P058 PERSISTENCE OF NEUTROPHIL ABNORMALITIES IN COVID-19 CONVALESCENCE

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Background/Aims
Patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may develop acute respiratory inflammation, due to an exaggerated immune response and some develop chronic complications. Neutrophils play a major role in the pathology of inflammatory diseases and have been shown to contribute to lung and vascular damage in COVID-19. Our aim was to establish a relationship between neutrophil phenotype and disease severity and to determine whether neutrophil abnormalities persist in convalescent patients.

Methods
Peripheral blood samples were obtained from acute COVID-19 patients ($n = 74$), follow-up (FU) patients discharged following inpatient admission ($n = 56$), a median of 87 days after discharge, and healthy controls (HCs, $n = 22$). Patients were stratified by disease severity based on inspired oxygen (FiO2) and admission to intensive care (ICU). Neutrophils were isolated from whole blood by negative selection for phenotyping and functional analysis. PBMC Isolation Tubes were used to quantify and phenotype low density neutrophils (LDNs) within the PBMC fraction. For quantification of reactive oxygen species (ROS) production, isolated neutrophils were incubated with a ROS reactive dye, DHR-123 and stimulated with PMA. All samples were stained and fixed prior to analysis by flow cytometry.

Results
There was a marked increase in neutrophils expressing the activation and degranulation markers, CD64 ($P < 0.0001$) and CD63 ($P < 0.0001$) and a reduction in neutrophils expressing the maturity markers, CD10 ($P < 0.0005$) and CD101 ($P < 0.0005$) in patients with acute COVID-19 compared to HCs. Increased frequency of neutrophils expressing CD64 ($P < 0.005$), CD63 ($P < 0.01$) and expressing decreased CD101 ($P < 0.0001$) were also detected in FU patients compared to HCs. Notably, $42.3 \pm 4.4\%$ of neutrophils were $CD101^{lo}$ in FU patients, compared to $29.0 \pm 3.7\%$ in acute patients and $9.6 \pm 4.1\%$ in HCs. These changes were most apparent in FU patients recovering from severe COVID-19 compared to mild or moderate disease. The frequency of LDNs in PBMCs from acute patients was significantly higher than HCs ($P < 0.0001$), and correlated with disease severity. Similarly, the frequency of LDNs in FU patients was significantly higher than in HCs ($P < 0.0005$). We found a trend towards higher basal ROS production in acute and FU patients, but a blunted response to PMA stimulated ROS production in neutrophils from acute patients versus HCs ($P < 0.0001$). Impaired ROS production persisted in FU patients compared to HCs ($P < 0.01$).

Conclusion
Circulating neutrophils in acute COVID-19 have an altered phenotype and comprise immature and activated cells. This altered phenotype persisted in convalescence and may contribute to the persistence of symptoms and an increased susceptibility to subsequent infections. Future work will aim to investigate the functional implications of these findings.

Disclosure
T.O. Williams: None. **V. Kästele:** None. **E.R. Mann:** None. **S.B. Knight:** None. **M. Menon:** None. **C. Jagger:** None. **S. Khan:** None. **J.E. Konkel:** None. **T.N. Shaw:** None. **M. Rattray:** Consultancies; M.R. has a paid consultancy with AstraZeneca. **L. Pearmain:** None. **A. Horsley:** None. **A. Ustianowski:** None. **I. Prise:** None. **N.D. Bakerly:** None. **P.M. Dark:** None. **G.M. Lord:** Corporate appointments; G.M.L. is cofounder and scientific advisory board member of Gritstone Oncology Inc., which is a public company that develops therapeutic vaccines (primarily for the treatment of cancer). **A. Simpson:** None. **T. Felton:** None. **L. Ho:** None. **M. Feldmann:** None. **I. Bruce:** None. **J.R. Grainger:** None. **T. Hussell:** None.