Comparison between non-pulmonary and pulmonary immune responses in a HIV decedent who succumbed to COVID-19

We read with interest the study by Manuel et al showing that chronic immunosuppression could protect against severe COVID-19 in liver transplant patients.\(^1\)\(^2\) Despite increased comorbidities, COVID-19 in liver transplant patients was not more severe than in non-transplant cohorts.\(^1\)\(^2\) We present findings from a COVID-19/HIV coinfected decedent who exhibited a significantly longer survival time (46 days) than that of three COVID-19 decedents (average 30 days) (figure 1A). Given the immunosuppressive effects of HIV,\(^2\) the prolonged survival of our COVID-19/HIV patient may reflect protection from severe COVID-19.

To investigate tissue-specific immune responses and viral load, we performed in-depth analyses of the patient’s immune landscape. Digital spatial profiling (online supplemental materials and methods) on liver, kidney and lung tissues from our study cohort (figure 1B) revealed that the COVID-19/HIV decedent had unique transcriptomic profiles, especially in the liver and kidney (figure 1C). The decedent exhibited a reduction of T-cells and elevation of macrophages only in the non-pulmonary tissues (figure 1D) and no difference in the abundance of natural killer cells and other immune cell types across tissue types (online supplemental figure 1A). Multiplex immunohistochemistry analysis (online supplemental materials and methods) further supported these findings (figure 1E) and additionally revealed increased T-cell and dendritic cell and decreased macrophage cell populations in the lung of the COVID-19/HIV decedent (figure 1E). Hence, at both transcriptomic and protein levels, prolonged survival of the COVID-19/HIV decedent involved differential immunological function in non-pulmonary tissues.

Differential gene expression analysis revealed an upregulation of myeloid cell lineage (CD68, CD63, CD163) and immunoregulatory (HLA-E, HLA-F) markers only in the non-pulmonary tissues of the COVID-19/HIV decedent while T-cell function-related markers (CD8, GZMB, MKI67) did not differ (figure 1F). Gene ontology enrichment analysis confirmed an enrichment in pathways related to myeloid cell activation exclusively in the non-pulmonary tissues (figure 1G, online supplemental figure 1B). Taken together, these imply that macrophages in the liver and kidney of the COVID-19/HIV decedent were activated and functional.

Liver-associated disease is a leading cause of death in patients with HIV.\(^4\)\(^5\) As liver injury has been reported in patients with COVID-19,\(^6\) we investigated the T-cell and myeloid cell responses in the

---

**Figure 1** Increased myeloid and reduced T-cell abundance characterises the liver and kidney but not the lungs of a COVID-19/HIV decedent. (A) Kaplan-Meier survival curve of COVID-19/HIV case (nCOVID-19/HIV = 1) and COVID-19 cases (nCOVID-19 = 3). P-value: Log-rank (Mantel-Cox) test. (B) Representative regions of interest (ROI) of the liver, kidney, and lung from the COVID-19/HIV decedent and one COVID-19 decedent. (C) Principal component analyses of transcriptional profiles of COVID-19/HIV and COVID-19 decedents from ROIs of the lung (nCOVID-19/HIV = 11, nCOVID-19 = 29), liver (nCOVID-19/HIV = 10, nCOVID-19 = 32), and kidney (nCOVID-19/HIV = 11, nCOVID-19 = 6). (D) Relative estimated levels of immune cells determined by deconvolution of digital spatial profiling ROIs using CIBERSORTx (https://cibersort.stanford.edu). Grey bars indicate the means. P-values were calculated by a two-tailed t-test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns (not significant). (E) Comparison of the abundance of various immune cell populations between ROIs of the COVID-19/HIV decedent and COVID-19 decedents by multiplex immunohistochemistry. P-values were calculated by a two-tailed U-test: *p<0.05, ns (not significant). (F) Differential gene expression of selected immune cell phenotypes and function-related genes between the COVID-19/HIV decedent and COVID-19 decedents. (G) Gene ontology analysis of pathways enriched in the COVID-19/HIV decedent compared with COVID-19 decedents.
These findings suggest that HIV as a comorbidity promotes virus-induced myeloid cell activation in response to SARS-CoV-2 infection in the absence of a viable T-cell response. Indeed, HIV-infected myeloid cells are long lived and resistant to the cytopathic effect. However, HIV infection renders them functionally impaired. Similarly, SARS-CoV-2-infected myeloid cells are also dysregulated and may not partake in the cascade signature of hyperinflammation. Particularly in the liver, where direct SARS-CoV-2 infection and high viral load likely contribute to liver injury, HIV-mediated immunosuppression may lower the risk of COVID-19 disease progression. Close monitoring of the immune status of myeloid cells in other organs such as the liver and further research on the COVID-19–HIV relationship is warranted to develop optimal treatment strategies.

Denise Goh,1 Justina Nadia Lee,1 Tracy Tien,1 Jeffrey Chun Tatt Lim,1 Sherlly Lim,1 An Sen Tan,2 Jin Liu,3 Benedict Tan,1 Joe Yeong4,5,6,7,8

1Institute of Molecular and Cell Biology, Agency of Science, Technology and Research, Singapore
2Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
3Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore
4Cancer Science Institute of Singapore, National University of Singapore, Singapore
5Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
6Department of Pathology, Pathology, University of Western Sydney, Greater Western Sydney, New South Wales, Australia
7Singapore Immunology Network, A*STAR, Singapore
8Department of Anatomical Pathology, Singapore General Hospital, Singapore

Correspondence to Dr Joe Yeong and Dr Benedict Tan, Institute of Molecular and Cell Biology, Agency of Science, Technology and Research, Singapore; yeongjo@imcb.a-star.edu.sg and Dr Jin Liu, Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore; jin.liu@duke-nus.edu.sg

Correction notice This article has been corrected since it published Online First. Author affiliations have been updated.

Contributors JY, BT and JL conceived and directed the study. DG, AST, BT and JL collated and interpreted the data and performed biostatistical analysis. JNL, TT, SL and JCTL performed immunohistochemical techniques and scoring. DG and BT drafted the manuscript and final approval of all authors.

Funding The authors received funding from A*STAR Career Development Award (C21112056).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by Agency of Science, Technology and Research, IRB: 2021 112.

Provenance and peer review Not commissioned; externally peer reviewed.

Gut Month 2021 Vol 0 No 0

Gut first published as 10.1136/gutjnl-2021-324754 on 10 August 2021. Downloaded from http://gut.bmj.com/ on April 26, 2022 by guest. Protected by copyright.
Supplemental material. This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

This article is made freely available for use in accordance with BMJ’s website terms and conditions for the duration of the covid-19 pandemic or until otherwise determined by BMJ. You may use, download and print the article for any lawful, non-commercial purpose (including text and data mining) provided that all copyright notices and trade marks are retained.

© Author(s) (or their employer(s)) 2021. No commercial re-use. See rights and permissions. Published by BMJ.

To cite Goh D, Lee JN, Tien T, et al. Gut Epub ahead of print: [please include Day Month Year]. doi:10.1136/gutjnl-2021-324754

Received 24 March 2021
Accepted 30 July 2021

Gut 2021;0:1–3. doi:10.1136/gutjnl-2021-324754

ORCID iD
Joe Yeong http://orcid.org/0000-0002-6674-7153

REFERENCES
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 illuminate 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 fIHC 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

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Survival since symptom onset (days)</th>
<th>Digital Spatial Profiling</th>
<th>mIHC</th>
<th>Clinical Diagnosis</th>
<th>Cause(s) of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt-HIV</td>
<td>Male</td>
<td>46</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19; HIV;</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV1</td>
<td>Male</td>
<td>20</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV2</td>
<td>Male</td>
<td>21</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV3</td>
<td>Male</td>
<td>30</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
</tbody>
</table>

Abbreviation: RFRWS: Respiratory failure related with SARS-CoV-2; mIHC: multiplex immunohistochemistry.
**Supplementary table 2: Antibodies used for digital spatial profiling and multiplex immunohistochemistry**

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


Supplementary figure 2

A

Gene expression in kidney ROIs

T cell activation

CD4
CD3E
CD8A
CD8B
CD83
GZMK
GZMB
NKp7
HAVCR2
LAIR1
LILRB1
CXCL13
LAG3

CD3-rich
CD8-rich

COVID-19/HIV
COVID-19

Relative gene expression
-2
2

T cell suppression

B

Correlation between T-cells and NP^+ cells in lung

Percentage of CD3^+ cells

Percentage of NP^+ cells

COVID-19/HIV
r = -0.73

COVID-19
r = 0.60

C

Abundance of activated MΦ in liver

Percentage of cells

HIV+

n=10

HIV-

n=25
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 fIHC 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/](https://immunoatlas.org/MIHC/210723-2/MIHC21048/) and [https://immunoatlas.org/MIHC/210723-2/MIHC21049/](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**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Survival since symptom onset (days)</th>
<th>Digital Spatial Profiling</th>
<th>mIHC</th>
<th>Clinical Diagnosis</th>
<th>Cause(s) of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt-HIV</td>
<td>Male</td>
<td>46</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19; HIV;</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV1</td>
<td>Male</td>
<td>20</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV2</td>
<td>Male</td>
<td>21</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV3</td>
<td>Male</td>
<td>30</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
</tbody>
</table>

*Abbreviation: RFRWS: Respiratory failure related with SARS-CoV-2; mIHC: multiplex immunohistochemistry.*
**Supplementary table 2: Antibodies used for digital spatial profiling and multiplex immunohistochemistry**

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


BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s).
**Supplementary figure 1**

**A**

Liver expression levels for COVID-19 and COVID-19/HIV groups. The bars represent relative expression levels, and the dots indicate individual patient data. The groups are as follows:

- **COVID-19**
- **COVID-19/HIV**

**B**

Enrichment scores for liver, kidney, and lung tissues comparing COVID-19 and COVID-19/HIV groups. The scores are categorized by biological processes:

- Myeloid cell activation involved in immune response
- Positive regulation of immune system process
- Innate immune response
- Viral process

Enrichment scores with their respective ES, NES, and P.adj values are provided for each group.
Supplementary figure 2

A  Gene expression in kidney ROIs

<table>
<thead>
<tr>
<th>T cell activation</th>
<th>Myeloid activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>CD63</td>
</tr>
<tr>
<td>CD3E</td>
<td>CD14</td>
</tr>
<tr>
<td>CD8A</td>
<td>CD68</td>
</tr>
<tr>
<td>CD8B</td>
<td>CD163</td>
</tr>
<tr>
<td>CD83</td>
<td>MRC1</td>
</tr>
<tr>
<td>GZMK</td>
<td>IL6</td>
</tr>
<tr>
<td>GZMB</td>
<td>IFNγR1</td>
</tr>
<tr>
<td>NKG7</td>
<td>IFNγR2</td>
</tr>
<tr>
<td>HAVCR2</td>
<td></td>
</tr>
<tr>
<td>LAIR1</td>
<td></td>
</tr>
<tr>
<td>LILRB1</td>
<td></td>
</tr>
<tr>
<td>CXCL13</td>
<td></td>
</tr>
<tr>
<td>LAG3</td>
<td></td>
</tr>
</tbody>
</table>

Relative gene expression
-2 to 2

B  Correlation between T-cells and NP+ cells in lung

C  Abundance of activated MΦ in liver

Correlation between T-cells and NP+ cells in lung
- Percentage of CD3+ cells vs Percentage of NP+ cells
  - COVID-19/HIV: r = -0.73
  - COVID-19: r = 0.60

Abundance of activated MΦ in liver
- HIV+: n=10
- HIV-: n=25
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 fIHC 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/](https://immunoatlas.org/MIHC/210723-2/MIHC21048/) and [https://immunoatlas.org/MIHC/210723-2/MIHC21049/](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**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Survival since symptom onset (days)</th>
<th>Digital Spatial Profiling</th>
<th>mIHC</th>
<th>Clinical Diagnosis</th>
<th>Cause(s) of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt-HIV</td>
<td>Male</td>
<td>46</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19; HIV;</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV1</td>
<td>Male</td>
<td>20</td>
<td>Lung, Liver, Kidney</td>
<td>Lung, Liver, Kidney</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV2</td>
<td>Male</td>
<td>21</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
<tr>
<td>Pt-CV3</td>
<td>Male</td>
<td>30</td>
<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
</tr>
</tbody>
</table>

*Abbreviation: RFRWS: Respiratory failure related with SARS-CoV-2; mIHC: multiplex immunohistochemistry.*
**Supplementary table 2: Antibodies used for digital spatial profiling and multiplex immunohistochemistry**

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

Supplementary figure 1

A

Liver

Kidney

Lung

Relative expression level

COVID-19 /HIV

COVID-19 /HIV

COVID-19 /HIV

COVID-19 /HIV

COVID-19 /HIV

NK Cells

Others

B

Liver

Kidney

Lung

Myeloid cell activation involved in immune response

Positive regulation of immune system process

Innate immune response

Viral process

Myeloid cell activation involved in immune response

Neutrophil activation involved in immune response

Positive regulation of immune system process

Innate immune response

Viral process

Viral process

Inflammatory response

Leukocyte chemotaxis

Regulation of immune response

ES

NES

P.adj

0.51

1.98

0.009

0.37

1.64

0.009

0.39

1.67

0.009

0.34

1.39

0.03

0.47

1.76

0.002

0.53

1.91

0.002

0.35

1.45

0.001

0.39

1.59

0.001

0.37

1.48

0.006

-0.39

-1.53

0.002

0.36

1.55

0.003

0.48

1.81

0.002

-0.36

-1.56

0.002
Supplementary figure 2

A

Gene expression in kidney ROIs

B

Correlation between T-cells and NP+ cells in lung

C

Abundance of activated MΦ in liver