

Oral Presentation

## Spatial heterogeneity of the immune compartment within the lungs of critical COVID-19 patients

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Acute respiratory distress syndrome (ARDS) is a defining feature of severe infection with the SARS-CoV-2 virus. Approaches to understand the immune response during COVID-19 are largely confined to characterisation of circulating leukocytes, however this approach excludes the most relevant cells that are active at the site of infection and injury.

The aim of this study was to characterise the immune landscape across the lungs of COVID-19 patients. Lung samples from three critical COVID-19 patients were assessed for histopathology, viral load, and distribution using qPCR, in situ hybridisation and immunohistochemistry. Leukocyte distribution was then assessed, and the transcript profile of selected areas examined against the >1800 genes in the Cancer Transcriptome Atlas panel on the NanoString GeoMx Digital Spatial Profiling platform.

Lung samples exhibited a spectrum of typical COVID-19 pathology with diffuse alveolar damage consistent with hyaline membrane and

type II pneumocyte hyperplasia, interstitial inflammation, organising pneumonia and thrombi. All tissues tested positive for SARS-CoV-2 RNA using qPCR, whilst spatially resolved techniques revealed only few and sparsely distributed cells carrying the viral nucleocapsid protein. Multiplexed immunofluorescence for lymphocytes (CD3+) and macrophages (CD68+) was used to select areas of immune enrichment for spatial transcriptomic profiling. These targeted analyses highlighted functional pathways involved in the interferon gamma response, TCR activation and antigen presentation. Comparison across immune-enriched areas identified a heterogeneity in lung infiltrates with spatial separation of chemokine and complement production. Our data identify pathological immune pathways that are amenable to therapeutic intervention in critical disease.