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# SEVERE ACUTE RESPIRATORY SYNDROME (SARS): A Year in Review

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■ **Abstract** Severe acute respiratory syndrome (SARS) emerged from China as an untreatable and rapidly spreading respiratory illness of unknown etiology. Following point source exposure in February 2003, more than a dozen guests infected at a Hong Kong hotel seeded multi-country outbreaks that persisted through the spring of 2003. The World Health Organization responded by invoking traditional public health measures and advanced technologies to control the illness and contain the cause. A novel coronavirus was implicated and its entire genome was sequenced by mid-April 2003. The urgency of responding to this threat focused scientific endeavor and stimulated global collaboration. Through real-time application of accumulating knowledge, the world proved capable of arresting the first pandemic threat of the twenty-first century, despite early respiratory-borne spread and global susceptibility. This review synthesizes lessons learned from this remarkable achievement. These lessons can be applied to re-emergence of SARS or to the next pandemic threat to arise.

## INTRODUCTION

An unexplained, atypical pneumonia emerged in Guangdong Province of China in November 2002. This was followed by independent clusters of cases in seven provincial municipalities through mid-January 2003 (1, 2). The first official report of the outbreak to the World Health Organization (WHO) on February 11, 2003 cited 305 affected persons, five of whom had died and 30% of whom were health care workers (2). In late February, the illness made a dramatic escape from China, capturing international attention. An infected physician, who had cared for ill patients in Guangdong, stayed on the ninth floor of Hotel M in Hong Kong on February 21, 2003. Within 24 h he infected at least 16 other guests before being hospitalized. These infected guests then seeded multi-country outbreaks of a severe acute respiratory syndrome (SARS) that persisted through the spring of 2003 (Figure 1) (1). In response to these outbreaks, the WHO issued a global alert on March 12, 2003, followed by a travel advisory on March 15, 2003 (3). Such travel advisories have only rarely been issued by the WHO in response to the emergence and spread of an infectious disease.

Faced with a novel but severe, untreatable, and rapidly spreading respiratory illness of unknown etiology, the WHO invoked traditional public health measures to contain it. This included heightened vigilance, exit and entry screening for international travelers, isolation of affected persons, and quarantine of their close

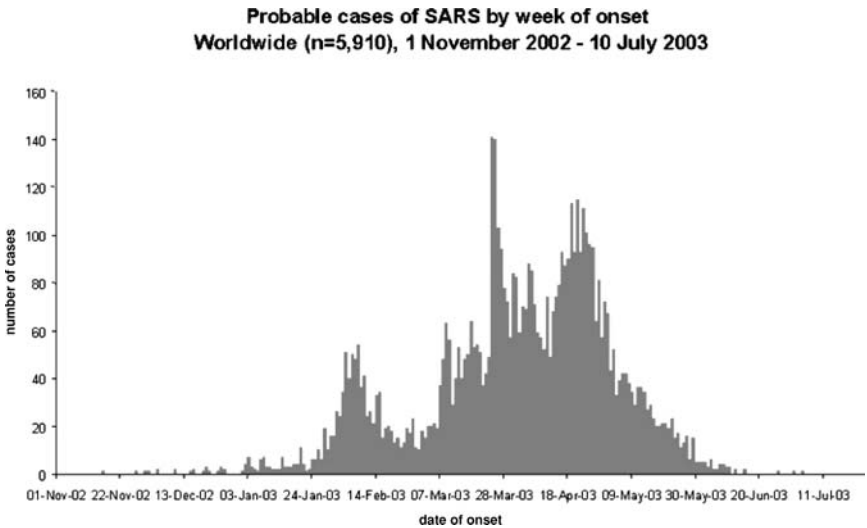


**Figure 1** Pandemic curve of probable cases of severe acute respiratory syndrome. This graph does not include 2,527 probable cases of SARS (2,521 from Beijing, China), for whom no dates of onset are currently available. Source: http://www.who.int/csr/sars/epicurve/epiindex/en/index1.html.

**TABLE 1** World Health Organization case definitions for surveillance of SARS in 2003[a]

| |
|---|
| **Probable case of SARS** |
| A suspect case with radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) on chest X-ray (CXR) |
| A suspect case that is positive for SARS coronavirus by one or more assays |
| A suspect case with autopsy findings consistent with the pathology of RDS without an identifiable cause |
| A case should be excluded if an alternative diagnosis can fully explain the illness |
| **Suspect case of SARS** |
| A person presenting after November 1, 2002[b] with history of<br>high fever (>38°C) AND<br>cough or breathing difficulty AND<br>one or more of the following exposures during the 10 days prior to onset of symptoms<br>i. close contact[c] with a person who is a suspect or probable case of SARS<br>ii. history of travel to an area with recent local transmission of SARS<br>iii. residence in an area with recent local transmission of SARS |
| A person who had an unexplained acute respiratory illness resulting in death after November 1, 2002 (but on whom no autopsy has been performed) AND one or more of the following exposures within 10 days prior to onset of symptoms<br>i. close contact[c] with a person who is a suspect or probable case of SARS<br>ii. history of travel to an area with recent local transmission of SARS<br>iii. residence in an area with recent local transmission of SARS |

[a]Adapted on April 23, 2004 from Reference 51 (version dated May 1, 2003): http://www.who.int/csr/sars/casedefinition/en/

[b]The surveillance period begins on November 1, 2002 to capture cases of atypical pneumonia in China now recognized as SARS. International transmission of SARS was first reported in March 2003 for cases with onset in February 2003.

[c]Close contact: having cared for, lived with, or had direct contact with respiratory secretions or body fluids of a suspect or probable case of SARS.

contacts (1, 2). At the same time, advanced technologies among expert networks were applied to identify the causative agent and to inform prevention and treatment options. A novel coronavirus was identified in patient specimens in late March 2003, and on April 16 the WHO announced that this coronavirus was the definitive cause of SARS (4–7). Without reliable laboratory diagnostic methods, surveillance and control measures depended on clinical case ascertainment throughout most of the pandemic (Table 1).

On July 5, 2003, four months after the initial WHO global alert, an exhaustive public health effort culminated in cessation of human-to-human transmission of SARS (3). All told, 8096 cases of SARS were reported from 26 countries, and 774 persons died as a result (case fatality of 10%) (8). The localities most strongly affected were China (Guangdong, Beijing, Hong Kong, and Taiwan); Singapore; Hanoi, Viet Nam; and the Greater Toronto Area, Canada. All but Taiwan had received cases prior to WHO alerts. Approximately two thirds of the cases (63%) and one third of the deaths (38%) were reported from mainland China (1). Economic

impact, largely attributed to reduced travel and investment in Asia, was estimated in the range of US$30–140 billion (2).

SARS was the first pandemic threat of the twenty-first century. Its sudden emergence in 2003 tested modern capacity to recognize and respond to unexpected communicable disease threats on a global scale. This review synthesizes key epidemiologic, scientific, and clinical lessons learned through the emergence, identification, and control of SARS.

## ETIOLOGY

### Agent

Infectious pathogens, such as metapneumovirus or chlamydia, were initially identified in the specimens of some affected patients and considered as potential etiologic agents in SARS. Only a novel coronavirus, however, was consistently found and fulfilled Koch's postulates for SARS causation—most notably, reproduction of illness following viral challenge in nonhuman primates (4–7). Final notation for this SARS-associated coronavirus has yet to be assigned. In the interim, the Coronaviridae Study Group of the International Committee on the Taxonomy of Viruses (ICTV) has recommended either SARS-CoV or SARS-HCoV. SARS-CoV has been the name most often used in publications to date.

Isolated in the mid-1960s, coronaviruses are a group of large, enveloped, positive-sense, single-stranded RNA viruses that are well-known agents of respiratory, enteric, and neurologic diseases in humans and domestic animals (cattle, pigs, dogs, cats, rabbits, rats, chickens, turkeys, etc.). (9, 10). Coronaviruses can be divided into three serologically distinct groups: two groups of predominantly mammalian coronaviruses (Group II also includes turkey coronavirus), and a third group of avian coronaviruses (chicken and turkey). Only two coronaviruses, HCoV-229E (Group I) and HCoV-OC43 (Group II), had previously been known to cause illness in humans (9, 10). Coronaviruses are responsible for 10%–35% of the upper respiratory tract infections known as the common cold and have been implicated in nosocomially acquired respiratory infections, possibly related to fomite transmission (10–12). Report of yet another Group I human coronavirus in 2004—HCoV-NL63—as a cause of upper respiratory illness in the Netherlands underscores that other, as yet unidentified coronaviruses may well be present in the human population (13, 14). There is also some evidence for uncharacterized human gastrointestinal coronaviruses (9, 10). These can escape detection by standard diagnostic tests unless specifically sought or dramatically manifest. Persistence of coronaviruses within a given host following infection is a well-known phenomenon.

It has been proposed that SARS-CoV represents an unrecognized animal coronavirus that became infectious to humans sometime in the past. Repeat transmissions from animals to humans in southern China are speculated to have occurred (15).

## Animal Reservoir and Sero-Archaeology

More than one third of early cases of SARS in Guangdong were animal or food handlers (16). Seroprevalence studies reinforced this zoonotic link: 13% of asymptomatic animal handlers at markets in Guangdong Province had antibodies against SARS-CoV, compared with 1%–3% of persons in three control groups ($p < 0.01$) (17). Apparently healthy Himalayan masked palm civet cats (family Viverridae), Chinese ferret badgers, and a raccoon dog, all sold in a market in Shenzhen, China, harbored a SARS-like coronavirus with >99% identity in the surface spike (S) protein to that of human SARS isolates (18). A mass cull of civet cats was undertaken in China in early 2004 following re-emergence of human cases with a possible link to these animals, but before establishing civets as the definitive reservoir (3). Other animal species, including fruit bats, snakes, and wild pigs, have also tested positive by reverse transcription polymerase chain reaction (RT-PCR) or serology, but issues related to test specificity and cross-reactivity must be considered in the interpretation of these results (1). Domestic pigs and chickens have not been reported as susceptible to SARS-CoV. Experimental infection of cynomolgus macaques has been reported to produce pneumonia pathologically similar to SARS in humans (6, 7). SARS-CoV infection of domestic cats and ferrets, disease in the latter and efficient transmission between them suggest the virus may be carried by a range of animals (2, 7, 19). The natural reservoir for SARS-CoV remains unknown.

The extent to which SARS-CoV may have infected people prior to 2003 is also unknown, but recent serologic studies in Hong Kong suggest that SARS-like viruses may have circulated in human populations previously (20, 21). Zheng and colleagues postulate that SARS may be the result of rapid evolution of a related coronavirus that had previously taken root in the human population and then experienced mutations (21).

## Evidence in the Genome

The RNA genome of coronaviruses represents the longest viral RNA known (27–32 kb). Coronaviruses replicate by a unique, discontinuous transcription mechanism that results in a high frequency of recombination (9, 10). This recombination generally occurs within groups rather than between groups of coronaviruses, although cross-group recombination has been observed (10). Like most RNA viruses, coronaviruses are also thought to mutate at a high frequency because of the high error rate of RNA-dependent RNA polymerases and the lack of an excision repair mechanism found in DNA polymerases (9, 10). Although this may lead to the accumulation of silent or conservative mutations, evidence suggests that most mutations are unlikely to yield competitive variants. Genetic stability of HCoV-OC43 isolates has recently been reported, for example, but the extent to which this stability may also apply to SARS-CoV is not yet known (22). The significance of observed mutations is ultimately determined by the combined effects of constraining and selective pressures exerted on the virus.

The first complete sequence of the SARS-CoV genome was reported by Canada's Michael Smith Genome Sciences Center at the BC Cancer Agency on April 12, 2003. Purified RNA from the second SARS fatality in Toronto, Canada, known as Tor2, was used to create two cDNA libraries of 1000–4000 base inserts using an RT-PCR approach. A second sequence of another isolate (Urbani) was reported by the U.S. Centers for Disease Control shortly after the sequence from Canada was released. These sequences differed by only eight nucleotides (23, 24).

Preliminary analysis of the SARS-CoV genome sequence identified genes for typical coronavirus proteins arranged in the same spatial organization as found in other coronaviruses. Lack of homology between SARS and representative members of previously sequenced coronaviruses, however, indicated that SARS-CoV was not the product of a simple recombination event. The largest open reading frames (ORFs) in coronaviruses, frequently referred to as the "Replicase" (Rep) gene (ORFs 1a and 1ab), are translated into large polypeptides. ORF 1a can be expessed alone as a 4000-amino-acid polypeptide. In addition, a translational frameshift mechanism can occur which allows translation to continue from ORF 1a through ORF 1b, resulting in a 7000-amino-acid protein (1ab, representing a fusion of ORF1a and ORF1b sequences). There are 14 putative cleavage sites on these two polypeptides, resulting in 16 predicted proteins. These mature proteins, along with the other SARS-CoV genes, have been grouped into 51 orthologous families available at the SARS Bioinformatics Suite (http://www.sarsresearch.ca). Many of the proteins do not have known functions.

Detailed analysis of the SARS-CoV genome identified an additional 9 ORFs (Tor2 ORFs 3, 4, 7–11, 13, 14) that are unique to this virus and have the potential to encode proteins ranging in size from 39 to 274 amino acids (23). The function of the proteins is unknown although convalescent sera have been shown to react with peptides from ORFs 3, 4, 9, 10, 13, and 14 (25). ORF 10, which encodes a putative 39–amino acid peptide, is particularly interesting. The fragmentation of this ORF into ORF 10 and 11 in SARS-CoV by a 29-nucleotide deletion at nucleotide 27,869 on the SARS-CoV genome appears to be the most significant difference between the SARS and the civet cat coronaviruses (18). It is unclear whether the 29-nucleotide deletion preceded or followed the transfer of the SARS coronavirus from its animal to human host, but subsequent analysis has identified five early human isolates (GZ02, HGZ8L1-A, HSZ-A, HSZ-B, and HSZ-C) that contain these additional 29 nucleotides (18, 26). This suggests that an animal virus may have transferred to humans and then subsequently lost these nucleotides. It is notable that several 82- and 415-nucleotide deletion variants have been detected in the same region of the RNA secondary structure of the genome; this area may be particularly susceptible to such events (26). The extent to which these minor deletional events may have conferred virulence or facilitated human-to-human spread is also unknown.

The predicted amino acid sequences of the structural proteins of SARS-CoV have only 20%–40% identity with those of the previously known three coronavirus groups. Unrooted phylogenetic analyses of the SARS-CoV proteins [Rep,

S, Membrane (M) and N] suggest that SARS-CoV may warrant assignment to a new, fourth Group within the coronavirus family (23, 24). Torovirus-rooted phylogenetic analysis of ORF 1b has more recently shown it to be related to Group II viruses, albeit very distantly, as may be the case for SARS-CoV (27, 28). Although SARS-CoV does not appear to be the simple product of a recent recombination, two reports have found evidence for recombination events in the evolution of its genome (23, 24, 27). In addition, the presence of the s2m sequence at the 3′ terminus (known only to be conserved in avian coronaviruses) supports a possible avian origin of this region. The neutral mutation rate for SARS-CoV during the epidemic is considered to have been constant at $\sim 8.26 \times 10^{-6}$ nt$^{-1}$ day$^{-1}$; this is similar to the rate of other known RNA viruses and is about one third that of the human immunodeficiency virus (HIV). The predicted domains of the S protein, responsible for receptor specificity and for recognition by neutralizing antibodies, showed the most numerous substitutions (26).

In aggregate, the evidence suggests that SARS-CoV arose by ancient recombination(s) with subsequent evolution, including deletional mutation events, over time.

## EPIDEMIOLOGY

Fifty-three percent of probable cases of SARS reported to the WHO were female, and all age groups were affected (age range 0–100 y) (8). Worldwide, SARS was strikingly a nosocomially acquired infection. Health care workers comprised 22% of reported cases in Hong Kong and Guangdong, China and >40% in Canada and Singapore (8, 29). A complex mix of agent, host-biologic, and behavioral factors and environmental context determine the magnitude and spread of outbreaks. Not all cities or countries that received even the earliest SARS importations experienced sustained transmission or outbreaks. For example, in Canada, both the Greater Toronto Area, Ontario and the city of Vancouver, British Columbia received critically ill patients from the Hotel M cluster. Whereas the Greater Toronto Area experienced an extensive outbreak, no secondary spread ensued from the case in Vancouver (29). Similarly, no sustained transmission occurred in the United States despite multiple importations (8). Why some areas experienced sustained outbreaks and others did not has yet to be fully explained.

### Incubation Period

The estimated incubation period for SARS is 2–10 d. An incubation period of as low as 1 d was reported from China (four cases) and Singapore (three cases). Incubation periods of 10–14 d have been reported in a small number of cases from China, but case ascertainment and a well-defined exposure interval for these cases are incomplete. Most countries reported a median incubation period of 4–5 d and a mean of 4–6 d. It remains unclear whether the route of transmission influences the incubation period (1).

## Infectious Period

There has been no evidence to date of SARS-CoV transmission prior to symptom onset, and transmission from asymptomatically infected persons has not been observed (20). There have been no reports of transmission beyond 10 d of fever resolution (1). Transmission appears to be greatest from severely ill patients and those experiencing rapid clinical deterioration, usually during the second week of illness (1). Patients with SARS are most infectious at around day 10 of illness (2). In this regard SARS is unlike most other respiratory-borne diseases, with the notable exception of smallpox.

## Route and Efficiency of Transmission

SARS-CoV is generally not very transmissible, with an average of 2–4 secondary cases stemming from each primary case (basic reproduction number or Ro) (1, 30, 31). Infected individuals have on occasion generated a disproportionately large number of transmissions, and the relative contributions of agent, host, and environmental factors to these so-called "superspreading" events require further study (3, 31–33). It is not known whether some virus lineages are more prone to transmission than others. Children only very rarely transmit the infection (20).

SARS-CoV is spread primarily through respiratory droplets in the context of close contact with very ill persons in the hospital or household setting (1). Aerosol generating procedures (intubation, nebulization, bronchoscopy, suction, and ventilation) have facilitated transmission during hospital care (1, 34, 35). There have been no reports of food- or waterborne transmission and no reports of blood or vertical transmission of SARS. The role of fecal-oral transmission is unknown but cannot be ruled out: A number of other coronaviruses are transmitted by this route; diarrhea is a symptom associated with SARS; and viral excretion is prolonged in stool (1, 9, 10, 36).

Among 329 residents affected during an outbreak of SARS in a single housing estate (Amoy Gardens) in Hong Kong, watery diarrhea was a prominent symptom (1, 36). This localized outbreak, in which 42 persons died, has been attributed to contaminated sewage droplets and faulty bathroom drains (1, 2). An animal vector (e.g., rodents, cockroaches) has also been postulated (37). Recent airflow-dynamics studies explain the curious pattern of spread at Amoy Gardens on the basis of a rising plume of virus-laden aerosol in the air shaft generated from a middle-level apartment unit (38).

Fomites may also play a role in transmission of SARS (1). SARS-CoV is surprisingly hardy for an enveloped RNA virus; it survives in urine and feces for 1–2 d and in diarrheic stools at higher pH for up to 4 d (1). The virus loses infectivity after exposure to various commonly used disinfectants and fixatives, and heat as low as 56°C inactivates SARS-CoV within 15 min (1).

Infection control measures to reduce contact, droplet, and aerosol spread are effective in mitigating transmission (39, 40). Spread in hospitals has generally occurred where these precautions have not been fully implemented. Patients with

atypical presentations may go unrecognized and have been important in facilitating surreptitious spread (41). A secondary attack rate in households of 5%–6% is estimated (42). Transmission has occurred on rare occasions after close contact with a symptomatic SARS patient in the workplace, on an airplane, in a taxi, or on a train (1, 20). Community-acquired infection was associated with a religious gathering in Toronto through close social contact (43).

The contribution of seasonality to the spread and subsequent disappearance of SARS remains unknown. Seasonality of other known human coronaviruses, HCoV-229E and HCoV-OC43, has been established in several large-scale investigations. These viruses are predominant in late winter and early spring. In one U.S. study of young adults, 97% of all serologically detected HCoV-229E infections occurred between January and May (43a). In children, two peaks in late autumn and early summer have been described (10). As with other respiratory pathogens, some geographic variation in the seasonal distribution of coronavirus activity is likely. Worldwide, SARS transmission ended abruptly in June 2003 (8).

## CLINICAL DISEASE

### Overview

A given strain of coronavirus, although possibly replicating in both respiratory and enteric tracts, generally causes disease in only one and is only occasionally more generalized (neurotropic, hepatotropic, nephrotropic) (9, 10). HCoV-229E and HCoV-OC43 produce respiratory symptoms of the common-cold type but have also occasionally been associated with more severe diseases (pneumonia, enteritis, myocarditis or neurologic symptoms), particularly in the extremes of age (9, 10). SARS-CoV replicates and causes severe disease in both respiratory and enteric tracts as well as in other systems in adults of all ages (44–47).

SARS is characterized by fever, chills, malaise, headache, cough, and dyspnea, along with radiologic evidence of pneumonia (29, 36, 48–51). Up to 70% of patients develop diarrhea, which has been described as voluminous and watery without blood or mucus (50). Illness is of insidious onset, and upper respiratory symptoms are uncommon. Lower respiratory symptoms progress slowly but steadily in severity for the first 10–15 d (29, 36, 48, 49). SARS-CoV pneumonia in most patients demonstrates slow spontaneous resolution, usually by the third week of illness (36, 52, 53).

### Presentation

The clinical profile of SARS at the onset of illness is summarized in Table 2 (54). The prodrome includes influenza-like symptoms such as fever, myalgias, headache, and diarrhea (53). The fever can vary from low to high grade or rarely can be absent, particularly in elderly persons.

The respiratory phase starts 2–7 d after the prodrome (36, 53, 54). The early respiratory stage includes a dry, nonproductive cough and mild dyspnea. Patients

**TABLE 2** Clinical profile at admission to hospital for SARS (adapted from Reference 54)[a]

| Clinical profile | Frequency in Toronto $n = 144$ | Frequency in Hong Kong $n = 138$ | Frequency in Hong Kong $n = 75$ |
|---|---|---|---|
| Age (years) | 45/34–57 (median/IQR) | 39.3/16.8 (mean/SD) | 39.8/12.2 (mean/SD) |
| Fever | 99.3% | 100% | 100% |
| Nonproductive cough | 69.4% | 57.3% | 22% |
| Myalgias | 49.3% | 60.9% | 68% |
| Dyspnea | 41.7% | NR | 4% |
| Headache | 35.4% | 55.8% | 15% |
| Malaise | 31.2% | NR | NR |
| Chills and rigors | 27.8% | 73.2% | 65% and 56% |
| Diarrhea | 23.6% | 19.6% | 1% |
| Nausea and vomiting | 19.4% | 19.6% | NR |
| Sore throat | 12.5% | NR | 11% |
| Arthalgia | 10.4% | NR | NR |
| Chest pain | 10.4% | NR | NR |
| Productive cough | 4.9% | 29.0% | NR |
| Dizziness | 4.2% | 42.8% | 4% |
| Abdominal pain | 3.5% | NR | NR |
| Rhinorrhea or coryza | 2.1% | 22.5% | NR |

[a]Abbreviations: SARS, severe acute respiratory syndrome; IQR, interquartile range; SD, standard deviation; NR, not reported.

may only have prodromal or early respiratory symptoms at the time of presentation, making the diagnosis of SARS difficult. Chest radiographic and laboratory findings may help the physician make an early diagnosis. Early chest radiographs often show subtle peripheral pulmonary infiltrates that can be more readily detected as consolidation or ground glass appearance using high-resolution computed tomographic lung scans (55, 56). Atypical presentations of the disease have also been described, further complicating the diagnosis (41).

## Natural History

Although branded on the basis of severity, SARS, like other infectious diseases, includes a spectrum of illness (54, 56). The disease is rare in children but when it occurs is classically mild (57). Asymptomatic illness has been described in a few persons (58). A small subset of persons experience febrile illness without a

respiratory component. More frequent is a mild variant of the disease that includes mild respiratory symptoms with fever. Within this category is a variant that presents with a persistent, intractable cough. The classic moderate-severe variant of SARS is characterized by a more serious later respiratory phase with dyspnea on exertion or at rest, and hypoxia. This later respiratory phase often occurs 8–12 d after the onset of symptoms (36, 53). In 10%–20% of hospitalized patients, persistent or progressive hypoxia requires intubation and mechanical ventilation (53, 59, 60). Subtle but progressive declines in oxygen saturation are often indicative of impending respiratory failure and should prompt more intensive monitoring and preparation for intubation under controlled circumstances. In total, the respiratory phase lasts ~1 week. The recovery phase begins ~14–18 d after the onset of symptoms with resolution of lymphopenia and thrombocytopenia.

### Prognosis

Although 6%–20% of patients may have some degree of respiratory impairment at hospital discharge, lung function returns to normal in most patients (20, 61). The overall case fatality is estimated at 10% (8). Advanced age is the most important risk factor for a fatal outcome. Children younger than 12 y of age with SARS experience good outcomes, whereas case fatality exceeds 50% for patients older than 65 y of age (1, 2, 20, 48, 52, 57, 62). Comorbid conditions, especially diabetes mellitus and heart disease, have been consistently found as independent predictors of SARS mortality (1, 2, 20, 36, 48, 52–54, 59, 60). Hepatitis B virus infection has also been reported to increase the risk of death (20). In some studies, high admission neutrophil count, high initial lactate dehydrogenase, and low CD4 and CD8 lymphocyte counts were associated with poor prognosis (20, 48, 63). Some studies report that males have higher SARS-related mortality (1).

## IMMUNE RESPONSE

### Cellular

A number of pro- and anti-inflammatory cytokines and chemokines have been detected in plasma during SARS (64). In particular, interleukin (IL)-13, IL-16, tumor necrosis factor alpha (TNF$\alpha$), and transforming growth factor beta (TGF$\beta$) are found at high levels during the initial phase of illness, and TNF$\alpha$ and IL-16 are found at high levels in plasma during the recovery phases (days 15–27). IL-18 levels are consistently suppressed throughout SARS. Antigen-specific cell-mediated immune responses to SARS have not been reported, although roles for both CD4 and CD8 T cells are likely. Specific human leukocyte antigen (HLA) class I alleles have been correlated with SARS susceptibility and resistance, indirectly suggesting a role for CD8 T cells (65). In vitro, SARS-CoV is susceptible to the influence of human interferons, with interferon-beta being the most active (66). Susceptibility to interferon-gamma depends on which cell type is tested.

## Humoral

Serum antibodies are detectable at a mean time of 20 d after disease onset (36). Seroconversion is characterized by the transient appearance of IgM antibodies, which is quickly followed by IgG antibodies that persist for >4 months (67). Antibodies recognize the internal viral nucleocapsid (N) protein, as shown by Western blot and by recombinant N protein enzyme-linked immunosorbent assay (ELISA) (68). Neutralizing antibodies are frequently produced on seroconversion and probably recognize epitopes on the heavily glycosylated SARS-CoV S protein, the target for such neutralizing antibodies in other coronaviruses (9, 10). The role of these antibodies in clearing plasma viremia and in contributing to resolution of SARS-CoV pneumonia is as yet unstudied but seems plausible. With other coronaviruses there is an inverse relationship between severity of disease and preexisting serum antibodies.

# PATHOLOGY

## Hematology

SARS produces characteristic changes in peripheral blood counts including leucopenia and lymphopenia (5, 7, 29). In one study the mean lymphocyte count ($\times 10^9$/L) for 38 SARS cases was $0.91 \pm 0.44$ compared to $3.00 \pm 1.00$ for normal controls ($p < 0.001$). Striking declines in both CD4 and CD8 T cells were noted along with more modest declines in B cells and natural killer cells (69). The reason for lymphopenia is unclear but may be related to limited SARS-CoV replication and induced apoptosis in peripheral blood mononuclear cells (70).

## Pathology

Few publications document findings from antemortem or postmortem examination of tissues from patients with SARS. In aggregate, pathologic features appear to represent a complex interplay of direct viral damage to cells and viral triggering of innate inflammatory and adaptive immune responses with some contribution of cytokine dysregulation. SARS-CoV has tissue tropism for the human respiratory and gastrointestinal epithelial surfaces, due in part to the specificity of the primary viral ligand, the S protein and its cognate receptor, angiotensin-converting enzyme 2 (ACE2) (5, 45, 71–73). ACE2 is expressed in lung, gastrointestinal, and renal tissue, and nucleic acid testing has detected SARS-CoV in all these sites (73).

Acute pulmonary SARS-CoV infection causes diffuse alveolar damage, desquamation of alveolar and bronchiolar epithelial cells, airspace edema, and bronchiolar fibrin deposition that involves nearly 50%–75% of the lung parenchyma and varies in grade from mild to marked. Multinucleate giant cells involving both macrophages and type II alveolar cells are seen. Multinucleate cells appear to represent virus-induced cell fusion secondary to S protein–ACE2 interactions (73).

Hemophagocytosis also occurs in the early phases of lung injury and has been attributed to cytokine dysregulation (74). Later in the reparative phase, lung pathology shows fibrosis and organizing pneumonia. Secondary features of bacterial or fungal bronchopneumonia are also noted in fatal cases of >10 d duration and may represent superinfection secondary to use of high-dose steroids (66).

Colonoscopy investigations from one patient with diarrhea and postmortem intestinal investigations from five cases revealed normal findings. By electron microscopy, viral particles (60–90 nm in size) that were consistent with coronavirus were detected in the small-intestine tissue and in the colonic biopsy specimens. Viral particles were confined to the epithelial cells, primarily in the apical surface enterocytes and rarely in the glandular epithelial cells (45).

## DIAGNOSTIC APPROACHES

Guidelines for appropriate collection, safe handling, and reliable processing of specimens should be consulted when a diagnosis of SARS is pursued (3, 50, 75, 76). Specimens from SARS patients have generally been handled in a containment level 2 laboratory by personnel wearing containment level 3 personal protective equipment. Growing or working with amplified virus, however, is a high-risk procedure to be undertaken only in a biologic containment level 3 laboratory (76). Laboratory-acquired SARS has already been reported in separate incidents in late 2003 and in 2004 from Singapore, Taiwan, and mainland China (Beijing). These underscore the importance of exquisite adherence to SARS-specific biosafety standards (3, 77).

In the postepidemic period, the specificity of assays has become an especially important consideration; a false positive finding has major economic implications as well as consequences for infection control and public health. Confirmatory testing is performed on multiple specimens (at least two sites or sequential samples from the same site) and in multiple laboratories (50). A definitive diagnosis also requires serologic evidence of infection either by documented seroconversion or high antibody titers (the latter if the specimen is obtained late in the illness or after recovery). A person under investigation for SARS should be excluded as a case if an alternative diagnosis can fully explain the illness and/or the results of sequential testing for SARS-CoV using validated methods are consistently negative or if a validated serologic test is negative 21 d or more after symptom onset (28 d if steroids have been used).

### Specimen Collection

SARS-CoV can be detected in secretions of the upper respiratory tract, such as sputum and secretions of the nasopharynx, and in the plasma fraction of the blood (78, 79). Viral loads in plasma are highest in the first week of illness and rapidly decline during the second week (78, 80). If respiratory symptoms progress and the patient is intubated, the virus can be readily detected in endotracheal secretions

and bronchoalveolar lavage specimens, as well as in postmortem lung in the event of death (29, 36, 75).

Peak viral shedding from the respiratory tract, stool, and urine occurs on about day 10 of illness and then declines (1, 36, 45, 75, 81). At its peak, nasopharyngeal viral shedding of $\sim 10^7$ genome copies per milliliter of nasopharyngeal aspirate has been detected (36). Urine has been reported as an acceptable but not optimal specimen (36, 82). In the study by Chan et al. (81), nasopharyngeal aspirates and throat and nose swabs were the most productive specimens in the first four days of disease, but stool samples were more useful after the fifth day of illness. In this study, although RNA was detectable $>30$ d after onset of symptoms from multiple sites, isolation of the virus was reported as difficult from any site after the third week.

## Tests for SARS-CoV

Laboratory confirmation of SARS can be on the basis of virus isolation, detection of viral RNA, or serologic assays.

DETECTION OF VIRUS/VIRAL RNA Although cells such as Vero can support the growth of SARS-CoV, the virus replicates most successfully in Vero-E6 cells and fetal rhesus monkey kidney cells (FRhK-4) (5, 81, 83). Cytopathic effects induced by the virus begin as a well-circumscribed hole in the monolayer surrounded by detaching cells in which sizable vacuoles can be discerned by phase contrast microscopy. Within 48 h, cytopathic effects may involve the entire monolayer. The virus can be readily detected in cell lysates by negative contrast electron microscopy. The virions have a corona or halo of large round peplomers about 10 nm in diameter (5). Isolation of the virus in cell culture is not the diagnostic method of choice, but it is important for subsequent viral characterization. Growth of the virus in cell culture is a prerequisite for determination of virus-neutralizing antibody.

Viral RNA for nucleic acid tests is extracted through conventional commercial extraction procedures. Numerous procedures based on nucleic acid amplification have been reported (84, 85). These include RT-PCR and nucleic acid sequence–based amplification (NASBA). The former generally targets sequences in the 5′ replicase (Rep) 1b gene or the 3′ N gene. Primers directed to the N gene are generally SARS-CoV specific. A NASBA assay has been developed by Biomerieux with proprietary primers and beacons. Several commercial RT-PCR assays have been produced; these real-time RT-PCRs use primers directed to the Rep1b region and, in the case of an assay developed by Eragen, also to the N region. NASBA and real-time RT-PCR have similar relative sensitivities and detect 10–100 genome copies or 0.1–100 plaque-forming units (pfu) of virus, with the real-time RT-PCR assays being more sensitive (82). RT-PCRs whose primers are directed to the N gene have potentially higher sensitivity because in the replication of coronaviruses all messenger RNAs are 3′ coterminal (9).

RT-PCR assay or other nucleic acid tests are the preferred methods for laboratory confirmation of SARS. The approach has been reported to be 100% specific

and 75%–79% sensitive compared to seroconversion when at least two distinct specimens are tested (85). SARS-CoV was detected in up to 80% of patients by RT-PCR on respiratory specimens collected between days 9 and 11 of illness, but in <40% of patients before day 5 or after day 15 of illness.

DETECTION OF ANTIBODY TO SARS-CoV Antibodies to SARS-CoV may be detectable in a subset of acutely ill patients, but in most cases (>90%) these become detectable only in convalescence, 3–4 weeks after onset of illness (36, 84, 85). Serologic assays may be based on immunologic or biologic (functional) indicators. Immunologic-based assays rely on the detection of antibody bound to variably defined SARS-CoV proteins, whereas biologic-based assays measure the ability of antibody to inhibit the growth of SARS-CoV in cell culture.

Immunologic assays for SARS include ELISAs with substrates varying from SARS-CoV–infected cell lysates to recombinant SARS-CoV N protein, and Western blots based on lysates of SARS-CoV infected cells (5, 68, 84). ELISA and Western blot detect immunoglobulin specifically bound to native and denatured viral proteins, respectively (5, 84). Because there is evidence that the N protein may have some immunodominant epitopes, and because the amino acid sequence of the N protein shares some epitopes with other coronaviruses, there is potential for a limited degree of cross-reactivity among the coronaviruses at the level of the immune response (23, 84). A lateral flow-colloidal gold adaptation of the ELISA has been described (84).

Virus-specific antibody can also be readily detected by immunofluorescence (IF) microscopy using SARS-CoV–infected and control cells fixed on a slide (5, 84). The cells are incubated with an appropriately diluted preparation of patient serum and subsequently with an antihuman IgG or IgM conjugate and are examined with a fluorescence microscope. A commercial assay based on infected and control cells grown on biochips is provided by Euroimmune (Euroimmune GmbH, Luebeck, Germany). Among the immunologic assays for antibody to SARS-CoV, the IF microscopy assay is perhaps the simplest to perform (84). It has, however, the potential drawback of being invalid if sera have antinuclear antibody.

The most applicable biologic assay is the microneutralization test (84). This test is, of all the serologic tests for SARS-CoV, the most specific. As a biologic assay, it measures the presence of SARS-CoV–specific immunoglobulin indirectly by its ability to neutralize the virus. It measures biologically relevant antibody, which makes it a definitive test for determining immunity to infection and response to a potential vaccine. Serial twofold dilutions of serum are incubated for 2 h with 100 pfu of virus, and the preparations are transferred to respective cell-culture wells on a microtiter plate. The cultures are examined within 3 d for the development of cytopathic effects. The reciprocal of the highest dilution of serum that protects the cells from developing cytopathic effects is designated the antibody titer. In this assay, only titers of 16 or greater are acceptable, since titers of 8 have been shown to occur nonspecifically. Because it incorporates a dilution principle, the assay readily

documents infection through seroconversion consisting of a fourfold or greater rise in antibody titer. Using a similar principle, the antibody titer can be determined in terms of plaque reduction, although the plaque-reduction neutralization test is considerably more complex and time-consuming.

## TREATMENT

Currently, no evidence points toward any specific therapy for SARS aside from supportive care.

### Antiviral

Antiviral agents used in the treatment of SARS include interferons, ribavirin, and lopinavir/ritonavir.

Interferons, particularly interferon-beta, inhibit SARS-CoV in vitro (86). An open-label study compared interferon-alfacon-1 and high-dose methylprednisolone against a historic cohort that received a lower dose of corticosteroid alone. This study demonstrated more rapid improvement in radiographic appearance and oxygenation among the interferon/methylprednisolone group (87). A complex four-arm trial that examined ribavirin, interferon, and differing doses of corticosteroids also demonstrated improvement in surrogate endpoints such as radiographic appearance, but only in the interferon arm that also received high-dose corticosteroids (88).

Ribavirin is a nucleoside analogue with in vitro activity against a number of RNA and DNA viruses, including some animal coronaviruses (73). Ribavirin was widely used for the treatment of SARS. Initial reports noted improvement in surrogate markers of outcome, such as resolution of fever and improvement in oxygenation and radiographic appearance (29). Subsequent reports, however, failed to identify improvement (53, 89). There was a high frequency of adverse events in patients treated with high-dose ribavirin, including severe hemolysis (53, 90). Knowles et al. (90) reported common adverse events in 110 patients with suspected or probable SARS who were treated with ribavirin. Sixty-one percent of the patients had evidence of hemolytic anemia, and hypocalcemia and hypomagnesemia were reported in 58% and 46% of patients, respectively (90). Finally, in vitro testing of SARS-CoV indicated that ribavirin is not active against this virus at clinically achievable concentrations (91). Postmortem findings demonstrated that high viral loads persisted in most patients despite treatment with ribavirin (92).

Lopinavir/ritonavir is a combination drug consisting of two protease inhibitors with proven efficacy in the treatment of HIV. Lopinavir/ritonavir used in a nonrandomized open-label study in Hong Kong as initial and rescue therapy was added to the local standard therapy, which consisted of ribavirin and corticosteroids (93). No data from this trial have yet been published.

### Anti-Inflammatory

Anti-inflammatory or immunomodulatory therapies include the use of corticosteroids and intravenous immunoglobulin (IVIG). Corticosteroids have been widely used for SARS therapy. Initial case reports described fever resolution and improvements in oxygenation and radiographic appearance in some patients treated with ribavirin and corticosteroids (74). Subsequently, clinicians noted that many patients progressed despite treatment with corticosteroids and that higher doses or pulsed steroid regimens were required as rescue therapy (94). A trial that compared early use of pulsed versus nonpulsed corticosteroids noted no difference in mortality or the requirement for ventilation but did see improvements in oxygenation and radiographic appearance (94).

Concerns have been raised about the use of prolonged high-dose and pulsed corticosteroid regimens in the treatment of a new viral infection (95). Pathology exams have detected high viral loads in patients who died >50 d into their illness, which suggests that persistent viral replication is occurring and probably contributing to the pathophysiology of lung damage in SARS-CoV infection (89). Corticosteroids are also associated with several well-known adverse outcomes including immunosuppression and avascular necrosis. Invasive fungal infections have been observed in patients treated for SARS with high-dose corticosteroids (96). Avascular necrosis of bone, a process that is characterized pathologically by bone marrow ischemia and eventual death of trabecular bone, has been reported from Hong Kong in a large number of patients who were treated with high-dose corticosteroids (61).

Standard IVIG has been used in the treatment of SARS in Taiwan, and IVIG derived from SARS patients' convalescent serum has been used to treat SARS in Hong Kong and elsewhere in China. However, no published data on these modalities, or on the use of human monoclonal antibodies in the clinical treatment of SARS, are currently available. Plasma exchange was used as salvage therapy in Hong Kong but no data exist on which to assess its efficacy.

## PREPARING FOR POSSIBLE RE-EMERGENCE

### Likelihood of Return

Although it is impossible to predict, there are reasons to be concerned about the possible return of SARS. In 2003, >8000 persons were affected in more than 25 countries, and worldwide susceptibility persists in the absence of an effective vaccine. No other zoonotic, respiratory-borne pathogen, disseminated on such a large scale, has been fully and lastingly driven from its human niche once so established—at least not through the use of traditional public health measures alone. An unidentified animal reservoir exists, and some epidemiologic and virus-sequencing evidence indicates that transmission from animals to humans continues in China in 2004 (3). Even without an animal reservoir, a human reservoir may now

exist; persistence of infection and periodic shedding from previously affected hosts has been documented with other coronaviruses and may also occur with SARS-CoV (1, 2, 9, 10). The contribution of seasonality to the sudden disappearance of SARS in 2003 is a possibility, as is its cyclical return, although this has not yet been evident. Exposure in laboratories where the virus is used or stored for diagnostic or academic purposes is also a potential ongoing source for SARS re-emergence. A number of confirmed SARS-CoV infections have already resulted from laboratory accidents in late 2003 and 2004 in Singapore, Taiwan, and China (Beijing), with sustained but limited transmission and fatality associated with the latter (3, 77).

## Updated Surveillance and Public Health Measures for the Interepidemic Period

The WHO has updated case definitions for surveillance, laboratory confirmation of infection, and SARS alert in the interepidemic period. Terminology related to "suspect" cases has been removed (3, 50). These changes reflect the absence of epidemiologic links to cases or to areas reporting recent local transmission. Instead, a clinical case definition (fever, lower respiratory symptoms, and radiographic evidence of pneumonia or respiratory distress syndrome) and a laboratory case definition for confirmation are recommended for surveillance purposes (50). Because of the public health and economic implications and the low predictive value of laboratory testing during the interepidemic period, vigilance must be balanced with clinical judgment and based on risk assessment. Risk assessment is defined by the WHO on the basis of three major areas: potential zones of re-emergence (origins of previous outbreaks or animal reservoirs); nodal areas (areas that previously experienced sustained transmission or currently receive large numbers of persons from potential zones of re-emergence); and low-risk areas. SARS alerts in potential zones of re-emergence and nodal areas are directed toward the more sensitive detection of nosocomial clusters and other enhanced surveillance indicators of re-emergence. This is to ensure that appropriate infection control and public health measures are implemented early and maintained until SARS has been ruled out as a potential cause of illness. Initial laboratory identification requires confirmation by a member of the WHO SARS International Reference and Verification Laboratory Network. Early implementation of infection control measures, isolation of persons under investigation for SARS, and monitoring of their close contacts remain the cornerstone of public health response until a vaccine becomes available.

## Vaccine Development

Reappearance of SARS in the human population would constitute a global public health emergency, and the exhaustive effort to contain it in 2003 would not be sustainable in the long term should it become more firmly established. In anticipation of this possibility, global efforts have been mobilized to develop a vaccine against SARS-CoV. Veterinary vaccines that target coronaviruses in chickens, cattle, pigs,

cats, and dogs already exist (10). Inactivated vaccines, though suboptimal, are widely administered to egg-producing chickens as a booster dose following application of live attenuated vaccine (10, 97). In most cases, vaccines in animals have been safe and cost-effective from the perspective of food-producing commercial outcomes. Disease enhancement, however, has been reported in cats in the case of live attenuated vaccine for feline infectious peritonitis (FIP) (21, 98). The molecular mechanisms by which disease exacerbation occurs in FIP are not fully understood, but specific classes of antibodies against the S protein can accelerate the disease process through their effect on Fc-receptor-mediated uptake of the virus by macrophages (99, 100). Although there is no evidence that a vaccine against SARS will induce enhanced disease, this will be a necessary and specific area of investigation for any SARS-CoV vaccine candidate.

Pursuit of a SARS-CoV vaccine has focused on conventional inactivated formulations as the most rapid, economical, and familiar approach. Other approaches include adenovirus or vaccinia virus vector recombinants expressing S with or without N viral proteins that, when delivered intranasally, may also induce mucosal immunity. Because the S protein of coronavirus is critical for attachment and initiation of infection and is a target for neutralizing antibodies, efforts are also under way to produce large quantities of S protein in insect or mammalian cell cultures for use as vaccine candidates. The S protein alone has been shown to induce immunity in chickens; S protein expressed in fowl adenovirus as a single oral application protected 90%–100% of vaccinated birds (97). The greatest challenge in the design of recombinant S protein is to ensure maintenance of its conformational integrity—a feature that is critical for the preservation of conformational antibody-directed epitopes and that may require expression in eukaryotic cells. DNA-based vaccine expressing S protein is also in development (101).

Passive immunization for postexposure prophylaxis of contacts is being developed in the form of neutralizing monoclonal antibody preparations for intramuscular administration. Passive transfer of mouse immune serum has been shown to reduce pulmonary viral titers in infected mice. In addition, human monoclonal antibodies against the S protein can inhibit virus replication in vitro in cell cultures and in vivo in ferret models (102). The delayed incubation period of SARS may facilitate success of antibody prophylaxis, although antibody preparations can be costly to administer.

Regardless of the vaccine candidate that is chosen, animal models are needed as evidence of baseline safety and efficacy before moving to trials in humans. Several animals have demonstrated virus replication and some level of pathology, including nonhuman primates (rhesus and cynomolgus macaques), ferrets, and cats (6, 19). Mice, hamsters, and other species are also being evaluated as potential models, and preliminary vaccine investigations using one or more of these are in progress. Human vaccine effectiveness studies will only be possible in the context of SARS re-emergence. This will require rapid implementation of trial protocols wherever in the world SARS may reappear with sufficient attack rate to power the interpretation of results. For this reason, vaccine trial protocols that are portable should be

developed as an international collaborative effort. In the event that immunization programs become an urgent public health need, agreement on licensing standards should ideally be sought in advance from authorities worldwide, so that qualifying studies do not have to be repeated on a country-by-country basis.

## CONCLUSION

In the 15 months since SARS first emerged internationally, >1500 SARS-related publications have appeared in peer-reviewed journals. This staggering catalogue represents an impressive array of epidemiologic, scientific, and clinical exploration whose focus has been the urgent control of a novel respiratory-borne pathogen. Such urgency facilitated global collaboration and the coordination of scientific endeavors. Ultimately, despite early spread and global susceptibility, the world proved capable of arresting the first pandemic threat of the twenty-first century. This remarkable achievement occurred at the frontier of public health tradition and scientific innovation. Re-emergence of SARS now looms as an uncertain possibility. Maintaining interest in vaccine development against that uncertainty becomes an ongoing challenge. What is most certain, however, is that the lessons learned from SARS will have future applications—whether related to re-emergence of SARS-CoV itself or the next pandemic threat to arise.

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