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RESEARCH LETTER

TITLE:
Inhaled corticosteroids downregulate SARS-CoV-2-related genes in COPD: results from a RCT

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In a RCT of 63 patients with COPD, 12 weeks of ICS/LABA therapy downregulated bronchial epithelial expression of the SARS-CoV-2-related genes **ACE2** and **ADAM17** compared to LABA alone. This may have implications for COVID-19 susceptibility or severity in COPD.
To the Editor:

Observational studies show that chronic obstructive pulmonary disease (COPD) is associated with increased COVID-19 severity and mortality [1]. Inhaled corticosteroids (ICS), which are commonly used to treat COPD, have been associated with increased risk of bacterial pneumonia in COPD and impaired immune response to viruses. Whether this class of medication affects the airway expression of SARS-CoV-2 receptors and cofactors – changes which may modify COVID-19 susceptibility and outcomes – is currently unclear. We therefore examined the effects of ICS treatment on SARS-CoV-2-related gene expression in lower airway bronchial epithelial cells (BECs) in a randomised controlled trial of COPD patients.

We conducted the DISARM trial (recruitment October 2015-June 2019; protocol at clinicaltrials.gov #NCT02833480; UBC/PHC ethics approval H14-02277) to examine the effects of two long-acting beta-2 agonist (LABA)/ICS combinations on the airway microbiome in people with COPD. After a two-week formoterol (FOR) run-in, we randomised participants to receive ongoing FOR 12 µg, formoterol/budesonide (FOR/BUD) 12/400 µg, or salmeterol/fluticasone propionate (SAL/FLU) 25/250 µg, twice daily for 12 weeks. We collected bronchial brush specimens according to a standard protocol (6th-8th-generation airways, right upper lobe) before and after treatment, and measured BEC gene expression by RNA-sequencing as previously described [2]. The co-primary outcomes (change in total bacterial population and diversity at 12 weeks) are yet to be reported. However, in response to the urgency of the COVID-19 pandemic, we performed an ad hoc analysis of genes encoding
SARS-CoV-2 entry receptors (ACE2, BSG) and host co-factors (TMPRSS2, ADAM17, FURIN).

Sixty-three participants (median age 64 years, 83 percent male, 46 percent current smokers, mean forced expiratory volume in 1 second 61.36 percent predicted) were randomised. There were no differences between the treatment groups with regard to demographics, lung function, comorbidities, or recent ICS use (Kruskal-Wallis and Fisher’s exact tests p>0.05). Principal component analysis of overall pretreatment gene expression showed no differences between the treatment groups, or between pre-enrolment ICS users and non-users. Fifty-four participants had both pre- and post-treatment gene expression data available (seven participants failed to attend the post-treatment bronchoscopy; and two had insufficient pre-treatment RNA). After 12 weeks of treatment, the FOR/BUD and SAL/FLU arms both showed significantly lower changes in ACE2 expression relative to FOR monotherapy (Wilcoxon rank sum test, p=0.049 and p=0.041, respectively), and the FOR/BUD arm showed significantly lower changes in ADAM17 expression relative to FOR monotherapy (Wilcoxon rank sum test, p=1.36x10^-4) (Figure). This suggests that ICS have a repressive effect on the transcription of these genes. There was no effect of ICS treatment on BEC expression of BSG, TMPRSS2, or FURIN. When stratified by baseline smoking status, the results were similar for ADAM17 (FOR/BUD versus FOR, p=0.01 in former smokers and p=0.002 in current smokers) but were not significant for ACE2.

We also qualitatively explored how ICS treatment affects transcriptome-wide BEC gene expression in COPD using a clustered heat map (Figure). LABA-only and
LABA/ICS treatment had modest but opposite effects on gene expression: upregulation in the FOR arm was met with downregulation in the FOR/BUD and SAL/FLU arms, and vice versa, suggesting an ICS class effect. Overall, FOR/BUD appeared to have a greater effect on gene expression than SAL/FLU despite similar doses (in beclometasone-equivalents). The reason for this is unknown, but may be due to the greater relative retention of BUD in airway epithelial cells [3].

Next, we determined how genes that were co-expressed with each of the key SARS-CoV-2-related genes – determined by a weighted gene correlation network analysis [4] – changed with ICS treatment. ACE2 was contained in a module of 444 genes; using Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) annotations, this module was highly enriched for genes related to type I interferons (IFN-I) and viral infections. ADAM17 and FURIN were in the same module of 1,900 genes, which was enriched for genes related to the regulation of innate immunity, cytokine production, infectious and autoimmune diseases. BSG and TMPRSS2 were each contained in modules of 788 and 985 genes, respectively, both of which were enriched for genes related to intracellular processes. We then annotated the clustered heat map according to each gene’s co-expression module membership. ACE2 and ADAM17/FURIN module genes were clustered in areas of the heat map where genes were downregulated by FOR/BUD and SAL/FLU treatment, suggesting that ICS downregulates genes that are highly connected to these key SARS-CoV-2-related genes.

Our analysis extends our previous findings that ACE2 gene and protein expression was increased in BECs [2] and lung tissue [5] of people with COPD, by showing that
ACE2 was downregulated by ICS treatment. Our data, which are from a randomised controlled trial of ICS/LABA therapy, confirm a recent study that indicated a downregulatory effect of ICS on ACE2 expression in the sputum of COPD patients (BUD and FLU) and in mouse lungs (BUD, FLU and beclomethasone) [6]. In this study, in concordance with our results, ICS did not affect the expression of BSG or TMPRSS2 [6]. Another group has recently conducted a post hoc analysis (currently in preprint [7]) of the GLUCOLD trial, showing that ACE2 expression in airway biopsy specimens was downregulated following treatment with FLU in patients with COPD. Our data complement these results by showing that ACE2 downregulation is likely the result of an ICS class effect, and can occur more acutely (after only 12 weeks of treatment, compared to 26 weeks in the GLUCOLD study).

The relative importance of differences in ACE2 expression for COVID-19 is debated. Increased availability of ACE2 protein in the airways may increase COVID-19 susceptibility and severity; in theory, the ICS-mediated downregulation of ACE2 reported here and by others [6, 7] could therefore be protective. On the other hand, since ACE2 is a critical negative regulator of the renin-angiotensin system, its downregulation could predispose to lung injury [8]. Our gene network analysis showed that ACE2 was co-expressed with genes related to the innate immune response to viruses, particularly IFN-I, and that genes in this module tended to be suppressed by ICS therapy. Animal models suggest that a delayed IFN-I response to SARS-CoV infection may lead to excessive inflammation and death [9]. Indeed, COPD patients already have impaired IFN-I responses following viral infection [10]. ACE2 expression in COVID-19 may therefore represent a double-edged sword, but
any interaction between COPD and ICS treatment in COVID-19 is likely more complex than can be explained by alterations in ACE2 expression alone.

A novel, truncated isoform of ACE2 that is transcriptionally-independent and highly expressed in lung epithelium has recently been reported [11]. This isoform does not have an extracellular domain and does not bind SARS-CoV-2 spike protein, meaning changes in its expression may have no effect on COVID-19 risk. This important finding challenges the notion that functional ACE2 is an interferon-stimulated gene, since it appears to be only the truncated isoform that is induced by IFN-I. We attempted exon-level analysis to quantify this isoform, but our sequencing depth was insufficient to produce reliable results. Future investigation of this novel isoform will be critical to understand the implications of our current findings.

Our finding that ICS therapy downregulates ADAM17 expression in human BECs is, to our knowledge, novel. The SARS-CoV-2 spike protein induces ADAM17-dependent shedding of the ACE2 ectodomain, creating the soluble form of ACE2 and facilitating fusion of the viral and cell membranes [12]. Inhibition of ADAM17 at least partially blocks SARS-CoV entry in cultured epithelial cells [12, 13]. ADAM17 also plays a crucial role in interleukin-6 (IL-6) signaling, which is activated in severe COVID-19 [14]; it has been described as a “master switch” between the pro-inflammatory trans- and anti-inflammatory classical (i.e. via membrane-bound IL-6 receptor) IL-6 signaling pathways [15]. However, any impact the ICS-mediated downregulation of ADAM17 may have on COVID-19 susceptibility or outcomes would be speculative at this stage.
Despite the limitations (including relatively small sample size, short follow-up period, and a lack of accompanying protein expression or functional data), our results show that ICS modifies lower airway BEC expression of genes relevant to SARS-CoV-2 and COVID-19 biology. The relative importance of upper (nasal) versus lower (bronchial) expression of these genes for SARS-CoV-2 transmission needs further study. However, our results provide potential mechanistic support for the results of the STOIC trial (clinicaltrials.gov #NCT04416399), which showed a reduction in urgent care/hospitalisation in early-stage COVID-19 with inhaled BUD [16]. The trial results require confirmation in a COPD population, which has increased BED ACE2 expression, before the clinical relevance of our findings can be determined. In the absence of any epidemiological evidence that ICS therapy increases COVID-19 severity or mortality [17], we agree with the international consensus that ICS treatment in COPD patients should be continued if clinically indicated until further evidence is available.

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Data acquisition: SM, FSSLF, TS, SFVE, JML, SL, DDS

Data analysis and interpretation: SM, XL, AIHC, CXY, FSSLF, CWTY, JML, DDS

Drafting of manuscript: SM, XL

Critical revision of draft manuscript: SM, XL, AIHC, CXY, FSSLF, CWTY, SFVE, JML, SL, DDS

All authors reviewed and approved the final version of the manuscript.

All authors agree to be accountable for all aspects of the work including data integrity.

**DATA SHARING STATEMENT:**

Individual participant gene expression data are publicly available via Gene Expression Omnibus (GEO) at [https://www.ncbi.nlm.nih.gov/geo/](https://www.ncbi.nlm.nih.gov/geo/) (accession no. GSE162120). The DISARM study protocol is available at [https://clinicaltrials.gov](https://clinicaltrials.gov) (identifier NCT02833480). Additional individual participant data that underlie the results reported in this article, after deidentification, will be made available immediately following publication to any investigators and for any purpose, upon reasonable request. Proposals should be made directly to the Principal Investigator, Dr. Don Sin ([don.sin@hli.ubc.ca](mailto:don.sin@hli.ubc.ca)).
REFERENCES:


**FIGURE CAPTION:**

**Bronchial epithelial cell (BEC) gene expression in the DISARM study.** Gene expression in BECs collected during bronchoscopy before and after treatment was determined by RNA sequencing (Illumina NextSeq 500, Illumina, San Diego, CA, with paired end 42bp × 42bp reads). Sequencing data was aligned to GENCODE genome reference assembly GRCh37 release 31 using Salmon. Low abundance genes (log$_2$ counts per million [log$_2$CPM] <1 or transcripts per million [TPM] <2 in more than 80% of the samples) were filtered out, leaving a total of 15,667 genes. (A) Box plots showing pre- to post-treatment change in expression (Δlog$_2$CPM) of ACE2 (encodes the SARS-CoV-2 receptor) and ADAM17 (encodes a metalloproteinase that cleaves the ACE2 protein and facilitates endocytosis of the ACE2-SARS-CoV-2 complex). Only participants with both pre- and post-treatment gene expression data available are shown (total n=54 out of 63 randomized participants). Between-group comparisons were by Wilcoxon rank-sum test. *p<0.05 ***p<0.001. (B) Heat map of pre- to post-treatment change in gene expression (median Δlog$_2$CPM) of genes with at least one significant between-group Wilcoxon rank-sum test at false discovery rate (FDR) <0.1 (977 out of the 15,667 total genes). Columns represent single genes, and are arranged using hierarchical clustering (`aheatmap` function in the NMF packing in R). Treatment with FOR/BUD and SAL/FLU tended to have the opposite direction of effect on gene expression compared to treatment with FOR, suggesting a class effect of ICS on the expression of these genes. Additionally, each plotted gene was annotated according to its membership of a SARS-CoV-2-related gene co-expression module determined by weighted gene correlation network analysis (WGCNA) of pre-treatment expression (as log$_2$[TPM+1], soft threshold power $\beta$=6,
minimum module size 50 genes). ICS treatment tended to decrease the expression of genes that are co-expressed with ACE2 and ADAM17/FURIN, whereas genes co-expressed with TMPRSS2 and BSG tended to be upregulated by ICS treatment.