still occurred when EDTA plasma was stored at −70 °C for 6 months compared with a 61.5% decrease at −20 °C.

There was no significant difference in OPG concentration before and after 6 weeks of storage at −20 or −70 °C when samples were collected into either lithium heparin or EDTA. Storage of EDTA-plasma samples for up to 6 months at −20 or −70 °C produced a 22.8% decrease in OPG when stored at −20 °C and a 19.7% decrease when stored at −70 °C.

We also investigated the effect of storing separated samples in glass or plastic tubes and observed no significant effect of tube type. When samples were collected into nonsiliconized plastic syringes, significant variability in sRANKL was observed compared with results obtained when siliconized tubes were used (SARSTEDT-MONOVETTE). OPG was not affected by the type of sampling technique used.

The variability in sRANKL concentration under different storage conditions suggests a possible explanation for previous discordant findings *(5, 8–11)*. The amount of sRANKL measured by the ELISA used in this study can reflect only the free, uncomplexed forms in human plasma; therefore, the assays may not accurately reflect the total concentrations of sRANKL and OPG proteins in the samples. Altered concentrations of the proteins could also result from fluctuations in the sRANKL:OPG ratio in human plasma, which may contribute to the previous discordant results for detection of sRANKL:OPG in human plasma. In some circumstances, determination of the expression of RANKL and OPG mRNA extracted from osteoblastic cells may provide a more accurate and precise estimate of changes in the RANKL:OPG ratio in metabolic bone disease than do measurements of plasma concentrations *(11–14)*.

We recommend that samples for measurement of sRANKL and OPG should be collected into siliconized syringes or collection tubes and that EDTA is the preferred anticoagulant. Separation within 1 h and measurement of sRANKL and OPG in plasma as soon as possible after collection are also recommended. If required, storage is best done at −70 °C, but even at this temperature significant decreases in OPG and sRANKL were observed at 6 months.

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Serial Analysis of the Plasma Concentration of SARS Coronavirus RNA in Pediatric Patients with Severe Acute Respiratory Syndrome, *Enders K.O. Ng,[1†] Pak-Cheung Ng,[2†] K.L. Ellis Hon,[2] W.T. Frankie Cheng,[2] Emily C.W. Hung,[2] K.C. Allen Chan,[1] Rossa W.K. Chiu,[1] Albert M. Li,[2] Leo L.M. Poon,[3] David S. Hui,[4] John S. Tam,[5] Tai-Fai Fok,[2] and Y.M. Dennis Lo[1*]* (Departments of [1] Chemical Pathology, [2] Paediatrics, [4] Medicine and Therapeutics, and [5] Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong SAR; [3] Department of Microbiology, The University of Hong Kong, Hong Kong Special Administrative Region; † these authors contributed equally to this work; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Room 38023, 1/F Clinical Sciences Building, Prince of Wales Hospital, 30--32 Ngan Shing St., Shatin, New Territories, Hong Kong Special Administrative Region, China; e-mail loym@cuhk.edu.hk)

The recent identification of a novel coronavirus, SARS coronavirus (SARS-CoV), as an etiologic agent for severe acute respiratory syndrome (SARS) has prompted many groups to develop rapid and accurate molecular assays for the detection of this virus *(1–4)*. To date, most of the assays have focused predominantly on samples taken from

nasopharyngeal aspirates, urine, and stools *(5, 6)*. Although detection of SARS-CoV RNA in the plasma of SARS patients has been reported *(1)*, the relatively low sensitivity of the ultracentrifugation-based approach for detecting SARS-CoV RNA in plasma has made this assay impractical. Recently we showed that a one-step real-time quantitative reverse transcription-PCR (RT-PCR) assay for the polymerase region of the SARS-CoV genome could detect viral RNA in 75–78% of nonultracentrifuged serum samples from adult SARS patients during the early stage of disease and that the serum SARS-CoV concentrations on admission were of prognostic significance *(7)*. This finding demonstrates that plasma/serum SARS-CoV quantification may potentially be useful for the early diagnosis of SARS.

Although most existing reports have focused on adult SARS patients, recent reports revealed that the clinical course was less severe in pediatric SARS patients than in adult SARS patients *(8, 9)*. On the whole, the outcomes of pediatric SARS patients have been favorable. In this study, we investigated whether SARS-CoV RNA can be detected in the plasma samples of pediatric patients during different stages of SARS and studied the serial variation in viral loads.

We quantified SARS-CoV RNA by real-time RT-PCR in the plasma of eight pediatric patients admitted to the New Territories East Cluster of Hospital Authority Hospitals in Hong Kong and who satisfied the WHO surveillance case definition for SARS *(9)*. These patients were recruited between March 13, 2003, and May 17, 2003. Informed consent was obtained from the patients or their parents, and ethics approval was obtained from the Institutional Review Board. The serial blood samples used in this study were collected from the patients during sample collection for routine blood tests for monitoring lymphocyte counts and biochemical indices and enzymes. The convalescent sera of these patients were tested for IgG antibody against SARS-CoV with SARS-CoV-infected cells in an indirect immunofluorescence assay *(6)*. All patients were serologically positive for SARS-CoV IgG antibody. As negative controls, blood samples from 15 pediatric patients who suffered from fever and infections other than SARS were collected. The plasma SARS-CoV RNA concentrations in the pediatric patients were compared with the results for adult SARS patients as reported previously *(7)*.

All eight studied patients satisfied the WHO surveillance case definition for SARS *(9)*. Seven of them had been in close contact with infected adults, but one patient had no SARS contact history. All patients had a fever, and the mean duration of the fever was 8 days (range, 4–10 days). During the course of hospitalization, all patients were initially treated with oral ribavirin (40–60 mg/kg daily), which was continued for a mean duration of 10 days (range, 3–14 days). Seven were treated with oral prednisolone starting at a mean of 7 days (range, 6–10 days) after fever onset, and the duration of prednisolone treatment was 14 days.

Blood samples were collected in EDTA-containing tubes and centrifuged at 1600*g* for 10 min at 4 °C. Plasma was then carefully transferred to plain polypropylene tubes. Viral RNA was extracted from 0.28 mL of plasma with use of a QIAamp viral RNA mini reagent set (Qiagen) as described previously *(7)*.

One-step real-time quantitative RT-PCR was used for SARS-CoV RNA quantification. A RT-PCR system specifically targeting the polymerase gene [orf1ab polyprotein; nt 15327–15398; GenBank accession no. AY278554 *(10)*] of the SARS-CoV genome was designed as described previously *(7)*. The RT-PCRs were set up in a reaction volume of 25 μL. The primers and fluorescent probe were used at concentrations of 300 and 100 nM, respectively, and 12 μL of extracted plasma RNA was used for amplification. The thermal profile used for the analysis was as follows: the reaction was initiated at 50 °C for 2 min for the included uracil N-glycosylase to act, followed by reverse transcription at 60 °C for 30 min. After a 5-min denaturation at 95 °C, 40 cycles of PCR were carried out with denaturation at 94 °C for 20 s and annealing/extension at 56 °C for 1 min. The sensitivity, linearity, and precision of the assay have been established, as described previously *(7)*. We were able to detect down to 5 copies of the synthetic oligonucleotide in the reaction mixture, which corresponds to 74 copies/mL. SARS-CoV concentrations are expressed as copies/mL of plasma/serum. Because no recovery experiments had been done, the reported concentrations (copies/mL) were minimum estimates. A semilogarithmic plot of different calibrator concentrations against the threshold cycles yielded a correlation coefficient (r) of 0.987. The CV of the copy number of these replicate analyses for this amplification system was 16% at 280 copies/mL *(7)*.

To investigate whether SARS-CoV RNA could be detected in the plasma of pediatric patients, we studied eight patients. The median age of this cohort was 10.3 years (range, 0.3–17.5 years). The earliest available plasma samples were taken from the patients at a mean of 5 days after fever onset (range, 3–7 days), representing a mean of 3 days after admission (range, 1–5 days). SARS-CoV RNA could be detected in seven of the eight (87.5%) pediatric patients (Fig. 1). The median plasma SARS-CoV RNA concentration was 357 copies/mL. As negative controls, SARS-CoV RNA was not detected in the plasma samples obtained from 15 pediatric patients who suffered from non-SARS-related infections.

To study the relative usefulness of plasma SARS-CoV measurement at different stages of the disease, we collected serial plasma samples from these eight pediatric SARS patients and subjected them to SARS-CoV quantification. The assay detected SARS-CoV RNA in the plasma of all patients (100%) at a mean of 7 days (range, 6–8 days) after fever onset, representing a mean of 4 days after admission (range, 2–6 days), and the median plasma SARS-CoV RNA concentration was 483 copies/mL. At a mean of 14 days (range, 12–15 days) after fever onset, the detection rate for plasma SARS-CoV dropped to 62.5% (5 of 8), and the median plasma SARS-CoV RNA concentration was 103 copies/mL.

To examine whether the plasma SARS-CoV viral load in pediatric SARS patients is different from that in adult SARS patients, we compared the data from the pediatric

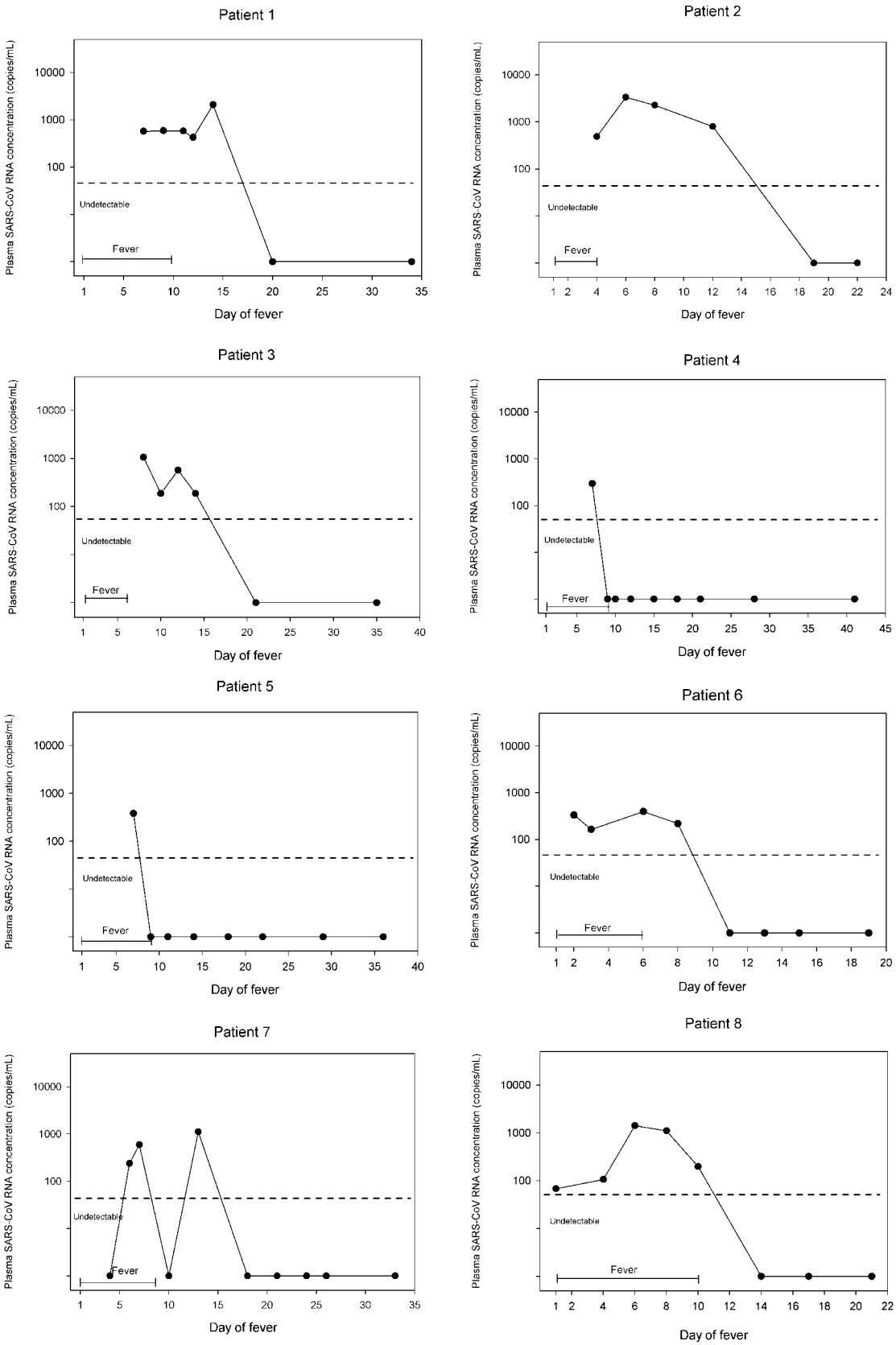

Fig. 1. Serial analysis of plasma SARS-CoV RNA concentrations in pediatric SARS patients.

Shown are plots of plasma SARS-CoV RNA concentrations in a common logarithmic scale (copies of SARS-CoV RNA/mL of plasma; *y axis*) against time after the onset of fever (*day 1* refers the day of fever onset; *x axis*). The *horizontal dashed line*s represent the detection limit of the assay.

patients with the data for 13 adult SARS patients who had been studied in a previous report *(7)*. The adult plasma samples were taken at a mean of 4 days after fever onset (range, 2–6 days) and exactly 7 days after fever onset. The median adult plasma SARS-CoV RNA concentration at a mean of 4 days after fever onset was 125 copies/mL. We observed no significant difference between the plasma SARS-CoV RNA concentration in the pediatric patients and that in the adult SARS patients (Mann–Whitney test, $P = 0.076$). In addition, we compared the plasma SARS-CoV RNA concentration at day 7 after fever onset. The median adult plasma SARS-CoV RNA concentration at day 7 was 84 copies/mL. Once again, we observed no significant difference between pediatric and adult SARS patients (Mann–Whitney test, $P = 0.076$). Because the number of patients in this study was limited, further study involving more patients may be necessary to address the difference of viral loads between adult and pediatric SARS patients.

In this study we demonstrated that SARS-CoV RNA is detectable in the plasma of pediatric SARS patients with a detection rate of 87.5–100% within the first week after fever onset and then dropped to 62.5% at a mean of 14 days after fever onset. These data are largely concordant with our previous data on adult SARS patients showing a high detection rate for serum SARS-CoV RNA within the first week of illness *(7)*. Taken together, these data suggest that plasma SARS-CoV measurement is a sensitive method for detecting SARS-CoV infection during the first week of fever onset.

The serial data presented here have demonstrated that SARS-CoV RNA in plasma from the studied patients became undetectable after a mean of 16 days of fever (range, 9–21 days). For patient 7, the undetectable plasma SARS-CoV RNA at days 4 and 10 might represent a fluctuation in the degree of viremia during the course of the illness as a result of intermittent shedding of virions. We did not observe any correlation between the plasma viral load and steroid or ribavirin treatment, and a larger scale study may be necessary to address this important question.

Recent studies have reported that the clinical course is less severe in pediatric SARS patients than in adult SARS patients *(8, 9)*. A logical question would be whether the plasma SARS-CoV viral load in pediatric SARS patients is different from that in adult SARS patients. When we compared the data from pediatric patients with data from adult SARS patients *(7)*, we observed no significant differences in plasma SARS-CoV viral load in samples taken from pediatric and adult SARS patients within the first week of admission and at day 7 after fever onset.

In conclusion, viremia appears to be a consistent feature in both pediatric and adult SARS patients. The relatively high detection of SARS-CoV in plasma during the first week of illness suggests that plasma-based RT-PCR may potentially be useful in the routine diagnostic work-up of patients with suspected or confirmed SARS in both adult and pediatric populations.

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Applicability of an Assay for Routine Monitoring of Highly Variable Concentrations of Olanzapine Based on HPLC with Mass Spectrometric Detection, *Guillermo Gervasini,[1] Sonia Vizcaíno,[1] Angustias G. Herráiz,[2] Julio Benítez,[1] and Juan Antonio Carrillo[1*]* ([1] Department of Pharmacology and Psychiatry, Extremadura University School of Medicine, E-06071 Badajoz, Spain; [2] Psychiatric Hospital Adolfo Díaz Ambrona, E-06800 Mérida, Spain; * address correspondence to this author at: Departamento de Farmacología y Psiquiatría, Facultad de Medicina, Universidad de Extremadura, Avda. de Elvas s/n, E-06071 Badajoz, Spain; fax 34-924-271100, e-mail carrillo@unex.es)

The thienobenzodiazepine derivative olanzapine (OLZ) is a commonly used antipsychotic drug that has demonstrated efficacy against both positive and negative symptoms of schizophrenia *(1)*.

OLZ shows variable pharmacokinetics, with induction of the CYP1A2 enzyme by cigarette smoking being one of the most important factors contributing to this variability *(2)*. Moreover, because of the potential toxicity of OLZ at relatively low concentrations, monitoring of OLZ has been suggested to be necessary *(3)*.