

Effect of Omalizumab on Blood Basophil Counts in Patients with Chronic Idiopathic/Spontaneous Urticaria

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TO THE EDITOR

Basophils are believed to play an important role in the pathophysiology of chronic idiopathic/spontaneous urticaria (CIU/CSU) (Vonakis and Saini, 2008). Notably, basopenia has been reported in patients with CIU/CSU (Rorsman, 1961) and is postulated to be the result of migration of basophils from the circulation into the skin (Caproni et al., 2005; Ito et al., 2011; Ying et al., 2002). Consistent with this hypothesis, the degree of basopenia has been shown to correlate with disease severity (Grattan et al., 2003) and improves during times of remission (Eckman et al., 2008; Kern and Lichtenstein, 1976). It is theorized that binding of free IgE by omalizumab, a humanized monoclonal antibody against IgE, may influence the behavior of basophils and mast cells via down-regulation of the high-affinity receptor (FcεRI). To evaluate the effect of omalizumab on circulating basophils in CIU/CSU, we conducted a post hoc analysis of randomized clinical trial data examining changes in blood basophil counts in relation to treatment.

Patient data were obtained from three pivotal trials conducted to evaluate the safety and efficacy of omalizumab in patients with CIU/CSU: ASTERIA I (NCT01287117), ASTERIA II (NCT01292473), and GLACIAL (NCT01264939). Written informed consent for participation in the trials was obtained from all patients or their parent or legal guardian, and study protocols were developed in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board or ethics committee at each center. Detailed information

regarding these studies has been previously reported (Kaplan et al., 2013; Maurer et al., 2013; Saini et al., 2015).

Patients received subcutaneous omalizumab (75 mg, 150 mg, or 300 mg in ASTERIA I and ASTERIA II; 300 mg in GLACIAL) or placebo every 4 weeks for 12 (ASTERIA II) or 24 (ASTERIA I, GLACIAL) weeks with a 16-week follow-up period. Blood samples for basophil analyses were collected at baseline and every 12 weeks. The presence of blood basophils was quantified using two different methods: whole blood histamine assay and basophil percentage by flow cytometry. Basophils are highly enriched for histamine content relative to plasma or other cellular components of blood. Evidence suggests that the cell-free fraction of histamine is minimal compared with the cell fraction in patients with CIU/CSU (Cho et al., 2013), and thus the measurement of histamine in whole blood lysate correlates closely with basophil levels in the blood (Sabroe et al., 1998; Siraganian, 1974, 1975). Histamine concentrations (measured in lysates only for patients participating in US-based sites) were determined using the method described by Siraganian (1974, 1975). Flow cytometric assessment of basophils was conducted using samples of sodium heparin anticoagulated peripheral blood. Basophils were identified through gating CD123⁺/HLA⁺DR⁺/CD303⁺ cells.

Overall, the three pivotal trials included a total of 766 patients; 586 received omalizumab and 180 received placebo. The histamine analysis included data from 578 patients (omalizumab, 440; placebo, 138). The basophil percentage by flow cytometry analysis

included data from 608 patients (omalizumab, 469; placebo, 139). Baseline demographic and clinical characteristics were similar across studies and treatment groups (Supplementary Table S1 online).

Mean $\pm$ standard deviation histamine concentrations were generally similar across treatment groups at baseline (Supplementary Table S2 online). Changes from baseline in histamine concentration are depicted in Figure 1a. The mean changes from baseline in histamine were greater in the omalizumab 300 mg group versus placebo for all three trials at weeks 12 and 24. Although this difference was not always statistically significant in individual trials, the difference was significant at week 12 when data from all three trials were pooled (omalizumab 300 mg, 4.38, vs. placebo, 0.54; $P < 0.001$). Changes in histamine concentration for the lower-dose omalizumab cohorts were less consistent and not statistically significant.

Mean $\pm$ standard deviation basophil percentages were generally similar across treatment groups at baseline (Supplementary Table S3 online). Changes from baseline in blood basophil percentage are shown in Figure 1b. As observed for histamine concentration, the mean change in basophil percentage was higher in the omalizumab 300 mg group versus placebo for all three trials at weeks 12 and 24. Although this difference was not always statistically significant in individual trials, the difference was significant at week 12 (omalizumab 300 mg, 0.16, vs. placebo, 0.03; $P < 0.001$) and at week 24 (omalizumab 300 mg, 0.19, vs. placebo, 0.08; $P = 0.018$) when data were pooled across trials. A similar trend of increased basophil percentage at week 12 was observed for the 75-mg group (omalizumab 75 mg, 0.08; $P = 0.072$) and the 150-mg group (omalizumab 150 mg, 0.10; $P = 0.039$). Across all three trials, histamine concentrations correlated well with

Abbreviations: CIU, chronic idiopathic urticaria; CSU, chronic spontaneous urticaria

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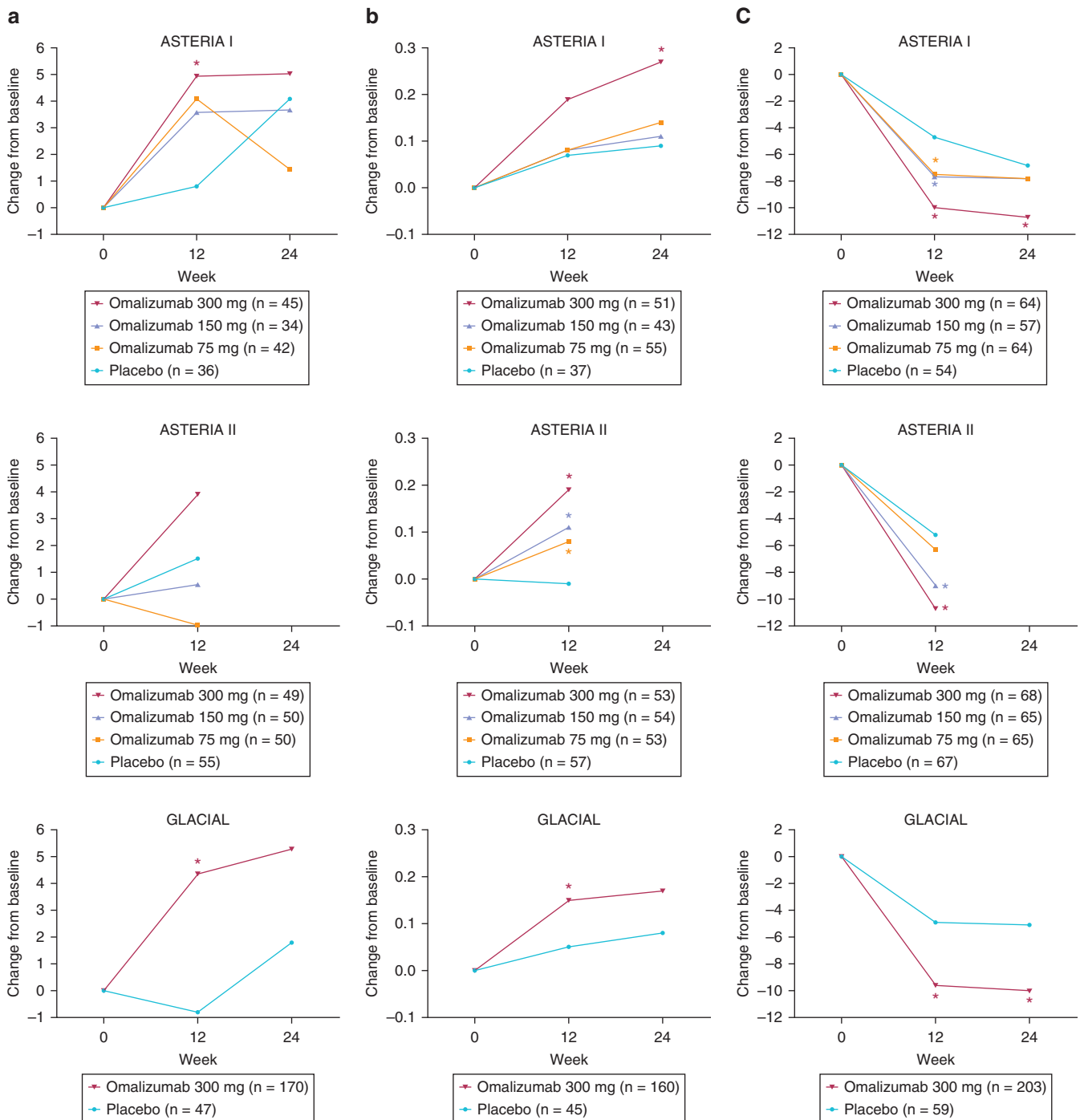


Figure 1. Effects of omalizumab on laboratory and clinical parameters. Change from baseline in (a) mean whole blood histamine concentration (ng/ml), in (b) mean blood basophil percentage (by flow cytometry), and in (c) mean weekly itch severity score in a subset of patients enrolled in ASTERIA I, ASTERIA II, and GLACIAL. * $P < 0.05$ versus placebo.

basophil percentages ($r = 0.614$, $P < 0.001$ at baseline and $r = 0.664$, $P < 0.001$ at week 12; Figure 2).

Among the subset of patients from the pivotal trials included in this post hoc analysis, we observed improvements in the weekly itch severity score that paralleled the improvements in blood basophil numbers (Figure 1c). Changes

in clinical efficacy, as measured by the weekly itch severity score, correlated weakly with changes in histamine concentration ($r = -0.2139$; $P \leq 0.001$; $n = 582$) as well as changes in basophil percentage ($r = -0.1486$; $P \leq 0.001$; $n = 637$).

To our knowledge, this is the first report to prospectively measure the

basophil presence in circulation relative to clinical measures of CIU/CSU activity, and also the first to examine basophil counts on a large scale within an interventional study. Our analysis of data from the pivotal trials of omalizumab for CIU/CSU showed that circulating blood basophils increased in response to treatment with omalizumab

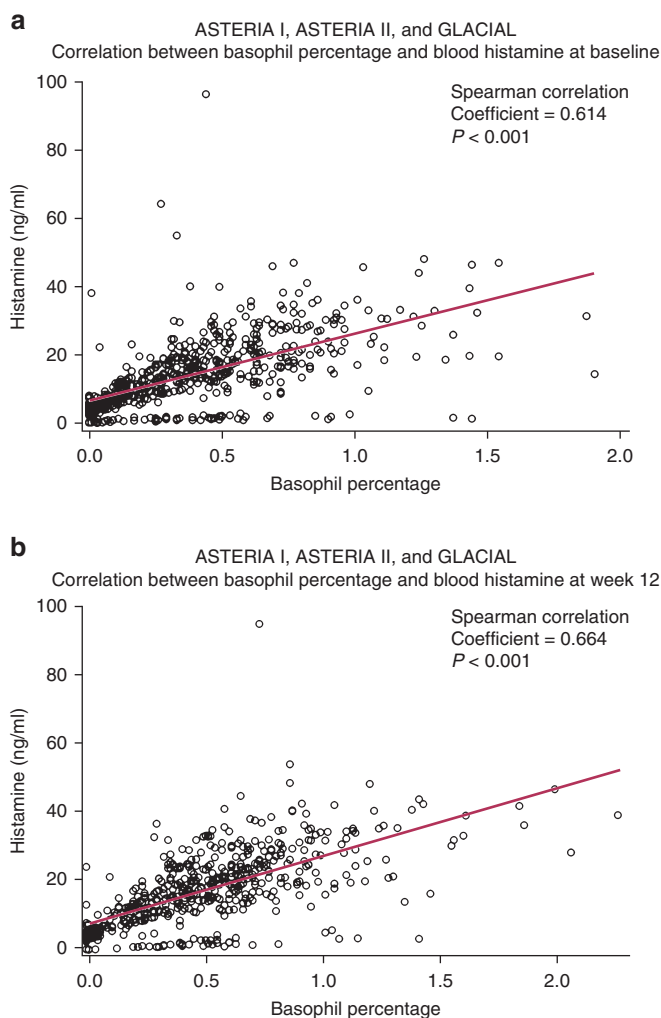


Figure 2. Whole blood histamine concentrations correlate with blood basophils. Correlation between basophil percentage and blood histamine at (a) baseline and (b) week 12 for pooled phase 3 trial patients.

300 mg. Parallel improvements in disease severity, as measured by the weekly itch severity score, were observed across all treatment groups, with the omalizumab 300 mg group demonstrating the greatest clinical benefit. Correlations between the weekly itch severity score and blood basophil measures were weak but significant, suggesting a possible relationship. Taken together, our data suggest that the clinical improvement observed with omalizumab treatment may be related to its effect on basophils.

This analysis is subject to the same limitations of any post hoc analysis of clinical trial data. Whole blood histamine concentrations and flow cytometry data were available for only a subset of patients; thus, power to detect smaller changes in these measures was limited. Although statistical

significance was not achieved at every individual time point, the trends observed were consistent between trials as well as between the two methods used to assess basophils, supportive of an overall treatment-related effect.

In summary, our findings lend further evidence to the hypothesis that basophils play an important role in pathobiology of CIU/CSU. Future investigation is needed to determine whether circulating basophils could serve as a potential useful biomarker for clinical response to omalizumab in patients with CIU/CSU.

CONFLICT OF INTEREST

SSS has received research support from Astra-Zeneca, Genentech, the National Institutes of Health, and Novartis, and has served as a consultant to Array, Genentech, Kendle, Med-Immune, Novartis, Pharmacyclics, and Teva. TAO, BT, KR, and HC are all employees of Genentech and receive stock options from Roche. HNH is a paid consultant to Genentech. PMS and J-PC state

no conflict of interest. AL is an employee of LabCorp Clinical Trials, which received payment for performing the flow cytometry assay described in this paper.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <http://dx.doi.org/10.1016/j.jid.2016.11.025>.

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Reply to Meisel et al.

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TO THE EDITOR

A recent article by Meisel et al. (2016) and related commentary (Kong, 2016) emphasized the crucial role of experimental design in 16S marker gene sequencing of skin samples. The authors reported that the specific primer pair used for sequencing the hypervariable region 1–3 (V1–V3) of the bacterial 16S ribosomal RNA (rRNA) gene performed substantially better than the primer pair used for sequencing the V4 region. Unfortunately, it is claimed that the failure of a specific primer combination (515F-806R) targeting the V4 region to amplify typical constituents of the skin microbiota (e.g. *Propionibacterium*, a genus very dominant on skin) can be generalized to the entire region of the 16S rRNA gene. In fact, it has already been reported that this specific primer combination poorly amplifies *Propionibacterium* (Kuczyński et al., 2012), because of a single mismatch at the 3' end of the primer that prevents efficient binding to a very specific group of bacteria, thereby causing underestimation of its presence. Furthermore, Peptostreptococcaceae, a clade including the peptoniphilic genera *Finegoldia* and *Peptoniphilus*, is detected by this V4 primer pair as analyzed by Meisel et al. but not by the V1–V3 primer pair (see Supplementary Figure S2 online from

the article by Meisel et al., panel “Umbilicus”). Others have reported the inefficiency of the 27F primer used for the V1–V3 region amplification to detect *Gardnerella* and *Lactobacillus* genera (Frank et al., 2008), which we have previously identified to be associated typically with females, on the basis of 454 pyrosequencing of the V3–V4 region from an amplicon that was generated with V3–V6 primers (338F-1061R) (Zeeuwen et al., 2012). We argue that a minor change in experimental design, that is, optimizing the primers for bacteria relevant in skin microbiomes, can overcome this major primer-specificity issue. For example, changing the single nucleotides responsible for the mismatch of the commonly used 16S rRNA gene universal primers (802R and 806R) with the *Propionibacterium* genus enables effective PCR amplification of this taxon (see Supplementary Table S1 online). This in silico analysis was validated for the primer pair combinations 338F-1061R and 357F-802RV2 on skin samples derived from the lower back of the same healthy volunteer. As shown in Figure 1, *Propionibacterium* is amplified by these primers. Also, note the identification of the genus *Finegoldia*. Rather than disqualifying the V4 region, we would suggest that (i) skin microbiome surveys are strongly

influenced by experimental design and (ii) the specific primer pair (515F-806R) used for the sequencing of the V4 region is not suitable, in particular for the skin microbiome. Overall, we do agree with the main conclusion as phrased in the title of the article and the concluding sentence of the summary: solid experimental design is key for generating valuable and accurate scientific data to improve the current understanding of (skin) biology.

We would also like to briefly address the ability of the two 16S rRNA gene regions to discriminate between different species of *Staphylococcus*, as raised by the authors. The “resolving power” of an amplicon (in this case, the ability to correctly differentiate between different species of the same genus) is a property of the sequenced region, and in that sense is not dependent on the specific primers that are used to target it. Still, selecting a primer pair that encompasses a larger region can be expected to have more resolving power, as shown in the Meisel et al. (2016) study for the primer pair targeting the V1–V3 region compared with V4 (±500 vs. 300 base pairs, respectively). More importantly, one should realize that both amplicon pairs are unsuitable for confidently classifying different *Staphylococcus* species. Although correctly assigning 11% (V1–V3) of *Staphylococcus* genus-level sequences to the species level is better than 1% (V4), as reported by Meisel et al., it still leaves 89% unresolved. We would argue that identifying specific species (or even phylotypes and strains) can more

Abbreviations: rRNA, ribosomal RNA; V, hypervariable region of the 16S rRNA gene

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