

Materials and Methods

Study cohort and approval. The specific use of each autopsy sample in each experiment is provided in [supplementary table 1](#). All safety precautions were in line with recently published guidelines.[1] The study of autopsy samples does not meet the definition of Human Subject Research in Singapore, and all samples were anonymously coded in accordance with the Helsinki Declaration. The Agency of Science, Technology and Research, Singapore, provided approval for the use of control tissue materials in this study IRB: 2020 112.

Digital spatial profiling. NanoString spatial profiling[2, 3] was used to analyze 3 protein-level and 1,800 transcriptomic-level immune-markers simultaneously on formalin-fixed paraffin-embedded (FFPE) tissue slides. Following deparaffinization and antigen retrieval procedures, sections were simultaneously incubated overnight with fluorescent-labeled antibodies against CD3 (Origene), CD68 (Santa Cruz), and Pan-Cytokeratin (Novus Biologicals) or cytokeratin 8/18 (Novus Biologicals) to visualize morphological features in the regions of interest (ROIs). After staining, the tissues were scanned using a GeoMx DSP instrument to generate digital fluorescent images and select individual ROIs inside a geometric section. To carry out high-resolution multiplex profiling, each ROI was assigned as a CD3⁺ T-cell-rich or CD68⁺ macrophage-rich region. UV light was directed through a programmable digital micromirror device (DMD) or dual DMD (DDMD) to accurately illumine the ROI and cleave the photocleavable oligos (PC-oligos) from the selected region, which were then collected by microcapillary tube inspiration and dispensed into a 96-well plate. Inside the microplate, the single-molecule counting nCounter System enabled the digital counting of released oligos.[4, 5] Individual counts were normalized against the 75th percentile of the signal from their own ROI (Q3 normalization).[6]

Multiplex immunohistochemistry. Multiplex immunohistochemistry was performed using an Opal Multiplex IHC kit (Akoya Biosciences, California), as previously described.[7, 8, 9, 10] In brief, FFPE tissue sections were cut onto Bond Plus slides (Leica Biosystems, Richmond) and heated at 60°C for 20 min.[11] The tissue slides were subjected to deparaffinization, rehydration, and heat-induced epitope retrieval using a Leica Bond Max autostainer (Leica Biosystems, Melbourne) before endogenous peroxidase blocking (Leica Biosystems, Newcastle). Next, the slides were incubated with primary antibodies followed by incubation with polymeric HRP-conjugated secondary antibodies (Leica Biosystems, Newcastle) (supplementary table 2). The samples were incubated with Opal fluorophore-conjugated tyramide signal amplification (TSA) (Akoya Biosciences, California) at 1:100 dilution. The slides were rinsed with wash buffer (BOND Wash Solution 10X Concentrate) after each step. Following TSA deposition, the slides were again subjected to heat-induced epitope retrieval to strip the tissue-bound primary/secondary antibody complexes before further labeling. These steps were repeated until the samples were labeled with all six markers and spectral DAPI (Akoya Biosciences, California) at a 1:10 dilution. Finally, the slides were mounted in ProLong Diamond Anti-fade Mountant (Molecular Probes, Life Technologies, USA) and developed in the dark at room temperature for 24 h. Images were captured for each case under a Vectra 3 pathology imaging system microscope (Akoya Biosciences, California) and then analyzed and scored by a pathologist using inForm software (version 2.4.2; Akoya Biosciences) and HALO™ (Indica Lab). Raw images have been deposited in <https://immunoatlas.org/MIHC/210723-2/MIHC21048/> and <https://immunoatlas.org/MIHC/210723-2/MIHC21049/>.

Supplementary figure 1: Biological pathways enriched in COVID-19/HIV co-infection

(A) Relative estimated levels of immune cells determined by deconvolution of digital spatial profiling ROIs using CIBERSORTx (<https://cibersort.stanford.edu/>). (B) Biological pathways enriched in the liver, kidney, and lung ROIs of the COVID-19/HIV decedent compared with the COVID-19 decedents identified by gene set enrichment analysis. A positive enrichment score indicates enrichment of the process in the COVID-19/HIV decedent.

Supplementary figure 2: Increased macrophage response in the liver of the COVID-19/HIV decedent is associated with COVID-19 viral load

(A) Expression heatmap of genes related to T-cell activation or suppression in CD3-rich ROIs (*left*) and myeloid activation in CD68-rich ROIs (*right*) of the kidney. (B) Correlation between NP⁺ cells and T-cells in ROIs of the lung of the COVID-19/HIV (red; $p=0.06$) and COVID-19 (blue; $p=0.0004$) decedents. p -values were calculated by Wald tests. (C) Comparison of the abundance of activated macrophages in the liver between the COVID-19/HIV decedent and COVID-19 decedents. The p -value was calculated by a two-tailed U-test: *(<0.05).

Supplementary table 1: Study cohort and tissues obtained for analysis

| Patient ID | Sex | Survival since symptom onset (days) | Digital Spatial Profiling | mIHC | Clinical Diagnosis | Cause(s) of Death |
|-------------------|------------|--|----------------------------------|---------------------|---------------------------|--------------------------|
| Pt-HIV | Male | 46 | Lung, Liver, Kidney | Lung, Liver, Kidney | COVID-19; HIV; | RFRWS |
| Pt-CV1 | Male | 20 | Lung, Liver, Kidney | Lung, Liver, Kidney | COVID-19 | RFRWS |
| Pt-CV2 | Male | 21 | Lung, Liver | Lung, Liver | COVID-19 | RFRWS |
| Pt-CV3 | Male | 30 | Lung, Liver | Lung, Liver | COVID-19 | RFRWS |

Abbreviation: RFRWS: Respiratory failure related with SARS-CoV-2; mIHC: multiplex immunohistochemistry.

Supplementary table 2: Antibodies used for digital spatial profiling and multiplex immunohistochemistry

| Usage | Primary antibody | Company | Clone |
|--------------------------------|------------------|-------------------|----------------------|
| Digital Spatial Profiling | CD3 | Origene | UMAB54 |
| | CD68 | Santa Cruz | KP1 |
| | PanCK | Novus Biologicals | AE1/AE3 |
| | CK8/18 | Novus Biologicals | KRT8/803 + KRT18/835 |
| Multiplex immunohistochemistry | BATF3 | Abcam | Polyclonal |
| | BDCA2 | Merck | 10E6.1 |
| | CD206 | Santa Cruz | D-1 |
| | CD3 | Dako | Polyclonal |
| | CD80 | Abcam | EPR1157(2) |
| | CD83 | BioLegend | HB15e |
| | SARS-CoV-2 (NP) | Novus Biologicals | Polyclonal |

References

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