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# Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study

## 以快利佳/諾億亞治療嚴重急性呼吸系統綜合症：多個中心的回顧性對照組別研究

**Objectives.** To investigate the possible benefits and adverse effects of the addition of lopinavir/ritonavir to a standard treatment protocol for the treatment of severe acute respiratory syndrome.
**Design.** Retrospective matched cohort study.
**Setting.** Four acute regional hospitals in Hong Kong.
**Patients and methods.** Seventy-five patients with severe acute respiratory syndrome treated with lopinavir/ritonavir in addition to a standard treatment protocol adopted by the Hospital Authority were matched with controls retrieved from the Hospital Authority severe acute respiratory syndrome central database. Matching was done with respect to age, sex, the presence of co-morbidities, lactate dehydrogenase level and the use of pulse steroid therapy. The 75 patients treated with lopinavir/ritonavir were divided into two subgroups for analysis: lopinavir/ritonavir as initial treatment, and lopinavir/ritonavir as rescue therapy. These groups were compared with matched cohorts of 634 and 343 patients, respectively. Outcomes including overall death rate, oxygen desaturation, intubation rate, and use of pulse methylprednisolone were reviewed.
**Results.** The addition of lopinavir/ritonavir as initial treatment was associated with a reduction in the overall death rate (2.3%) and intubation rate (0%), when compared with a matched cohort who received standard treatment (15.6% and 11.0% respectively, P<0.05) and a lower rate of use of methylprednisolone at a lower mean dose. The subgroup who had received lopinavir/ritonavir as rescue therapy, showed no difference in overall death rate and rates of oxygen desaturation and intubation compared with the matched cohort, and received a higher mean dose of methylprednisolone.
**Conclusion.** The addition of lopinavir/ritonavir to a standard treatment protocol as an initial treatment for severe acute respiratory syndrome appeared to be associated with improved clinical outcome. A randomised double-blind placebo-controlled trial is recommended during future epidemics to further evaluate this treatment.

**目的：**研究在嚴重急性呼吸系統綜合症的標準治療方案之上，加入快利佳/諾億亞的潛在好處及副作用。
**設計：**回顧性對照組別研究。
**安排：**香港四間提供急症服務的地區醫院。
**患者及方法：**75名接受快利佳/諾億亞配合醫院管理局標準治療方案的嚴重急性呼吸系統綜合症對照病人資料，與從醫院管理局嚴重急性呼吸系統綜合症中央數據庫獲取的病人資料，就年齡、性別、其他病患、乳酸脫氫酶水平，以及接受靜脈滴注類固醇的情況，進行配對。75名接受快利佳/諾億亞為療法的病人，被分為以快利佳/諾億亞為首要療法，及以之作為拯救療法兩組，進行分析。將這兩組病人分別與634名及343名對照病組別人進行比較。回顧的結果包括總死亡率、氧不飽和度、插管比率，以及使用靜脈滴注甲基潑尼松龍的比率。
**結果：**加入快利佳/諾億亞為首要療法的病人，其總死亡率（2.3%）及插管比率（0%）較接受標準療法（分別為15.6%及11.0%，P<0.05）的對照病人為低；使用

甲基潑尼松龍的比率及平均劑量亦較低。以快利佳/諾億亞為拯救療法的病人，其總死亡率、氧不飽和度及插管比率，均與對照組病人沒有分別；而使用甲基潑尼松龍的平均劑量較高。

**結論：**在標準治療方案中加入快利佳/諾億亞，作為嚴重急性呼吸系統綜合症的首要療法，似乎能改善臨床結果。建議在未來的流行病中使用抽樣雙盲安慰劑對照劑試驗，以進一步評估此療法。

## Introduction

Severe acute respiratory syndrome (SARS) has created global alarm, with significant impact on the health care and economy of affected areas. At the time of writing, at least 30 regions had been affected with over 8099 probable cases and 774 deaths.[1] The intubation rate for patients with SARS in many centres was over 20%,[2,3] and the overall case fatality rate was 9.6%.[1] Both human and animal studies have now confirmed that SARS is caused by a novel coronavirus (SARS-CoV),[4-6] satisfying Koch's postulations for causation.[7,8]

In the early days of the epidemic, histopathological changes in lung tissue from patients suggested a viral aetiology possibly associated with immuno-dysregulation in pathogenesis as evident by the pronounced activation of macrophages within the alveoli.[9] Various treatment regimens were recommended for SARS, ranging from supportive therapy to intensive immunomodulation by corticosteroids, with the majority being empirical.[2,3,10-12] Before SARS-CoV was identified as the causative agent, ribavirin, a purine nucleoside analogue which prevents replication of a large number of RNA and DNA viruses, was suggested for treatment in view of its broad spectrum activity.[2,13] Based on this theoretical background and the low fatality rate reported in early cases,[2,10] the combination of corticosteroid and ribavirin therapy was adopted by the Hospital Authority of Hong Kong (HA) as the interim standard treatment in the early phase of the SARS epidemic.[14] With isolation of SARS-CoV, in vitro anti-viral susceptibility testing demonstrated that the virus was inhibited by ribavirin at a level which was difficult to achieve in the clinical setting.[15] As the epidemic progressed, the case fatality rate in Hong Kong was projected to be around 13% for patients younger than 60 years and 43% for patients aged 60 years or older,[16] indicating an urgent need to find an effective therapy.

A previous prospective study of the clinical progression of SARS and the viral load of patients demonstrated a progressive increase in viral load in the respiratory tract, reaching a peak in the second week of the illness.[17] The results implied a window period of lower viral load during which anti-viral therapy might confer significant clinical benefit. A number of treatment modalities, including anti-viral peptides inhibiting viral fusion, anti-sense RNA, and immunomodulators have been suggested. However, these options are not clinically available at the time of writing.

Among the licensed anti-viral drugs screened in our laboratory, lopinavir was demonstrated to have clinically relevant in vitro activity against the prototype SARS-CoV HKU39849; synergism was also demonstrated for lopinavir and ribavirin.[15] Given this information, a number of physicians in the HA had used lopinavir/ritonavir (LPV/r) in addition to the standard treatment protocol. This was given either as an initial treatment, or as a rescue treatment after failure of standard treatment, as previous data had suggested patients might have persistently high viral loads.[17] The aim of this study was to analyse retrospectively the outcomes of patients with SARS treated with LPV/r compared with a matched cohort who received standard treatment only.

## Methods

### *Diagnosis and standard treatment protocol*

During the epidemic, all SARS patients were admitted to HA hospitals as policy. A modified World Health Organization definition was adopted as the case definition of probable SARS. This included the presence of the following: fever of 38°C or higher; new radiological infiltrates compatible with pneumonia; two of the following: chills, cough, general malaise, physical signs of lung consolidation; and the absence of an alternative diagnosis to explain the clinical presentation. Probable SARS cases were treated according to the principles of the HA SARS guideline.[10,14,18] These included a trial of broad spectrum antibiotics, consisting of a combination of a β-lactam plus a macrolide, or levofloxacin, according to current recommendations.[19] As soon as the diagnosis of SARS was established, ribavirin was given for 10 to 14 days (2.4 g oral loading dose, followed by 1.2 g orally every 8 hours, or 8 mg/kg intravenously every 8 hours, if the patient could not tolerate oral treatment), together with a tailing regimen of corticosteroid therapy for 21 days (starting dose: hydrocortisone 100-200 mg every 6-8 hours, or methylprednisolone 3 mg/kg/day, depending on severity).[10,14,18] If patients developed increasing shortness of breath, oxygen desaturation, and radiological worsening, pulses of methylprednisolone 500-1000 mg daily given intravenously were used as rescue therapy, if not contraindicated.[10] Patients were considered for intubation if they developed acute respiratory distress syndrome (ARDS) as defined by a partial pressure of arterial oxygen to fraction of inspired oxygen ($PaO_2/FiO_2$) ratio of <200 mmHg,[20] or had persistent and severe hypoxaemia with oxygen saturation of less than 85%, despite oxygen supplementation.

### *Lopinavir/ritonavir treatment protocol*

From 16 April 2003, physicians from four participating hospitals (United Christian Hospital, Princess Margaret Hospital, Tuen Mun Hospital, and Caritas Medical Centre)

added lopinavir 400 mg/ritonavir 100 mg orally every 12 hours to the above standard regimen after obtaining consent from patients. Patients with contra-indications, such as pre-existing liver disease, known hypersensitivity, or who were pregnant were excluded from treatment with LPV/r. Approval for off-license use of LPV/r was obtained from the HA. Lopinavir/ritonavir were given for 10 to 14 days, depending on disease severity and patient tolerance. Physicians either gave LPV/r as an initial treatment in combination with ribavirin for sequential patients newly diagnosed to have SARS; or it was given as rescue therapy later in the course of the illness when patients had worsening oxygen saturation, shortness of breath, and relevant radiological findings, and after patients were judged to have failed pulse steroid treatment. In the latter group, administration of LPV/r may not have been given concurrently with ribavirin, depending on the time of the decision to use LPV/r as rescue therapy.

### *Data collection*

A standardised data capture form was developed for collecting clinical data for all probable SARS cases admitted to HA hospitals. The information captured included the following:

(1) important dates (onset of fever, onset of symptoms, contact with SARS patients, admission, discharge or death);
(2) presence of pre-defined co-morbidities (asthma, chronic obstructive pulmonary disease, ischaemic heart disease, cerebrovascular disease, cancer, diabetes mellitus, chronic renal failure, and chronic liver disease);
(3) daily observations of clinical parameters (temperature, pulse, respiratory rate, bowel movement, oxygen saturation, fraction of inspired oxygen); and
(4) details of drug treatment, invasive and non-invasive ventilation.

The clinical information was merged with selected laboratory and pharmacy information from the HA central database.

Patients treated with LPV/r who fulfilled the criteria for probable SARS were recruited from the four hospitals. Case matching was performed between the LPV/r-treated group and the HA standard treatment group. Patients treated with LPV/r were divided into two subgroups for analysis: LPV/r as initial treatment and LPV/r as rescue therapy, as previously described. Matched cohorts, who were treated with the standard treatment adopted by the HA, were retrieved from the database for the two subgroups. Matching was done with respect to the reported prognostic factors for poor outcome: age, sex, presence of co-morbidities and level of lactate dehydrogenase (LDH).[2,3] Age was matched according to five defined age-groups (15-24 years, 25-44 years, 45-64 years, 65-84 years, and 85 years or older). Co-morbidity was matched according to the presence or absence of significant medical illnesses. Lactate dehydrogenase was matched according to six defined ranges of LDH levels (<300 IU/L, 300-399 IU/L, 400-499 IU/L, 500-699 IU/L, 700-899 IU/L, and ≥900 IU/L).

For the subgroup that received LPV/r as initial treatment, matching with patients who received standard treatment was performed on four variables—age, sex, co-morbidity, and the initial LDH level within 5 days of onset of symptoms. For the subgroup that received LPV/r as rescue therapy after pulse steroid treatment, matching was performed on five variables—age, sex, co-morbidity, maximal LDH level before the administration of pulse methylprednisolone, and the use of pulse methylprednisolone. Patients with SARS who had received standard treatment and who matched with the respective study groups on the relevant prognostic variables were included as controls. This approach was adopted to strengthen statistical power and avoid sampling variation due to random selection of control patients from the database. The distribution of patients in the two LPV/r-treated subgroups and their matched cohorts across the prognostic strata are shown in Tables 1a and 1b.

The following outcomes were compared for each of the LPV/r-treated subgroups and their matched cohorts: death rate, percentage of patients with oxygen desaturation (inability to maintain normal saturation of ≥96% despite supplemental oxygen), airway intubation rate, and use of rescue pulse methylprednisolone (proportion and mean dose). Adverse effects in terms of raised serum transaminase levels (a three-fold rise in alanine aminotransferase [ALT]) and a raised serum amylase level (two-fold rise) were compared between the treatment subgroups and matched cohorts.

The age-standardised mortality rates for all patients with SARS aged 15 years or older admitted to the HA during the epidemic were retrieved. These were then classified into five cohorts according to their time of symptom onset. We assessed whether there was a general trend towards declining mortality through the epidemic as clinical knowledge, experience and acumen improved.

### *Statistical analysis*

To adjust for the differing distribution of patients across the prognostic strata between the LPV/r-treated and the standard treatment group, the direct standardisation method was employed to compute the standardised outcome rates for each of the two matched cohorts, which were then compared with the corresponding crude rates of the LPV/r-treated subgroups. The outcome rates for the matched cohorts were weighed against the proportion of LPV/r-treated cases in each prognostic stratum as shown in Tables 1a and 1b.

The outcome rates of the LPV/r-treated group were considered to be significantly different from the respective matched cohort's standardised rates if their 95% confidence intervals (95% CIs) did not overlap. The exact 95% CIs for binomial variables were calculated for the LPV/r-treated subgroups whereas the 95% CIs of the directly standardised

**Table 1a. Matching of the LPV/r initial treatment group**

| Sex | Co-morbidity | Age-group (years) | First lactate dehydrogenase result (IU/L) | LPV/r as initial treatment No. (%) | Matched cohort No. (%) |
|---|---|---|---|---|---|
| F | No | 15-24 | 1-299 | 1 (2.3) | 49 (7.7) |
| F | No | 25-44 | 1-299 | 12 (27.3) | 209 (33.0) |
| F | No | 25-44 | 300-399 | 3 (6.8) | 65 (10.3) |
| F | No | 45-64 | 1-299 | 7 (15.9) | 69 (10.9) |
| F | No | 45-64 | 300-399 | 1 (2.3) | 25 (3.9) |
| F | No | 45-64 | 400-499 | 2 (4.5) | 13 (2.1) |
| F | No | 45-64 | ≥500 | 1 (2.3) | 18 (2.8) |
| F | No | 65-84 | 1-299 | 1 (2.3) | 12 (1.9) |
| F | No | ≥85 | 1-299 | 1 (2.3) | 1 (0.2) |
| F | Yes | 25-44 | 1-299 | 1 (2.3) | 6 (0.9) |
| F | Yes | 65-84 | ≥500 | 1 (2.3) | 4 (0.6) |
| F | Yes | ≥85 | 1-299 | 1 (2.3) | 4 (0.6) |
| F | No | 25-44 | 1-299 | 4 (9.1) | 114 (18.0) |
| M | No | 65-84 | 1-299 | 1 (2.3) | 7 (1.1) |
| M | Yes | 25-44 | 300-399 | 1 (2.3) | 2 (0.3) |
| M | Yes | 45-64 | 300-399 | 3 (6.8) | 10 (1.6) |
| M | Yes | 45-64 | ≥500 | 1 (2.3) | 4 (0.6) |
| M | Yes | 65-84 | ≥500 | 2 (4.5) | 22 (3.5) |
| (Overall) | | | | 44 (100.0) | 634 (100.0) |

**Table 1b. Matching of the LPV/r rescue treatment group***

| Sex | Co-morbidity | Age-group (years) | First lactate dehydrogenase result (IU/L) | LPV/r as rescue treatment No. (%) | Matched cohort No. (%) |
|---|---|---|---|---|---|
| F | No | 15-24 | 300-399 | 1 (3.2) | 8 (2.3) |
| F | No | 15-24 | 700-899 | 1 (3.2) | 3 (0.9) |
| F | No | 25-44 | 1-299 | 1 (3.2) | 84 (24.5) |
| F | No | 25-44 | 300-399 | 2 (6.5) | 33 (9.6) |
| F | No | 25-44 | 400-499 | 2 (6.5) | 16 (4.7) |
| F | No | 25-44 | 500-699 | 4 (12.9) | 25 (7.3) |
| F | No | 25-44 | 700-899 | 1 (3.2) | 9 (2.6) |
| F | No | 45-64 | 1-299 | 2 (6.5) | 27 (7.9) |
| F | No | 45-64 | 400-499 | 1 (3.2) | 7 (2.0) |
| F | No | 65-84 | ≥900 | 1 (3.2) | 1 (0.3) |
| F | Yes | 45-64 | 300-399 | 1 (3.2) | 3 (0.9) |
| F | Yes | 65-84 | 500-699 | 1 (3.2) | 2 (0.6) |
| M | No | 15-24 | 300-399 | 1 (3.2) | 3 (0.9) |
| M | No | 15-24 | 700-899 | 1 (3.2) | 1 (0.3) |
| M | No | 25-44 | 1-299 | 2 (6.5) | 49 (14.3) |
| M | No | 25-44 | 300-399 | 3 (9.7) | 22 (6.4) |
| M | No | 25-44 | 400-499 | 1 (3.2) | 20 (5.8) |
| M | No | 25-44 | 500-699 | 2 (6.5) | 17 (5.0) |
| M | No | 45-64 | 300-399 | 1 (3.2) | 6 (1.7) |
| M | No | 45-64 | 500-699 | 1 (3.2) | 6 (1.7) |
| M | Yes | 45-64 | 700-899 | 1 (3.2) | 1 (0.3) |
| (Overall) | | | | 31 (100.0) | 343 (100.0) |

* All patients received pulse methylprednisolone

rates for the matched cohorts were approximated using the method for weighted sums of independent Poisson variables proposed by Dobson et al.[21] Two sample *t*-tests were used to test the between-group difference in the mean dose of pulse methylprednisolone.

## Results

The initial database included 1521 probable cases of SARS who were admitted into HA hospitals. There were 676 males and 845 females, and the mean age was 42.5 years (standard deviation [SD], 19.5 years). Eighty-one LPV/r-treated patients were identified from the four centres. Of the 81 LPV/r-treated patients, five patients did not have LDH readings at an appropriate time-point and one patient did not have a matched control, and were therefore excluded from the analyses. Data from 75 patients were analysed, and all had microbiological confirmation of SARS-CoV infection by serology or reverse-transcriptase polymerase chain reaction (RT-PCR) tests. Among the 75 patients, LPV/r was given as initial therapy to 44 patients and as rescue therapy to 31 patients. The prognostic profiles of these patients are reported in Table 2.

For the subgroup that received LPV/r as initial treatment, a total of 634 patients were retrieved from the standard treatment group as controls after matching on the four variables of age, sex, co-morbidity, and initial LDH level.

**Table 2. Profiles of the two LPV/r-treated subgroups according to prognostic indicators**

| Prognostic factor | Data | LPV/r as initial treatment, n=44 No. (%) | LPV/r as rescue treatment, n=31 No. (%) |
|---|---|---|---|
| Sex | Male | 12 (27.3) | 13 (41.9) |
| | Female | 32 (72.7) | 18 (58.1) |
| Age (years) | 15-24 | 1 (2.3) | 4 (12.9) |
| | 25-44 | 21 (47.7) | 18 (58.1) |
| | 45-64 | 15 (34.1) | 7 (22.6) |
| | 65-84 | 5 (11.4) | 2 (6.5) |
| | ≥85 | 2 (4.5) | 0 |
| Co-morbidity | Yes | 10 (22.7) | 3 (9.7) |
| | No | 34 (77.3) | 28 (90.3) |
| Lactate dehydrogenase (LDH) level (IU/L)* | <300 | 29 (65.9) | 5 (16.1) |
| | 300-399 | 8 (18.2) | 9 (29.0) |
| | 400-499 | 2 (4.5) | 4 (12.9) |
| | 500-699 | 5 (11.4) | 8 (25.8) |
| | 700-899 | 0 | 4 (12.9) |
| | ≥900 | 0 | 1 (3.2) |

* The LDH level refers to the initial level for the LPV/r as initial treatment group and to the maximal LDH level before pulse methylprednisolone for the LPV/r as rescue treatment group

**Table 3. Comparison of outcomes for the group given LPV/r as initial treatment and a matched cohort***

| | LPV/r as initial treatment, n=44 Crude rate or mean (95% CI) | Matched cohort, n=634 Standardised rate or mean† (95% CI) | P value |
|---|---|---|---|
| Death rate (%) | 2.3 (0-6.8) | 15.6 (9.8-22.8) | <0.05 |
| Intubation rate (%) | 0 | 11.0 (7.7-15.3) | <0.05 |
| Desaturation rate (SaO2≤95%) [%] | 68.2 (52.3-81.8) | 84.5 (74.4-95.2) | NS‡ |
| Proportion requiring pulse methylprednisolone rescue (%) | 27.3 (11.4-40.9) | 55.4 (47.6-63.9) | <0.05 |
| Mean pulse methylprednisolone dose (g) | 1.6 (1.1-2.0) | 3.0 (2.8-3.2) | <0.05 |

* Matched on age, sex, presence/absence of co-morbidity, and initial lactate dehydrogenase level
† Standardised based on the percentage distribution of subjects of the treated group across the prognostic strata in Table 1
‡ NS not significant

**Table 4. Comparison of outcomes of the group given LPV/r as rescue treatment and a matched cohort***

| | LPV/r as rescue, n=31 Crude rate or mean (95% CI) | Matched cohort, n=343 Standardised rate or mean† (95% CI) | P value |
|---|---|---|---|
| Death rate (%) | 12.9 (0-25.8) | 14.0 (5.2-26.3) | NS‡ |
| Intubation rate (%) | 9.7 (0-22.6) | 18.1 (9.0-29.7) | NS |
| Desaturation rate (SaO2≤95%) [%] | 93.5 (80.6-100) | 92.1 (75.9-100) | NS |
| Mean pulse methylprednisolone dose (g) | 3.8 (3.5-4.2) | 3.0 (2.9-3.2) | <0.05 |

* Matched on age, sex, presence/absence of co-morbidity, lactate dehydrogenase level before pulse methylprednisolone, and use of pulse methylprednisolone
† Standardised based on the percentage distribution of subjects of the treated group across the prognostic strata in Table 1
‡ NS not significant

In this control group, 576 (90.9%) patients had positive SARS-CoV serological or RT-PCR tests. Treatment with LPV/r was started at a median of 5.5 days after symptom onset, and one day after the commencement of ribavirin. Of the 44 patients who received LPV/r as initial therapy, 30 patients (68.2%; 95% CI, 52.3-81.8) had episodes of oxygen desaturation and 12 patients (27.3%; 95% CI, 11.4-40.9) were given pulse methylprednisolone. None were intubated and one patient died (2.3%; 95% CI, 0-6.8). In the matched cohort, the respective standardised rates for various outcomes were as follows: oxygen desaturation in 84.5% (95% CI, 74.4-95.2); use of pulse methylprednisolone in 55.4% (95% CI, 47.6-63.9); intubation in 11.0% (95% CI, 7.7-15.3); and death in 15.6% (95% CI, 9.8-22.8). Comparing the 95% CIs of the LPV/r-treated group and the matched cohort, there was a statistically significant difference evident in the overall death rate and intubation rate, with reductions seen with LPV/r treatment. As shown in Table 3, the mean dose of pulse methylprednisolone in the subgroup who received LPV/r as initial treatment was lower (1.6 g; 95% CI, 1.1-2.0) than the matched cohort (3.0 g; 95% CI, 2.8-3.2). Regarding drug toxicity as evidenced by a three-fold rise in ALT, there was no significant difference in rate between the group who received LPV/r as initial therapy and the matched cohort (9.1%; 95% CI, 0-18.2 versus 6.9%; 95% CI, 4.5-9.9). Raised serum amylase levels (a two-fold rise) were reported in 5% (95% CI, 0-15) in the group given LPV/r as initial treatment compared

with 2.4% (95% CI, 0-4.8) of the matched cohort (non-significant). No patient in either group had serum amylase levels greater than 1000 IU/L.

For the LPV/r rescue subgroup, a total of 343 patients were retrieved from the standard treatment group as controls after matching of the five variables of age, sex, co-morbidity, peak LDH level, and the use of pulse methylprednisolone. In this control group, 329 (95.6%) patients had positive SARS-CoV serological or RT-PCR tests. Treatment with LPV/r was started at a median of 18 days after symptom onset, and did not overlap with ribavirin treatment (median time-lag, 1 day). As shown in Table 4, there were no significant differences in overall oxygen desaturation, intubation, and death rates between the treatment and control groups. The mean dose of pulse methylprednisolone given was significantly higher (3.8 g; 95% CI, 3.5-4.2) compared with the matched cohort (3.0 g; 95% CI, 2.9-3.2). Regarding drug toxicity as evidenced by a three-fold rise in ALT, there was no significant difference between the group receiving LPV/r as rescue therapy and the matched cohort (25.8%; 95% CI, 9.7-41.9 versus 9.1%; 95% CI, 5.8-13.4). Raised serum amylase levels (two-fold rise) were reported in 11.8 % (95% CI, 0-29.4) in the LPV/r as rescue treatment group versus 2.6% (95% CI, 0-6) of the matched cohort (not significant). In the LPV/r as rescue therapy group, one patient was reported to have a serum amylase level greater than 1000 IU/L, but none was reported in the matched controls. Treatment with LPV/r was discontinued in this affected patient, with subsequent normalisation of amylase levels.

The age-standardised mortality rates for 1667 SARS cases aged 15 or more were retrieved from the HA SARS central database. The rates are reported for the five time periods according to the dates of symptom onset: 15.0% for 15 March 2003 or before; 18.1% for 16 to 31 March; 17.8% for 1 to 15 April; 15.1% for 16 to 30 April; and 20.8% for 1 to 31 May. These age-standardised mortality rates did not suggest a trend towards declining mortality throughout the epidemic.

## Discussion

Genomic analysis of the SARS-associated coronavirus genome revealed dfferent types of enzymatic targets—the RNA replicase, and the proteases.[22-24] The discovery of a putative mRNA cap-1 methyltransferase may suggest another potential target for anti-viral therapy.[25] Although a chemically synthesised cysteine proteinase inhibitor (E64d),[26] a natural salivary cysteine proteinase inhibitor (cystatin D),[27] and a natural serine proteinase inhibitor (leupeptin)[28] are known to inhibit mouse or human coronaviruses in cell culture systems at low concentrations, none of these has been used in the treatment of coronavirus infection in animals or humans.

With respect to nucleoside analogues, success has been achieved by ribavirin in treating fulminant hepatitis caused by mouse coronavirus.[29] Ribavirin is active against mouse coronavirus, mostly due to its indirect immunomodulatory effect rather than its weak anti-viral activity.[29] The only anti-viral agent that has been used against coronavirus in human is alpha-interferon, which is given intranasally as a prophylaxis rather than a treatment for common cold.[30] Recently, alpha-interferon and beta-interferon have been shown to inhibit SARS-CoV in vitro.[31] Hitherto, alpha-interferon has not been used in the treatment of SARS because it is known to be pro-inflammatory and associated with pulmonary toxicity, including causing interstitial pneumonitis, and bronchiolitis obliterans-organising pneumonia.[32] Glycyrrhizin, an active component of liquorice root, has been found to inhibit SARS-CoV replication in vitro,[33] but no clinical data are available on its use.

Both lopinavir and ribavirin individually have a weak in vitro inhibitory effect on the prototype SARS-CoV. In vitro anti-viral susceptibility testing showed that the cytopathic effect (CPE) of SARS-CoV was inhibited by lopinavir at 4 μg/mL and by ribavirin at 50 μg/mL after 48 hours of incubation.[15] This is in keeping with the fact that lopinavir is specific for aspartate proteases.[34] Using the checkerboard assay for synergy, CPE inhibition was achieved down to a concentration of lopinavir 1 μg/mL plus ribavirin 6.25 μg/mL when the viral inoculum was reduced below 50 $TCID_{50}$ (tissue culture infectious dose).[15] Thus, both peak (9.6 μg/mL) and trough (5.5 μg/mL)[35] serum concentrations of lopinavir may inhibit this virus. Synergism between lopinavir and ribavirin might be a reason for the relatively large therapeutic benefits seen in this study, despite the weak anti-viral activities of the individual drugs. Ritonavir has little in vitro activity against SARS-CoV, but it inhibits the CYP3A-mediated metabolism of lopinavir, thereby increasing the serum drug concentration of lopinavir.

Our results showed that early use of lopinavir/ritonavir with ribavirin was associated with a reduced use of pulse methylprednisolone, and a reduction in intubation and mortality rates. They also showed that this treatment was not associated with abnormal liver function or with raised serum amylase levels. These benefits were not evident in those patients who received LPV/r rescue therapy, initiated later in the course of the disease when patients had deteriorated further. It is also noteworthy that patients who received LPV/r as rescue treatment received higher doses of pulse corticosteroid, suggesting that physicians had selected patients for LPV/r rescue therapy who were in poorer clinical condition. This might have masked any possible benefits of LPV/r treatment. Furthermore, any benefit from synergism between the two anti-viral agents might have been eliminated in the rescue treatment group since ribavirin was already discontinued when LPV/r was added.

A previous study on the sequential changes in viral load and disease progression of SARS suggested that there was an initial viral replicative phase that led to a maximal viral load at around day 10. Thereafter, the disease progressed to ARDS and severe end-organ damage in some patients.[11] This suggests that in the treatment of SARS, a reasonable strategy is to reduce the peak viral load by an effective anti-viral agent, as this might decrease the need for salvage therapy with immunosuppressants, and hence reduce the risk of nosocomial infections.[36] Our results are in keeping with this hypothesis, showing that early use (median, 5.5 days after symptom onset, ie before peak viral replication at day 10), but not rescue use of LPV/r (median, 18 days after symptom onset), was beneficial in terms of reduction in use of pulse corticosteroid therapy, rates of intubation and death.

Our study was limited in being retrospective, with patients not randomly assigned to treatment and control groups. Attempts were made to match all the known prognostic factors for the LPV/r-treated subgroups and the controls, however. It is noteworthy that co-morbidities were not classified according to severity, as this may have confounded results. Physicians had possibly selected sicker patients for LPV/r as rescue treatment, as the mean dose of pulse steroid received prior to LPV/r was higher for this group than the matched cohort. This might have masked some of the possible benefits of LPV/r. It is also possible that the apparent benefits of LPV/r seen were due to declining mortality towards the end of the epidemic as clinical experience grew. However, the age-standardised mortality rates were fairly constant throughout the epidemic, arguing against this contention.

This study was not designed to evaluate whether ribavirin and/or steroid therapy are effective treatments for SARS. Both the LPV/r-treated and the standard treatment groups were given ribavirin and steroid therapy according to the same standard protocol. Future studies are needed to evaluate these drugs separately.

## Conclusion

The addition of LPV/r to a standard treatment protocol for SARS as an initial treatment appeared to be associated with a significant reduction in the need for rescue pulse corticosteroid therapy, avoidance of intubation, and a reduction in mortality rate. A randomised, double-blind, placebo-controlled trial for the use of anti-viral agents is recommended during future SARS epidemics to fully evaluate this potential treatment.

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