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In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds

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Abstract

Effective antiviral agents are urgently needed to combat the possible return of severe acute respiratory syndrome (SARS). Commercial antiviral agents and pure chemical compounds extracted from traditional Chinese medicinal herbs were screened against 10 clinical isolates of SARS coronavirus by neutralisation tests with confirmation by plaque reduction assays. Interferon-beta-1a, leukocytic interferon-alpha, ribavirin, lopinavir, rimantadine, baicalin and glycyrrhizin showed antiviral activity. The two interferons were only active if the cell lines were pre-incubated with the drugs 16 h before viral inoculation. Results were confirmed by plaque reduction assays. Antiviral activity varied with the use of different cell lines. Checkerboard assays for synergy were performed showing combinations of interferon beta-1a or leukocytic interferon-alpha with ribavirin are synergistic. Since the clinical and toxicity profiles of these agents are well known, they should be considered either singly or in combination for prophylaxis or treatment of SARS in randomised placebo controlled trials in future epidemics.

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1. Introduction

Although the SARS epidemic has been successfully contained with quarantine and infection control measures, the presence of this virus in wild game food animals (Guan et al., 2003), stocks in laboratories and possible seasonality of this disease suggest that recurrence of such an epidemic is not unlikely in the coming winters. Since all age groups are affected and a high fatality is noted in the elderly and those with co-morbidities (Donnelly et al., 2003), there is an urgent need to find a cure. Prospective clinical and viral load studies in nasopharyngeal secretions from SARS patients showed that viral replication peaked at the 10th day after the onset of symptoms with subsequent clinical deterioration in 30% of the cases despite a decreasing viral load (Peiris et al., 2003). Therefore the key facet of management should include respiratory support, immuno-modulation in selected cases and early institution of an effective antiviral agent. Such an antiviral agent, if given early, may decrease the peak viral load and the associated immuno-dysregulatory damage. At the moment, there are no commercially available antiviral agents tailored-made for SARS coronavirus. There is an urgent need to search for an agent with a known in use clinical and toxicity profile so that a randomised placebo control trial can be conducted if the epidemic recurs in one of the coming winters. We report in this study on the in vitro antiviral susceptibility of 10 isolates of SARS coronavirus to commercially available antiviral agents and pure chemical compounds including baicalin, glycyrrhizin, and chlorogenic acid extracted from traditional Chinese herbs.

2. Methods

Ten isolates of SARS coronavirus from 10 different SARS patients who satisfied the revised WHO criteria for SARS are listed in Table 1. The drugs used for antiviral susceptibility
Table 1
Clinical data of 10 isolates of SARS coronavirus in 10 patients suffering from SARS

| Patient number | Isolate number | Clinical specimen | Sex/age | Day of specimen after onset of symptoms | RT-PCR result | Day of seroconversion after onset of symptoms | Antibody titer by immunofluorescence staining | Hospital |
|----------------|----------------|-------------------|---------|------------------------------------------|---------------|-----------------------------------------------|----------------------------------------------|----------|
| 1              | M39849         | Lung tissue biopsy | M/54    | 9                                        | Positive      | 9                                             | 1:160 KWH                                    | KWH      |
| 2              | M36871         | NPA               | F/41    | 6                                        | Positive      | 12                                            | 1:1280 PMH                                   | PMH      |
| 3              | M35189         | NPA               | F/41    | 6                                        | Positive      | 20                                            | 1:640 PYNEH                                  | PYNEH    |
| 4              | M70221         | NPA               | M/30    | 10                                       | Positive      | 22                                            | 1:2560 PYNEH                                  | PYNEH    |
| 5              | M71749         | NPA               | F/40    | 11                                       | Positive      | 24                                            | 1:160 PYNEH                                  | PYNEH    |
| 6              | M51776         | NPA               | F/39    | 6                                        | Positive      | 10                                            | 1:640 TKOH                                   | TKOH     |
| 7              | M61558         | NPA               | F/53    | 10                                       | Positive      | 18                                            | 1:40 UCH                                     | UCH      |
| 8              | M61556         | Urine             | M/40    | 12                                       | Positive      | 18                                            | 1:40 UCH                                     | UCH      |
| 9              | M61565         | NPA               | M/38    | 10                                       | Positive      | 18                                            | 1:40 UCH                                     | UCH      |

Note: KWH, Kwong Wah Hospital; NPA, Nasopharyngeal aspirates; PMH, Princess Margaret Hospital; PYNEH, Pamela Youde Nethersole Eastern Hospital; TKOH, Tseung Kwan O Hospital; UCH, United Christian Hospital.

* Prototype virus.

The pharmacological properties of baikalin, glycyrrhizin, and chlorogenic acid are summarized in Table 2. Artesunate is not included in this table since it is already well known as an anti-malarial drug in Western Medicine (Price, 2000). They were extracted as we have previously reported (Lu et al., 2003). The concentrations of baikalin, glycyrrhizin,
Table 2
Summary on three natural compounds from traditional Chinese medicines

| Compounds                  | Bacalain (黄芩苷) | Glycyrrhizin (甘草甜素) | Chlorogenic acid (茶多酚) |
|----------------------------|-------------------|-------------------------|--------------------------|
| Name of herbs              | Scutellaria baicalensis (Huang Qin) | Glycyrrhiza uralensis (Gan Cao) | Lonicera japonica (金银花) |
| Chemical structure         | C_{21}H_{18}O_{11} | C_{42}H_{62}O_{16} | C_{16}H_{18}O_{9} |
| Molecular weight           | 354.3             | 822.9                   | 348.3                    |
| Thermal stability          | Stable at boiling temperature (a typical extraction method) | Stable at a temperature below 120 °C | Stable after long time boiling |
| Serum level (after oral administration) | C_{\text{max}} = 60 μg/ml (189.8 mg per person, in human) | No glycyrrhizin in plasma is found after oral administration of 100 mg glycyrrhizin in healthy persons, presumably glycyrrhizin is metabolized to glycyrhetinic acid by intestinal bacteria which contain β-glucuronidase or the amount consumed is too little | Only traces expected (1000 mg per person, in human); this may be due also to the amount consumed is much lower than that for animals |
| Standard doses in oral administration in humans | ~1500 mg bacalain (as tablets); also can be up to ~6000 mg bacalain (calculated from herb, assuming 30 g herb used; the herb may contain up to 26% as bacalain) | ~222 mg (calculated from the herb assuming that the herb contains 7.4% chlorogenic acid) | ~1700 mg chlorogenic acid (calculated from the herb assuming that the herb contains 5.6% glycyrrhizin) |
| Serum level (after intravenous administration in humans) | C_{\text{max}} = 74 μg/ml (360 mg per person) | C_{\text{max}} = 80 μg/ml (200 mg per person) | C_{\text{max}} = 34 μg/ml (24 mg per person) |
| Standard doses in intravenous administration in humans | ~600 mg bacalain | ~240 mg | ~74 mg, when used together with bacalain in injection preparation, 180 mg for muscle injection |
| Half life (in humans)      | ~3 h              | ~10 h                   | ~10 h                    |
| Antiviral effect           | Inhibition of HIV-1 | Inhibition of SARS-associated virus | Inhibition of various viruses |

chlorogenic acid, and lopinavir in the cell culture system were also monitored by HPLC (Lu et al., 2003) whereas the concentration of others were monitored by neutralization assays with the Vesicular Stomatitis Virus Indiana strain and a laboratory strain of Influenza A H1N1. The procedures used for in vitro antiviral susceptibility testing are as follows. Initial screening of all compounds against the prototype SARS coronavirus strain no. 39849 was performed in 96-well microtitre plates seeded with foetal rhesus kidney-4 cells. Two-fold dilutions of antiviral agents starting from more than four times the peak serum concentration after the maximum therapeutic dose to less than one-quarter of the trough serum concentration were tested in quadruplicate against 100 TCID$_{50}$ of SARS coronavirus. A corresponding set of cell controls with drug but without virus inoculation was used as controls for drug toxicity. The cells were scored for the inhibition of the cytopathic effect (CPE) at 48 and 72 h. Those compounds with demonstrable in vitro inhibitory activity were re-assayed against the other nine strains of SARS coronavirus collected from different patients from different hospitals of the Hong Kong Special Administrative Region (HKSA). Their antiviral activities were also compared in both foetal rhesus kidney-4 (fRhK-4) and Vero-E6 cell lines. Those likely to have clinically significant inhibitory activity were tested by the plaque reduction assay.

For selected agents with consistent activity in the plaque reduction assay, checkerboard assays for synergy were performed for combinations of interferons and ribavirin using the same neutralization test in 96 well microtitre plates seeded with Vero cell line. Cells were not incubated with the interferons before viral inoculation. Vero cells were used instead of Vero E6 and fRhK-4 cell lines because better antiviral effect can be demonstrated in Vero but less so in the other two cell lines for ribavirin and the interferons.

3. Results

Ten isolates of SARS coronavirus from 10 different patients with SARS admitted to different hospitals in HK-SAR showing seroconversion towards the prototype virus infected rRhK-4 cell line were used in this study (Table 1). They were isolated from the lung tissue biopsy (prototype virus, M39949), urine, and nasopharyngeal aspirates. Initial screening of 20 commercially available antimicrobial agents against the prototype virus grown in rRhK-4 cell line did not reveal inhibitory activities for acyclovir, ganciclovir, cidofovir, foscamet, interferon-alpha-2a, interferon-alpha-2b, amantadine, zidovudine, stavudine, nevirapine, abacavir, and ritonavir. Inhibitory activities were not detectable for glycyrrhizin, artecmate and chlorogenic acid in rRhK-4 cell line. Glycyrrhizin was still included for further testing because this was reported to be active in Vero-E6 cell lines (Cinatl et al., 2003a). Further testing by neutralization tests...
Table 3
Comparison of antiviral activity of 10 compounds against 10 strains of SARS-CoV in fRhK4 cell line, against the prototype strains (39849) of SARS-CoV in fRhK4 and Vero-E6 cell lines by neutralization test

|            | fRhK4 cell line (against 10 strains of SARS-CoV) | IRhK4 cell line (against 39849) | Vero-E6 cell line (against 39849) |
|------------|-------------------------------------------------|---------------------------------|----------------------------------|
|            | EC50 (µg/ml) at 48 h                            | EC50 (µg/ml) at 72 h            | SI = CC50/EC50 at 48 h           |
| Ribavirin  | 12.5 to 200                                     | 50 to 200                       | 5 to >80                         |
| Interferon alpha (natural multi-subtype) added at and after viral adsorption | 5000 IU a | >1000 IU a | >10,000 IU a |
| Interferon alpha (natural multi-subtype) pre-incubation for 16h | 39 to 625 IU a | >10,000 IU a | >16 to 250 |
| Interferon beta 1a added at and after viral adsorption for 16h | 2500 to 10,000 IU a | 10,000 IU a | >10,000 IU a |
| Interferon beta 1a pre-incubation for 16h | 625 IU a | 10,000 IU a | >10,000 IU a |
| Rimantadine | 8 to 16                                         | 32                             | 4 to 8                           |
| Lopinavir  | 1 to 4                                          | 4 to 8                          | 8 to 32                          |
| Bucalain   | 12.5 to 25                                      | 25 to 50                        | >100                             |
| Glycyrrhizin | >400                                        | >400                            | NA                               |

Note: EC50, effective concentration of compound required to inhibit the cytopathic effect to 50% of control value; CC50, cytotoxic concentration of compound that reduced cell viability to 50%; SI, selectivity index.

4. Discussion

Control of SARS may be achieved by epidemiological measures, antiviral prophylaxis or treatment, and vaccination. During the last pandemic of SARS, the only available means for control were public health measures such as isolation of suspected cases, quarantine of contacts, and personal protective infection control procedures for high-risk individuals such as health care workers. There is an urgent need to find effective antiviral agents with acceptable side effect profiles. In developing countries such as China, commercially available western antiviral medicine is unlikely to be affordable by most people. Moreover, the SARS mortality of Mainland China was only 7% comparing favourably with the 15% to 27% of other areas (WHO, 2003). China
Table 4
Checkerboard assay for synergism between interferons and ribavirin by neutralization test without pre-incubation

| Interferon alpha | 72h |  |  |  |  |  |  |  |  |  |  |  |  |
|------------------|-----|---|---|---|---|---|---|---|---|---|---|---|---|
| Ribavirin         |     | 200| 100| 50 |25 |12.5| 0  | 200| 100| 50 |25 |12.5| 0  |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| 96h              | 1000| 500| 250| 1250|625|312.5|156| 78| 39|19.5|0  |  |  |
| Ribavirin         |     | 200| 100| 50 |25 |12.5| 0  | 200| 100| 50 |25 |12.5| 0  |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| Interferon beta | 1a  |84h | 1000| 500| 250| 1250|625|312.5|156|78|39|19.5|0  |  |
| Ribavirin         |     | 200| 100| 50 |25 |12.5| 0  | 200| 100| 50 |25 |12.5| 0  |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |
| +                | +   | + | + | + | + | + | + | + | + | + | + | + | + |

+ ≥50% inhibition; − ≤50% inhibition.

is also the only place where traditional Chinese medicinal herbs were extensively used for treatment of SARS. The development of vaccine will take a much longer time. Therefore, we undertook these antiviral susceptibility tests for all commercially available antiviral agents in the HKSAR and pure chemicals purified from traditional Chinese herbs known to have antimicrobial activity. These chosen herbs were included in a standard formula used for the treatment of SARS in China and the HKSAR.

Only interferon-beta and glycyrrhizin were reported to have significant antiviral activity against SARS coronavirus (Cinatl et al., 2003a,b). Using the RhK-4 cell line, we have shown that ribavirin, rimantadine, lopinavir, and baicalin also have detectable antiviral activities. However, like the interferons and glycyrrhizin, their activities tend to decrease with incubation beyond 48 h (Table 3). Judging from the achievable serum levels with standard oral or parenteral dosing, rimantadine, ribavirin, glycyrrhizin, and even the two interferons are unlikely to have clinically significant in vivo activities. Moreover, lopinavir, and rimantadine have a relatively inferior selectivity index of 4 to 32. Upon subsequent testing with Vero-E6 cell line, both leukocytic interferon-alpha and interferon-beta-1a were more active and especially after pre-incubation for 16 h before viral inoculation. The findings suggest that prophylaxis with the interferons should be considered. Though ribavirin was much less active in the Vero cell line, it is highly synergistic with either two interferons. Therefore, a combination of ribavirin with either of these two interferons should be considered for the treatment of SARS.
Interferon-gamma was reported not to possess antiviral activity against SARS coronavirus (Cinatl et al., 2003b), whereas interferon-beta was confirmed to be active in this study. What is interesting was the demonstration of activity of leukocytic interferon-alpha despite the lack of activity of the recombinant interferon-alpha-2a and interferon-alpha-2b. This was not unexpected because this preparation of leukocytic interferon-alpha is a multi-subtype natural interferon with predominantly interferon alpha-1 and alpha-2 in contrast to the other commercial preparation with a single subtype of recombinant interferon-alpha-2.

In vitro studies, different subtypes have been found to have different antiviral activities as well as immunological effects (Foster et al., 1996). It was also demonstrated that leukocytic interferon-alpha had a superior antiviral effect than that of recombinant interferon on Human immunodeficiency Virus infection (Fan et al., 1993).

It is important to know that in vitro findings may not correspond with clinical efficacy. Despite its in vitro activity, topical or systemic interferon-alpha did not produce a consistent reduction in symptoms or lesion duration of genital herpes (Eron et al., 1987; Lebowohl et al., 1992). And interferon-alpha was not effective in preventing CMV infections or treating CMV pneumonia in bone marrow transplant patients (Meyers et al., 1980). Despite its broad-spectrum antiviral activities against respiratory viruses in vitro, prophylactic intranasal interferon-alpha is only protective against rhinovirus-induced common cold under natural condition (Douglas et al., 1986). This was unexpected because intranasal leucocyte or recombinant interferon-alpha protect against experimental human infection by rhinovirus, coronavirus, and respiratory syncytial virus (Hayden and Gwaltney, 1984; Higgins et al., 1983; Higgins et al., 1990).

Besides the high cost of interferons, the high incidence of pancytopenia may also be confused with markers of SARS activity such as a decrease in platelets and occasionally neutrophils (Raanani and Ben-Bassat, 2002). Although interstitial pneumonitis and bronchiolitis obliterans organisating pneumonia are rare complications of prolonged use of interferons (Karim et al., 2001; Ogata et al., 1994), there is always a fear that their proinflammatory effect may worsen the viral pneumonitis caused by SARS.

As for the less expensive option, baicalin but not glycyrrhizin is considered for treatment in randomised placebo control trials. The serum level after 100 mg of glycyrrhizin orally was not detectable. Even with a 200 mg dose of intravenous administration, the peak serum level is only 30 μg/ml which is still below the EC50 of glycyrrhizin. Although an oral dose of 1.5 gms of baicalin can only achieve a serum concentration of 0.47 μg/ml, intravenous administration of a 360 mg dose of baicalin in human can achieve a peak serum concentration of 74 μg/ml. Thus intravenous baicalin should be considered for treatment in randomised placebo control trials in developing countries where such formulations are available and affordable. Baicalin was shown to inhibit HIV-1 by two mechanisms (Kitamura et al., 1998; Li et al., 2000). At the level of cellular entry, baicalin can conjugate with selected chemokines such as MIP-1α and SDF-1α, and interfere with their capacity to activate cellular receptors CCR5 and CXCR4 respectively. These two co-receptors are essential elements for HIV-1 infection and therefore baicalin can inhibit Env-protein mediated fusion of HIV with cells expressing CCR5 or CXCR4. Baicalin has also been shown to inhibit HIV-1 reverse transcriptase probably by interfering with the binding of viral RNA to the RT molecule near the active site of the enzyme.

In terms of prophylaxis against SARS short of an effective vaccine, intranasal leukocytic interferon-alpha or interferon-beta-1a are likely to be effective. However the local side effect of nasal irritation can decrease compliance. It could also be considered for randomised placebo-control trials.

As for the antiviral treatment of symptomatic SARS, it is important to have a rapid and reliable diagnostic test since early institution of antiviral therapy is important to decrease the peak viral load (Poon et al., 2003). Interferon-beta-1a or leukocytic interferon-alpha plus ribavirin appear to be the most effective combination. Since interferons may not be effective in inducing an antiviral state in the uninfected host cells during the first 24 h, a combination with a short course of ribavirin appears to be reasonable. This will also reduce the side effects and fluid volume associated with a full course of ribavirin. Despite the superiority of interferons in the in vitro assays, there is little clinical data of its use in the treatment of acute viral respiratory infection in human. Thus the combination of ribavirin with lopinavir/ritonavir should still be considered since some positive clinical data has already been accumulated in a historical controlled treatment trial (Chu et al., 2004).

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References

Cinatl J, Morgenstern B, Bauer G, Chandak P, Rabenau H, Deen HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 2003a;361:2045–6.
Cinatl J, Morgenstern B, Bauer G, Chandak P, Rabenau H, Deen HW. Treatment of SARS with human interferons. Lancet 2003b;362:293–4.
Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, et al. The role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 2004;59:252–6.
Donnelly CA, Ghani AC, Leung GM, Hellewell J, Fraser C, Riley S, et al. Epidemiological determinants of spread of casual agent of severe acute respiratory syndrome in Hong Kong. Lancet 2003;361:1761–6.
Douglas RM, Moerz BW, Miles HB, Davies LM, Graham NM, Ryan P, et al. Prophylactic efficacy of intranasal alpha 2-interferon against rhinovirus infections in the family setting. N Engl J Med 1986;314:65–70.

Eisen LJ, Toy C, Saloiz B, Schoen RR, Wood DL, Nadler PE. Therapy of genital herpes with topically applied interferon. Antimicrob Agents Chemother 1997;41:1375–9.

Fan SX, Skillman DR, Liao MJ, Tosta D, Melzak MS. Increased efficacy of human natural interferon alpha (IFN-alpha n3) versus human recombinant IFN-alpha 2 for inhibition of HIV-1 replication in primary human monocytes. AIDS Res Hum Retroviruses 1993;9:1115–22.

Foster GR, Rodrigues O, Ghosez F, Schulte-Frohlinde E, Tosta D, Liao MJ, et al. Differential relative activities of human cell-derived interferon-alpha subtypes. IFN-alpha 8 has very high antiviral potency. J Interferon Cytokine Res 1996;16:1027–33.

Guan Y, Zheng BJ, He YQ, Liu XL, Zhang ZX, Cheung CL, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. Science 2003;302:276–8.

Hayden FG, Gwaltney Jr JM. Intranasal interferon-alpha 2 treatment of experimental rhinoviral colds. J Infect Dis 1984;150:174–80.

Higgins PG, Phipps RJ, Scott GM, Wallace J, Bernhardt LL, Tyrrell DA. Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrob Agents Chemother 1983;24:713–8.

Higgins PG, Barrow GL, Tyrrell DA, Isaacs D, Gauci CL. The efficacy of intranasal interferon alpha-2a in respiratory syncytial virus infection in volunteers. Antiviral Res 1999;40:1–10.

Karim A, Ahmed S, Khan A, Steinberg H, Mattana J. Interstitial pneumonitis in a patient treated with alpha-interferon and ribavirin for hepatitis C infection. Am J Med Sci 2001;322:213–5.

Kitamura K, Honda M, Yoshikawa H, Yamamoto S, Nakane H, Fukushima M, et al. Baculain, an inhibitor of HIV-1 production in vitro. Antiviral Res 1998;37:131–40.

Lebwohl M, Sacks S, Comnar M. Recombinant alpha-2 interferon gel treatment of recurrent herpes genitalis. Antiviral Res 1992;17:235–43.

Li BQ, Fu T, Dongyan Y, Mikovits JA, Russett PW, Wang JM. Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. Biochem Biophys Res Commun 2000;276:534–8.

Lu HT, Jiang Y, Chen F. Application of high-speed counter-current chromatography to the preparative separation and purification of baicalin from the Chinese medicinal plant Scitellaria baicalensis. J Chromatogr A 2003;1017:117–23.

Meyers JD, McGuffin RW, Neiman PE, Singer JW, Thomas ED. Toxicity and efficacy of human leukocyte interferon for treatment of cytomegalovirus pneumonia after marrow transplantation. J Infect Dis 1980;141:555–62.

Ogita K, Kogo T, Yaya K. Interferon-related bronchiolitis obliterans organizing pneumonia. Chest 1994;106:612–3.

Pesins JS, Chua CM, Cheng VC, Chan KS, Hung IF. Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003;361:1767–72.

Poon LL, Chan KH, Wong OK, Yum WC, Yum KY, Guan Y, et al. Early diagnosis of SARS Coronavirus infection by real time RT-PCR. J Clin Virol 2003;28:233–8.

Price RN. Artemisinin drugs: novel antimalarial agents. Expert Opin Invest Drugs 2003;9:1813–27.

Rausch P, Ben-Bassat I. Immune-mediated complications during interferon therapy in hematological patients. Acta Haematol 2002;107:133–44.

World Health Organization. Summary table of SARS cases by country. 1 November 2002–7 August 2003. Available at: http://www.who.int/csr/sars/country/table20030923/en/. Accessed 29 October 2003.