**Materials and Methods**

***Study cohort.*** Patient 1 presented with acute symptoms (fever of 37.3°C, pharyngitis, choking, bronchospasm and dysphagia, loss of smell and taste, anorexia, expectoration, migraine headache, chills in the spinal cord, palate petechiae, nausea and diarrhoea, weight loss by 8.5%, etc.) on 7 March 2020, which corresponded with the first wave of the COVID-19 pandemic, when polymerase chain reaction (PCR) diagnostic testing was not available yet. On 11 May 2020, the patient had a negative test result on diagnostic PCR, although their symptoms persisted (Supplementary Table 1). A year later, on 4 May 2021, the patient presented with generalized abdominal pain, loss of appetite, and nausea. Urgent exploratory laparotomy and appendectomy was carried out, and tissue histology showed reactive lymphoid hyperplasia. A biopsy of the skin of the lower limb was also obtained, and the patient was diagnosed with superficial and deep perivascular dermatitis. Before the procedures, the patient had a negative test result for SARS-CoV-2.

Patient 2 presented with acute symptoms (intensive headache, upper stomach pain, nausea, diarrhoea, myalgias and fatigue) on 14 March 2020 (Supplementary Table 1) and received a positive result on diagnostic PCR from a nasopharyngeal swab on 26 March 2020. Over the next two months, the patient reported that several symptoms worsened. On 8 May 2020, the patient underwent a PCR test with a negative result for SARS-CoV-2, although symptoms persisted. On 12 August 2020 and 1 September 2020, the patient underwent partial breast resection and margin control surgery, respectively. Before the procedures, the patient underwent preoperative PCR testing for SARS-CoV-2 and received a negative result.

***Study approval.*** We obtained tissue samples from two patients who were confirmed to have COVID-19 infection and who subsequently underwent surgery for unrelated conditions (Supplementary Table 1). The age of the patients ranged from 44 to 45 years. Both patients tested negative for SARS-CoV-2 using two consecutive PCR nasopharyngeal swabs at the time of surgery. The Agency of Science, Technology and Research (A\*STAR) granted approval for the use of control tissue materials in this study (IRB: 2021 161).

***Specimen collection.*** The type of tissues obtained from the patients is included in Supplementary Table 1. Tissues from more than 20 patients not affected by COVID-19 were obtained in 2018 or earlier for use as a negative control. All explanted fresh tissues samples were sent to the Department of Anatomical Pathology of the Singapore General Hospital for further formalin-fixed paraffin-embedded (FFPE) processing and analysis.

***Multiplex immunohistochemistry.*** mIHC was performed using an Opal Multiplex fIHC kit (Akoya Biosciences, USA), as previously described.[1, 2, 3] In brief, 4-µm thick FFPE tissue sections were subjected to deparaffinization, rehydration and heat-induced epitope retrieval using a Leica Bond Max autostainer (Leica Biosystems, Melbourne) before peroxidase blocking (Leica Biosystems, Newcastle).[4] Next, the slides were incubated with primary antibodies against the SARS-CoV-2 nucleocapsid protein (Novus Biologicals, Cat# NB100-56576, Polyclonal), CD68 (Agilent-Dako, Cat# M0876, PG-M1), CD45 (Agilent-Dako, Cat# M0701, 2B11 + PD7/26) or cytokeratin/EpCAM (Agilent-Dako, Cat# M3515, AE1/AE3; BioLegend, Cat# 324202, 9C4), followed by incubation with polymeric HRP-conjugated secondary antibodies (Leica Biosystems, Newcastle). Then, the samples were incubated with Opal tyramide signal amplification (TSA) (Akoya Biosciences, USA). Following TSA deposition, the slides were again subjected to the extraction of heat-induced epitopes to remove tissue-bound complexes of primary/secondary antibodies before further labelling. These steps were repeated until the samples were labelled with all four markers and a spectral DAPI (Akoya Biosciences, USA) mounting in ProLong Diamond Anti-fade Mountant (Molecular Probes, Life Technologies, USA). Images were captured for each case under the Vectra 3 pathology imaging system microscope (Akoya Biosciences, USA), and then analysed and scored by a pathologist using inForm software (version 2.4.2; Akoya Biosciences) and HALO (Indica Labs). Raw images have been deposited in <https://immunoatlas.org/MIHC/211022-2/MIHC21711/> and <https://immunoatlas.org/MIHC/211022-1/MIHC21710/>.

***RNAscope.***  RNAscope in situ hybridisation (Advanced Cell Diagnostics, USA) assay was performed according to standard manufacturer protocol,[5] on FFPE tissue sections. Deparaffinised tissues were subjected to peroxidase inhibition and pre-treatments, followed by incubation with SARS-CoV2 anti-sense specific probe v-nCoV2019-S (Cat# 848561), which targets the positive-sense viral RNA, and SARS-CoV2 sense specific probe v-nCoV2019-orf1ab-sense (Cat# 859151), which targets the negative-sense genomic viral RNA. Incubation was performed prior to haematoxylin counterstain and mounting in VectaMount Mounting Medium (Vector Labs, Cat# H-5000). RNAscope 2.5 HD Duplex Reagent Kit (Cat# 322430) was used for probe/RNA detection.[6, 7] The corresponding positive and negative controls were included in the assay as per the manufacturer’s recommendation. Images were acquired using an Axio Scan.Z1 (Carl Zeiss, Germany).

**Supplementary Table 1. Cohort characteristics.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Patient Profile** | **ID** | 1  | 2  |
| **Age/Sex** | 44 /Female | 45/Female |
| **Pertinent medical history / comorbidities** | Peritonitis, appendiceal lymphoid hyperplasia, and adjacent colon lesion  | Ductal carcinoma in situ |
| **COVID-19 History** | **Date of symptom onset** | 07/03/2020  | 14/03/2020 |
| **Hospitalisation (Y/N)** | N | Y |
| **ICU admission (Y/N)** | N | N |
| **Symptomatic (Y/N)** | Y | Y |
| **Post COVID-19 symptoms and complication(s)** | **Otorhinolaryngology:**Lingual tonsil hyperplasia, mucositis, outbreaks of tongue inflammation, laryngospasm, recurrent pharyngitis with secondary bacterial infection, and tinnitus**Ocular:**Loss of near vision, conjunctivitis, and dry eye**Respiratory:** Bronchospasm, bronchial hyperresponsiveness to respiratory effort, inspiratory peak to outbreaks**Cardiac:** Reactive sinus tachycardia with minimal effort**Digestive:**Inflammatory bowel disease **Neurological:** Chronic fatigue syndrome/post-COVID-19 encephalomyelitis, headaches, dizziness, mental fog, and loss of spatial orientation**Osteomuscular:** Myalgias, cervicalgia, and dorsalis with outbreaks**Dermatology:** Flare-up skin eruptions for 18 months co-occurring with the acute phase of COVID-19**Gynaecological:**Menstrual disorders | **Respiratory:**Mild paralysis of right hemidiaphragm and dyspnoea**Cardiac:**Tachycardias and high blood pressure**Digestive:**Stomachache, loss of appetite, and pain in the liver and spleen area**Neurological:**Headaches, mental confusion, talking difficulties, mood swings, sleeping disorders, and lack of concentration**Osteomuscular:**Muscular aches, arthralgias, asthenia, and extremity debilitation**Dermatology:**Spontaneous bruises |
| **Surgical History and Sample Collection** | **Type of Surgery** | Exploratory laparotomy and appendectomy  | Partial breast resection |
| **Surgery date****(Days upon symptom onset)** | 06/05/2021(426 days) | 04/09/2020(175 days) |
| **Tissue(s) obtained** | Appendix, skin | Breast, Sentinel lymph nodes |
| **Investigation and Results** | **RNAscope for SARS-CoV-2 (+/-)** | + (appendix) | + (breast) |
| **IHC for SARS-CoV-2 (+/-)** | + (appendix) | + (breast) |
| **Multiplex IHC for SARS-CoV-2 (+/-)** | + (appendix) | + (breast) |

**Supplementary Table 2: Antibodies used for multiplex immunohistochemistry**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primary antibody** | **Manufacturer** | **Clone** | **Catalog number** |
| SARS-CoV-2 nucleocapsid protein  | Novus Biologicals | Polyclonal  | NB100-56576 |
| CD68 | Agilent-Dako | PG-M1 | DKO.M0876 |
| CD45 | Agilent-Dako | 2B11 + PD7/26 | DKO.M0701 |
| Cytokeratin | Agilent-Dako | AE1/AE3 | M3515 |
| EpCAM | BioLegend | 9C4 | Biolegend 324202 |

**References**

1 Lim JCT, Yeong JPS, Lim CJ, Ong CCH, Wong SC, Chew VSP*, et al.* An automated staining protocol for seven-colour immunofluorescence of human tissue sections for diagnostic and prognostic use. Pathology 2018;**50**:333-41.

2 Ng HHM, Lee RY, Goh S, Tay ISY, Lim X, Lee B*, et al.* Immunohistochemical scoring of CD38 in the tumor microenvironment predicts responsiveness to anti-PD-1/PD-L1 immunotherapy in hepatocellular carcinoma. J Immunother Cancer 2020;**8**.

3 Yeong J, Lim JCT, Lee B, Li H, Ong CCH, Thike AA*, et al.* Prognostic value of CD8 + PD-1+ immune infiltrates and PDCD1 gene expression in triple negative breast cancer. Journal for ImmunoTherapy of Cancer 2019;**7**:34.

4 Yeong J, Lim JCT, Lee B, Li H, Chia N, Ong CCH*, et al.* High Densities of Tumor-Associated Plasma Cells Predict Improved Prognosis in Triple Negative Breast Cancer. Frontiers in immunology 2018;**9**:1209-.

5 Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A*, et al.* RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 2012;**14**:22-9.

6 Chandrashekar A, Liu J, Martinot AJ, McMahan K, Mercado NB, Peter L*, et al.* SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science (New York, NY) 2020;**369**:812-7.

7 Tostanoski LH, Wegmann F, Martinot AJ, Loos C, McMahan K, Mercado NB*, et al.* Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. Nature medicine 2020;**26**:1694-700.