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LETTER

A signature of differential gene expression in bronchoalveolar lavage fluid predicts mortality in influenza-associated pulmonary aspergillosis

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Dear Editor,

Influenza-associated pulmonary aspergillosis (IAPA) is a severe co-infection with the fungus *Aspergillus*, affecting critically ill influenza patients. Mortality of IAPA patients reaches 45%, more than twice as much as observed in influenza patients admitted to intensive care unit (ICU) without aspergillosis [1]. Importantly, the effect of antifungal treatment on patient outcome is limited, while prognostic biomarkers tailored for this patient group are lacking.

We performed screening for predictors of IAPA mortality using a dataset of Nanostring nCounter-measured expression of 755 genes linked to innate immunity in bronchoalveolar lavage (BAL) fluid of 38 IAPA patients [2]. The BAL samples were the first available samples with mycological evidence for IAPA (positive culture and/or galactomannan optical density index ≥ 1.0, Supplementary Table 1). We refer to the original paper for further information on ethical approval, included patients, sample processing and nCounter methods [2].

We determined the differentially expressed genes (DEG) in these BAL samples between IAPA patients who had survived ($n=20$) versus those who had died ($n=18$) 90 days after ICU admission (Fig. 1A, Supplementary Fig. 1).

Strikingly, eight of the 11 downregulated DEGs were implicated in antifungal immunity, with three downregulated DEGs (*CLEC7A, SIGLEC15, LGALS3*) encoding proteins that are directly linked to recognition of *Aspergillus* (Supplementary Table 2) [4–6]. Hypergeometric enrichment pathway analysis in Cytoscape using the ClueGO plug-in using two separate pathway libraries (GO biological process and WikiPathways) showed downregulation of pathways linked to superoxide anion generation and several cellular differentiation and effector functions (Fig. 1C). Kaplan–Meier analysis using a score calculated from the geometric mean expression of *CLEC7A, SIGLEC15* and *LGALS3* confirmed the association between lower expression of these genes and IAPA mortality in our cohort (Fig. 1D).

Among the 23 significantly upregulated DEGs, several genes were linked to the vascular endothelial growth factor (VEGF) signaling, such as *FLT1* (encoding VEGFR-1). VEGFR-1 signaling has been implicated in acute respiratory distress syndrome (ARDS) development while downregulation of *FLT1* promoter activity has been associated with protective effects in ARDS [3]. Pathway analysis confirmed upregulation of this signaling pathway in non-survivors (Fig. 1B). Given this finding, we assessed whether the protein VEGF is associated with IAPA mortality as well using a dataset of BAL VEGF levels obtained from 40 patients, of whom 38 were included in the gene expression dataset [2]. We found a significant association

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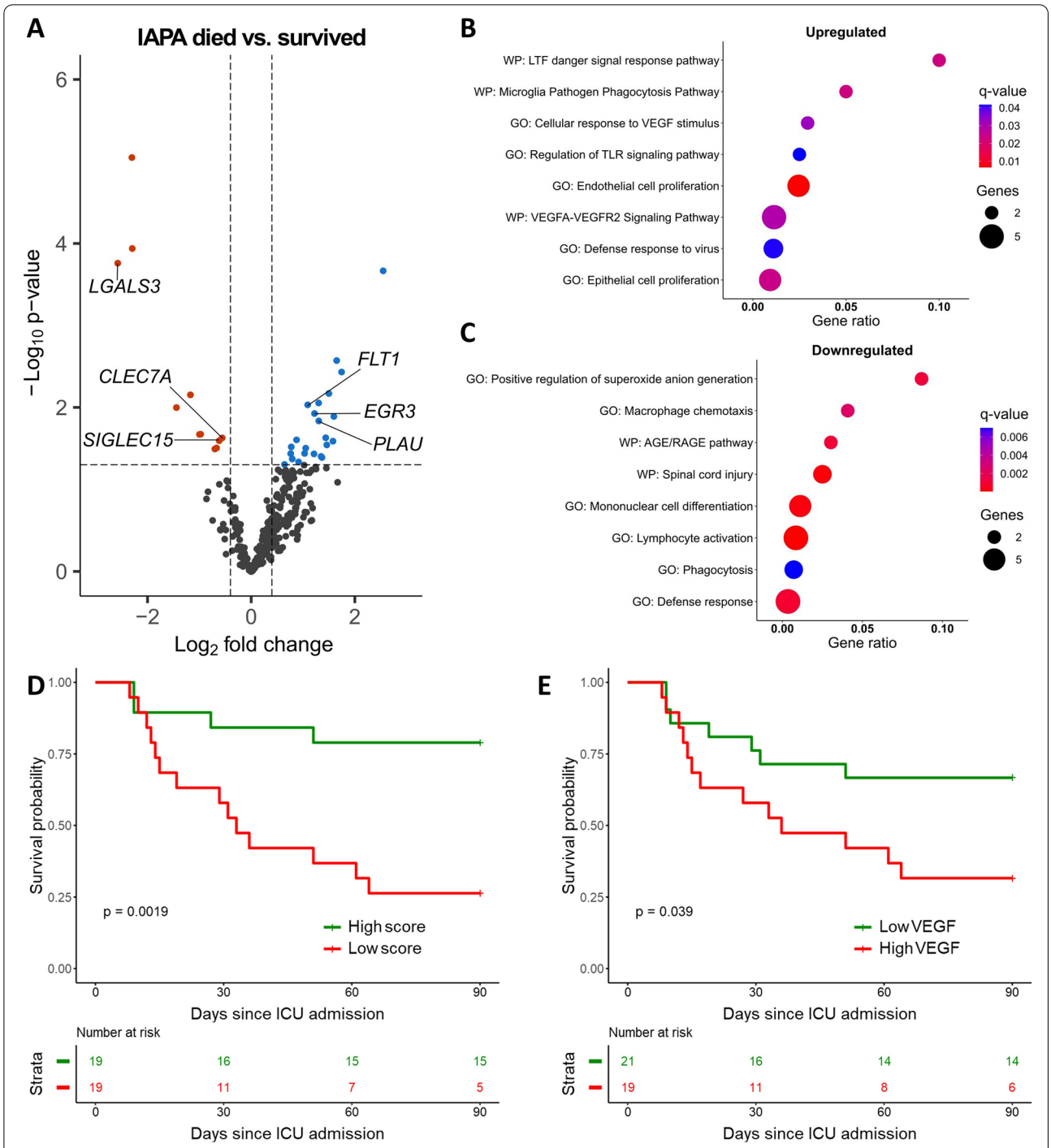

Fig. 1 Panel **A** Volcano plot of differentially expressed genes (DEGs) between IAPA patients who died vs. survived. Several genes of interest for *Aspergillus* recognition or VEGF signaling are pointed out. For the full volcano plot, see Supplementary Fig. 1. Panels **B** and **C** Dot plots showing a selection of significant pathways obtained by hypergeometric enrichment pathway analysis using GO biological process (GO) and WikiPathways (WP) as ontology libraries in Cytoscape using the ClueGO plug-in. Benjamini–Hochberg correction for multiple testing was used to obtain *q* values. Panel **E** Kaplan–Meier analysis of the impact of high or low expression of three genes implicated in *Aspergillus* recognition (*LGALS3*, *SIGLEC15*, *CLEC7A*). Score for each patient was constructed by calculating the geometric mean expression of these three genes. Cut-off value for low vs. high recognition score was determined by Weka OneR, which uses the minimum-error attribute for prediction, discretizing numeric attributes. Panel **F** Kaplan–Meier analysis of the impact of high or low VEGF levels on 90-day mortality in IAPA patients. Cut-off value (396 pg/mL) for low vs. high VEGF was determined by Weka OneR

between higher BAL VEGF levels and 90-day mortality in Kaplan–Meier analysis (Fig. 1E).

We conclude that decreased lower respiratory tract expression of genes related to anti-*Aspergillus* immunity and increased VEGF levels and VEGF-related signaling are associated with increased mortality in IAPA patients. The biological plausibility of the identified actors in IAPA mortality strengthens their validity. Further research in larger and separate validation cohorts (not confined to IAPA patients only) is needed to explore the clinical potential of these findings, which may translate in identification and development of prognostic biomarkers. The mechanisms of the transcriptional differences in fungal recognition mediators (e.g. due to underlying genetic variation) and ways to correct these should be investigated, as these may be potential targets for adjuvant immunomodulatory therapy in IAPA.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1007/s00134-022-06958-w.

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Declarations

Conflicts of interest
SF received travel grants from Pfizer. JW received investigator-initiated grants from Pfizer, Gilead and MSD and speakers' and travel fees from Pfizer, Gilead and MSD, and declares participation in advisory boards of Pfizer and Gilead, and receipt of study drugs from MSD. CV received speaker fees from Pfizer. KL received consultancy fees from Gilead, speaker fees from FUJIFILM Wako, Pfizer and Gilead and a service fee from TECOmedical. YD reports speakers' and travel fees from Pfizer and participation in advisory boards of Pfizer. The other authors declare no conflict of interest.

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