Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

DOI: 10.1111/all.15378

Innate lymphoid cell subsets in obese asthma patients: Difference in activated cells in peripheral blood and their relationship to disease severity

To the Editor,

The parallel increase in the prevalence of obesity and asthma has suggested a relationship between these two diseases in recent years. The identification of common or distinct pathophysiological mechanisms associated with these two chronic diseases has not yet been clarified. Although Type 2 asthma has classical characteristics of Th2 cells, group 2 innate lymphoid cells (ILC), their cytokines, IL-4, IL-5, and IL-13 and eosinophilia, the classical Type 2 immune response is more complex in obese patients, mainly due to the involvement of fat tissue in immune responses. In obese asthmatics, asthma is generally nonatopic, and ILC3s play a critical role in obesity-induced airway hyperreactivity. However, it has recently been shown that eosinophils are also predominant in the lung tissue of obese asthma patients. In addition, increased T2 response and ILC2s are observed in obese asthmatic patients.[1,2]

Under homeostatic conditions, adipose tissue is infiltrated by ILC2, but ILC2 and ILC3 levels decrease, whereas ILC1 levels increase in response to the chronic ongoing inflammation in obesity.[1] In obese asthmatics, ILC2s and ILC3s are decreased in the adipose tissue and increased in lungs potentially secondary to the redistribution to lungs. Because of this trafficking, they can also be detected in peripheral blood.[1] It was reported that ILC3s mediate obesity-associated asthma through IL-1β production by macrophages, leading to the release of IL-17.[2] Similarly, ILC2s are found to be activated in the blood or sputum of obese asthmatics.[3] Particularly, total numbers of ILC2s were significantly increased in allergic and/or eosinophilic asthma and ILC2s were related to corticosteroid unresponsiveness in T2 asthma.[3,4] While increased numbers of ILC2s may provide benefits for obesity by creating metabolic homeostasis, it is known to have detrimental effects in terms of asthma. These conflicting results call for further research. In the present study, we aimed to investigate the differences in the distributions of the main ILC groups (Lin^- $CD45^+CD127^+CD161^+$total ILC, c-kit^-CRTH2^- ILC1, c-kit^-CRTH2^+ ILC2, and c-kit^+CRTH2^- ILC3) and the activated subgroups of the ILCs ($CD25^+$ total ILC, ILC1, ILC2, ILC3; $CD69^+$ total ILC, ILC1, ILC2, ILC3; $Nkp44^+$ILC3) between obese asthma (OA) and normal-weight asthma (NWA) patients in comparison with obese control (OC) and normal-weight control (NWC). The c type lectin receptor CD69 is the earliest activation and tissue residency marker. The most prominent cellular activation marker CD25 is the alpha chain of the trimeric IL2 receptor. We isolated peripheral blood mononuclear cells (PBMC) in total of 80 women (Figure S1) and counted main and activated subgroups of ILCs with flow cytometry (Figure S2). Demographic specificities of the patient groups were given at Table S1.

Obese asthma patients tended to have higher $CD69^+$ILC subsets, while lower $CD25^+$ILC subsets than OCs. However, significant results were found only in the percentages of $CD69^+$total ILCs, $CD69^+$ILC3, $CD25^+$total ILCs, and $CD25^+$ILC3s (Figure 1). On the contrary, in terms of cell counts in the viable $CD45^+$lymphocytes, we showed that OA patients had lower $CD161^+$total ILC, ILC1, $CD69^+$ILC1 than NWCs. In addition, we found that both $CD25^+$ and $CD69^+$ innate lymphoid cell counts tended to be lower in NWA patients than NWCs. However, only significant results were detected in $CD69^+$ILC1 and $CD25^+$ILC3 groups (Figure 2A).

In terms of asthma severity, $CD69^+$total ILCs, ILC2s, $CD69^+$ILC2, $CD69^+$ILC3 were higher in severe asthma patients than mild asthmatics in this study. In addition, we showed that $CD161^+$total ILCs, $CD69^+$ total ILCs, ILC2, $CD69^+$ ILC2, ILC3, $CD69^+$ ILC3 were higher in the circulation of moderate asthma patients compared to mild asthmatics (Figure 2B). Our results failed to show any significance between moderate and severe asthma, although there was a tendency.

Supporting our findings of low CD25 and high CD69 expression in total ILCs, ILC1, ILC2, and ILC3s in OA patients, it was found that under non-pathological conditions, the majority of ILCs expressed CD25 even in PBMCs, whereas CD69 was found to be upregulated in pathological tissues, such as omentum adipose tissue, lung tumor, and colorectal tumor.[5] Later, it was reported that circulating ILCs did not express CD69, which is known as a marker of early activation and tissue residence, but the demonstration of $CD69^+$ILCs in the peripheral blood may exhibit that these cells are redistributed from tissues after local tissue activation.[6]

Low peripheral ILC1 levels in OA may be secondary to the increased accumulation of ILC1s in the adipose tissue. In particular, ILCs accumulate and are activated in inflamed tissues. In NWA, ILCs, particularly ILC2s and ILC3s, accumulate and become activated in lungs which may be the reason of the lower $CD25^+$ and $CD69^+$ILC subsets in the peripheral blood of NWA than in NWC.

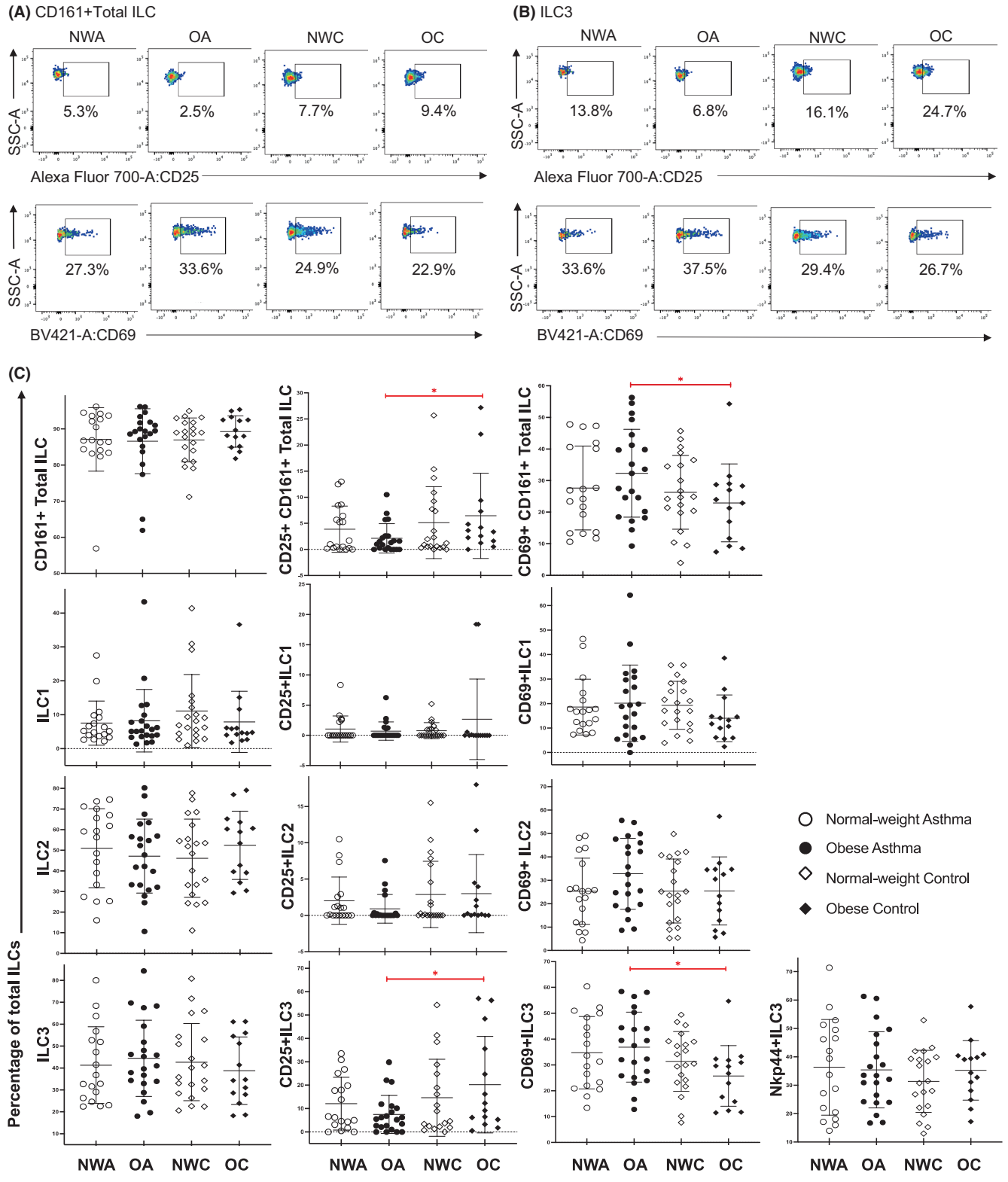

FIGURE 1 Innate lymphoid cell subsets in peripheral blood. (A) Representative data of $CD25^{+}CD161^{+}$total ILCs and $CD69^{+}CD161^{+}$total ILCs according to NWA, OA, NWC, OC study groups. (B) Representative data of $CD25^{+}$ ILC3s and $CD69^{+}$ ILC3s according to NWA, OA, NWC, OC study groups. (C) Cumulative data of the ILCs according to 4 main study groups. $CD25^{+}CD161^{+}$ total ILCs and $CD25^{+}$ILC3s were lower in OA than OC ($p = .04$, $p = .04$, respectively). $CD69^{+}CD161^{+}$ total ILCs and $CD69^{+}$ ILC3s were higher in OA than OC ($p = .02$, $p = .02$, respectively). (NWA, Normal-weight asthma; OA, Obese asthma; NWC, Normal-weight control; OC, Obese control)

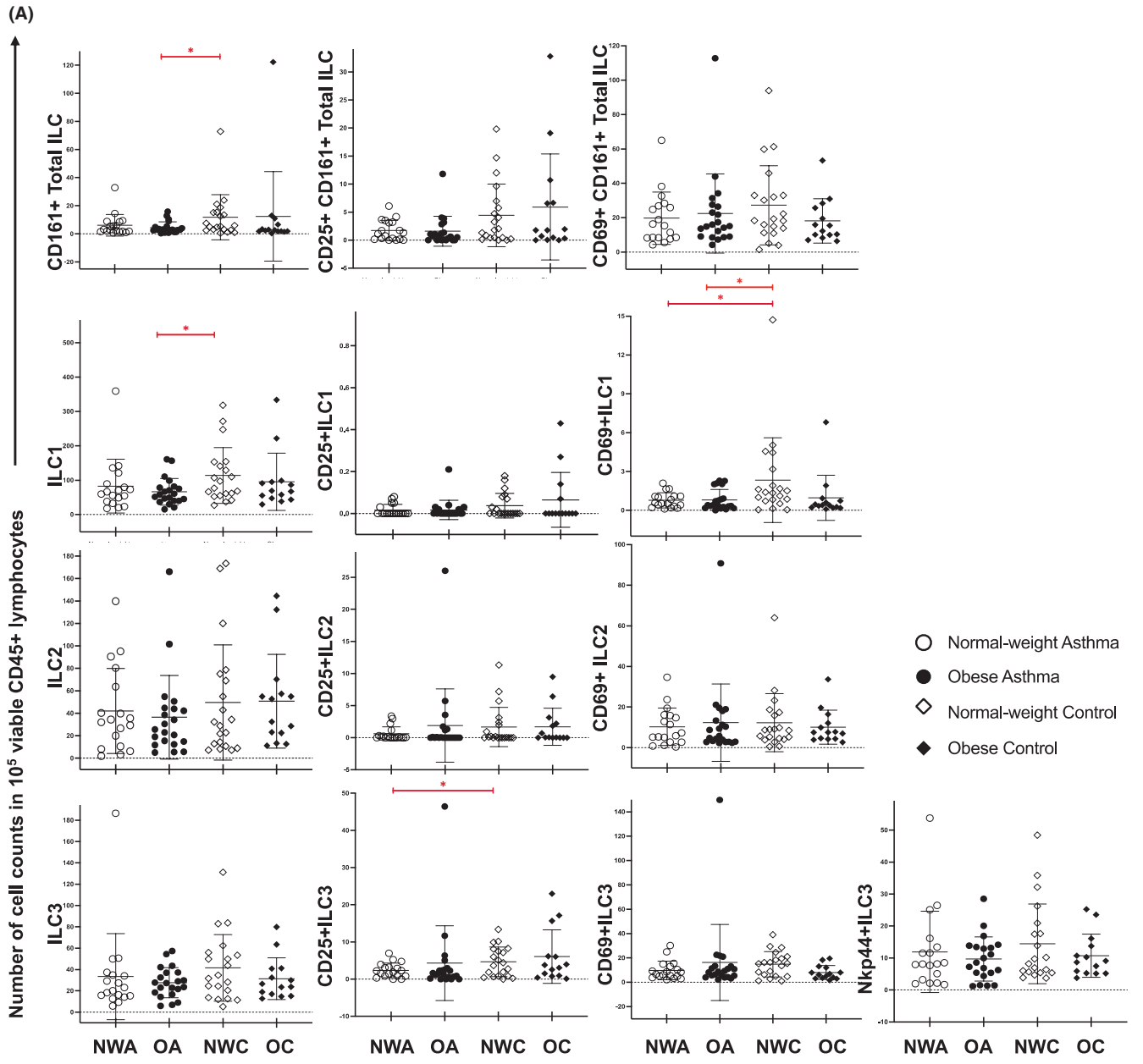

FIGURE 2 (Continued)

ILCs and their relationship to asthma severity has been studied several times. It was shown that circulating ILC2s were higher in severe asthmatics compared with patients with mild asthma.[7] Moreover, increased expression of IL-17 in lungs of severe asthma patients suggested ILC3s as well as ILC2s are involved in inflamation.[2] Consistent with these results, we found higher activated $^{+}$total ILCs, ILC2s, ILC3s in severe asthma patients than mild asthmatics.

Our results suggest that total ILCs and ILC3s are activated and migrated to the lungs in OA patients as they were low in the circulation. In addition, asthma severity was associated with an increase in ILCs, particularly in CD69^{+} activated CD161^{+}total ILC, ILC2, and ILC3 subgroups. Indeed, altered epithelial barrier functions seem to play an essential role in the activation and upregulation of ILCs. Because of chronically persistent inflammation and impaired epithelial barriers in adipose and lung tissue in OA patients, it is not surprising that increased and activated ILCs are found in inflamed tissues. Altogether, the present study demonstrates that total ILCs and ILC3 and activated ILCs show major changes in chronic ongoing inflammation in OA patients.

FUNDING INFORMATION

This research was funded by Scientific Research Projects Office of Ankara University (Project No:19B0230002) and Swiss National Science Foundation No. 310030-201053/320030-159870.

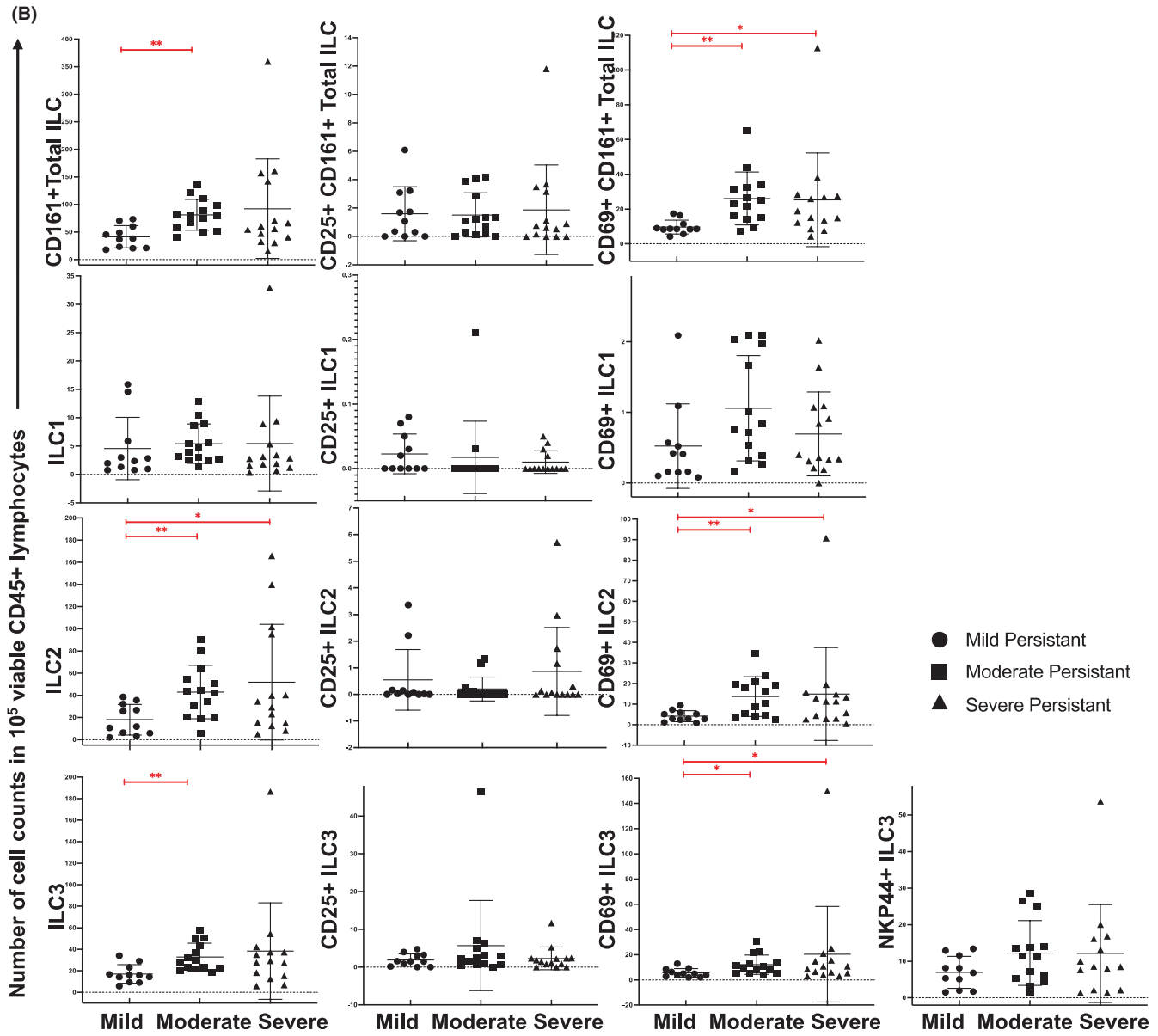

FIGURE 2 ILC subsets in peripheral blood show a relationship to asthma severity. Cell counts in 10^5 viable $CD45^+$lymphocytes. (A) $CD161^+$total ILC, ILC1, $CD69^+$ILC1 were lower in OA than NWC ($p = .04$, $p = .03$, $p = .03$, respectively). $CD69^+$ ILC1 and $CD25^+$ILC3 were lower in NWA than NWC ($p = .04$, $p = .03$, respectively). (B) $CD161^+$total ILCs, $CD69^+$ total ILCs, ILC2, $CD69^+$ ILC2, ILC3, $CD69^+$ ILC3 were higher in moderate asthma patients than mild asthmatics ($p = .001$, $p = .001$, $p = .006$, $p = .003$, $p = .002$, and $p = .01$, respectively). $CD69^+$total ILCs, ILC2s, $CD69^+$ILC2, $CD69^+$ILC3 were higher in severe asthma patients than mild asthmatics ($p = .01$, $p = .03$, $p = .03$, $p = .02$)

ACKNOWLEDGEMENT

Open access funding provided by Universitat Zurich.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

DOI: 10.1111/all.15379

Safety of combining biologics in severe asthma: Asthma-related and unrelated combinations

To the Editor,

Monotherapies with antibodies approved for severe asthma treatment were reported to be safe, with side effects close to placebo[1]. However, the safety of concomitant treatments with several biologics in asthma is poorly understood. Two scenarios for treatment with two or more biologics in asthma exist. Firstly, patients may receive an additional biologic approved for severe asthma, either to treat insufficiently controlled typical co-morbidities, or as an add-on treatment for insufficiently controlled asthma. Secondly, patients may receive another biologic not approved for asthma for the treatment of an unrelated disease. Concomitant treatment with 2 immunomodulating antibodies is approved in oncologic diseases such as melanoma [2] or mesothelioma [3]; however, possible autoimmune toxicities remain a concern. In rheumatoid arthritis or inflammatory bowel diseases, concomitant treatment with two or more biologics is currently avoided, because of concerns related to serious infections [4,5]. In contrast, the safety of concomitant treatments with two or more biologics in asthma is unclear. There was no safety signal (but also no additive efficacy) in a trial investigating concomitant treatment with the anti-interleukin 4 receptor antibody dupilumab and the anti-interleukin 33 antibody itepekimab [6]. However, despite several single case reports [7–10], there are no larger case series investigating this issue.

Therefore, seven German academic severe asthma centres (Rostock, Hannover, Mainz/Heidelberg, Berlin, Magdeburg, Kiel, Munich) were asked to report all severe asthma cases documented in their databases with a concomitant treatment (for at least 3 months) with two or more biologics. In order to minimise biases, there were

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