Mini Review

Severe Acute Respiratory Syndrome: Clinical and Laboratory Manifestations

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Abstract

Severe acute respiratory syndrome (SARS) is a recently emerged infectious disease with significant morbidity and mortality. An epidemic in 2003 affected 8,098 patients in 29 countries with 774 deaths. The aetiological agent is a new coronavirus spread by droplet transmission. Clinical and general laboratory manifestations included fever, chills, rigor, myalgia, malaise, diarrhea, cough, dyspnoea, pneumonia, lymphopenia, neutrophilia, thrombocytopenia, and elevated serum lactate dehydrogenase (LD), alanine aminotransferase (ALT) and creatine kinase (CK) activities. Treatment has been empirical; initial potent antibiotic cover, followed by simultaneous ribavirin and corticosteroids, with or without pulse high-dose methylprednisolone, have been used. The postulated disease progression comprises (1) active viral infection, (2) hyperactive immune response, and (3) recovery or pulmonary destruction and death. We investigated serum LD isoenzymes and blood lymphocyte subsets of SARS patients, and found LD1 activity as the best biochemical prognostic indicator for death, while CD3+, CD4+, CD8+ and natural killer cell counts were promising predictors for intensive care unit (ICU) admission. Plasma cytokine and chemokine profiles showed markedly elevated Th1 cytokine interferon (IFN)-γ, inflammatory cytokines interleukin (IL)-1β, IL-6 and IL-12, neutrophil chemokine IL-8, monocyte chemotactant protein-1 (MCP-1), and Th1 chemokine IFN-γ-inducible protein-10 (IP-10) for at least two weeks after disease onset, but there was no significant elevation of inflammatory cytokine tumor necrosis factor (TNF)-α and anti-inflammatory cytokine IL-10. Corticosteroid reduced IL-8, MCP-1 and IP-10 concentrations from 5 - 8 days after treatment. Measurement of biochemical markers of bone metabolism demonstrated significant but transient increase in bone resorption from Day 28 - 44 after onset of fever, when pulse steroid was most frequently given. With tapering down of steroid therapy, there was a decrease in bone resorption marker together with an increase in bone formation markers round Day 50, suggesting that some of the bone loss might be reversed. Our research studies on the chemical pathology and clinical immunology of SARS should have implications for the pathophysiology and therapy of this potentially lethal infection. (Clin Biochem Rev 2004; 121-132)

Introduction

SARS is a recently emerged infectious disease that is highly contagious with significant morbidity and mortality. From 1 November 2002 to 31 July 2003, 8,098 cases have been diagnosed in 29 countries with 774 attributed fatalities reported.¹ The very first SARS patient was probably a 46-year-old government official in Fushan city of the Guangdong province in southern China, who presented on 19 November 2002 together with three members of his family.² Subsequently, cases were reported in several nearby cities between November 2002 and January 2003. A major cluster of cases then occurred in Guangzhou, the capital of Guangdong. The international outbreak began when a 64-year-old emeritus professor of medicine in Guangzhou who had been treating pneumonia cases arrived in Hong Kong on 21 February 2003 and checked into a local hotel.³ He was admitted to hospital on 22 February with about one week's symptoms of respiratory tract infection, and died from respiratory failure several days later.⁴ The infection was passed onto eight key persons who had either stayed or visited friends in that hotel. These secondary cases
subsequently brought the infection back to their home countries and started the epidemic in North America, Europe and other Asian countries. In Hanoi, Vietnam, Dr Carlo Urbani, the 46-year-old World Health Organisation (WHO) infectious disease specialist and president of the Italian branch of Medecins sans Frontieres, whose work defined SARS, died on 29 March 2003 of SARS. Since the infection did not respond to anti-microbial treatment for community-acquired or atypical pneumonia, and bacteriological and virological pathogens known to cause pneumonia were not identified, this new disease was designated SARS by WHO on 15 March 2003.

The index patient at our Prince of Wales Hospital in Hong Kong was a 26 year old man who had visited a friend in the above hotel. He was admitted on 4 March 2003 with febrile pneumonia that progressed initially despite antibiotics, but improved after 7 days without additional treatment. Six days after his admission, 18 healthcare workers from the same ward fell ill and 50 potential cases among staff were identified. More staff, patients and visitors became ill over the next several days and there was subsequent infection to their contacts. By 25 March, 156 patients including 69 healthcare workers and 16 medical students had been admitted with SARS, all traceable to the initial case. From 24 March to 15 April 2003, a single community outbreak of SARS involving 329 residents with 42 deaths occurred in a high-rise housing estate; the index case being a 33-year-old renal patient on haemodialysis. He had also been admitted to the same index ward at the Prince of Wales Hospital during the same time period. His main symptom was diarrhoea and he had visited relatives several times in that apartment complex. The most likely route of infection in this outbreak was via leaky or faulty sewage pipes allowing an aerosol of infectious faecal materials to travel up the narrow light well between buildings in rising air currents. During our three-month (101 days) epidemic, community measures taken by the Hong Kong Government included public alert on personal protection (17 March), addition of SARS as a notifiable disease (26 March), two-week suspension of schools and introduction of health declaration for all incoming residents and visitors (29 March), isolation of residents of the community outbreak to rural camps for 10 days (31 March), home quarantining of close contacts (10 April), and body temperature check for all air passengers (17 April). By 23 June 2003, when WHO deleted Hong Kong from the list of SARS-affected areas, there had been 1,755 cases with 299 deaths in our population of 6.9 million. A summary of SARS cases with onset of illness from 1 November 2002 to 31 July 2003 in the five most affected areas (Canada, China, Hong Kong, Singapore, and Taiwan) and Australia is provided in Table 1.

Several groups in Hong Kong, Canada and Germany have identified the causative agent of SARS as a new coronavirus spread by droplet transmission, since designated SARS-CoV. Human metapneumovirus has also been isolated as a second pathogen in SARS patients and it may potentiate the progression and severity of coronavirus infection. SARS-CoV is a member of the Coronaviridae family of enveloped, positive-stranded RNA viruses, which have a broad host range. Some coronavirus infections in man, cattle, and birds cause respiratory disease, while other infections in rodents, cats, pigs and cattle result in enteric disease. The 27-32 kB genomes of the coronaviruses encode 23 putative proteins, including four major structural proteins: nucleocapsid, spike, membrane, and small envelope. The spike protein, a glycoprotein projection on the viral surface, is crucial for viral attachment and entry into the host cell. In addition, variations of this protein among strains of coronaviruses are responsible for host range and tissue tropism. The genomic sequencing of SARS-CoV, as well as its diagnostic testing, representing very intelligent and speedy scientific research and developments, are reviewed separately in this issue.

Clinical and Laboratory Manifestations of SARS

Case Definition

The WHO case definition (latest revision 1 May 2003) for probable SARS comprises (1) fever > 38 °C or history of such in the past 2 days, (2) radiological evidence of new infiltrates consistent with pneumonia, (3) chills or cough or malaise or myalgia or known history of exposure, and (4) positive test for SARS-CoV by one or more assays. Before the inclusion of the laboratory criterion, SARS was a diagnosis of exclusion. Some patients may not have all the clinical features rendering the guidelines insufficiently sensitive, while others may present differently (e.g. in the community outbreak in Hong Kong, 73% of 75 patients had watery diarrhoea).

Clinical and Laboratory Manifestations

The clinical and general laboratory manifestations of SARS in adult patients are summarized in Table 2. The infection affected men and women of all ages with an incubation period of 2 - 16 days. Fever, chills and rigor, malaise and myalgia were the most common presenting symptoms. Physical examination was unremarkable except for inspiratory crackles and percussion dullness of the chest. On admission all patients had abnormal chest radiography or thoracic computer tomography: the air space consolidation with ground-glass opacification ranged from unilateral focal to bilateral multi-focal involvement with pleural effusion.
The pulmonary infiltrates may resolve spontaneously or in response to therapy or, in some patients, continue to worsen resulting in progressive oxygen de-saturation, acute respiratory distress, multi-organ failure, and death. Common presenting haematology findings included lymphopenia, thrombocytopenia with prolonged activated partial thromboplastin time (APTT) and elevation of serum D-dimer concentration. Our more detailed laboratory study of 157 patients documented transient leucopenia in 64% of patients during the first week of illness, 61% leucocytosis from second to third week, 82% neutrophilia that was associated with episodes of bacterial infection, and in 61% patients, a decrease of blood haemoglobin by >20 g/L from baseline. Biochemical abnormalities included hyponatraemia, hypokalaemia, elevated serum LD, ALT and CK activities and, not unexpectedly, increased C-reactive protein concentration. Advanced age (>60 years), co-morbidity (diabetes, chronic lung disease, cardiovascular disease, chronic viral hepatitis, malignancy), and an elevated serum LD have been shown by most studies to be important independent risk factors for high oxygen dependency, admission to ICU, assisted ventilation, and death.

Most adult SARS patients in Hong Kong have been treated according to the following Hospital Authority protocol. On the first day of admission, ribavirin, a broad-spectrum antiviral agent, was given either orally at 1.2 g three times daily after a loading dose of 2.4 g, or intravenously 400 mg every 8 hours for a complete 14-day course. Levofloxacin or cefotaxime was supplemented to cover any bacterial chest infection. If there was persistent fever >38°C on the third day of admission, a maintenance steroid therapy would be started using either oral prednisolone 0.5–1.0 mg/kg/day, or intravenous hydrocortisone 100 mg every 8 hours, with step-down titration starting from the third week of admission. For patients with clinical deterioration including further persistent fever, de-saturation (oxygen saturation <90% using pulse oximetry), or development of new infiltrates in chest radiograph, pulse steroid using methylprednisolone 0.5 g daily for 3 days was given. In selected cases, intravenous human IgM-enriched immunoglobulin (Pentaglobin), convalescent serum, and protease inhibitor (Kaletra) have been tried. Other regimes have been used in other countries. The rationale, dosage, timing, efficacy, and side effects of using ribavirin and steroids have been much discussed (e.g. ribavirin is known to cause haemolytic anaemia, and early administration of steroids may promote viral replication, enhance infectivity and aggravate lymphopenia ).

There have not been many reports on SARS in children and adolescents (<18 years old), perhaps partly because there has been far less number of such patients, (e.g. only 88 in Hong Kong). The predominant and most consistent symptom in all patients so far studied is fever. Other symptoms include coryza and running nose. Chills, rigor, myalgia, and malaise, which are common in adult patients, might also be present in teenagers and adolescents, but were absent in younger children. In one study of ten children aged 1 to 16 years, nine had abnormal chest radiographs (primarily air-space opacification) on presentation that resolved within two weeks. Clinically important lymphopenia, occurring between days 3-7 after onset of fever, was reported in all patients, but was less severe among younger children. Mild thrombocytopenia and elevated serum LD and ALT were manifested in some but not all children. As for adults, treatment was started with antibiotics covering common bacterial and atypical pneumonia. Oral ribavirin was added if there was a definite contact history. Oral prednisolone was given if fever persisted after 48 hours. Intravenous pulse methylprednisolone was used for persistent fever, progressive clinical deterioration, or worsening radiology. There was no clinically significant decrease in blood haemoglobin concentration during treatment, and all children recovered in three weeks without intubation or admission to the ICU. Eight of the ten children had been attending school at the time of presentation. There was no evidence that they had spread the infection to their classmates. Compared to adults and teenagers, younger children seemed to have a milder disease with lower infectivity, less aggressive clinical course, and more speedy resolution. Elucidation of the underlying mechanisms for this disparity would foster very interesting multidisciplinary studies.

The Hong Kong Hospital Authority’s hypothetical disease model for SARS comprises three phases: (1) acute viral infection with fever, chills, myalgia and minimal respiratory symptoms, (2) hyperactive immune response with clinical, radiological and laboratory manifestations of severe acute pneumonia and other tissue inflammation consequent to a possible cytokine and chemokine storm, and (3) recovery or, sometimes, pulmonary destruction and death. We review below some of our research work on the chemical pathology and clinical immunology of SARS that should have implications for the pathophysiology and therapy of this potentially lethal infection.

 Serum LD1 Isoenzyme and Blood Lymphocyte Subsets

The pathophysiology of SARS is at present poorly understood, but advanced age and elevated serum total LD activity have been associated with adverse clinical outcomes and death in three separate studies of 138, 227 and 151 patients in Hong Kong. Blood leucocytes and lymphocyte subtypes were reported to decrease, respectively,
Table 1. Summary of SARS cases with onset of illness from 1 November 2002 to 31 July 2003.\textsuperscript{12}

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of Cases</th>
<th>Sex</th>
<th>Median age (range)</th>
<th>Number of Deaths (%)</th>
<th>Number of HCW* infected (%)</th>
<th>Date onset of first probable case</th>
<th>Date onset of last probable case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>6</td>
<td>M 2</td>
<td>F 4</td>
<td>15 (1-45)</td>
<td>0 (0)</td>
<td>26 Feb 03</td>
<td>1 Apr 03</td>
</tr>
<tr>
<td>Canada</td>
<td>251</td>
<td>100</td>
<td>151</td>
<td>49 (1-98)</td>
<td>43 (17)</td>
<td>23 Feb 03</td>
<td>12 Jun 03</td>
</tr>
<tr>
<td>China</td>
<td>5281</td>
<td>2607</td>
<td>2674</td>
<td>Pending</td>
<td>349 (6.6)</td>
<td>16 Nov 02</td>
<td>3 Jun 03</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1755</td>
<td>778</td>
<td>977</td>
<td>40 (0-100)</td>
<td>299 (17)</td>
<td>15 Feb 03</td>
<td>31 May 03</td>
</tr>
<tr>
<td>Singapore</td>
<td>238</td>
<td>77</td>
<td>161</td>
<td>35 (1-90)</td>
<td>33 (14)</td>
<td>25 Feb 03</td>
<td>5 May 03</td>
</tr>
<tr>
<td>Taiwan</td>
<td>346</td>
<td>128</td>
<td>218</td>
<td>42 (0-93)</td>
<td>37 (11)</td>
<td>25 Feb 03</td>
<td>15 Jun 03</td>
</tr>
<tr>
<td>Others</td>
<td>221</td>
<td></td>
<td></td>
<td></td>
<td>13 (5.9)</td>
<td>44 (17)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8098</strong></td>
<td></td>
<td></td>
<td><strong>774 (9.6)</strong></td>
<td><strong>1707 (21)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* HCW = healthcare workers.
### Table 2. Clinical and general laboratory manifestations of SARS in adult patients.

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Incubation period</th>
<th>Clinical presentations</th>
<th>Radiological findings</th>
<th>General Laboratory findings</th>
<th>Admission to ICU</th>
<th>Independent predictors of adverse outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Chinese University of Hong Kong&lt;sup&gt;27&lt;/sup&gt;</td>
<td>2-16 days (median 6 days)</td>
<td>Fever (100%) Chills ± rigor (73.2%) Myalgia (60.9%) Cough (57.3%) Headache (55.8%) Dizziness (42.8%) Sputum production (29.0%) Sore throat (23.2%) Coryza (22.5%) Nausea and vomiting (19.6%) Diarrhoea (19.6%)</td>
<td>Progressive air-space disease (90%)</td>
<td>Lymphopenia (69.6%) Thrombocytopenia (44.8%) Prolonged APTT (42.8%) ↑D-dimer (45.0%) ↑ALT (40.0%)</td>
<td>32 patients (23.2%)</td>
<td>Advanced age (odds ratio (OR) 1.8) High peak LD (OR 2.1) High absolute neutrophil count on presentation (OR 1.6)</td>
</tr>
<tr>
<td>The University of Hong Kong&lt;sup&gt;28&lt;/sup&gt;</td>
<td>2-11 days</td>
<td>Fever (100%) Rigor (90%) Cough (80%) Headache (70%) Malaise (70%) Dyspnoea (60%) Myalgia (50%) Pleurisy (30%) Sputum production (10%)</td>
<td>Infiltrate on CXR (100%)</td>
<td>Lymphopenia (90%) ↑ALT (40%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Canadian SARS Study Team&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3-10 days</td>
<td>Fever (100%) Non-productive cough (100%) Dyspnoea (80%) Malaise (70%) Diarrhoea (50%) Chest pain (30%) Headache (30%) Sore throat (30%) Myalgia (20%) Vomiting (10%)</td>
<td>On admission, 96% patients had pneumonia on CXR, remaining 4% detected by thoracic computer tomography. (CT)</td>
<td>Oxygen saturation on room air &lt;95% (78%) Leucopenia (22%) Lymphopenia (89%) Thrombocytopenia (33%) ↑ALT (56%) ↑AST (78%) ↑LD (80%) ↑CK (56%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Princess Margaret Hospital, Hong Kong&lt;sup&gt;29&lt;/sup&gt;</td>
<td>-</td>
<td>Fever (99%) Chills (74%) Myalgia (52%) Cough (43%) Rigor (41%) Headache (33%) Shortness of breath (20%) Sputum (20%) Diarrhoea (15%)</td>
<td>-</td>
<td>Lymphopenia (73%) Thrombocytopenia (50%) Leukopenia (27%) Prolonged APTT (18%) Hyponatraemia (60%) Hypokalaemia (47%) ↑LD (47%) ↑ALT (31%) ↑CK (19%)</td>
<td>69 patients (26%)</td>
<td>Mortality 12% Age&gt;60 years (OR 5.1 LD&gt;3.8 µkat/L (OR 2.2)</td>
</tr>
<tr>
<td>Queen Elizabeth Hospital, Hong Kong&lt;sup&gt;30&lt;/sup&gt;</td>
<td>-</td>
<td>Fever (100%) Chills (55%) Cough (46%) Myalgia (38%) Malaise (35%) Sputum (15%) Headache (11%) Diarrhoea (11%) Dyspnoea (10%)</td>
<td>At presentation, 87% patients had infiltrate on CXR, remaining 13% detected by thoracic CT.</td>
<td>Lymphopenia (common) ↑LD (common) Thrombocytopenia (mild) ↑ALT (uncommon)</td>
<td>39 patients (34%)</td>
<td>Mortality 15.7% Age&gt;60 years (hazards ratio (HR) 6.8) co-morbidity (HR 9.0) LD&gt;450 U/L (HR 4.8)</td>
</tr>
</tbody>
</table>
in 47% and up to 100% of 38 patients in Beijing, China. However, their prognostic implications have not been investigated. We studied serum total LD, LD isoenzymes and other parameters including age and blood lymphocyte subsets as prognostic indicators in 109 adult SARS patients for adverse clinical outcomes in terms of admission to ICU and death. Serum total LD was measured using the lactate-to-pyruvate assay (DP Modular Analyser, Roche Diagnostics Corp, IN, USA), and isoenzymes (LD1 to LD5) were separated by agarose gel electrophoresis before densitometry (Paragon Electrophoresis System, Beckman Coulter Inc, CA, USA). Total T lymphocytes (CD3+), T helper lymphocytes (CD4+), T suppressor and cytotoxic T lymphocytes (CD8+), natural killer (NK) lymphocytes (CD56+) and B lymphocytes (CD19+) were enumerated by immunofluorescence flow cytometry (MultiTest IMK kit, FACSCalibur Flow Cytometer, Becton Dickinson Corp, CA, USA). Receiver-operator-characteristic (ROC) curve analysis was used to determine and compare different cutoffs for various biochemical and immunological parameters at peak serum total LD activity in predicting adverse clinical outcomes.

Figure 1. Multiple ROC curve comparison of age, serum total LD activity, serum LD1 activity, serum LD1/LD2 ratio, blood haemoglobin concentration, and blood total lymphocyte count for the prediction of death.

Of the 109 (43 male and 66 female) patients aged 21 to 100 years (median 43, interquartile range 29-69) who were monitored daily for up to 21 days, 41 were admitted to ICU and 42 died (24 in ICU and 18 in medical ward). Lymphopenia was noted in 98% of patients, most of them had reduced T helper, T suppressor and NK cell counts during the early phase of their illness, which reached lowest values on Day 5 - 7 from disease onset (fever), and recovered gradually. B-lymphocyte count and the ratio of T-helper to T-suppressor lymphocytes (CD4:CD8) remained normal and stable. Age was found to be an independent prognostic indicator for death with an area-under-curve (AUC) of 0.96 [95% confidence interval (CI) = 0.90-0.99] but not for admission to ICU [AUC = 0.61 (CI = 0.51-0.70)]. Whilst serum total LD could only achieve an AUC of 0.68 (CI = 0.59 – 0.77) for predicting death, LD1 was the best biochemical prognostic indicator for predicting death, with an AUC of 0.84 (CI = 0.75-0.90), sensitivity of 62% (CI = 46-76%) and specificity of 93% (CI = 83-98%) at cutoff activity of ≥80 U/L (Figure 1). CD3+, CD4+, CD8+, and natural killer cell counts were promising immunological prognostic indicators for predicting admission to ICU with AUC of 0.94 (CI = 0.86-0.98), 0.91 (CI = 0.81-0.96), 0.93 (CI = 0.85-0.98), and 0.87 (CI = 0.76-0.94), respectively.

LD is a cytoplasmic zinc-containing enzyme of ubiquitous tissue distribution. The increase in serum total LD activity in SARS has been postulated to be a result of massive tissue destruction during the acute phase of SARS-CoV infection with immune hyperactivity. Since LD1 is the major contributor of elevated total LD in our patients, it is likely that the former has also originated from tissue destruction. Contribution of LD1 from the myocardium is unlikely since only 9% of all samples had serum cardiac troponin T concentration ≥ 0.1 µg/L (cutoff for acute myocardial infarction). However, as LD1 is abundantly distributed in erythrocytes, the haemolytic effect of ribavirin may be another cause of serum LD elevation. Whilst full evaluation of all five LD isoenzymes requires electrophoresis, routine measurement of serum LD1 is as easy as total LD assay using automated analysis. SARS patients with increased serum LD1 activity should be closely monitored to ensure timely management.

Lymphocyte subsets are actively involved in humoral and cell-mediated immunity against viral infection. The markedly suppressed CD3+, CD4+, CD8+, and CD56+ cell counts in our SARS patients could possibly be due to direct viral cytocotoxicity. After steroid therapy, these immunological markers started to rise from their trough values. In acquired immunodeficiency syndrome (AIDS), only a selected population of T-lymphocytes (T-helper cells) is attacked and destroyed by the human immunodeficiency virus (HIV). Published recommendations by the Center for Disease Control (CDC) exist on the use of CD4+ cell count for monitoring the disease course and therapeutic efficacy in HIV infection and AIDS. During the initial outbreak of SARS in our hospital, we encountered difficulty in the allocation of the limited number of ICU beds because SARS patients requiring ICU care could be as high as 20%.
our study, measurement of the above immunological markers should be useful for the management of SARS patients; preparation for admission to ICU or other serious clinical outcomes could be planned ahead for those with markedly suppressed lymphocyte subsets.

Plasma Cytokines and Chemokines

Cytokines are extracellular proteins capable of mediating intercellular communication through their binding onto target cell surface to initiate diverse responses. The term was used originally to designate such molecules produced by immune cells: lymphocytes, monocytes, mast cells, neutrophils and eosinophils, before other cell types were found to communicate similarly. Chemokines are chemotactic cytokines that facilitate trafficking (recruitment and migration) of leukocytes for inflammation, angiogenesis, tumor immunity, graft rejection, and allergic reaction. In severe acute infections, markedly elevated cytokine and chemokine concentrations are frequently manifested in the circulation and tissues. For example, pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α, and anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1ra) were increased in plasma and bronchoalveolar lavage fluid of patients with community-acquired pneumonia. The H5N1 “avian flu” influenza virus has been shown to be a potent inducer of pro-inflammatory cytokines interferon (IFN)-γ, IL-6 and, particularly, TNF-α. These so-called cytokine and chemokine storms are a two-edged weapon. They are a physiological host reaction for accelerating viral clearance. But the consequent exaggerated inflammation, especially in severe acute infections, can also cause pulmonary destruction, disseminated intravascular coagulation, and multi-organ failure, terminating in acute respiratory distress syndrome and death.

We have investigated daily changes in plasma inflammatory cytokines and chemokines in 20 adult SARS patients [19 men and 1 woman, mean (SD) age 33 (12) years, range 21 – 58] for 19 consecutive days upon hospital admission (from ≤ 2 days after disease onset). Because the more commonly used enzyme-linked immunosorbent assay may generate infectious droplets and aerosol, cytokines and chemokines were measured by cytometric bead array using a FACSCalibur Flow Cytometer (Becton Dickinson) installed in a Biosafety Level II laboratory. The cytokine profile showed marked elevation of T-helper lymphocyte type 1 (Th1) cytokine IFN-γ, inflammatory cytokines IL-1β, IL-6 and IL-12 for at least two weeks after disease onset, but there was no significant increase in inflammatory cytokine TNF-α, anti-inflammatory cytokine IL-10, Th1 cytokine IL-2, and T-helper lymphocyte type 2 (Th2) cytokine IL-4. The chemokine profile demonstrated significant elevation of neutrophil chemokine IL-8, monocyte chemoattractant protein-1 (MCP-1), and Th1 chemokine IFN-γ-inducible protein-10 (IP-10). Corticosteroids reduced IL-8, MCP-1 and IP-10 concentrations significantly by 5 – 8 days after treatment. The above cytokine and chemokine profiles with a notable lack of TNF elevation were identical to those of the National Cheng Keng University Hospital and CDC in Taiwan (unpublished communication), but different from those described above for community-acquired pneumonia and H5N1 influenza. Our observation therefore does not support the use of TNF-α monoclonal antibody that was contemplated during the peak of the epidemic for the treatment of worsening pneumonia refractory to steroid therapy. We have also performed serial studies of plasma cytokine and chemokine profiles of 8 children with SARS (5 boys and 3 girls, age 0.3 – 6.2 years) and found that, similar to their other clinical and laboratory manifestations, these patients had a much milder cytokine and chemokine storm, rendering the use of corticosteroids more controversial if not unjustified.

Accordingly, we have attempted to postulate the immunopathogenesis of SARS in the following summary, which also serves as the legend for the schematic representation in Figure 2. SARS-CoV can induce the production of IL-12 by the viral antigen-presenting cells (e.g. macrophages), which can enhance the production of IFN-γ, the typical Th1 cytokine. IFN-γ can suppress the Th2 pathway and its production of cytokines (e.g. IL-4), and consequently shift the balance of Th1/2 cytokine profile to Th1 predominance. This forcefully switches on cellular immunity and cell-mediated cytotoxicity that constitute inflammation. The laboratory manifestations of SARS include the induction of lymphopenia. Simultaneously, the early secretion of pyrogenic cytokine IL-1β and the acute-phase cytokine IL-6, also by macrophages, can cause fever, lymphocyte activation, macrophage stimulation, enhancement of leucocyte and endothelial adhesion, and induction of other acute phase reactions. SARS-CoV may also instigate an excessive immune response through overproduction of chemokines IL-8, MCP-1 and IP-10, resulting in transendothelial infiltration and accumulation of neutrophils, alveolar macrophages and Th1 lymphocytes into lung tissue causing pulmonary inflammation and destruction. Corticosteroids can significantly reduce the markedly elevated IL-8, MCP-1 and IP-10 concentrations by 5 – 8 days after treatment, thereby suppressing leucocyte infiltration into pulmonary tissue, alleviating disease severity, and controlling the rapidly deteriorating clinical condition. In view of the prominent roles of cytokines and chemokines in the pathogenesis of SARS, anti-cytokine / chemokine
immunotherapy using specific monoclonal antibodies (e.g. anti-IL-8, anti-MCP-1) or cytokine / chemokine antagonists may represent an effective approach for the treatment of hyperactive inflammation in SARS. The elevated plasma cytokines and chemokines should also suggest the possibility of using them as markers and prognostic indicators of disease severity in SARS.

Biochemical Markers of Bone Metabolism

Use of massive doses of corticosteroids for the treatment of SARS has been criticized because of its potential adverse effects on various organs including the skeletal system. Steroid-induced osteonecrosis, most frequently involving only the hips, can impose very significant morbidity and devastating disability. Estimated prevalence of osteonecrosis in the last decade was 7.6% in 132 post-renal transplant recipients, and 10% in 69 SLE patients. 55 It may develop in patients who received steroids in very high short-term doses, in long-term doses, or even by intra-articular injection. The interval between corticosteroid administration and the onset of symptoms is rarely less than six months and may be more than three years. 55 At this early stage (about 7 months after the epidemic), the incidence of steroid-induced osteonecrosis among SARS patients treated at our hospital, as confirmed by magnetic resonance imaging, is less than 5%, and up to 15% in other Hong Kong hospitals (unpublished communication from the Department of Diagnostic Radiology & Organ Imaging, The Chinese University of Hong Kong). We have conducted a retrospective study on the effect of massive doses of corticosteroids (median = 499 mg hydrocortisone-equivalent per day, 95% CI = 369 – 636) in 51 adult SARS patients (20 males and 31 females, age 21 - 89 years) so treated for 6 - 41 days. Serum osteocalcin (OC) and C-terminal telopeptide (CTx) were measured by electrochemiluminescence immunoassay (E170 Analyzer, Roche Diagnostics Corp, IN, USA) as bone formation and resorption markers, respectively. Another bone formation marker, serum bone-specific alkaline phosphatase (BALP), was measured using chemiluminescent enzyme immunoassay (Access Analyzer, Beckman-Coulter Inc, FL, USA). Results showed a significant increase in bone resorption as indicated by a marked elevation of serum CTx that occurred from Day 28 - 44 after the onset of fever, when the greatest frequencies of pulse methylprednisolone were given (Figure 3a). With tapering down of steroid dosage, CTx started to return to previous baseline concentration from Day 51 onwards, while the bone formation markers, serum OC and BALP, began to rise (Figures 3b and 3c). The later effect was even more marked on Day 90 or later. No patient experienced any fracture during the time course of the study. We concluded
from this pilot investigation that the adverse effect of massive doses of corticosteroids on bone metabolism was transient, and that osteoblasts began to replenish the bone matrix round 50 days, resulting in bone formation after the peak bone loss. However, the adverse effects on other organ systems remain to be investigated.

**Summary Remarks**

Severe acute respiratory syndrome is a newly emerged infectious disease that is highly contagious with significant morbidity and mortality. The epidemic from February to June 2003 affected more than 8,000 patients in 29 countries with 774 fatalities. The SARS coronavirus infected both men and women of all ages; many were previously in good health and the wage earners of their families. The spread of the disease to healthcare workers was a serious problem in most countries dealing with SARS. The need for isolation prohibited patients from family support and social contact; some of them became stigmatized. Combined clinics staffed by physicians, orthopaedic surgeons and physiotherapists, psychiatrists / clinical psychologists, and social workers have been established to help patients recover. Two months into the epidemic in April 2003, WHO estimated a worldwide loss of US$30 billion from SARS. For a final estimation of financial losses, the Asia Development Bank in last October arrived at figures of US$180 billion for Asia, and US$46 billion for Hong Kong, equivalent to 2.9% of our hard-earned general domestic product. To our community, the SARS period was the worst of times as characterized by Charles Dickens, with little illusion that it could also be the best of times.

Compared to colleagues in genomic sequencing and molecular epidemiology who have admirably climbed Mount Everest under very demanding conditions, we hope to equate our studies of the chemical pathology and clinical immunology of SARS to the crossing of Blue Mountains
(NSW, Australia) by Gregory Blaxland, William Lawson and Charles Wentworth in May 1813. These explorers hacked through dense bush for 18 days to discover prosperous agricultural and grazing countries westward, with a rich river that surveyor George Evans subsequently named The Macquarie and the location of a first inland town that Governor Lachlan Macquarie christened Bathurst. Braving the risk of infection in blood analysis, we reached the waterfall of SARS immunopathology, to observe cytokines and chemokines as disease markers, and LD1 isoenzyme and lymphocyte subsets as prognostic indicators. Of course real men and women thrive as explorers at the frontier - they much prefer practicing clinical biochemistry in the laboratory to attending committee board meetings.

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