Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS)

G M-K Tse, K-F To, P K-S Chan, A W I Lo, K-C Ng, A Wu, N Lee, H-C Wong, S-M Mak, K-F Chan, D S C Hui, J J-Y Sung, H-K Ng

Background: Severe acute respiratory syndrome (SARS) became a worldwide outbreak with a mortality of 9.2%. This new human emergent infectious disease is dominated by severe lower respiratory illness and is aetiologically linked to a new coronavirus (SARS-CoV).

Methods: Pulmonary pathology and clinical correlates were investigated in seven patients who died of SARS in whom there was a strong epidemiological link. Investigations include a review of clinical features, morphological assessment, histochemical and immunohistochemical stainings, ultrastructural study, and virological investigations in postmortem tissue.

Results: Positive viral culture for coronavirus was detected in most premortem nasopharyngeal aspirate specimens (five of six) and postmortem lung tissues (two of seven). Viral particles, consistent with coronavirus, could be detected in lung pneumocytes in most of the patients. These features suggested that pneumocytes are probably the primary target of infection. The pathological features were dominated by diffuse alveolar damage, with the presence of multinucleated pneumocytes. Fibrogranulation tissue proliferation in small airways and airspaces (bronchiolitis obliterans organising pneumonia-like lesions) in subpleural locations was also seen in some patients.

Conclusions: Viable SARS-CoV could be isolated from postmortem tissues. Postmortem examination allows tissue to be sampled for virological investigations and ultrastructural examination, and when coupled with the appropriate lung morphological changes, valuable to confirm the diagnosis of SARS-CoV, particularly in clinically unapparent or suspicious but unconfirmed cases.

MATERIAL AND METHODS

Case selection

The chain of events that led to the outbreak in Hong Kong and various countries has been described.8–12 Our index patient was a 26 year old man with a history of contact with the first index case when staying in the same hotel.8 10 We describe seven patients who died of SARS, six of whom stayed in the same ward as our index patient and one who had previous contact with the index patient in the accident and emergency department. SARS was diagnosed according to the World Health Organisation criteria.13 Routine microbiological investigations, including serology, were performed on these patients. All seven patients eventually succumbed and necropsies were performed.

SARS-CoV isolation

Virus isolation was performed using African green monkey kidney (Vero) cells. When a diffuse, refractile, rounding, cytopathic effect was noted, the Vero cell culture supernatant was passaged to a fresh Vero cell culture tube to ensure reproducibility of the cytopathic effect. SARS-CoV in the supernatant was further confirmed by reverse transcription polymerase chain reaction using primers described previously.14

 Necropsy and histological assessments

Full necropsies (excluding the brain) were performed in patients 1–6. A limited necropsy (lung and heart) was performed in patient 7. Sections (4 μm thick) were prepared from the 10% formalin fixed, routinely processed, paraffin wax embedded blocks and were stained with haematoxylin and eosin. The following histochemical stainings were performed: periodic acid Schiff, Grocott’s hexamine silver, Gram, Ziehl Neelsen, Warthin Starry, and Masson’s trichrome. The standard avidin–biotin method was used for immunohistochemical study using markers for herpes simplex virus (HSV; BioGenex, San Ramon, California, USA; 1/200 dilution), cytomegalovirus (CMV; Dako, Glostrup, Denmark; 1/25 dilution), lymphoid marker (CD45; Dako; 1/150 dilution), B cell marker (L26; Dako; 1/25 dilution).

Abbreviations: BOOP, bronchiolitis obliterans organising pneumonia; CMV, cytomegalovirus; CoV, coronavirus; CXR, chest x-ray; DAD, diffuse alveolar damage; EM, electron microscopy; HSV, herpes simplex virus; SARS, severe acute respiratory syndrome.
Lung pathology of SARS

1/240 dilution), T cell marker (CD3; Dako; 1/200 dilution),
natural killer cell marker (CD56; Monosan, Uden, the
Netherlands; 1/200 dilution), histiocyte markers (CD68; clone
KP1; Dako; 1/2000 dilution; Mac 387; Dako; 1/400 dilution),
and epithelial marker (cytokeratin; AE1/AE3; Dako; 1/100
dilution). Postmortem lung tissues were also fixed in 2.5%
glutaraldehyde for routine transmission electron microscopy
(EM).

Statistical analysis

Spearman analysis was used to study the correlation between
duration of illness and various pathological features with
commercial statistical software (Statistical Package for Social
Science, SPSS, version 10.1.0) and p < 0.05 was considered
to be significant.

RESULTS

Clinical data

There were six male patients and one female patient, who
had an age range from 44 to 81 years (mean, 71); all had pre-
existing medical illnesses (table 1) and CXR abnormality at
disease onset. The duration of illness ranged from four to 20
days (median, 11). Six patients received high dose intrave-
nous ribavirin (range, 1–14 days; median, 9.5). Five patients
received intravenous steroids (range, 4–16 days; median, 10).
Six of the seven patients were intubated and mechanically
ventilated (range, 1–16 days; median, 10). Apart from the
isolation of SARS-CoV from nasopharyngeal aspirate speci-
mens of patients 1–3, 5, and 7 (five of six; table 1), other
microbiological investigations, including the isolation of
metapneumovirus, were negative.2 11 Bacterial cultures were
positive in five patients at the terminal stage. Staphylococcus
aureus (patients 2 and 3) and enterococcus (patient 6) were
isolated from blood culture. Klebsiella sp (patient 1) and
stenotropnomas (patient 7) were identified in tracheal
aspirate specimens. All patients died of respiratory failure,
with concurrent congestive heart failure, hepatic encephalo-
pathy, and acute renal failure in patients 1, 2, and 5,
respectively.

CoV isolation

Postmortem lung, heart, liver, spleen, kidney, and small
intestine tissues were available for viral isolation from
patients 1–6 and lung and heart tissues were available from
patient 7. SARS-CoV was isolated in culture from postmor-
tem lung tissues in patients 1 and 2 (two of seven) and from
small intestinal tissue in patients 1–5 (five of six). Transmission EM demonstrated viral particles in patients
1–4, 6, and 7 (six of seven; table 1).

Pulmonary pathology

The upper respiratory tract was unremarkable. The lungs
were heavy (650–1200 g), with a mild pleural effusion of
clear serous fluid (50–200 ml on each side), pronounced
pulmonary oedema, and extensive consolidation (fig 1A).
Focal haemorrhage was seen in some cases. Apart from one
case with apical pleural adhesion, pleuritis was not apparent.
Mild and small pulmonary thromboembolism was only noted
in patient 7. Peribronchial or hilar lymph nodes were not
enlarged.

Histologically, all patients had features of diffuse alveolar
damage (DAD) with pronounced pulmonary oedema and
hyaline membrane formation (table 2; fig 1B). In some areas,
there was interstitial thickening, with mild to moderate
fibrosis, but a disproportionately sparse infiltrate of inflam-
atory cells (mainly histiocytes, including multinucleated
forms, and lymphocytes). Dilatation of the airspaces was seen
(fig 1C), as was focal honeycomb fibrosis (patient 6; fig 1D).
Intra-alveolar organisation of exudates was seen and,
in four cases, there was formation of granulation tissues in
small airways and airspaces (fig 2A, B). These lesions were
typically located in the subpleural region and the cellular
component consisted mainly of histiocytes. The collection of
acute inflammatory exudates in the airspaces was noted only
in three patients with secondary bacterial infection (with
positive premortem bacterial blood cultures). More unu-
usually, atypical pneumocytes were seen in all seven patients,
although the distribution was focal (fig 3). These atypical
forms included multinucleated giant pneumocytes with
irregularly distributed nuclei (fig 3A) or pneumocytes with
large atypical nuclei, prominent eosinophilic nucleoli, and
granular amphiphilic cytoplasm (fig 3B). However, distinct
viral inclusions were not apparent. Among all the histologi-
ical features investigated (table 2), a positive correlation was
detected only between the duration of illness and the degree
of interstitial fibrosis (Spearman correlation, p = 0.019). In
the extrapulmonary organs, splenic white pulp lymphoid
depletion was seen in all patients. Focal individual muscle
fibre necrosis and regenerative changes were noted in four
patients. Apart from the comorbid conditions, no other
unique pathological features were found in other organs.

Histochemical and immunohistochemical studies

All the special histochemical stains for infective agents were
negative except that Gram positive cocci were detected in the
acute inflammatory cell infiltrate in patient 3. Immunohistochemical staining for CMV and HSV was negative. Some of the multinucleated cells with atypical
nuclei were positive for cytokeratin (AE1/AE3), confirming

Table 1: Clinical features of seven patients who died of severe acute respiratory syndrome (SARS)

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>69</td>
<td>64</td>
<td>76</td>
<td>81</td>
<td>44</td>
<td>79</td>
<td>81</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Premorbid disease</td>
<td>CVS</td>
<td>CLD</td>
<td>MDS</td>
<td>COAD</td>
<td>CLD</td>
<td>CVS</td>
<td>HT</td>
</tr>
<tr>
<td>Illness (days*)</td>
<td>4</td>
<td>9</td>
<td>11</td>
<td>6</td>
<td>16</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Ribavirin (days*)</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>No</td>
<td>12</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Hydrocortisone (days*)</td>
<td>No</td>
<td>4</td>
<td>7</td>
<td>No</td>
<td>10</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Intubation (days*)</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>No</td>
<td>12</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Viral isolation</td>
<td>NPA</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NPA</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Small intestine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Lung EM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
their epithelial nature as pneumocytes. The inflammatory cell infiltrate included histiocytes (CD68 and Mac387 positive). The lymphoid infiltrate was sparse and consisted mainly of T cells (CD3 positive) and a few B cells (CD20 positive); natural killer cells (CD56 positive) were largely lacking.

Ultrastructural study
Pneumocytes containing viral-like particles were noted in most cases (six of seven), although such cells were scanty (fig 4A). The particles measured 60–90 nm in size and were found within dilated cytoplasmic vesicles, reminiscent of endoplasmic reticulum. The appearance of the viral-like particles was similar to that seen in the Vero cell culture (fig 4B, C). These particles appeared to be the viral nucleocapsids. The surfaces of some of these particles were decorated with club shaped projections (arrows, fig 4A). In addition, a ring immediately underneath the envelope was also seen in some of the better preserved particles. This may represent the characteristic helical nucleocapsid of coronaviruses. The cross section of these particles also showed the typical electron lucent centre. Such viral-like particles were not detected in macrophages or other cell types in the lung.

DISCUSSION
We have reported the lung pathology of patients with microbiologically confirmed, fatal SARS. These patients were epidemiologically linked to the first index case in Hong Kong, which subsequently resulted in the world endemic of SARS.

The known human coronaviruses, types 229E and OC43, generally cause common cold symptoms and have only rarely been associated with more severe lower respiratory diseases, such as pneumonia in neonates, the elderly, or immunocompromised patients. However, SARS is a lower respiratory tract disease and upper respiratory tract symptoms are uncommon and mild. For all of our patients who died, and

Table 2 Summary of the pulmonary pathological features of patients with severe acute respiratory syndrome

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical pneumocytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diffuse alveolar damage</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pulmonary oedema</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hyaline membrane</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Organising phase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>BOOP-like</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>Pulmonary haemorrhage</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>Bronchopneumonic changes</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>

+, mild; ++, moderate; +++, severe; --, not present.
BOOP-like, bronchiolitis obliterans organising pneumonia-like lesion.
In other series, DAD was the dominant picture. DAD is a severe pattern of lung injury and could be secondary to various pulmonary and extrapulmonary insults. In addition, the formation of tissue plugs in terminal small airways and alveolar spaces may correlate with the early radiological findings. Such lesions are not specific to SARS, and may be idiopathic (termed BOOP), or be associated with post-infection states, drug effects, connective tissue diseases, post-organ transplant, and post-irradiation states. Such BOOP-like lesions in patients with SARS may indicate a non-specific response to lung injury.

“The formation of multinuclear cells is not unique to severe acute respiratory syndrome, and is seen in pneumonia caused by the family of Paramyxoviridae, including parainfluenza viruses, measles, mumps, respiratory syncytial virus and, perhaps, metapneumovirus.”

Despite the small numbers of cases, a positive correlation was detected between the duration of illness and the degree of interstitial fibrosis. The results suggested that the pulmonary fibrosis seen in these fatal cases may be related to SARS rather than pre-existing lung lesions. Follow up radiological studies indicated that 62% of surviving patients had pulmonary fibrosis. Thus, it is possible that at least in patients with severe SARS, the development of pulmonary fibrosis could be relatively rapid. However, because many patients had severely compromised respiratory function during the illness and required ventilation or oxygen supplementation, the exact role played by each factor in causing pulmonary fibrosis remains speculative.

In our current series, viral culture for coronavirus was positive in postmortem lung tissue in two patients and viral-like particles compatible with coronavirus were demonstrated ultrastructurally in the lung tissue in most of the patients (six of seven patients). These viral-like particles were noted in the pneumocytes, but not in the other cell types within the lung. These observations suggested that the primary target cells for SARS-CoV infection are probably pneumocytes. The atypical morphology of the pneumocytes was probably related to viral cytopathic effects or reactive changes. The presence of multinucleated pneumocytes in SARS has been noted by several other investigators. However, the formation of multinucleated cells is not unique to SARS, and is seen in pneumonia caused by the family of Paramyxoviridae, including parainfluenza viruses, measles, mumps, respiratory syncytial virus and, perhaps, metapneumovirus. Although foamy histiocytes and multinucleated histiocytes were seen, these probably reflect non-specific secondary changes.

Apart from lung tissues, postmortem small intestinal tissue was also valuable for viral isolation with a high yield (six of seven patients), suggesting viral intestinal tropism, which may be related to intestinal manifestations in some patients. Interestingly, gastrointestinal symptoms were not prominent in our series of patients. For those cases in which SARS-CoV was isolated, the time interval between the patients’ death and necropsy ranged from four to seven days, indicating the presence of viable SARS-CoV up to a week after the patients’ death.
In summary, we have presented the pulmonary pathology in a confirmed and well defined series of fatal SARS cases. The pathological features, in addition to DAD, included the presence of multinucleated pneumocytes and intrabronchial fibrogranulation tissue proliferation (BOOP-like lesions). Although each of these features is non-specific, their presence of multinucleated pneumocytes and intrabronchial fibrogranulation tissue proliferation (BOOP-like lesions) allows tissue to be sampled for virological investigations and/or ultrastructural examination enabling the diagnosis of SARS to be confirmed, and is particularly useful in clinically suspicious cases that do not fulfill the World Health Organisation criteria or in clinically unapparent cases. We have shown that viral particles can be successfully isolated from postmortem lung and small intestinal tissue samples by culture and also by ultrastructural examination, highlighting the importance of necropsy, particularly in those patients who die before the diagnosis is confirmed. At the time of necropsy, when minimal exposure is desirable, a limited dissection with sampling of tissues from the lungs and small intestine may allow for maximal diagnostic yield.

**Authors’ affiliations**

G M-K Tse, K-F To, A W I Lo, H-C Wong, K-F Chan, H-K Ng, Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong, Hong Kong SAR

P K-S Chan, K-C Ng, Department of Microbiology, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong SAR

**Take home messages**

- In severe acute respiratory syndrome (SARS), coronavirus (CoV) could be isolated from postmortem tissues up to one week after death.
- Pneumocytes are probably the primary target of infection.
- Postmortem examination is invaluable because it allows tissue to be sampled for virological investigations and ultrastructural examination.
- When coupled with the appropriate lung morphological changes, postmortem examination is extremely useful to confirm the diagnosis of SARS-CoV, particularly in clinically unapparent or suspicious but unconfirmed cases.

**REFERENCES**


14 World Health Organisation. PCR primers for SARS developed by WHO network laboratories (http://www.who.int/csr/sars/primer/en/).


Apoptotic gene variants do not predispose to primary Sjögren’s syndrome in Australians

A molecular genetic study has suggested that a common variant in the Fas gene promoter region prevents an autoimmune response to intracellular ribonucleoproteins in some patients with primary Sjögren’s syndrome. How this relates to pathogenesis is unclear, but testing polymorphisms against other autoantigens that relocate to the cell surface during apoptosis may be worthwhile.

The study in Australian patients tested whether genetic variants in this region were linked to the syndrome. Genotype distributions and allelic frequencies of the two common variants at positions −1377 and −670 were no different in patients and controls, but the allelic frequency of the −670 variant was significantly higher in patients without Ro/La autoantibodies than in those with, raising the possibility that this variant might somehow prevent immunological exposure to these intracellular antigens.

The study typed 101 patients with primary Sjögren’s syndrome and 108 ethnically matched controls for Fas gene promoter variants −1377G→A and −670A→G by PCR-SSP. Patients were grouped according to their autoantibody status to Ro/La ribonucleoproteins: Ro, La antibody negative; Ro positive only; Ro positive, precipitating La negative; and Ro positive, precipitating La positive.

Most patients with primary Sjögren’s syndrome have circulating autoantibodies to Ro/La ribonucleoproteins. Apoptosis may trigger the autoimmune response as these proteins migrate to the cell surface on blebs during the process. One theory is that abnormal apoptosis leads to the destruction of exocrine glands that is a feature of the syndrome, but the findings of this study indicate that this is not so—at least for these patients.

Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS)

G M-K Tse, K-F To, P K-S Chan, A W I Lo, K-C Ng, A Wu, N Lee, H-C Wong, S-M Mak, K-F Chan, D S C Hui, J J-Y Sung and H-K Ng

doi: 10.1136/jcp.2003.013276

Updated information and services can be found at:
http://jcp.bmj.com/content/57/3/260

*These include:*

**References**

This article cites 22 articles, 6 of which you can access for free at:
http://jcp.bmj.com/content/57/3/260#BIBL

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Interstitial lung disease (24)
- TB and other respiratory infections (72)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/