder" (Asthma Control Questionnaire [ACQ] total score <1 at Month 12 and/or with a reduction in number of exacerbation versus the previous year); "functional responder" (an increment of ≥ 0.1 L in forced expiratory volume in 1 s [FEV_1] at Month 12 versus baseline); and "super responder" (among clinical responders group, who also showed a functional response). Plasma galectin-3 (GAL-3) levels were quantified using a micro titer plate-based enzyme linked immunosorbent assay kit.

Results: The Majority of patients (86.36%) in sub-study population were identified as clinical responders. Of the total patients identified as clinical responders, 64.86% were identified as super responders. A statistically significant difference in the baseline plasma GAL-3 levels between responders and non-responders was observed only in the functional responders group ($P = 0.0446$). Patients with plasma GAL-3 level of ≥ 11 ng/mL had a greater probability of being a super responder ($P = 0.0118$) or a functional responder ($P = 0.0032$).

Conclusion: Our findings support the use of plasma GAL-3 as a predictive marker to stratify responders and identify super responders and functional responders to omalizumab treatment in patients with severe allergic asthma using less invasive sample like plasma.

Keywords: Severe allergic asthma, Biomarkers, Omalizumab responders, Galectin-3

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BACKGROUND

Asthma is a common chronic disorder affecting over 330 million worldwide.[1,2] In Italy, the prevalence of asthma increased by 38% over two decades from 1991 to 2010, based on data from 3 multicenter cross-sectional surveys carried out in the Italian adult population.[3]

Omalizumab is recommended as an add-on therapeutic approach for the treatment of patients with uncontrolled severe allergic asthma (SAA).[1] Results of several observational studies have confirmed the effectiveness of omalizumab in the real-life setting in moderate-to-severe and severe persistent allergic asthma,[4–9] however treatment response is indicated in a highly targeted population.

Biomarkers are quantitatively measurable indicators associated with biological processes that can predict the extent of treatment effectiveness in patients.[10] Identifying biomarkers in real-life settings can provide guidance to treating physicians on when to escalate or de-escalate treatment, thus facilitating personalized and targeted therapy by identifying patients who may gain the most benefit from the treatment, improving benefits for patients and optimizing medical resources.[11–13]

GAL-3 binding protein (GAL-3 BP) is a member of the macrophage scavenger receptor.[14] Galectin-3 (GAL-3) was first discovered as an immunoglobulin E (IgE)-binding protein on the surface of murine macrophages. Previously, Riccio et al. reported GAL-3 as a possible biomarker associated with airway remodeling reduction in response to omalizumab treatment.[15]

PROXIMA, an observational, multicenter two-phase study (cross-sectional and longitudinal phase) conducted in patients with SAA showed that omalizumab reduced exacerbation rates by 87% and effectively controlled asthma after 6 and 12 months of treatment, improving quality of life of these patients.[16] A high proportion of the study population (longitudinal phase) responded to the omalizumab treatment with a response rate of 89%–96%.

Though several studies have shown that a high proportion of patients respond to omalizumab treatment,[12,17] these studies have not attempted to identify and categorize patients as clinical, functional, or super responders to omalizumab treatment.

The PROXIMA[18] sub-study, was an exploratory analysis designed to identify protein biomarkers focusing on the role of GAL-3 as a predictor of clinical, functional, and super responders for omalizumab treatment in patients with SAA.

METHODS

Study design and patients

The PROXIMA study was a multicenter, two-phase, single-arm, observational study, conducted at 25 centers in Italy, in outpatient settings specialized in the treatment of asthma. Detailed inclusion/exclusion criteria have been described previously.[16] The study consisted of a cross-sectional phase and a 12-month longitudinal prospective phase. After screening, patients entered into the cross-sectional phase designed to estimate the pattern of allergic asthma (perennial versus seasonal). Further, as per the clinician's judgment, patients who required omalizumab treatment entered the longitudinal phase from the cross-sectional phase. All the patients who participated in the longitudinal study had the option to participate in this study.

Furthermore, ten healthy subjects were recruited as control group, and plasma was collected. Enrolled volunteers did not suffer from respiratory and immunological diseases. As this is an ancillary study with no *a priori* hypothesis, no formal sample size calculations were done. Blood samples from patients in this sub-study were collected before (i.e. baseline) and after 12 months of omalizumab treatment. Furthermore, urine analysis was conducted on a sub-set of these patients.

Objectives of the PROXIMA ancillary study

The main objective of the study was to explore the role of plasma GAL-3 as a predictive biomarker for functional response to omalizumab in patients with SAA. Additional analysis in urine samples was conducted to confirm the predictive value of GAL-3.

Sample collection and handling

Blood and urine samples were collected at baseline and after 12 months of omalizumab

treatment. The blood and urine samples were collected and stored either at 4C if shipped within 24/48 hours or stored at -20C and -80C, respectively, if the proteomics analysis was to be conducted later.

Sample preparation

Urine

Stored urine samples were thawed and centrifuged at 17,000×*g* for 10 minutes at 4C. Supernatants were separated and subjected to ultracentrifugation at 200,000×*g* for 1 hour at 4C to obtain exosome pellets. The protein concentration was assayed using the SPNTM Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA), and 50 ± 0.5 µg of protein from each sample was digested with trypsin using a 1:50 (w/w) enzyme/substrate ratio at 37C overnight. The next morning, an additional aliquot of enzyme was added at an enzyme/substrate ratio of 1:100 (w/w), and the digestion continued for 4 hours. Samples were then centrifuged at 13,000×*g* for 10 minutes, desalted by PepClean C-18 spin columns (Thermo Fisher Scientific, Waltham, MA, USA) and concentrated in a SpeedVac (Savant Instruments Farmingdale, NY, USA).

GAL-3 measurement

Plasma GAL-3 levels were quantified using a microtiter plate-based enzyme-linked immunosorbent assay (C) kit (BGM Galectin-3 Assay kit, BG Medicine, Inc., Waltham, MA, USA). Testing procedures were performed according to manufacturer protocol. Assay characteristics included a lower detection limit of 1.4 ng/mL and upper detection limit of 94.8 ng/mL, for clinical specimens.

Proteomics analysis

Trypsin-digested mixtures were analyzed by the Eksigent nanoLC-Ultra 2D System (Eksigent, AB SCIEX Dublin, CA, USA) combined with cHiPLC-nanoflex system (Eksigent) in trap-elute mode on a nano cHiPLC column (75 µm × 15 cm ChromXP C18-CL, 3 µm, 120 Å), through a 65 minute gradient of 5–45% of eluent B (eluent A, 0.1% formic acid in water; eluent B, 0.1% formic acid in acetonitrile), at a flow rate of 300 nL/min. Mass spectra were acquired using a QExactive mass spectrometer (Thermo Fisher Scientific, San José, CA, USA) recorded in positive ion mode over a 400–1600 *m/z* range and with a resolution setting of 70,000 FWHM (@ *m/z* 100) with 1 microscan per sec. For other details on data handling, see Supplementary Appendix 2.

Study assessments

Forced expiratory volume in 1 s (FEV_1), number of exacerbations, and the Asthma Control Questionnaire (ACQ) scores were evaluated at baseline (12 months prior to the start of the observation) and after 12 months of omalizumab treatment in each study population (longitudinal and sub-study [plasma and urine samples]. Patients were categorized in accordance with their response to treatment as clinical, functional, and super responders. In particular, a "clinical responder" was defined as a patient with an ACQ total score <1 at month 12 and/or with a reduction in number of exacerbation versus the previous year. Patients showing an increment of at least 0.1 L in FEV_1 at month 12 versus baseline were defined as "functional responders". Among the clinical responders group, patients who also showed a functional response were classified as "super responders".

To evaluate correlation with plasma GAL-3 levels, we also analyzed GAL-3 BP in the urine in sub-set of patients through proteomics technique.

Statistical analysis

Summary statistics were used to describe each variable. *P*-values were generated using Wilcoxon signed rank test or paired *t*-test for comparison within patients, and Wilcoxon nonparametric test for comparison between classes of responder. Clopper-Pearson method was used to calculate 95% confidence interval. The correlation between FEV_1 and GAL-3 was analyzed by means of a logistic regression model. Protein lists were evaluated by hierarchical clustering using in-house R-scripts, based on XlsReadWrite, clue and clValid library; Ward's method and Euclidean distance metric were applied. Protein lists were aligned, normalized and processed by linear discriminant analysis (LDA) applied by using a common covariance matrix for all groups, and the Mahalanobis distance (3) from each point to each group's multivariate mean; to select proteins

discriminating the analyzed groups we considered those with largest F ratio (≥3) and smallest *P* value (≤0.05).

RESULTS

Patient disposition and baseline characteristics

Of 365 patients who were enrolled in cross-sectional phase, 130 were included in the longitudinal phase, and of these, 58 were enrolled in the sub-study, and 44 (75.9%) completed the study and were considered for the analysis. Further from this sub-set, 19 patients were randomly selected for urine analysis (Fig. 1).

The patient demographics and baselines characteristics are described in Table 1. The mean age of patients was similar between overall study and sub-study populations. Patients were predominantly female and Caucasian in all populations. Patients included in the sub-study (plasma) population had a slightly lower duration of asthma history compared with the longitudinal patient population. No differences were observed in FEV_1 at baseline and number of exacerbations in the 12 months prior to study entry between the analysis populations. The proportion of women were slightly higher in sub-study populations; however, the difference was not clinically relevant ensuring that the population included in the sub-study was representative of the entire population. About 63% patients in longitudinal population and 42–50% of patients in the sub-study populations were reported to have had at least 1 comorbidity (Table 1).

Identification of clinical, functional, and super responders to omalizumab treatment

Patients were identified as clinical, functional, and super responders based on the changes from the baseline in FEV_1, number of exacerbations and ACQ total score after 12 months of omalizumab treatment.

Change in FEV_1

A statistically significant increase in FEV_1 from baseline was observed in the sub-study populations after 12 months of omalizumab treatment. Moreover the mean FEV_1 of the small population of patients selected for the proteomics analysis of urine sample was consistent with the entire population (Fig. 2a).

Change in number of exacerbations

A significant decrease in number of exacerbations from baseline in all populations was observed after 12 months of omalizumab treatment in all the 3 populations (Fig. 2b).

Change in ACQ total score

After 12 months of treatment, a significant decrease in ACQ total score from baseline was observed in the longitudinal population and its sub-study populations (Fig. 2c).

Based on the changes in ACQ score and number of exacerbations from baseline after 12 months of treatment, most of patients in the sub-study (plasma), 86.4%; and sub-study (urine), 89.5%, were identified as clinical responders. According to the changes in FEV_1, of the total patients identified as clinical responders, over half

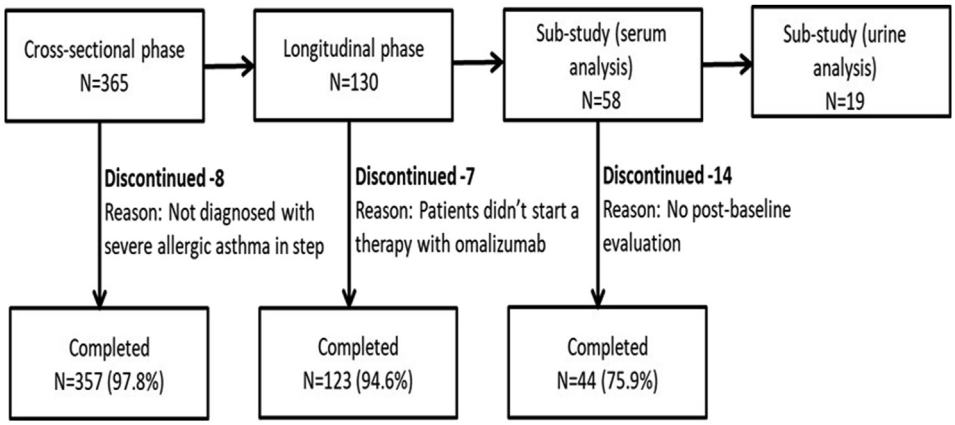

Fig. 1 Patient disposition

Variables	Cross-sectional population N = 357	Longitudinal population N = 123	Sub-study (plasma) population N = 44	Sub-study (urine) population N = 19
Age, years	50.5 ± 15.5	52.7 ± 13.6	51.7 ± 12.0	50.8 ± 12.3
Gender, n (%)				
Women	232 (65.0)	76 (61.8)	32 (72.7)	14(73.7)
Men	125 (35.0)	47 (38.2)	12 (27.3)	5 (26.3)
Race, n (%)				
Caucasian	340 (95.2)	117 (95.1)	41 (93.2)	17 (89.5)
Asthma duration (years)	18.4 ± 14.9	19.8 ± 14.5	16.3 ± 11.9	21.2 ± 17.39
Number of asthma exacerbations in the last 12 months	3.6 ± 4.2	4.6 ± 4.1	4.6 ± 4.0	4.4 ± 3.5
FEV_1 at baseline (L)	2.0 ± 0.8	1.7 ± 0.7	1.7 ± 0.7	1.6 ± 0.7
IgE assessment, n (%)	252 (70.6)	121 (98.4)	42 (95.5)	19 (100)
IgE serum level (IU/mL)	434.8 ± 556.4	409.3 ± 394.1	390.7 ± 299.3	521.6 ± 445.4
Number of patients with at least one comorbidity, n (%)	208 (58.3)	77 (62.6)	22 (50.0)	8 (42.1)

Table 1. Demographics and baseline characteristics. *Data presented as mean ± SD, unless specified.FEV_1, forced expiratory volume in 1 s; IgE, immunoglobulin E*

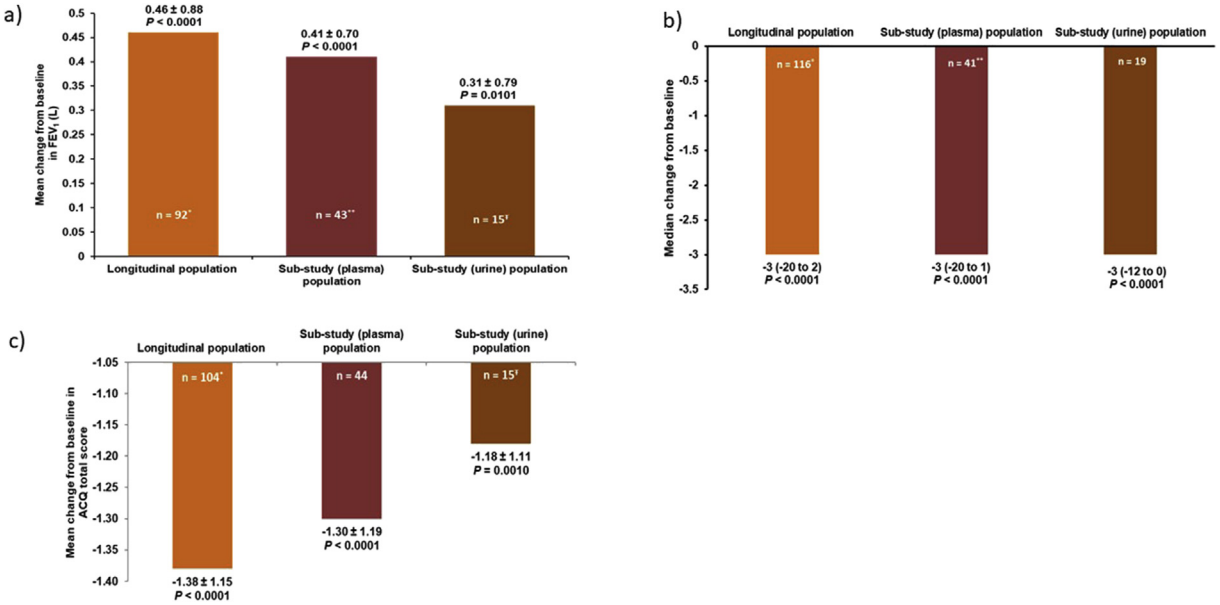

Fig. 2 a) Mean ± SD change from baseline in FEV_1 after 12 months of treatment with omalizumab; b) Median (min-max) change from baseline in number of exacerbations after 12 months of treatment with omalizumab; c) Mean ± SD change from baseline in ACQ total score after 12 months of treatment. Fig a: Data presented as mean ± SD. P values were calculated with signed-rank test. *Data not available for 31 patients; ** Data not available for 1 patient; ǂ Data not available for 4 patients. Fig b: Data presented as median (min to max). P values were calculated with signed-rank test. *Data not available for 7 patients; ** Data not available for 3 patients. Fig c: Data presented as mean ± SD. P values were calculated with paired *t*-test. *Data not available for 19 patients; ǂData not available for 4 patients. SD, standard deviation

were identified as super responders (sub-study [plasma], 64.9%; sub-study [urine], 57.1%; Table 2a).

Correlation of plasma GAL-3 (ng/mL) levels and the clinical, functional and super responders

The mean levels of plasma GAL-3 (ng/mL) at baseline were similar between the responders and non-responders with no significant differences, except for functional responders showing a statistically significant difference in the sub-study (plasma) population (responders, 11.80 ± 2.49; non-responders, 10.35 ± 2.45; $P = 0.0446$) (Table 2b).

A comparison of plasma GAL-3 levels in severe asthmatic patients versus healthy controls showed the levels of plasma GAL-3 were significantly higher in severe asthmatic patients compared with control ($P = 0.0019$; Supplementary Figure 1).

An analysis evaluating the correlation between baseline GAL-3 levels and FEV_1 change for functional responders showed a significant ($P = 0.0484$) trend between FEV_1 change and plasma GAL-3 levels (Supplementary Figure 2). A significant correlation was also observed showing that patients with a higher change in FEV_1 tend to have higher GAL-3 levels at baseline when adjusted by age, BMI and gender ($P = 0.0484$; odds ratio [95%CI], 0.795 [0.633 to 0.998]. However, no correlation between FEV_1 and demographic data was observed.

Patients were categorized into 2 groups according to the change from baseline in FEV_1 after 12 months of treatment; improved FEV_1 (all patients with a change in $FEV_1 > 0$) and not improved FEV_1 (all patients with a change in $FEV_1 \leq 0$). The GAL-3 level in plasma is significantly ($P = 0.0366$) higher in patients with improved FEV_1 (mean ± SD; 11.69 ± 2.50) versus patients with not improved

a)

Response		Longitudinal study (N = 123)	Sub-study (plasma) (N = 44)	Sub-study (urine) (N = 19)
Clinical responder	n/m (%)	105/120 (87.5)	38/44 (86.4)	17/19 (89.5)
	95% CI	80.2 to 92.8	72.6 to 94.8	66.9 to 98.7
Functional responder	n/m (%)	60/92 (65.2)	29/43 (67.4)	8/15 (53.3)
	95% CI	54.6 to 74.9	51.5 to 80.9	26.6 to 78.7
Super responder	n/m (%)	54/81 (66.7)	24/37 (64.9)	8/14 (57.1)
	95% CI	55.3 to 76.8	47.5 to 79.8	24.6 to 75.4

b)

Response	Sub-study (plasma) N = 44		
		GAL-3 ng/mL	*P*-value
Clinical responder	Responder: n = 38	11.17 ± 2.48	0.2664
	Non-responder: n = 6	12.45 ± 2.70	
Functional responders	Responder: n = 29	11.80 ± 2.49	**0.0446**
	Non-responder: n = 14	10.35 ± 2.45	
Super responders	Responder: n = 24	11.56 ± 2.44	0.1081
	Non-responder: n = 13	10.37 ± 2.54	

Table 2. a) Proportion of clinical, functional and super responders during the 12 months of treatment. b) Plasma GAL-3 levels at baseline by response. *95% CI is derived using Clopper-Pearson method.CI, confidence interval; n, number of patients with response; m, number of evaluable patients.P-values were calculated with Wilcoxon test*

FEV_1 (mean ± SD; 9.93 ± 2.33; Supplementary Table 1).

In order to find the cut-off of plasma GAL-3 level at baseline to identify patients who show improved FEV_1 response, 3 cut-off limits of plasma GAL-3 levels were considered; 10 ng/mL, 11 ng/mL and 12 ng/mL. There was a significant difference between patients with improved FEV_1 and not improved FEV_1 at a cut-off of 11 ng/mL plasma GAL-3 levels ($P = 0.0166$; Fig. 3). The analysis of change in FEV_1 by plasma GAL-3 level showed that there was a trend towards a predictability of lung function improvement at a cut-off of 11 ng/mL ($P = 0.0716$). However, this did not demonstrate a statistical significance, which may be due to low sample size and the wide FEV_1 variability.

Considering a cut-off limit of 11 ng/mL for plasma GAL-3 levels, the correlation between the GAL-3 level and treatment responders (clinical, functional, and super responders) was analyzed. The results showed that patients with a plasma GAL-3 level of ≥11 ng/mL have a greater probability of being a super responder ($P = 0.0118$) or a functional responder ($P = 0.0032$; Table 3).

Proteomics analysis

Cluster analysis of protein data

To obtain an unbiased proteomics stratification of patients and extract the discriminating factors (protein descriptors), unsupervized cluster analysis was performed for proteins identified in the urinary exosomes. Cluster was divided into 3 groups (Group 1, Group 2 [main groups] and Group 3) according to agglomerative coefficient of >0.8 at T0 (Supplementary Figure 3).

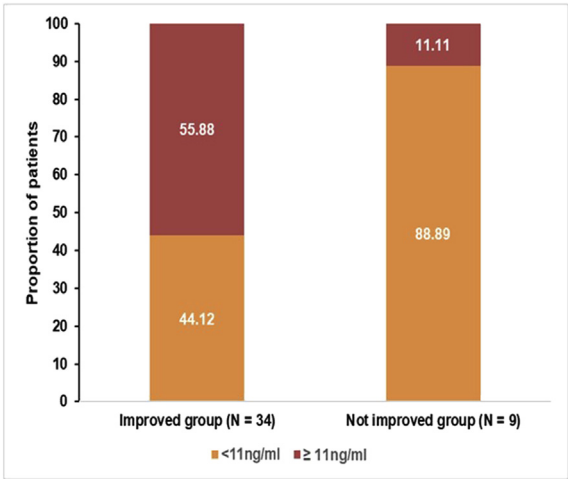

Fig. 3 Number of patients with improved and not improved FEV_1 at a cut-off of 11 ng/mL of plasma GAL-3 levels at baseline

Relationship between clinical parameters and GAL-3 BP

In the two main groups (Groups 1 and 2), the values of the different clinical parameters and GAL-3 BP level was evaluated. GAL-3 BP levels in the urinary exosomes for the two groups (1 and 2) were evaluated for the 14 patients who completed treatment. Levels of GAL-3BP were higher in Group 1 compared with Group 2 (Fig. 4).

The results confirm the segregation of patients into 2 identified groups by proteomics and suggest a relationship between GAL-3 BP and IgE level at T0 and the effect on FEV_1 (change of FEV_1) due to omalizumab treatment at 12 months (T12) (Table 4a). Patients in Group 1 with higher IgE levels showed more hits for GAL-3 BP. It is notable that this group reported higher change in FEV_1 after 12 months of omalizumab treatment showing response to the omalizumab treatment. However, patients in Group 2 showed lower levels of IgE and lesser number of hits for GAL-3 BP with no significant change in FEV_1 showing non-response to the treatment after 12 months.

Correlation between plasma GAL-3 level (cut-off of 11 ng/mL) and urine analyses

No difference in patient distribution between groups (Groups 1 and 2) and plasma GAL-3 levels (cut-off of 11 ng/mL) was observed. However, in term of frequencies, patients with GAL-3 ≥ 11 ng/mL were higher in Group 1 (33%) than in Group 2 (25%) (Table 4b).

DISCUSSION

This PROXIMA sub-study, an exploratory analysis aimed to identify and confirm the role of GAL-3 to predict the modulation of airway remodeling after omalizumab treatment, as it can be associated with an improvement of pulmonary function. In particular, GAL-3 level can identify super responder to omalizumab treatment, with both a clinical and a functional response, thereby facilitating personalization of medication to improve patient care and control healthcare costs. Our

Class of responder		Plasma GAL-3 level cut-off at baseline		*P* value	Odds ratio (95% CI)
		<11 ng/mL	≥11 ng/mL		
Clinical responder	Yes	21 (55.26%)	17 (44.74%)	0.3176	0.40 (0.07–2.48)
	No	2 (33.33%)	4 (66.67%)		
Functional responder	Yes	11 (37.93%)	18 (62.07%)	**0.0032**	9.82 (1.84–52.38)
	No	12 (85.71%)	2 (14.29%)		
Super responder	Yes	10 (41.67%)	14 (58.33%)	**0.0118**	7.70 (1.39–42.63)
	No	11 (84.62%)	2 (15.38%)		

Table 3. GAL-3 level by class of clinical, functional and super responder. *P-value calculated by Chi-square test.Percentage computed by row*

study confirmed the role of plasma GAL-3 levels as a predictive marker for omalizumab super responders (patients who are clinical responders and showed functional response), i.e. these patients would show improvement in ACQ score and reduction in exacerbations as well as improvement in lung function after omalizumab treatment. GAL-3 can also be used as a marker for functional responders showing improvement only in FEV_1 levels.

The study included patients from the longitudinal phase of the PROXIMA main study. The demographics and baseline characteristics of the patients were similar across the cross-sectional, longitudinal and sub-studies showing that the 44 patients analyzed for presence of GAL-3 in the plasma are representative of entire population of the longitudinal study. Additional the mean FEV_1 of the small population of patients selected for the proteomics analysis of urine was consistent with the entire population, establishing the generalizability of these results in wider population.

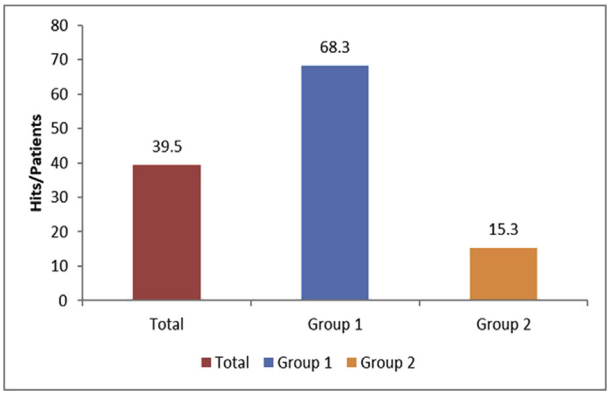

Fig. 4 GAL-3 BP hits average per patient in urine sample at T0

Patients were categorized as clinical, functional, and super responders to omalizumab treatment based upon change from baseline in FEV_1, number of exacerbations, and ACQ score after 12-months of treatment. Most patients were identified as clinical responders in all 3 groups, and of these, more than half the population were also identified as super responders. These results were consistent with the previous retrospective analysis showing that omalizumab treatment can lead to clinically meaningful improvements in the lung function.[19,20] Since severe asthma has been related to remodeling of airways, leading to airway obstruction, the improvement in lung function after omalizumab treatment has been attributed to its beneficial effect on airway remodeling.[21–25]

GAL-3 has been reported in earlier studies as a reliable marker that can predict the modulatory effect of omalizumab treatment on bronchial reticular basement membrane thickening, to identify responders.[26] GAL-3 was shown to be a useful tool for predicting clinical improvement in pulmonary function in patients treated with omalizumab for up to 3 years.[15] Proteomics analysis has shown that baseline GAL-3 in the bronchial tissue is predictive of the response to omalizumab,[15] as shown by the specific airway remodeling obtained after the treatment. Studies have confirmed that presence of GAL-3 at baseline can act as a modulator of airway remodeling upon treatment with omalizumab.[23,27,28]

a) Number of patients (T0/T12)	Group 1 7/6	Group 2 10/7	*P* value[a]
IgE at T0 (IU/mL)	807.86 (382.24)	266.11 (190.63)	0.0054
FEV_1 at T0 (L)	1.69 (0.78)	1.49 (0.62)	0.5259
FEV_1 at T12 (L)	2.37 (1.60)	1.53 (0.32)	0.5613
Change in FEV_1	0.71 (0.77)	0.02 (0.77)	0.2448
ACQ at T0	3.33 (1.65)	2.81 (1.20)	0.5565
ACQ at T12	1.74 (1.34)	1.91 (1.16)	0.6982
Changed ACQ	−1.40 (1.22)	−0.91 (1.08)	0.3324
Annualized Exacerbations at T0 (N)	6.43 (4.69)	3.20 (2.15)	0.2171
Injection/patient	19.43 (7.30)	12.60 (4.22)	0.0537
Score Gal-3 BP at T0	238.43 (98.06)	48.90 (49.29)	0.0015
Hit Gal-3 BP at T0	68.29 (32.16)	15.30 (14.85)	0.0021

b) Plasma GAL-3 level cut-off at baseline	Group 1 n (%)	Group 2 n (%)	2 n (%)	Total n
<11 ng/mL	4 (66.67)	6 (75.00)	1 (100.00)	11
≥11 ng/mL	2 (33.33)	2 (25.00)	0	4
Total	6 (100.00)	8 (100.00)	1 (100.00)	15

Table 4. a). Mean (SD) of clinical parameters and GAL-3 BP levels in the two identified groups and b) Patient distribution by plasma GAL-3 level (cut-off of 11 ng/mL) and cluster analysis Groups 1 and 2. *T0, start of treatment period; T12, after 12 months treatment period.ACQ, asthma control questionnaire; FEV_1, forced expiratory volume in 1 s; GAL-3 BP, galectin-3 binding protein.Chi-square P = 0.7744.Percentage computed by column. a. Wilcoxon test*

In our study, since no significant change in GAL-3 plasma levels was observed during the study, it was identified as a reliable marker to categorize treatment responders and its correlation with the treatment response was analyzed. Comparison of GAL-3 levels at baseline by response showed a statistical difference between functional responders and non-responders in the serum samples of the sub-study population with higher levels of GAL-3 in omalizumab responders.

Further, a statistical correlation was observed between the baseline GAL-3 levels in plasma and FEV_1 changes. It should be noted that although a cut-off of >0.1 L in FEV_1 represents a clinically relevant difference, we have considered a cut-off of >0 L in FEV_1 to indicate minimum improvement in these patients. The plasma GAL-3 level was significantly higher in patients with improved FEV_1 versus patients with not improved FEV_1. These results support use of GAL-3 as a predictive biomarker of lung function improvement in patients with severe allergic asthma after omalizumab treatment. A cut-off of 11 ng/mL of GAL-3 level in plasma at baseline can be considered as a marker for super responders and functional responders. However, a specific trend in the increase in FEV_1 may not be entirely predictable. These results support previous studies and suggest that

plasma GAL-3 levels can act as an effective biomarker in predicting functional response of omalizumab in patients with SAA.

Proteomics analyses of urine samples for a subset of patients were conducted to further confirm the reliability of GAL-3 as a biomarker. Stratification of patients in 2 main groups (Group 1 and Group 2) was done on the basis of the protein profiles of exosomes from urine samples of patients before omalizumab treatment, and characterized by a difference in clinical trend after treatment. The molecular stratification was in good agreement with those obtained by means of clinical parameters, mainly based on IgE level and change of FEV_1 after omalizumab treatment. Patients in Group 1 with higher IgE levels showed more hits for GAL-3 BP at baseline and reported higher change in FEV_1 after 12 months of treatment indicating response to omalizumab. The results support the role of GAL-3 level before the treatment as a biomarker for predicting omalizumab response. Previously, Riccio et al. demonstrated that GAL-3 level before treatment enabled stratifying patients and predict omalizumab responders at (relatively, 12 months); also, confirmed stable up to 36 months.[14,15]

The FEV_1 increase, after a 12 month omalizumab treatment, in Group 1 is quite remarkable as absolute value (0.7 L) and percentage (41.4%): such an improvement would be highly appreciated in clinical practice. We are aware that this improvement is not statistically significant, but we can possibly ascribe this result to the low number of patients. In Group 1 at T0, a significantly high value of GAL-3 BP level is also evident and thus substantiating the relation already shown between high GAL-3 serum levels and FEV_1 improvement.

The result of proteomics analysis is consistent with plasma analysis data, confirming the role of GAL-3 can be considered as a reliable marker for improvement in FEV_1.

Limitations

Limitations of the study include the single-arm, observational study design that requires results to be interpreted with due consideration that factors other than the treatment of interest may influence the findings; in addition to low number of control samples and lack of a comparator arm.

CONCLUSIONS

Our data support the previous studies indicating the role of plasma GAL-3 levels at baseline as a useful biomarker for identifying and predicting super responders, and functional responders (improvement in FEV_1) for omalizumab treatment among patients with severe persistent allergic asthma.

Abbreviations

Asthma Control Questionnaire: ACQ total score; CI: confidence interval; forced expiratory volume in 1 s: FEV_1; GAL-3: Plasma galectin-3; GAL-3 BP: GAL-3 binding protein; IgE: immunoglobulin E; SAA: severe allergic asthmas

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Consent for publication

Not applicable.

Ethics approval

The study was conducted according to the ethical principles of the Declaration of Helsinki. All patients provided informed consent before participating in the study.

Author contributions

GWC, AMR and PLM substantially contributed to conception and design of the study, analysis and interpretation of the data. MB and FS participated in the study design, analysis plan and interpretation of data. LDF, RR and DDS conducted experiments and data analysis. GWC and all the Proxima sub-study centers participated in the data collection process. All authors have read, revised the article critically for important intellectual content, and approved the final manuscript.

Availability of data and materials

Raw data of PROXIMA study are not available for public disclosure.

Declaration of competing interest

AMR, PLM, LDF, RR, DDS, GWC have no competing interests. FS and MB are employees of Novartis.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2019.100095.

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REFERENCES

1. *Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA)*; 2018. Available from: http://ginasthma.org.
2. *The Global Asthma Report 2018*. Auckland, N.Z.: Global Asthma Network; 2018, 2018.
3. de Marco R, Cappa V, Accordini S, et al. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. *Eur Respir J*. 2012;39(4):883–892.
4. Humbert M, Beasley R, Ayres J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy*. 2005;60(3): 309–316.
5. Ayres JG, Higgins B, Chilvers ER, Ayre G, Blogg M, Fox H. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with poorly controlled (moderate-to-severe) allergic asthma. *Allergy*. 2004;59(7):701–708.
6. Vignola AM, Humbert M, Bousquet J, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with concomitant allergic asthma and persistent allergic rhinitis: SOLAR. *Allergy*. 2004;59(7):709–717.
7. Buhl R, Soler M, Matz J, et al. Omalizumab provides long-term control in patients with moderate-to-severe allergic asthma. *Eur Respir J*. 2002;20(1):73–78.
8. Lanier BQ, Corren J, Lumry W, Liu J, Fowler-Taylor A, Gupta N. Omalizumab is effective in the long-term control of severe allergic asthma. *Ann Allergy Asthma Immunol*. 2003;91(2):154–159.
9. Bousquet J, Cabrera P, Berkman N, et al. The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe persistent asthma. *Allergy*. 2005;60(3):302–308.
10. Paone G, Leone V, Conti V, et al. Blood and sputum biomarkers in COPD and asthma: a review. *Eur Rev Med Pharmacol Sci*. 2016;20(4):698–708.
11. Bhakta NR, Woodruff PG. Human asthma phenotypes: from the clinic, to cytokines, and back again. *Immunol Rev*. 2011;242(1): 220–232.
12. Kallieri M, Papaioannou AI, Papathanasiou E, Ntontsi P, Papiris S, Loukides S. Predictors of response to therapy with omalizumab in patients with severe allergic asthma - a real life study. *PGM (Postgrad Med)*. 2017;129(6):598–604.
13. Bousquet J, Rabe K, Humbert M, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med*. 2007;101(7):1483–1492.
14. Gao P, Gibson PG, Baines KJ, et al. Anti-inflammatory deficiencies in neutrophilic asthma: reduced galectin-3 and IL-1RA/IL-1beta. *Respir Res*. 2015;16:5.
15. Anna Maria Riccio PM, De Ferrari Laura, Rossi Rossana, et al. Galectin-3: an early predictive biomarker of modulation of airway remodeling in patients with severe asthma treated with omalizumab for 36 months. *Clin Transl Allergy*. 2017;7(6).
16. Canonica GW, Rottoli P, Bucca C, et al. Improvement of patient-reported outcomes in severe allergic asthma by omalizumab treatment: the real life observational PROXIMA study. *World Allergy Organ J*. 2018;11(1):33.
17. Bousquet J, Siergiejko Z, Swiebocka E, et al. Persistency of response to omalizumab therapy in severe allergic (IgE-mediated) asthma. *Allergy*. 2011;66(5):671–678.
18. Canonica GW, Bartezaghi M, Marino R, Rigoni L. Prevalence of perennial severe allergic asthma in Italy and effectiveness of omalizumab in its management: PROXIMA - an observational, 2 phase, patient reported outcomes study. *Clin Mol Allergy*. 2015;13(1):10.
19. Busse WW, Stephenson MHP, et al. The effect of omalizumab on lung function in adolescents with moderate-to-severe allergic asthma. *Am J Respir Crit Care Med*. 2017;195:A5105.
20. Alfarroba S, Videira W, Galvao-Lucas C, Carvalho F, Barbara C. Clinical experience with omalizumab in a Portuguese severe asthma unit. *Rev Port Pneumol*. 2014;20(2):78–83.
21. Hoshino M. Effects of add-on omalizumab therapy on airway wall thickening in severe persistent asthma. *Eur Respir J*. 2011;38:267.
22. Anna Maria Riccio RWDN, De Ferrari Laura, Micheletto Claudio, Canonica Giorgio Walter, Folli Chiara, Chiappori Alessandra. Anti-IgE and remodeling: Omalizumab effects on reticular basement membrane thickness in severe asthma. *Eur Respir J*. 2011;38:3848.
23. Mauri P, Riccio AM, Rossi R, et al. Proteomics of bronchial biopsies: galectin-3 as a predictive biomarker of airway remodelling modulation in omalizumab-treated severe asthma patients. *Immunol Lett*. 2014;162(1 Pt A):2–10.
24. Hoshino M, Ohtawa J. Effects of adding omalizumab, an anti-immunoglobulin E antibody, on airway wall thickening in asthma. *Respiration*. 2012;83(6):520–528.
25. Michael Roth JZ, Chong Teck S'ng, Tamm Michael. IgE-induced pro-inflammatory extracellular matrix composition in airway smooth muscle cells is prevented by omalizumab. *Eur Respir J*. 2012;40:4832.
26. Moretta L, Pistoia V. Anti-IgE treatment in asthma: galectin-3 as a predictive marker. *Immunol Lett*. 2014;162(1 Pt A):1.
27. Samitas K, Delimpoura V, Zervas E, Gaga M. Anti-IgE treatment, airway inflammation and remodelling in severe allergic asthma: current knowledge and future perspectives. *Eur Respir Rev*. 2015;24(138):594–601.
28. Chiappori A, De Ferrari L, Folli C, Mauri P, Riccio AM, Canonica GW. Biomarkers and severe asthma: a critical appraisal. *Clin Mol Allergy*. 2015;13:20.