Review of: "Sputum Interleukin-32 in childhood asthma: correlation with IL-1β"

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Potential competing interests: No potential competing interests to declare.

In this work by Louhaichi et al, the authors describe the association between the proinflammatory cytokines IL-32 and IL-1b in childhood asthma. Both inflammatory cytokines were measured in induced sputum from a cohort of children with either mild, moderate, or severe conditions. Data presented in this report showed that total IL-32 protein (and RNA) are significantly higher in children with severe conditions and that IL-32 significantly correlate with IL-1b. The study is interesting given that earlier investigations on IL-32 and asthma were mostly carried out on cohorts of older adults. The association between IL-32 and IL-1b is also of high interest as increasing evidence point to the potential role of IL-1b in multiple allergic diseases including allergic rhinitis, asthma, and atopy, which turns this cytokine into a potential therapeutic target. The manuscript is overall well-written but could benefit from some edits and clarifications as follows:

1. There is significant introduction on Th subsets and their roles in the pathogenesis of asthma, although this manuscript does not present any data in this regard. It would be more relevant to focus on the inflammatory cytokines IL-32 and IL-1b and their link with macrophages and neutrophils.
2. Also in the introduction, the authors highlighted an important fact on the multiple roles of IL-32 as it might work as inflammatory, anti-inflammatory or regulatory cytokine, depending on the expressed isoform (keep in mind IL-32 is expressed in at least 10 different isoforms generated by alternative splicing). However, the current study measured only total IL-32 protein and used a set of primers to quantify the total IL-32 RNA. The authors should include a limitation section in the discussion to highlight that the current study did not identify which isoform is more dominant in the induced sputum and is likely to be associated with the sever asthma conditions. Please note that earlier studies showed that IL-32gamma (the longest and most inflammatory isoform) was the IL-32 candidate to be associated with disease severity and asthma exacerbation.
3. In the results section, age of children with severe conditions seems to be significantly higher compared to children with moderate or mild conditions or even compared with the control group. Since IL-32 was shown to be associated with age by other studies, the authors should discus it.
4. In Figure 1, I had a difficulty to understand panel A . What is the second group in Panel A (to the right of HC)?. Is it a sum of Athmatic groups (Severe+Moderate+Mild?). If yes, why this comparison was included in the figure since the levels of IL-32 in each of the three groups (Severe, Moderate and Mild) is also shown on the same graph?. Also, if it is a sum of the three groups, why the range of IL-32 in the sum of the three groups is lower than the range in the Severe group alone? Please indicate HC= Healthy Control in the Figure Legend.
5. Unclear how P values in Table 1 were generated. Is that a comparison between the sum of the three asthmatic groups

against the control group analyzed by Mann Whitney' U test or a comparison between the four groups analyzed by 1way ANOVA or Kruskal-Wallis?

6. The results section on Figure 1 says "IL-32 mRNA in severe asthma was more expressed than patients with mild and moderate asthma ($p < 0.0001$)". By looking at Figure 1b, the p value seems to be calculated on the difference between the severe group and the HC group not the mild and moderate groups. Please use the right analysis.
7. In the methods sections on IL-32 measures, the authors say that "The detection range was 5 ng/ml – 100 ng/ml. Values below this level were scored as 0 ng/mL for statistical analysis". However, in the results section, IL-32 data are presented in pg/ml, unclear whether these data are pgs or ngs?. Please keep in mind that the R&D Duo sets kit used have an IL-32 detection range of 17.8-5000pg, whereas the data in this manuscript are ranging 5-25pg/ml. Please discuss it.

Minor comments:

1. In the introduction, the authors write "Restoring the balance between the responses of Th2/Th1 as well as Treg cells, and their respective transcription factors T-bet/STAT6 and Foxp3 considerably improves asthma". Please correct the lineage transcription factors for T cells: T-bet for Th1, GATA3 for Th2 and Foxp3 for Treg.
2. Introduction: the authors write "The production of IL-1β, IL-6, IL-8, and TNF-α was down-regulated by silencing of IL-32 expression in monocytes [19]". Actually, the siRNA was used to silence IL-32 in endothelial cells not monocytes in the cited reference of Nold et al., 2009. Also, the effect of IL-32 silencing was tested by measuring IL-16, IL-8 and TNFa not IL-1b. Please be careful on citations.
3. In the discussion section, the authors say "The role of IL-32 in inflammation can be explained by the participation of the immune mechanisms of Th1 cells in an endotype according to the classification thus influencing angiogenesis [29] [32]". Unclear what the authors want to say and conclude here. In fact, the cited work describes the role of IL-32 on the suppression of proangiogenic signals from bronchial epithelial cells and not Th1 cells.