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. Despite increased comorbidities, COVID-19 in liver transplant patients was not more severe than in non-transplant cohorts. 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, 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-cell 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. As liver injury has been reported in patients with COVID-19, we investigated the T-cell and myeloid cell responses in the liver. T-cell suppression-associated genes (HAVCR2, LAIR1, LILRB1, CXCL13, LAG3) were upregulated, and T-cell activation-associated genes (CD3, CD4, CD8, GZMB, GZMK) were downregulated in CD3-rich regions of the liver (figure 2A). T-cell genes were also broadly down-regulated in the kidney of the COVID-19/HIV decedent (online supplemental figure 2A). In line with a deficient T-cell response due to HIV infection, T-cell abundance was unrelated to SARS-CoV-2 viral load (NP+) in the COVID-19/HIV decedent but was positively associated with viral load in the COVID-19 decedents (figure 2B, online supplemental figure 2B).

In contrast to the T-cell response, a comparison of CD68-rich regions revealed upregulated myeloid cell activation-associated genes (CD63, CD14, CD68, IL6) in the liver (figure 2A) and kidney (online supplemental figure 2A) of the COVID-19/HIV decedent. A greater abundance of activated macrophages was similarly observed (figure 2C, online supplemental figure 2C), together with a significantly higher viral load (NP+) (figure 2D). Interestingly, macrophage

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=11), liver (nCOVID-19/HIV = 10, nCOVID-19=10), and kidney (nCOVID-19/HIV = 11, nCOVID-19=11). (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: *(<0.05), **(<0.01), ***(<0.001), ****(<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: *(<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.
abundance positively correlated with viral load in the liver of the COVID-19/HIV decedent but not in the COVID-19 decedents (figure 2E).

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.7 8 However, HIV infection renders them functionally impaired.7 8 Similarly, SARS-CoV-2-infected myeloid cells are also dysregulated9 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,6 10 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.

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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, TL, SL and JCTL performed immunohistochemical techniques and scoring. DG and BT drafted the manuscript and final approval of all authors.

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DG, JNL, TT and JCTL contributed equally.


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The grant number C21112056 is incorrect and should be C210112056.

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Materials and Methods

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

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References

Supplementary figure 1

A

Liver

Kidney

Lung

Relative expression level vs. COVID-19 and HIV infection

NK Cells vs. Others

B

Liver

Kidney

Lung

Ranked list metric vs. Enrichment Score

Myeloid cell activation involved in immune response
Negative regulation of immune response process
 Innate immune response
 Viral process

ES NES P.adj

Liver

COVID-19/HIV COVID-19

Ranked in Ordered Dataset

0.51 1.98 0.009

0.37 1.64 0.009

0.39 1.67 0.009

0.34 1.39 0.03

Kidney

COVID-19/HIV COVID-19

Ranked in Ordered Dataset

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

Lung

COVID-19/HIV COVID-19

Ranked in Ordered Dataset

ES NES P.adj

Viral process

-0.39 -1.53 0.002

0.36 1.55 0.003

0.48 1.81 0.002

-0.38 -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
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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.

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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>
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<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>
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<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
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<tr>
<td>Pt-CV3</td>
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<td>Lung, Liver</td>
<td>Lung, Liver</td>
<td>COVID-19</td>
<td>RFRWS</td>
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</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>
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<tr>
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<tr>
<td>CD3</td>
<td>Origene</td>
<td>UMAB54</td>
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<tr>
<td>CD68</td>
<td>Santa Cruz</td>
<td>KP1</td>
<td></td>
</tr>
<tr>
<td>PanCK</td>
<td>Novus Biologicals</td>
<td>AE1/AE3</td>
<td></td>
</tr>
<tr>
<td>CK8/18</td>
<td>Novus Biologicals</td>
<td>KRT8/803 + KRT18/835</td>
<td></td>
</tr>
<tr>
<td><strong>Multiplex immunohistochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BATF3</td>
<td>Abcam</td>
<td>Polyclonal</td>
<td></td>
</tr>
<tr>
<td>BDCA2</td>
<td>Merck</td>
<td>10E6.1</td>
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</tr>
<tr>
<td>CD206</td>
<td>Santa Cruz</td>
<td>D-1</td>
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<tr>
<td>CD3</td>
<td>Dako</td>
<td>Polyclonal</td>
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<td>CD80</td>
<td>Abcam</td>
<td>EPR1157(2)</td>
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<td>CD83</td>
<td>BioLegend</td>
<td>HB15e</td>
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<tr>
<td>SARS-CoV-2 (NP)</td>
<td>Novus Biologicals</td>
<td>Polyclonal</td>
<td></td>
</tr>
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</table>
References


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