Running head: Mepolizumab for EGPA

Title: Mepolizumab for Eosinophilic Granulomatosis with Polyangiitis (EGPA): a European multicenter observational study

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Abstract

Objective: Mepolizumab proved efficacious for eosinophilic granulomatosis with polyangiitis (EGPA, former Churg-Strauss) at the dosage of 300mg/4 weeks in the randomized controlled MIRRA trial. Few successful real-life experiences with the dosage approved for severe eosinophilic asthma (100mg/4 weeks) were recently reported. We retrospectively assessed the effectiveness and safety of mepolizumab 100 and 300mg/4 weeks in a large European EGPA cohort.

Methods: We included all EGPA patients treated with mepolizumab at the recruiting centres in 2015-2020. Treatment response was evaluated from month 3 through 24 (T3-T24) after mepolizumab starting. Complete response (CR) was defined as no disease activity (Birmingham Vasculitis Activity Score, BVAS=0) and a prednisone dose ≤4mg/day. Respiratory outcomes included asthma and ear-nose-throat (ENT) exacerbations.

Results: We included 203 patients, of whom 191 at stable dosage (158 mepolizumab 100mg/4 weeks, 33 300mg/4 weeks). At T3, 25 patients (12.3%) had a CR. CR rates increased to 30.4% and 35.7% at T12 and T24 and were comparable between mepolizumab 100 and 300mg/4 weeks. Mepolizumab led to a significant reduction in BVAS, prednisone dose, eosinophil counts from T3 through T24, with no significant differences between 100 and 300 mg/4weeks. Eighty-two patients (40.4%) experienced asthma exacerbations [57/158 (36%) on 100mg/4 weeks; 17/33 (52%) on 300mg/4 weeks]. Thirty-one (15.3%) experienced ENT exacerbations. Forty-four patients (21.7%) experienced adverse events, most being non-serious (38/44).

Conclusion: Mepolizumab both at 100 and 300mg/4 weeks is effective for EGPA. The two dosages should be compared in the setting of a controlled trial.

Keywords: ANCA-associated Vasculitis; Biologicals; Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss); Epidemiology; Glucocorticoids

INTRODUCTION

Eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg–Strauss syndrome) is an anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) characterized by asthma, ear-nose-throat (ENT) involvement, blood and tissue eosinophilia and systemic vasculitic manifestations. (1,2) The treatment mainly relies on systemic glucocorticoids and inhaled

therapies for respiratory symptoms (3). Its course is usually chronic-relapsing, thus patients are at risk for permanent tissue or organ damage, also due to glucocorticoid-related toxicity; thus, immunosuppressive treatments are often required, also to spare glucocorticoids. (3,4)
Among novel therapeutic options, mepolizumab is a monoclonal antibody targeting interleukin (IL)-5, a cytokine involved in eosinophil maturation, differentiation and survival. Increased serum levels of IL-5 are observed in eosinophilic disorders, including EGPA (5), and a genome-wide association study identified the *IL5* region as one of the main EGPA-associated loci. (6)
Mepolizumab is approved at the dosage of 100mg/4 weeks subcutaneously for the treatment of severe eosinophilic asthma (7), and at 300mg/4 weeks for hypereosinophilic syndrome (HES) (8). After encouraging studies, (9,10) the phase 3 MIRRA trial proved the efficacy of mepolizumab 300mg/4 weeks subcutaneously for relapsing or refractory EGPA. (11,12), leading to its approval by the Food and Drug Administration (FDA), while in Europe it is currently used off-label.
Recently, small studies reported the successful use of mepolizumab 100mg/4 weeks for EGPA, especially for the control of respiratory manifestations. (13–15) However, the benefits and side-effects of mepolizumab 100 vs. 300mg/4 weeks for systemic and respiratory EGPA involvement have never been compared, thus its optimal dosage is still debated. (16) This study aimed to investigate the effectiveness and safety of mepolizumab 100 vs 300mg/4 weeks in a large European cohort of patients with EGPA.

MATERIALS AND METHODS

Study design and setting

This multicenter, retrospective study was conducted on a cohort of EGPA patients treated with mepolizumab between May 2015 and February 2020 at 38 EGPA referral centres from eight European countries (Italy, France, Germany, United Kingdom, Russia, Spain, Switzerland, Sweden). The study received ethical approval (University of Florence Ethics Committee; ref.16821_OSS).

Study population and treatment

The cohort included adult patients meeting the American College of Rheumatology classification criteria for EGPA (17) or the criteria proposed in the MIRRA trial, (11) who received mepolizumab 100mg/4 weeks or 300mg/4 weeks, according to local practice. Patients with a

follow-up of less than three months after the first mepolizumab dose or those enrolled in clinical trials were excluded.

Data collection and outcome assessment

Demographic, clinical, biological and treatment-related data were retrospectively collected from medical charts at the time of mepolizumab starting (T0) and at 3, 6, 12 and 24 months of follow-up (T3-T24). The effectiveness of mepolizumab in controlling systemic disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS). (18) Complete response (CR) was defined as no disease activity (BVAS=0) and a prednisolone or prednisone dose (or equivalent) ≤4.0 mg/day, as defined by the MIRRA trial. (11) Partial response (PR) was defined as no disease activity and a prednisolone or prednisone dose >4.0 mg/day.

Relapse was assessed only for patients who had achieved a CR and was defined, as in the MIRRA trial, by at least one the following criteria: a) active vasculitis (defined as BVAS>0) and/or b) worsening asthma and/or ENT manifestations leading to an increase in the prednisolone or prednisone dose to more than 4.0 mg/day, an initiation of a new immunosuppressive therapy, or hospitalization. (11)

As for respiratory outcomes, we assessed asthma exacerbations, defined as any of the following events: asthma attack needing an increase in oral prednisone dose, emergency department admission related to asthma, and/or use of acute oral glucocorticoids, antibiotics, or short-acting beta-agonists (SABA).

In addition, the effect of mepolizumab on lung function was monitored by the variation in pre-bronchodilator forced expiratory volume in 1 second (FEV1). ENT relapse was defined as the reappearance of ENT symptoms, following their complete control at the previous timepoint. Additional outcomes included changes in organ manifestations (assessed separately from BVAS items), the glucocorticoid- and DMARD-sparing effect, the variation in the proportion of ANCA-positive patients, and the reduction in the eosinophil count.

During the follow-up, variations in mepolizumab monthly dosage or treatment discontinuation were recorded. All adverse events (AEs) occurring during treatment were also recorded and their seriousness was assessed according to the World Health Organization criteria. (19) All study outcomes were analysed in the whole cohort and compared between patients on stable treatment with mepolizumab 100mg/4 weeks vs. 300mg/4 weeks. Stable treatment was defined as no change in mepolizumab monthly dosage during the whole follow-up.

Statistical analysis

Data are presented as median and interquartile range (IQR) for continuous variables, and as absolute number and percentage for qualitative variables. Continuous endpoints were compared between T3-T24 and T0 using the Wilcoxon signed-rank test, whereas qualitative variables using the McNemar test. Non-parametric tests were used as the distribution of the data was not normal. CR and PR rates and AEs rates were compared using the Fisher exact test between patients on stable treatment with mepolizumab 100 and those on 300mg/4 weeks. Cox regression models were fitted to derive Kaplan-Meier curves and to estimate hazard ratios (HR) and their 95% confidence intervals (CIs) for the occurrence of asthma and ENT exacerbations over time.

If a patient was still on mepolizumab treatment at a given follow-up timepoint, but had missing data regarding EGPA manifestations, BVAS and/or glucocorticoid daily dosage, the data were imputed using the method of the last observation carried forward, as these parameters were necessary to assess the primary outcome of this study. For all other clinical and laboratory parameters, the analyses were conducted only on subjects with available data at the given timepoint.

Statistical analyses were performed using the software Stata, version 14. P-values <0.05 were considered statistically significant.

Patient and public involvement: Patients were not involved in this study.

RESULTS

We included 203 patients (**table 1**), of whom 57.1% were female. Their median age at mepolizumab beginning was 55.1 years (46.7–62.5) and their median disease duration 4.8 years (4.9-9.2). At diagnosis, 70 patients (34.5%) tested ANCA-positive, most of them showing either P-ANCA or MPO-ANCA (84.3%). Before mepolizumab beginning, 150/203 patients (73.9%) had received traditional DMARDs, 51 (25.1%) biologic DMARDs and 18 (9.0%) intravenous immunoglobulins; 120 patients had achieved disease remission according to clinical judgement after induction therapy. At the time of mepolizumab starting (T0), 92.1% of the patients had active disease, the median BVAS being 4 (2-8). The most common manifestations were pulmonary (89.7%), ENT (71.4%), constitutional (27.6%) and peripheral neurological (22.7%). Ten patients had cardiac involvement at T0, including one case of pericarditis, one of myocarditis, and eight of cardiomyopathy with cardiac failure. Out of 190 patients with available ANCA tests, 38 (20.0%)

were ANCA-positive at the time if mepolizumab beginning, most showing P-ANCA/MPO-ANCA (89.5%). At T0, almost all patients (95.6%) had been on stable glucocorticoid treatment in the previous three months, at a median prednisone dose of 10 mg/day (5-20). Additional therapies included conventional DMARDs, mostly methotrexate (18.7%), azathioprine (11.3%), rituximab (11.3%) or intravenous immunoglobulins (5.9%). Ninety-five percent of the patients (n=192) were taking inhaled therapy for asthma.

One hundred and sixty-eight patients initially started mepolizumab at the dosage of 100mg/4 weeks and 35 at the dosage of 300mg/4 weeks. During the follow-up, 10 switched from 100 to 300mg/4 weeks, due to inefficacy. Another two patients switched from 300 to 100mg/4 weeks, due to personal reasons (**supplementary figure 1**). Conversely, 158 (77.8%) and 33 (16.3%) patients maintained over the entire follow-up a stable treatment with mepolizumab 100 and 300 mg/4 weeks, respectively.

Baseline demographic and clinical characteristics were comparable between these two groups, except for constitutional and ENT manifestations, which were more frequent among patients receiving mepolizumab 100 mg than among those receiving 300 mg/4 weeks (31.7% vs 9.1%, p=0.009; 76.6% vs 51.5%, p=0.005, respectively) (**table 1**).

Mepolizumab effectiveness on systemic disease activity

At T3, 25/203 patients (12.3%) had already achieved a CR, while 64 (31.5%) had a PR (**supplementary table 1**). CR rates increased to 23.6% at T6, 30.4% at T12, and 35.7% at T24. Response rates were similar between patients on mepolizumab 100 and 300mg/4 weeks (**figure 1**). In particular, 12.0% and 18.2% of patients on 100 and 300 mg/4 weeks achieved CR at T3, respectively, while 32.9% and 36.4% of patients achieved PR (p= 0.474). CR rates further increased during follow-up for both treatments (p=0.204 and p=0.809 for mepolizumab 100 vs 300mg/4 weeks at T6 and T12, respectively). At T24, only 39 and 12 patients on mepolizumab 100 and 300mg/4 weeks had available follow-up data; a greater proportion of patients on mepolizumab 300mg/4 weeks had CR (58.3% vs 33.3%) or PR (33.3% vs 30.8%), but this difference was not statistically significant (p=0.168). Of note, the small numbers of patients, particularly on mepolizumab 300mg/4 weeks, at the different follow-up timepoints did not allow to achieve a sufficient power to detect statistical significant differences in the proportion of CR between the two dosages at the different timepoints (**supplementary table 2**).

Twenty-two of the 71 patients who had achieved CR (31.0%) relapsed after a median time of 6 (6-9) months from CR. At all time-points, relapse rates were comparable between mepolizumab 100 and 300mg/4 weeks (p=1.000 at T6 and T12; p=0.642 at T24), the overall relapse rates being 32.1% (17/53) and 25.0% (4/16) for mepolizumab 100 vs 300mg/4 weeks, respectively. The median time to relapse was 6 (3-9) and 10 (9-12) months in the mepolizumab 100 vs 300mg/4 weeks groups, respectively (p=0.081). Response rates were higher among ANCA-negative patients, especially at T24, but the differences were not statistically significant (**supplementary table 3**).

The efficacy outcomes for the 10 patients who switched from mepolizumab 100 to 300mg/4 weeks are summarized in the **supplementary figure 2**. Follow-up data suggested no clear benefit in terms of EGPA control following the increase in the monthly dosage.

The impact of mepolizumab on the different disease manifestations is summarized in **table 2** and in the **supplementary table 4.** A significant reduction in all active manifestations was observed in patients on stable mepolizumab 100mg/4 weeks already at T3. The control of constitutional, pulmonary, ENT, and peripheral neurological manifestations was maintained during the follow-up. For mepolizumab 300mg/4 weeks, a significant reduction in the proportion of patients with pulmonary and ENT manifestations was observed at all time-points, whereas no clear effect was observed on non-respiratory manifestations.

Systemic disease activity also decreased during the follow-up, both for mepolizumab 100 and 300mg/4 weeks, with the median BVAS of the whole cohort decreasing from 4 (IQR 2-8) at T0 to 2 (IQR 0-4) at T3 (p<0.001), and further to a median of 0 at the subsequent timepoints (p<0.001 for both regimens at T6, T12 and T24) (**figure 2a**). Similarly, both mepolizumab dosages were associated with a significant reduction in the daily glucocorticoid dose (**figure 2b**), with a significant proportion of patients who were able to discontinue glucocorticoids (at T24 29.2% and 41.7% respectively) (**supplementary table 5**). Concomitantly, a DMARD-sparing effect was observed in both treatment regimens, although statistical significance was only achieved for mepolizumab 100mg/4 weeks (**supplementary table 5**).

Mepolizumab effectiveness on respiratory outcomes

Respiratory outcomes are reported in **figure 2c-f** and in the **supplementary table 6**. Overall, 82 patients (40.4%) experienced asthma exacerbations after a median time of 12 (12-24) months: asthma exacerbations occurred in 36.1% of patients on stable mepolizumab 100mg/4 weeks, and

in 51.5% on 300mg/4 weeks (p=0.139) (**figure 2c**). ENT relapses occurred after a median of 12 (6-12) months in twenty-five patients on mepolizumab 100mg/4 weeks (15.8%), four on 300mg/4 weeks (12.2%) and two who switched mepolizumab dosage [unadjusted HR 0.67 (0.23-1.91) for mepolizumab 300mg/4 weeks as compared to 100mg/4 weeks, p=0.450] (**figure 2d**).
As for lung function, a significant improvement in FEV1 was already observed three months after the initiation of mepolizumab 100mg/4 weeks (**figure 2e**). FEV1 also improved in patients receiving mepolizumab 300mg/4 weeks, although the statistical significance was not reached.

Additional outcomes

Both mepolizumab regimens were associated with a dramatic reduction in the eosinophil count, already at T3, which was maintained during the whole follow-up (**figure 2f**).
Although ANCA testing was available for a small subgroup of patients during the follow-up, a significant reduction in the proportion of ANCA-positive patients was observed (**supplementary figure 3a**), both for patients on stable mepolizumab 100mg/4 weeks (**supplementary figure 3b**) and 300mg/4 weeks (**supplementary figure 3c**).

Treatment persistence and safety

Twenty-three patients discontinued mepolizumab: 16 discontinued mepolizumab 100mg/4 weeks due to AEs in six cases (two due to malaise, one arthralgias, one reactivation of Herpes Zoster, two not reported) and inefficacy in three; in the remaining seven patients, the reason for treatment discontinuation was unknown. Seven patients discontinued mepolizumab 300mg/4 weeks, due to inefficacy in four and unknown reasons in three (**figure 2**).
Forty-four patients (21.7%) experienced AEs, mostly related to lower respiratory tract infections or to myalgias or arthralgias. At all time-points, AEs were more frequent among patients receiving mepolizumab 300mg/4 weeks (**table 3**). Overall, six AEs required hospitalization: four occurred on mepolizumab 100mg/4 weeks and included lower respiratory tract infection, secondary adrenal insufficiency, transient ischemic attack and infection of the central venous catheter. The other two occurred in patients on mepolizumab 300mg/4 weeks and consisted of lower respiratory tract infection and myocarditis.

DISCUSSION

In this study, conducted on the largest series of mepolizumab-treated EGPA patients reported so far, we observed that mepolizumab at either 100mg/4 weeks or 300mg/4 weeks is effective and safe for the control of the systemic and respiratory disease manifestations.

The use of mepolizumab in EGPA has solid evidence. Indeed, the randomized controlled MIRRA trial proved the superiority of mepolizumab 300mg/4 weeks to placebo for relapsing and/or refractory EGPA, (11,12) leading to the FDA approval of mepolizumab 300mg/4 weeks for EGPA.

Despite this, our data show that, in the real practice, most EGPA patients received mepolizumab 100mg/4 weeks, the dosage licensed for severe eosinophilic asthma, rather than 300mg/4 weeks. This prescription was probably based on the rationale that mepolizumab 100mg/4 weeks effectively controls severe eosinophilic asthma, which is an invariable feature of EGPA, and was also driven by regulatory reasons, as mepolizumab 300mg/4 weeks is not currently approved in Europe.

In the MIRRA trial, the dosage choice was based on the phase 2b/3 dose range–finding study on mepolizumab in severe eosinophilic asthma, (7) and on a trial in HES. (20,21) This choice was also supported by the concept that EGPA, similarly to HES, is a more aggressive condition as compared to eosinophilic asthma (14). After the FDA approval of mepolizumab 300mg/4 weeks for EGPA, a growing body of literature from the real clinical practice suggested that mepolizumab 100mg/4 weeks might also be used for EGPA.[13–15, 22] Notably, all patients included in these studies were in remission (13,15) or had low disease activity (14) at treatment beginning, mepolizumab being started mainly for the control of asthma.

Our results indicate that both mepolizumab 100 and 300mg/4 weeks were associated with an effective control of respiratory EGPA manifestations and an improvement in systemic disease activity, and also allowed glucocorticoid sparing.

Unexpectedly, also the proportion of ANCA-positive patients significantly decreased; nevertheless, given the small number of patients with ANCA (re)testing, this finding should be taken with caution. Though the exact mechanisms of ANCA negativization are unknown, this may be accounted for by anti-IL5-mediated eosinophil depletion. Indeed, eosinophils have been shown to promote B cell survival, T-independent and T-dependent B cell activation, proliferation, and immunoglobulin secretion (23). B cells and their progeny produce and release ANCA; thus, eosinophil depletion following mepolizumab treatment may account for the reduction in antigen-presentation and plasma cell survival, with a consequent reduction in ANCA titres.

The proportion of CR steadily increased throughout follow-up, reaching 31.2% and 37.9% at 12 months and 33.3% and 58.3% at 24 months for mepolizumab 100 and 300mg/4 weeks, respectively, with only a minor proportion of patients experiencing disease relapse. However, response rates at 24 months must be taken with caution, as only 39 and 12 patients on mepolizumab 100 and 300mg/4 weeks had available follow-up data.

Notably, CR rates observed for both dosages were similar to that reported in the MIRRA trial for mepolizumab 300mg/4 weeks, where 32% of patients achieved CR at both weeks 36 and 48. (11) The response rates of our study were lower to that reported in the observational study by Canzian *et al*. (14) in a small EGPA cohort (76% and 82% of CR at T12 on mepolizumab 100 and 300mg/4 weeks, respectively, as defined by a BVAS=0 and a prednisone dose ≤5 mg/day). (14)

Of note, in our study CR rates seemed higher among ANCA-negative patients, although the subgroups were too small to draw conclusions. We speculate that these findings reflect the different nature of ANCA-positive and ANCA-negative EGPA, the latter being traditionally associated with a more prominent eosinophilic phenotype. (24–26)

The control of systemic disease activity was paralleled by the improvement in asthma and lung function, in both mepolizumab regimens. Interestingly, the lower mepolizumab dosage was not associated with an increased risk of asthma re-exacerbation during the follow-up. Additionally, both mepolizumab dosages were associated with a good control of ENT manifestations, according to recent data. (27) Moreover, we also observed a remarkable reduction in peripheral neuropathy during treatment with mepolizumab. In EGPA, neuropathy seems to have not only a vasculitic but also a neurotoxic aetiology, mainly due to eosinophil products. (28,29) Thus, eosinophil depletion via mepolizumab could effectively counteract this pathogenetic mechanism. To date, the possible role of mepolizumab for the control of EGPA neurological manifestations was reported only in a retrospective study on six patients. (30) Our results, however, must be taken with caution, as other factors may contribute to the improvement of neuropathy, including progressive nerve function recovery or delayed effects of previous and concomitant therapies.

In our study, mepolizumab was generally well tolerated. Around one fifth of patients reported AEs, and the 100mg/4 weeks dosage appeared associated with a lower rate of AEs. Most AEs were related to infections or to myalgias/arthralgias, as observed in the MIRRA trial. (11) Only few AEs required treatment discontinuation or hospitalization. However, as in all retrospective studies, underreporting of AEs cannot be excluded.

Our study has other limitations, mostly related to its retrospective nature. First, as data were retrospectively captured from medical charts, missing data occurred, and the assessment of clinical parameters was not systematic. Second, the heterogeneity in clinical management among centers cannot be excluded. Third, in line with the MIRRA trial, the BVAS calculation was used to retrospectively assess disease activity and treatment outcomes, as no standard assessment tool is validated specifically for EGPA. Nevertheless, it cannot be excluded that items related to chronic or persistent damage were erroneously counted in the BVAS. Fourth, the disparity in sample size between the 100 and 300mg/4 weeks groups did not allow us to draw definite conclusions. Finally, given the small sample size, the effect of mepolizumab dose escalation in patients with inappropriate response to 100mg/4 weeks could not be ascertained. Despite these limitations, our study finds its strengths in the long follow-up, the large sample size representative of the European clinical setting, and the availability of detailed longitudinal clinical data.

In conclusion, this large European real-world study shows that mepolizumab is associated with an effective control of respiratory EGPA manifestations, with a good safety profile. Our results further suggest a role of mepolizumab also for systemic manifestations, though the retrospective assessment of systemic disease activity requires cautious interpretation of these findings.

Our data also suggest that mepolizumab 100mg/4 weeks could be an acceptable dosage for EGPA patients, and a valid alternative to the dosage licensed for this therapeutic indication (300mg/4 weeks). Nevertheless, caution is needed as some reports suggest a risk of systemic disease flare in patients on anti-IL5 treatments used at the dose for asthma control. (31,32) Randomized clinical trials are advocated to compare the efficacy and safety of these two treatment regimens for EGPA, to assess whether dose escalation from 100 to 300mg/4 weeks can be effective in case of unsatisfactory clinical responses, as well as to compare the efficacy of mepolizumab as an alternative or sequential treatment to other biological therapies for EGPA.

DATA AVAILABILITY STATEMENT

Deidentified individual participant data will be made available upon reasonable request to the corresponding author.

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FIGURE LEGENDS

Figure 1. Complete and partial responses during stable treatment with mepolizumab a) 100mg/4 weeks or b) 300mg/4 weeks.

Complete response (CR) was defined as no disease activity (BVAS=0) and daily prednisone dose ≤4mg/day. Partial response was defined as no disease activity (BVAS=0) and daily prednisone dose>4mg/day. No response was defined as active disease (BVAS>0).

Figure 2. a-b) Variation in a) disease activity and b) daily dose of prednisone equivalents during mepolizumab treatment; c-f) Respiratory outcomes during mepolizumab treatment:

c) Kaplan-Meier curves for the occurrence of asthma exacerbations and d) of ENT exacerbations, e) variations in the FEV1, expressed as percentage of the predicted value, and f) variations in eosinophil count.

BVAS: Birmingham Vasculitis Activity Score; ENT: ear-nose-throat; FEV1: Forced Expiratory Volume in the first second

**p<0.05 as compared to baseline; **p<0.01 as compared to baseline*

Table 1. Baseline characteristics at the time of mepolizumab beginning

	Overall	On stable Mepolizumab 100mg/4 weeks	On stable Mepolizumab 300mg/4 weeks	p-value
	n=203	n=158	n=33	
Gender				
Female	116 (57.1)	88 (55.7)	22 (66.7)	0.333
Smoking				
Former	44 (21.7)	36 (22.8)	5 (15.2)	0.640
Current	3 (1.5)	3 (1.9)	0	
Median age at diagnosis, years	49.1 (IQR 37.7-57.1)	48.7 (IQR 37.9-57.5)	49.2 (IQR 39.8-53.4)	0.380
Median age at mepolizumab beginning, years	55.1 (IQR 46.7 – 62.5)	55.1 (IQR 46.7 – 62.8)	53.0 (IQR 47.3 – 59.3)	0.426
Median disease duration at mepolizumab beginning, years	4.8 (IQR 4.9-9.2)	4.9 (IQR 1.6-8.9)	3.9 (IQR 1.1-14.1)	0.921
Patients with active organ involvement at mepolizumab beginning:				
Systemic manifestations	56 (27.6)	50 (31.7)	3 (9.1)	0.009
Purpura	15 (7.4)	11 (7.0)	2 (6.1)	1.000
ENT	145 (71.4)	121 (76.6)	17 (51.5)	0.005
Pulmonary	182 (89.7)	141 (89.2)	29 (87.9)	0.765
Cardiac	10 (4.9)	8 (5.1)	1 (3.0)	1.000
Gastrointestinal	9 (4.4)	8 (5.1)	1 (3.0)	1.000
Renal	5 (2.5)	5 (3.2)	0	n.a.
Peripheral neuropathy	46 (22.7)	36 (22.8)	6 (18.2)	0.650
Active disease at	187 (92.1)	144 (91.1)	31 (93.9)	0.792

mepolizumab beginning (BVAS>0)				
Median BVAS at mepolizumab beginning	4 (IQR 2-8)	4 (IQR 2-8)	4 (IQR 2-7)	0.163
Laboratory parameters at mepolizumab beginning	N obs 190	N obs 148	N obs 33	
ANCA positivity	38 (20.0)	28 (18.9)	9 (27.3)	0.339
p-ANCA	34 (17.9)	26 (17.6)	8 (24.2)	
c-ANCA	4 (2.1)	2 (1.4)	1 (3.0)	
Anti-MPO	34 (17.9)	27 (18.2)	8 (24.2)	
Anti-PR3	4 (2.1)	2 (1.4)	1 (3.0)	
Eosinophil count	610 (IQR 200-1040) [n obs 194]	700 (IQR 200-1080) [n obs 152]	440 (IQR 200-910) [n obs 32]	0.328
Pharmacological therapies administered before mepolizumab beginning				
Oral corticosteroids	201 (99.0)	156 (98.7)	33 (100.0)	n.a.
Azathioprine	91 (44.8)	69 (43.7)	17 (51.5)	0.446
Methotrexate	78 (38.4)	56 (35.4)	18 (54.6)	0.050
Cyclophosphamide	57 (28.1)	44 (27.9)	11 (33.3)	0.531
Mycophenolate	39 (19.2)	29 (18.4)	6 (18.2)	1.000
Cyclosporine	21 (10.3)	18 (11.4)	1 (3.0)	0.206
Rituximab	39 (19.2)	36 (22.8)	3 (9.1)	0.097
IvIg	18 (8.9)	17 (10.8)	1 (3.0)	0.321
Omalizumab	17 (8.4)	13 (8.2)	2 (6.1)	1.000
Other immunosuppressants	16 (7.9)	13 (8.2)	1 (3.0)	0.471
Pharmacological therapies at time of mepolizumab beginning				
Median prednisone	10 (IQR 5-20) [n	10 (IQR 5-20) [n	10 (IQR 5-22.5)	0.854

equivalent daily dose in the previous 3 months	obs 195]	obs 151]	[n obs 32]	
Oral corticosteroids	194 (95.6)	149 (94.3)	33 (100.0)	n.a.
Median prednisone equivalent daily dose	10 (IQR 5-20)	10 (IQR 5-20)	10 (IQR 5-25)	0.511
Methotrexate	38 (18.7)	29 (18.4)	9 (27.3)	0.240
Azathioprine	23 (11.3)	19 (12.0)	3 (9.1)	0.772
Mycophenolate	18 (8.9)	12 (7.6)	4 (12.1)	0.486
Cyclosporine	2 (1.0)	1 (0.6)	0	n.a.
Rituximab	23 (11.3)	20 (12.7)	3 (9.1)	0.771
IvIg	12 (5.9)	11 (7.0)	1 (3.0)	0.695
Other immunosuppressants	5 (2.5)	3 (1.9)	1 (3.0)	0.535
Inhaled therapy for asthma	192 (95.0)	150 (94.9)	30 (90.9)	0.407

ANCA: Anti-neutrophil cytoplasmic antibodies; c-ANCA: cytoplasmic ANCA; p-ANCA: perinuclear ANCA; anti-MPO: anti-myeloperoxidase ANCA; anti-PR3: anti-proteinase 3 ANCA; BVAS: Birmingham Vasculitis Activity Score; ENT: ear nose throat; IQR: interquartile range; IvIg: intravenous immunoglobulin; LABA: long-acting beta-2 adrenergic receptor agonists; n.a.: not assessable

Table 2. Organ involvement among patients on stable treatment with mepolizumab 100 or 300mg/4 weeks

	Mepolizumab dosage	MEPO beginning (t0)	3 months	p-value (t3 vs t0)	6 months	p-value (t6 vs t0)	12 months	p-value (t12 vs t0)	24 months	p-value (t24 vs t0)
N patients	***100mg/4 weeks***	*158*	*158*		*151*		*122*		*39*	
	300mg/4 weeks	*33*	*33*		*32*		*29*		*12*	
Constitutional	**100mg/4 weeks**	50 (31.7)	25 (15.8)	<0.001	23 (15.2)	<0.001	15 (12.3)	<0.001	6 (15.4)	0.035
	300mg/4 weeks	3 (9.1)	0	n.a.	2 (6.3)	0.564	2 (6.9)	1.564	0	n.a.
Purpura	**100mg/4 weeks**	11 (7.0)	6 (3.8)	0.025	4 (2.7)	0.014	3 (2.5)	0.008	0	n.a.
	300mg/4 weeks	2 (6.1)	1 (3.0)	0.317	1 (3.1)	0.317	2 (6.9)	1.000	0	n.a.
Ear nose throat	**100mg/4 weeks**	121 (76.6)	64 (40.5)	<0.001	55 (36.4)	<0.001	34 (27.9)	<0.001	8 (20.5)	<0.001
	300mg/4 weeks	17 (51.5)	12 (36.4)	0.025	7 (21.9)	0.003	8 (27.6)	0.034	0	n.a.
Pulmonary	**100mg/4 weeks**	141 (89.2)	61 (38.6)	<0.001	46 (30.5)	<0.001	37 (30.3)	<0.001	7 (18.0)	<0.001
	300mg/4 weeks	29 (87.9)	10	<0.00	5	<0.001	9 (31.0)	<0.001	1 (8.3)	0.005

			(30.3)	1	(15.6)					
Cardiac	**100mg/4 weeks**	8 (5.1)	4 (2.5)	0.046	4 (2.7)	0.046	3 (2.5)	0.046	1 (2.6)	0.317
	300mg/4 weeks	1 (3.0)	0	n.a.	0	n.a.	0	n.a.	0	n.a.
Gastrointestinal	**100mg/4 weeks**	8 (5.1)	0	0.005	5 (3.3)	0.257	4 (3.3)	0.257	0	0.083
	300mg/4 weeks	1 (3.0)	1 (3.0)	n.a.	0	n.a.	0	n.a.	0	n.a.
Renal	**100mg/4 weeks**	5 (3.2)	1 (0.6)	0.046	0	n.a.	1 (0.8)	0.180	0	0.317
	300mg/4 weeks	0	2 (6.1)	0.157	0	n.a.	1 (3.5)	0.317	0	n.a.
Peripheral neurological	**100mg/4 weeks**	36 (22.8)	23 (14.6)	0.005	21 (13.9)	0.001	15 (12.3)	0.001	2 (5.1)	0.005
	300mg/4 weeks	6 (18.2)	6 (18.2)	n.a.	3 (9.4)	0.157	2 (6.9)	0.157	0	n.a.

n.a.: not assessable

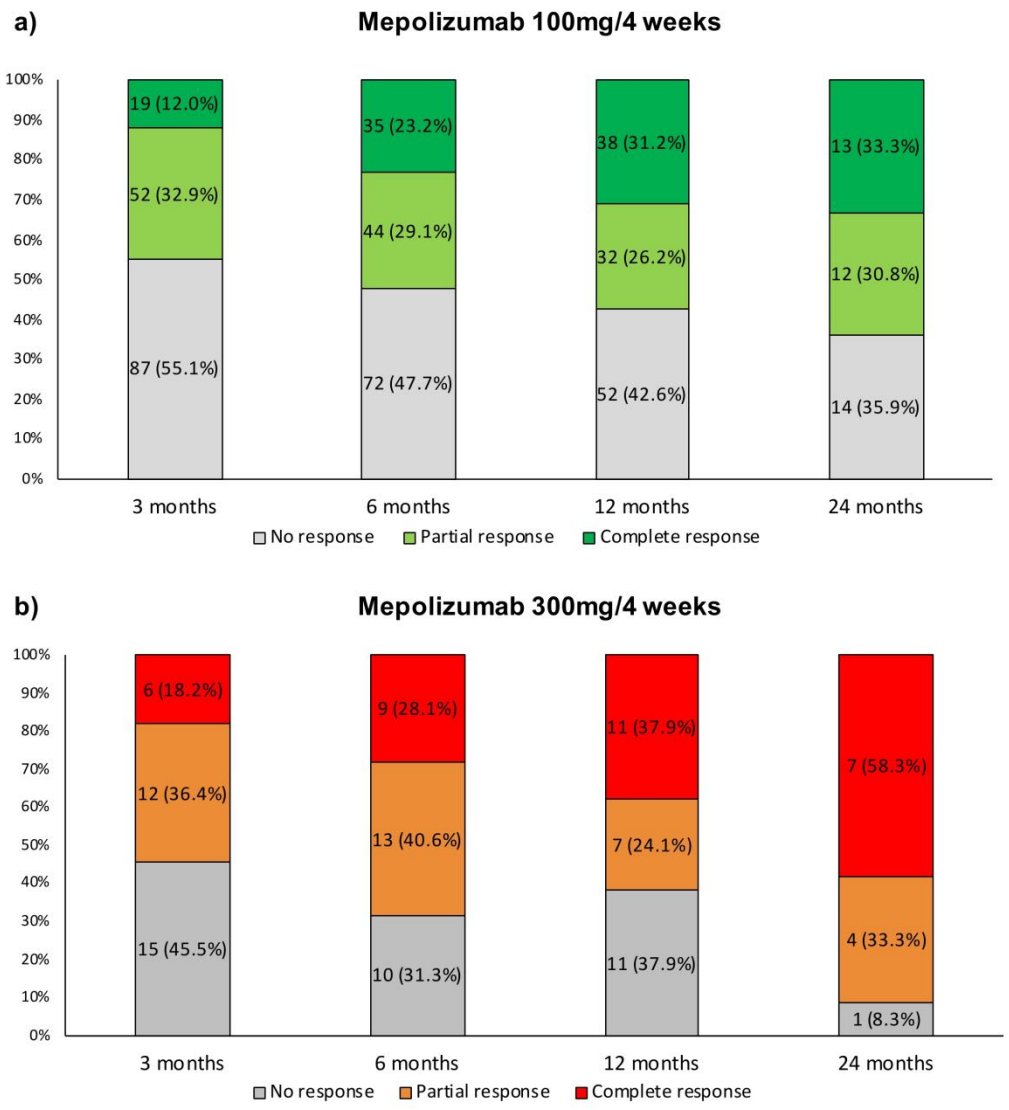
Table 3. Adverse events occurred during mepolizumab treatment

	0-3 months	**4-6 months**	**7-12 months**	**13-24 months**
N patients experiencing at least one adverse event (AE)	21/203 (10.3%)	20/195 (10.3%)	16/161 (9.9%)	9/56 (16.1%)
Among patients on stable treatment with 100mg/4 weeks	10/158 (6.3%)	13/151 (8.6%)	6/122 (4.9%)	3/39 (7.7%)
Among patients on stable treatment with 300mg/4 weeks	9/33 (27.3%)	5/32 (15.6%)	10/29 (34.5%)	6/12 (50.5%)
p-value	<0.001	0.322	<0.001	0.003
AE requiring hospitalization	0	2	2	2
Among patients on stable treatment with 100mg/4 weeks	0	1	2	1
Among patients on stable treatment with 300mg/4 weeks	0	1	0	1
AE requiring treatment discontinuation	2	3	1	0
Among patients on mepolizumab 100mg/4 weeks	2	3	1	0
Among patients on mepolizumab 300mg/4 weeks	0	0	0	0
Type of AEs and number of cases				
SOC: Infections and infestations				
Lower respiratory tract infections	4	3 (1*)	7 (1*)	2
Upper respiratory tract infections	2			1
Other infections		2 (1*)	1	1
SOC: Musculoskeletal and connective tissue disorders				
Myalgia/arthralgia	3	1	1	
Osteoporosis/fractures	1	1	1	1
Epicondylitis		1		
SOC: Nervous system disorders				

Dizziness	1		1	
Headache	2	1		
Transient color vision disorder		1		
SOC: Skin and subcutaneous tissue disorders				
Eczema/urticaria	2	1		
Papillary edema			1	
SOC: General disorders and administration site conditions				
Malaise	2			
Swelling at injection site	1			
SOC: Endocrine disorders				
Secondary adrenal insufficiency				1*
SOC: Blood and lymphatic system disorders				
Sialoadenitis		1		
SOC: Cardiac disorders				
Myocarditis				1*
SOC: Hepatobiliary disorders				
Acute hepatitis			1	
SOC: Renal and urinary disorders				
Renal colic		1		
SOC: Respiratory, thoracic and mediastinal disorders				
Lung consolidation			1	
SOC: Vascular disorders				
Transient ischemic attack (TIA)			1*	

*Adverse events requiring hospitalization

SOC: system organ class classification



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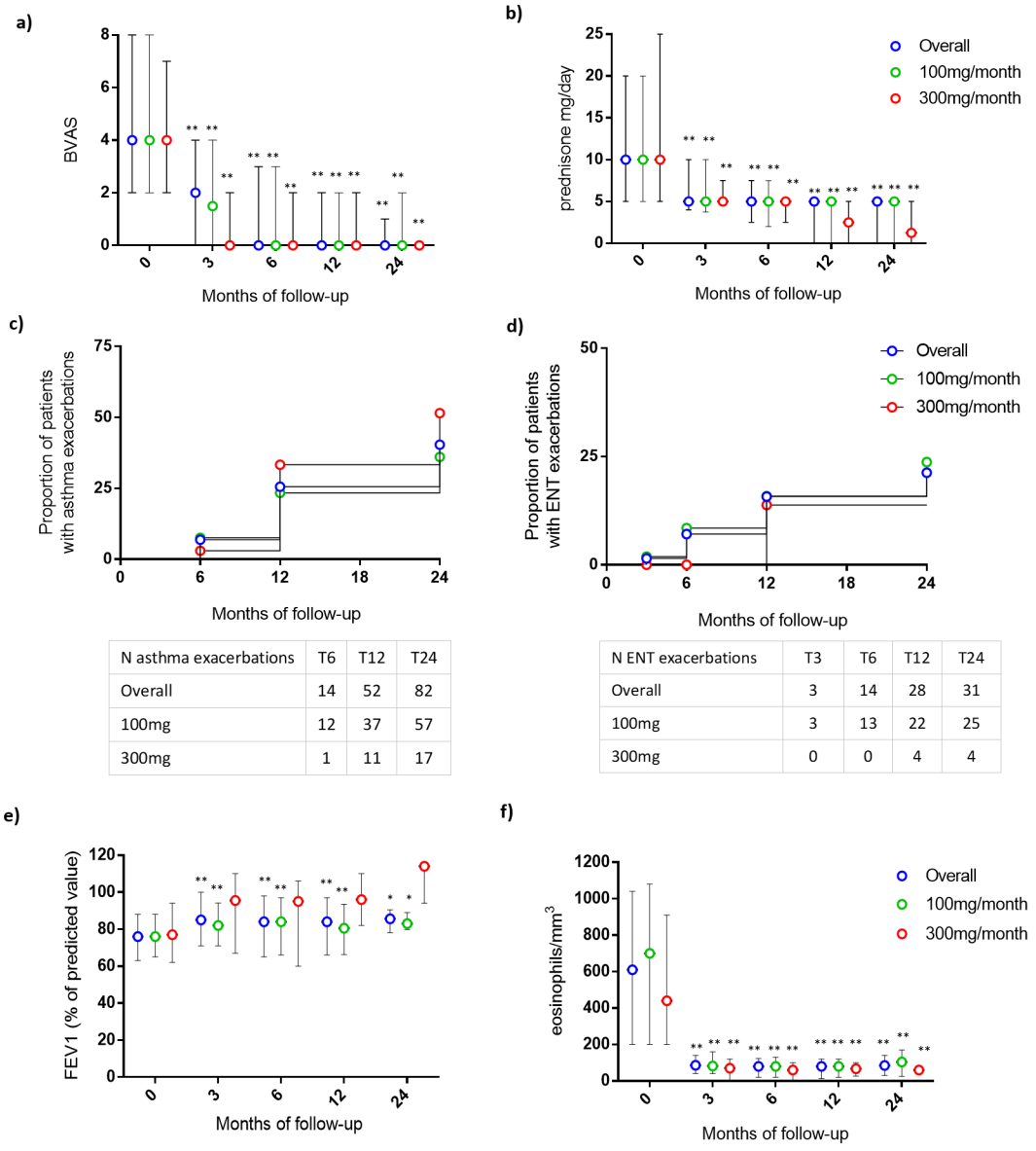

N asthma exacerbations	T6	T12	T24
Overall	14	52	82
100mg	12	37	57
300mg	1	11	17

N ENT exacerbations	T3	T6	T12	T24
Overall	3	14	28	31
100mg	3	13	22	25
300mg	0	0	4	4

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