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## CHEMICAL PATHOLOGY

### Steroid-induced osteonecrosis in severe acute respiratory syndrome: a retrospective analysis of biochemical markers of bone metabolism and corticosteroid therapy

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#### Summary

**Aim:** We investigated the effect of massive doses of corticosteroid therapy on bone metabolism using specific biochemical markers of bone metabolism, and the prevalence of osteonecrosis in severe acute respiratory syndrome (SARS) patients at a university teaching hospital in Hong Kong.

**Methods:** Seventy-one patients with a clinical diagnosis of SARS were studied according to the modified World Health Organization case definition of SARS who were involved in the SARS epidemic between 10 March and 20 June 2003. The clinical diagnosis was confirmed by serological test and/or molecular analysis. Biochemical markers of bone metabolism were analysed retrospectively using serial clotted blood samples collected from each patient during the course of hospital admission to discharge and subsequent follow-up at out-patient clinic using the arbitrary time periods: (i) Day <10; (ii) Day 28–44; (iii) Day 51–84; and (iv) Day >90 after the onset of fever. Magnetic resonance imaging of the knee and hip joints were performed post-admission to evaluate the prevalence of osteonecrosis amongst these SARS patients. Various risk factors for the development of osteonecrosis were assessed using receiver operating characteristics curve comparison with appropriate test statistics and Spearman's coefficients of rank correlation with biochemical bone markers.

**Results:** Biochemical markers of bone metabolism showed significant bone resorption as evidenced by a marked increase in serum C-terminal telopeptide concentration (CTX) from Day 28–44 after the onset of fever. With tapering down of corticosteroid dosage, CTx started to return to previous baseline level from Day 51 onwards, while other bone formation markers, serum osteocalcin and bone-specific alkaline phosphatase concentrations (OC and BALP, respectively), started to increase. The latter effect was even more marked after Day >90. Seven patients developed radiological evidence of osteonecrosis. The prevalence of osteonecrosis in this cohort was 9.9%. A total corticosteroid dosage of >1900 mg hydrocortisone, >2000 mg methylprednisolone, >13 340 mg hydrocortisone-equivalent corticosteroid therapy, and >18 days on corticosteroid

therapy were found to be significant risk factors for the subsequent development of osteonecrosis. There were also significant positive correlations amongst various biochemical bone markers in this patient cohort.

**Conclusions:** Both bone resorption and formation markers were unable to predict the subsequent development of osteonecrosis. The use of high dose of hydrocortisone or methylprednisolone for an extended duration was shown to be a significant risk factor for osteonecrosis. Its prevalence in this cohort is comparable to those reported in the literature for SARS patients with high-dose corticosteroid therapy. The Day 28–44 increase in the serum CTx coincided with the timing of corticosteroid use. The Day >51 increase in serum OC and BALP coincided with the timing of corticosteroid withdrawal.

**Key words:** Steroid-induced osteonecrosis, severe acute respiratory syndrome (SARS), biochemical markers, bone metabolism, corticosteroid therapy.

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#### INTRODUCTION

Use of massive doses of corticosteroid has been criticised because of its potential adverse effects on various organ systems including the cardiovascular, endocrine, skeletal, and central nervous systems apart from the targeted immunosuppression. High-dose corticosteroid has been used as salvage therapy for refractory adult respiratory distress syndrome (ARDS), with the typical dosage ranging from 1–2 mg of methylprednisolone per kg of body weight daily.<sup>1</sup> For a 70 kg patient with ARDS, the amount of corticosteroid received would be equivalent to 350–700 mg of hydrocortisone per day, that is 17–35 times the normal daily production by the adult adrenal glands. High-dose inhaled corticosteroid in prepubertal asthmatic children was associated with compromised bone mineral density that may potentiate the risk of fractures in the future.<sup>2</sup>

In patients with systemic lupus erythematosus (SLE), the role of daily oral corticosteroid therapy in the pathogenesis of osteonecrosis is well recognised. Oral non-bolus corticosteroid therapy for the treatment of SLE was shown to correlate strongly with osteonecrosis.<sup>3</sup> More recently, a 5-year prospective study on patients with SLE showed that 14% developed osteonecrosis. Both intravenous pulse corticosteroid and oral prednisolone therapy at a dose of more than 30 mg/day for at least 1 month were risk factors for osteonecrosis.<sup>4</sup> However, it is unclear whether short-term high-dose methylprednisolone pulse therapy is a risk factor.

During the recent outbreak of severe acute respiratory syndrome (SARS) in Hong Kong,<sup>5</sup> corticosteroids were prescribed during the acute phase of infection. Maintenance corticosteroid using either intravenous hydrocortisone or oral prednisolone was offered to SARS patients who did not respond to conventional anti-viral and antimicrobial agents, while high-dose pulse methylprednisolone was prescribed in addition to maintenance therapy to those who deteriorated clinically. The use of corticosteroid was to withhold or at least to alleviate the alleged cytokine and chemokine storm induced by the SARS-associated coronavirus (SARS-CoV),<sup>6</sup> thereby preventing massive tissue destruction and organ damage.<sup>7</sup> However, the dosage of methylprednisolone used was about 3.5–7.0-fold of those documented in the literature for the salvage therapy of ARDS.<sup>1</sup> While short-term high-dose glucocorticoid therapy is ineffective in ARDS,<sup>8</sup> steroid usage in SARS has also been questioned.<sup>9,10</sup>

In this study, biochemical markers of bone metabolism were used retrospectively to investigate the effect of massive doses of pulse and maintenance corticosteroid therapies on patients with SARS. After discharge from hospital, patients were followed up at the medical outpatient clinic, with magnetic resonance imaging for assessment of knee and hip joints to document any osteonecrotic changes.

## MATERIALS AND METHODS

### *Study population*

Seventy-one patients from the Prince of Wales Hospital in Hong Kong with clinical presentations fulfilling the World Health Organization criteria<sup>11</sup> for probable SARS, who were involved in the SARS epidemic from 10 March to 20 June 2003 were included in the study. All of them presented with fever ( $>38^{\circ}\text{C}$ ), cough or breathing difficulty, a contact history within 10 days prior to the onset of symptoms, and lymphopenia, with subsequent development of pneumonia evidenced by chest radiography or computed tomography (CT) of the thorax. The diagnosis of SARS was subsequently confirmed either serologically by detection of specific antibody against the SARS-CoV or by demonstration of SARS-CoV using the reverse-transcriptase polymerase chain reaction technique.<sup>12</sup> They did not have any previous history of bone diseases including Paget's disease of bone, fractures, and osteoporosis, or diseases that affect the bone metabolism such as end-stage renal failure, thyrotoxicosis, and hyperparathyroidism.

### *Treatment of SARS*

All SARS patients were given the following treatment according to our hospital protocol.<sup>13</sup> On the first day of admission, ribavirin was given either orally 1.2 g three times daily after a loading dose of 2.4 g, or intravenously 400 mg every 8 h for a complete 14-day course. Levofloxacin or cefotaxime was supplemented to cover any bacterial chest infection. If there was persistent fever  $>38^{\circ}\text{C}$  on the third day of admission, a maintenance

steroid therapy was started using either oral prednisolone 0.5–1.0 mg/kg of body weight daily, or intravenous hydrocortisone 100 mg every 8 h with step-down titration starting at the third week of admission. For patients with clinical deterioration including desaturation (oxygen saturation  $<90\%$  using pulse oximetry) or development of new infiltrates in chest radiograph, pulse steroid using methylprednisolone 0.5 g daily for 3 days was given. Pulse methylprednisolone therapy was repeated if clinically indicated.

### *Laboratory study*

Serum samples were retrieved from the SARS Serum Bank of our university hospital during the following time periods using the day of fever onset as Day 1: (i) Day  $<10$  (baseline), (ii) Day 28–44 (receiving corticosteroid therapy), (iii) Day 51–84 (discharge from hospital), and (iv) Day  $>90$  (during outpatient follow-up). These samples were studied depending on their availability. All blood samples arrived at the laboratory in an ice-water bath and were centrifuged immediately with sera aliquoted for storage at  $-70^{\circ}\text{C}$  until analysis. Serum osteocalcin (OC) and C-terminal telopeptide of type I collagen (CTX) concentrations were measured using electrochemiluminescence immunoassay (E170 Modular Analyser; Roche Diagnostics, USA) as bone formation and resorption markers, respectively. The antibody used in the measurement of OC detects only the intact osteocalcin (OC [1–49]) and its N-mid fragment (OC [1–43]). Another bone formation marker, serum bone-specific alkaline phosphatase (BALP) concentration, was measured for all patient samples using chemiluminescent enzyme immunoassay (Access Analyser; Beckman-Coulter, USA). The inter-assay coefficients of variation at low, normal, and high concentrations were 3.8, 3.9, and 2.8% at 13.7, 102.5, 195.3  $\mu\text{g/L}$  for serum OC; 4.3, 4.5, and 2.4% at 0.39, 0.75, 3.1  $\mu\text{g/L}$  for serum CTx; 3.7, 3.3, and 4.5% at 10.8, 25.5, 46.0  $\mu\text{g/L}$  for serum BALP, respectively. The least significant change values at these concentrations were 1.5, 11.2, and 15.3  $\mu\text{g/L}$  for serum OC, 0.05, 0.10, and 0.21  $\mu\text{g/L}$  for serum CTx, 1.1, 2.4, and 5.8  $\mu\text{g/L}$  for serum BALP, respectively. Other markers of bone metabolism using enzyme-linked immunosorbent assay (ELISA) method were not performed because of the potential infectious hazard due to aerosol generation.

### *Radiological investigation*

Magnetic resonance imaging (MRI) was performed on all SARS patients as part of a screening program for post-SARS follow-up assessment in our institute using a 1.5-T Magnetom instrument (Siemens, Germany). The median time from admission to MRI examination was 6.7 months (range 3.3–9.7). For the hips, a T1-weighted (TR590, TE20), spin-echo coronal sequence was obtained, slice thickness 3 mm, intersection gap 0.3 mm, field of view 350 mm with a  $256 \times 512$  matrix. For the knees, a T1-weighted (TR520, TE20), spin-echo coronal sequence was obtained, slice thickness 3 mm, intersection gap 0.3 mm, field of view 300 mm with a  $256 \times 512$  matrix. A more detailed MRI examination was performed within 2 weeks for patients showing evidence of osteonecrosis on the screening MRI. This detailed MRI examination individually evaluated the affected hip or knee joint using standard coils and orthogonal planes. For lesions of the hip, T1-weighted (TR590, TE20) and T2 SPIR (TR5170, TE66) oblique coronal and sagittal images were obtained. For lesions of the knee, T1-weighted (TR520, TE20) and T2-weighted SPIR (TR3970, TE74) coronal, intermediate-weighted (TR3500, TE43) and T2-weighted (TR3970, TE74) sagittal images, as well as intermediate-weighted SPIR (TR3500, TE43) axial images were obtained.

MRI examinations were interpreted by two experienced musculoskeletal radiologists (JFG and GEA) and findings were reached by consensus. Osteonecrosis was defined as a subchondral or intramedullary area demarcated by a distinct T1 hypo-intense marginal rim, and encompassing medullary fat in its centre.

### *Statistical analysis*

Concentrations of all biochemical bone markers measured for all patient samples were divided into four groups according to the timing of blood collection as described previously. Non-parametric Mann-Whitney U statistics for paired data were computed for all bone markers measured at different time periods against one another. Area under receiver operating

characteristics (ROC) curve (AUC) with 95% confidence intervals (CI), cut-off values, and positive likelihood ratios (LR+), as well as one-way analysis of variance (ANOVA) for continuous variables, or Fisher's exact test statistics for categorical variables, were computed for various risk factors for osteonecrosis. Spearman's coefficients of rank correlation with 95%CI amongst all biochemical bone markers and between various risk factors for osteonecrosis were also computed. Data analyses were performed using MedCalc statistical programme version 8.0 (MedCalc, Belgium). All probabilities (*p*) were two-tailed. A *p* value <0.05 was considered to be statistically significant.

**RESULTS**

*Patient demographics and treatment*

Patient demographic data, number of patients admitted to intensive care unit (ICU), number of patients with and without pulse methylprednisolone therapy, cumulative doses of maintenance and pulse corticosteroid therapies, and the average dose of hydrocortisone-equivalent corticosteroid per hospital day are summarised in Table 1. There were 29 males and 43 females aged from 17–89 years (median 33, inter-quartile range [IQR] 26–47) included in this retrospective study. Most of them were health care workers, including 10 medical doctors, 23 nurses, 12 health care assistants, and one occupational therapist. The rest consisted of nine medical students, 13 existing hospital patients, and three relatives of health care professionals, or confirmed SARS patients. They were all involved in the initial outbreak of SARS in Hong Kong.<sup>5</sup> There were six males and seven females who required admission to intensive care unit. All except 12 patients received pulse methylprednisolone therapy. The median (IQR) doses for intravenous hydrocortisone and oral prednisolone as maintenance therapy as well as oral methylprednisolone as pulse therapy were 0 (0–1600), 500 (360–675), and 1500 (500–2500)mg, respectively. The median (IQR) dose of hydrocortisone-equivalent corticosteroid therapy prescribed to these patients was calculated to be 9520 (4580–16600)mg using a conversion factor of 1mg of methylprednisolone equal to 5mg of hydrocortisone and 1mg of prednisolone equal to 4mg of hydrocortisone.<sup>14</sup> The median (IQR) hospital-day on maintenance and/or pulse corticosteroid therapies was 17 (15–21) days which resulted in the daily average of hydrocortisone-equivalent dose (IQR) being 572 (326–749)mg/day, that is, about 29 times the normal daily production by our adrenal glands.

*Biochemical markers of bone metabolism*

Plots of percentage change from baseline serum biochemical markers of bone metabolism are shown in Fig. 1. Medians are joined by straight line. Error bars define 95% confidence intervals for the medians. Asterisks denote significant change from baseline (Day <10) value using non-parametric Mann–Whitney U test statistics with *p*<0.05.

Compared with the baseline concentration on Day <10, there was a significant increase (change of median concentration by about 75%) in serum CTx during Day 28–44. This finding coincided with the greatest frequencies of pulse corticosteroid use. With the withdrawal of pulse and maintenance corticosteroids and commencement of the replacement dosage, serum CTx started to return to previous baseline concentration from Day 51 onwards, which was significantly different compared with that of Day 28–44 when the peak bone loss was prominent. There was no significant difference in serum CTx among Day <10, Day 51–84, and Day >90.

Serum OC started to increase and reached a statistically significant difference from Day 51 onwards; its elevation was even more marked at Day >90. Serum BALP essentially followed the same trend of serum OC. Its concentration started to rise earlier during Day 28–44 to reach a statistically significant difference. From Day 51–84, serum BALP appeared to increase continuously but without reaching a statistically significant difference. However, from Day 90 onwards, the increase in serum BALP was marked with a significant difference compared with baseline and Day 51–84 concentrations.

*Radiological investigation*

During the follow-up period, one patient died of existing medical illness and one patient refused MRI for assessment of osteonecrosis due to absence of symptoms. Therefore, the total number of corticosteroid-treated patients followed up with repeated MRI examination was 69. Of these 69 patients, there were 28 patients (about 41%) complaining of symptomatic bone or joint pain. Seven of these (about 10%) developed radiological evidence of osteonecrosis including three males and four females aged 17–53 years with a median (IQR) of 31 (30–48). The MRI findings of osteonecrosis for one 36-year-old female post-SARS patient are presented in Fig. 2, showing subchondral

TABLE 1 Patient demographics, number of patients admitted to intensive care unit, cumulative doses of maintenance and pulse corticosteroid therapies, and the daily average of hydrocortisone-equivalent dose of corticosteroid

Variable	Median (inter-quartile range)
Age (years)	33 (26–47)
Sex (M : F)	28 : 43
Occupation (Doctor : Nurse : Allied Health : Medical Student : Patient : Others)	10 : 23 : 13 : 9 : 13 : 3
Number of admissions to intensive care unit (M : F)	6 : 7
Total dose of hydrocortisone (mg)	0 (0–1600)
Total dose of prednisolone (mg)	500 (360–675)
Number of patients with and without pulse methylprednisolone therapy	59 : 12
Total dose of methylprednisolone (mg)	1500 (500–2500)
Hydrocortisone-equivalent for all corticosteroids (mg)*	9520 (4580–16600)
Number of hospital days on corticosteroid therapy (day)	17 (15–21)
Daily hydrocortisone-equivalent (mg/day)	572 (326–749)

\* Conversion factors:<sup>14</sup> 1 mg methylprednisolone=5 mg hydrocortisone; 1 mg prednisolone=4 mg hydrocortisone.

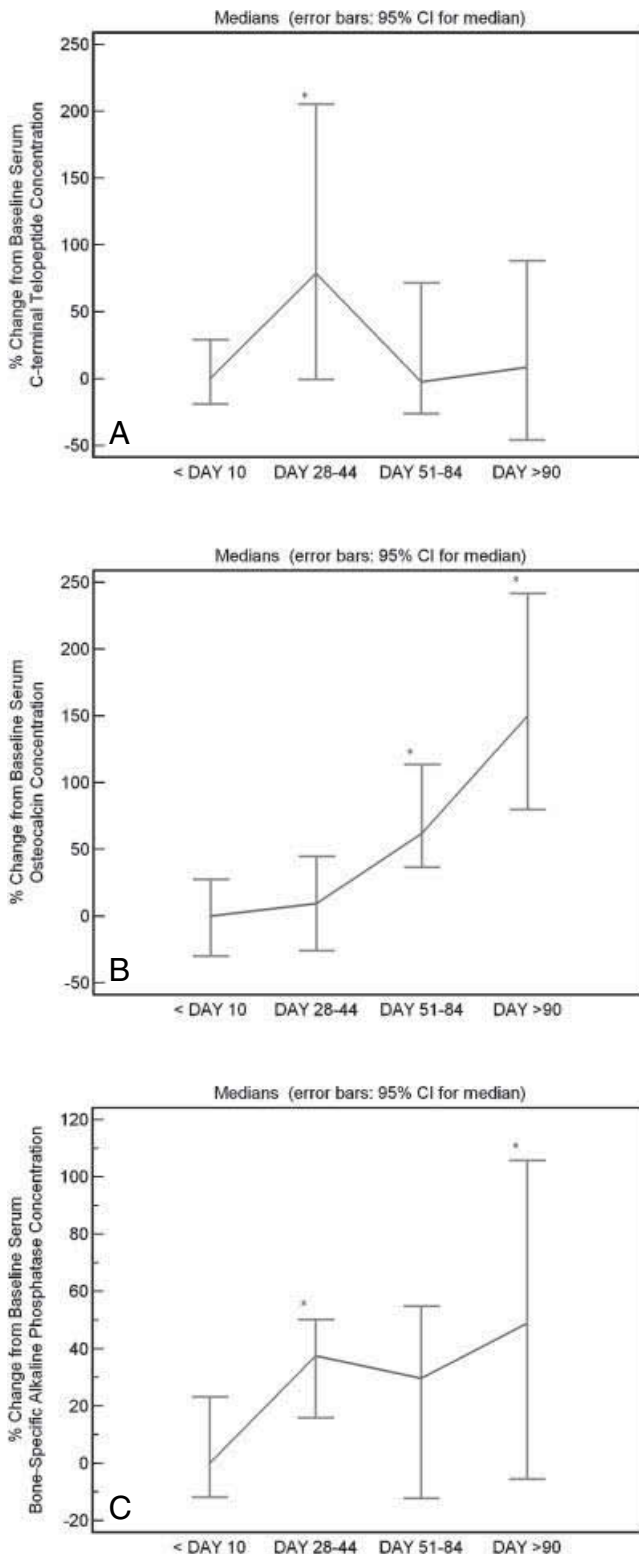


Fig. 1 Plot of percentage change from baseline serum biochemical markers of bone metabolism at different time periods of SARS infection. x-axis, day from onset of fever (Day 1). y-axis, (A) C-terminal telopeptide of type I collagen, (B) osteocalcin, and (C) bone-specific alkaline phosphatase concentrations. \* $p$  value < 0.05.

abnormalities of the antero-superior aspect of both femoral heads demarcated by hypo-intense rims and similar intramedullary and subchondral areas of osteonecrosis of the right knee.

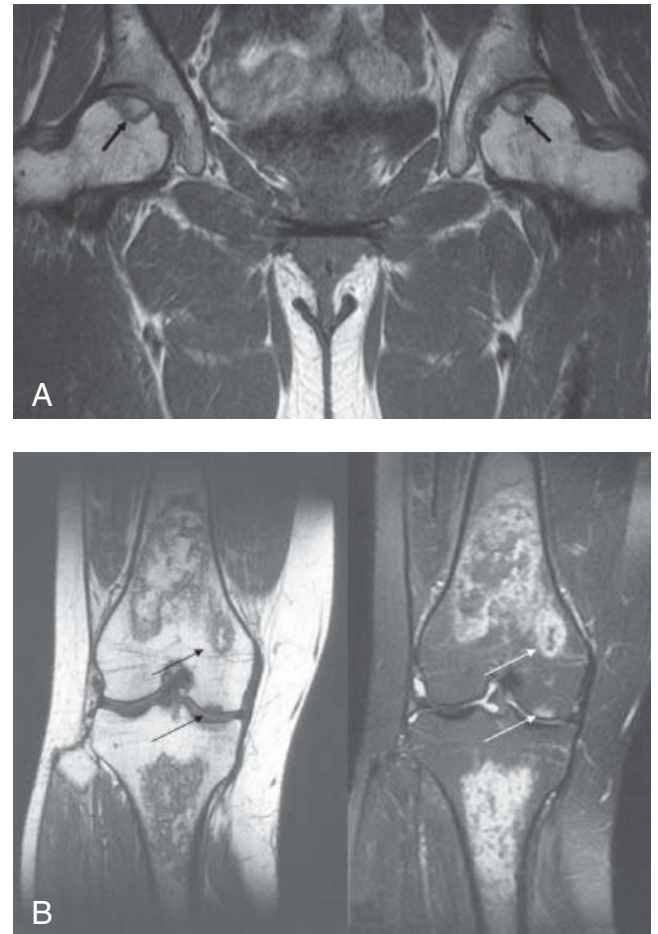


Fig. 2 Coronal images of a 36-year-old female post-SARS patient. (A) T1-weighted (TR590, TE20) image of both hips showed sub-chondral abnormalities of the antero-superior aspect of both femoral heads demarcated by hypo-intense rims (arrows) consistent with osteonecrosis. (B) T1-weighted (TR520, TE20) (left) and T2-weighted SPIR (TR3970, TE74) (right) coronal images of the right knee showed similar intramedullary and subchondral areas of osteonecrosis (arrows).

#### Statistical analyses

Table 2 summarises the results of AUC with 95%CI, cut-off values, LR+ as well as ANOVA or Fisher's exact test statistics for various risk factors of osteonecrosis after high-dose corticosteroid therapy for the treatment of SARS. A total dose of >1900 mg of intravenous hydrocortisone (AUC 0.626, 95%CI 0.499–0.742, LR+ 3.33,  $p=0.004$ ), total dose of >2000 mg of oral methylprednisolone (AUC 0.889, 95%CI 0.787–0.953, LR+ 5.00,  $p<0.001$ ), total dose of >13 340 mg of hydrocortisone-equivalent corticosteroid therapy (AUC 0.908, 95%CI 0.812–0.965, LR+ 4.29,  $p<0.001$ ), and >18 days on corticosteroid therapy (AUC 0.849, 95%CI 0.699–0.899, LR+ 3.00,  $p=0.008$ ) were found to be significant risk factors for the subsequent development of osteonecrosis. However, there was no significant difference in the change of the turnover markers between patients with and without osteonecrosis.

On further analysis, comparison was made for the serial changes of biochemical bone markers on individual SARS patient. Among the seven osteonecrotic patients, three showed a significant difference in the Day 28–44 CTx results compared with their respective baseline values, four showed a significant difference in the Day 51–84 OC results

TABLE 2 Results of area under receiver operating characteristics curve with 95% confidence intervals, cut-off values, positive likelihood ratio, and one-way analysis of variance or Fisher's exact test statistics for various risk factors of osteonecrosis after high-dose corticosteroid therapy for the treatment of SARS

Variable	Area under ROC curve (95%CI)	Cut-off	LR+	p-value
Sex*	0.514 (0.393–0.634)	Male sex	1.07	1.000
Age (years)	0.491 (0.371–0.612)	>28	1.39	0.813
History of admission to intensive care unit*	0.637 (0.516–0.747)	Positive history	2.79	0.106
Total dose of hydrocortisone (mg)	0.626 (0.499–0.742)	>1900	3.33	0.004
Total dose of prednisolone (mg)	0.712 (0.588–0.817)	>535	2.38	0.342
History of pulse methylprednisolone use*	0.600 (0.472–0.719)	Positive history	1.25	0.582
Total dose of methylprednisolone (mg)	0.889 (0.787–0.953)	>2000	5.00	<0.001
Hydrocortisone-equivalent for all corticosteroids (mg)	0.908 (0.812–0.965)	>13 340	4.29	<0.001
Number of hospital days on corticosteroid therapy (day)	0.849 (0.739–0.925)	>18	3.00	0.008
Daily hydrocortisone-equivalent (mg/day)	0.814 (0.699–0.899)	>585	2.40	0.142
Serum peak CTx concentration (µg/L)	0.581 (0.458–0.698)	>0.39	2.61	0.151
Serum peak OC concentration (µg/L)	0.516 (0.394–0.636)	>8.1	1.31	0.852
Serum peak BALP concentration (µg/L)	0.581 (0.458–0.698)	≤12.1	1.66	0.484
Serum peak OC to CTx ratio	0.567 (0.444–0.684)	≤59.4	1.83	0.730
Serum peak BALP to CTx ratio	0.598 (0.475–0.713)	≤44.5	1.69	0.555

\*Fisher's exact test.

ROC, receiver operating characteristic; CI, confidence interval; LR+, positive likelihood ratio; CTx, C-terminal telopeptide; OC, osteocalcin; BALP, bone-specific alkaline phosphatases.

compared with their respective baseline values, and two showed a significant difference in the Day >90 BALP results compared with their respective baseline values.

Results of Spearman's coefficients of rank correlation ( $\rho$ ) with 95%CI amongst all biochemical bone markers are summarised in Table 3. There were significant correlations between CTx and BALP ( $\rho$  0.241, 95%CI 0.008–0.449,  $p=0.044$ ), OC and BALP ( $\rho$  0.400, 95%CI 0.184–0.579,  $p<0.001$ ), as well as significant negative correlation between OC and age ( $\rho$  -0.408, 95%CI -0.585 – -0.193,  $p<0.001$ ). However, there was no significant correlation between serum CTx and OC or between various bone markers and other risk factors of osteonecrosis.

DISCUSSION

To the best of our knowledge, this is the first report on specific biochemical markers of bone metabolism to study SARS patients treated with short-term high-dose corticosteroids. We have shown that there has been a close temporal relationship between the serial changes in serum biochemical bone markers in SARS patients and pulse corticosteroid therapy. The initial marked increase in serum

CTx from Day 28–44 could represent the immediate osteoporotic effect of corticosteroid on bone metabolism after pulse methylprednisolone was given. With the withdrawal of all corticosteroid therapies and the commencement of the replacement dose around Day 51 onwards, serum CTx started to return to baseline level.

The majority of our study patients were formerly healthy individuals who did not have a previous history of bone disease such as Paget's disease of bone, fractures, osteoporosis, or diseases that affect bone metabolism, for example end-stage renal failure, thyrotoxicosis and hyperparathyroidism. None of them were previously on any corticosteroid therapy. During the acute phase of SARS infection, they were treated with high-dose corticosteroid therapy.<sup>5,13</sup> On subsequent follow-up, only seven of 69 patients developed osteonecrosis, even though nearly half of them complained of bone or joint pain. This indicated that symptoms alone were not a good predictor for the subsequent development of osteonecrosis. Therefore, various factors for risk assessment of osteonecrosis were studied. The AUC (95%CI) for peak serum CTx, OC, and BALP for predicting the subsequent development of osteonecrosis were 0.581 (0.458–0.698), 0.516 (0.394–0.636), and 0.581 (0.458–0.698), respectively. The AUC

TABLE 3 Results of Spearman's coefficient of rank correlation ( $\rho$ ) amongst peak serum C-terminal telopeptide of type I collagen, osteocalcin, bone-specific alkaline phosphatase against various risk factors of osteonecrosis

Variable	$\rho$ for CTx (95%CI)	p-value	$\rho$ for OC (95%CI)	p-value	$\rho$ for BALP (95%CI)	p-value
Age (years)	0.072 (-0.164–0.300)	0.546	-0.408 (-0.585 – -0.193)	<0.001	-0.025 (-0.257–0.210)	0.835
Total dose of hydrocortisone (mg)	0.071 (-0.174–0.308)	0.567	0.061 (-0.183–0.299)	0.621	0.070 (-0.175–0.307)	0.573
Total dose of prednisolone (mg)	0.098 (-0.147–0.332)	0.429	-0.201 (-0.422–0.043)	0.101	-0.162 (-0.389–0.083)	0.191
Total dose of methylprednisolone (mg)	0.141 (-0.104–0.370)	0.255	-0.133 (-0.364–0.112)	0.282	-0.165 (-0.391–0.080)	0.183
Hydrocortisone-equivalent for all corticosteroids (mg)	0.134 (-0.112–0.364)	0.280	-0.130 (-0.360 – -0.116)	0.296	-0.144 (-0.373–0.101)	0.244
Number of hospital days on corticosteroid therapy (day)	0.132 (-0.114–0.362)	0.290	-0.045 (-0.284–0.199)	0.717	-0.047 (-0.286–0.197)	0.705
Daily hydrocortisone-equivalent (mg/day)	0.109 (-0.137–0.342)	0.380	-0.109 (-0.342–0.137)	0.381	-0.145 (-0.374–0.101)	0.244
Serum peak CTx concentration (µg/L)	Not applicable		0.136 (-0.100–0.358)	0.254	0.241 (0.008–0.449)	0.044
Serum peak OC concentration (µg/L)	0.136 (-0.100–0.358)	0.254	Not applicable		0.400 (0.184–0.579)	<0.001
Serum peak BALP concentration (µg/L)	0.241 (0.008–0.449)	0.044	0.400 (0.184–0.579)	<0.001	Not applicable	

CI, confidence interval; CTx, C-terminal telopeptide; OC, osteocalcin; BALP, bone-specific alkaline phosphatase.

(95%CI) for the ratios of bone formation to bone resorption markers (peak serum OC to CTx and BALP to CTx ratios) were 0.567 (0.444–0.684) and 0.598 (0.475–0.713), respectively. None of them was a significant risk predictor for the development of osteonecrosis. There was also no significant difference in the change of bone turnover markers between osteonecrotic and non-osteonecrotic patient groups.

On the contrary, a cumulative dose of >2000 mg of methylprednisolone was found to be the best predictor for osteonecrosis amongst all other significant risk factors, as it has the highest positive likelihood ratio of 5.0. Therefore, it is suggested that short-term high-dose pulse methylprednisolone, not history of pulse corticosteroid use, is one of the important risk factors for osteonecrosis not reported previously. However, there was no statistical significant difference in AUC amongst all other risk factors, namely, total doses of >1900 mg of hydrocortisone, >13 340 mg of hydrocortisone-equivalent, and >18 days on corticosteroid therapy after re-analysis of their AUC with that of methylprednisolone treatment using multiple ROC curve comparison. Interestingly, neither a positive history of admission to intensive care unit with high degree of immobilisation due to the need for respiratory support, nor a positive history of pulse methylprednisolone use, were found to be significant risk factors for the subsequent development of osteonecrosis.

Steroid-induced osteonecrosis, most frequently (>25% of cases) involving only the hips, can impose very significant morbidity and devastating disability.<sup>15</sup> Estimated prevalence in the last decade was 7.6% in 132 renal transplant recipients,<sup>16</sup> and 10% in 69 SLE patients.<sup>17</sup> It may develop in patients who received steroids in very high short-term doses, in long-term doses, or even by intra-articular injection. The interval between corticosteroid administration and the onset of symptoms is rarely less than six months and may be more than three years.<sup>18</sup> In a Korean study, the time for the development of osteonecrosis with steroid therapy was reported to be 1 to 16 months (median 5.3).<sup>19</sup> The median time from admission to MRI examination in our cohort was 6.7 months (range 3.3–9.7) which is comparable with the literature data. The prevalence of steroid-induced osteonecrosis is around 10% for this cohort of SARS patients involved in the earliest outbreak and treated at our hospital. This is also comparable with the prevalence reported in the literature<sup>15,16</sup> but higher than the overall results of 5% when more SARS patients from other hospitals are included.<sup>20</sup> A plausible explanation could be the more judicious use of ribavirin-corticosteroid combination therapy during the respiratory decompensation stage towards the end of the SARS epidemic in Hong Kong when much clinical experience had been accumulated. At a later stage of the SARS epidemic in Hong Kong, convalescent sera donated from recovered SARS patients were available to serve as another treatment modality; the corticosteroid dose could thereby be lowered accordingly to minimise the risk of osteonecrosis.<sup>21</sup>

The subsequent increase in serum concentrations of both bone formation markers (OC and BALP) suggests that the osteoblasts were activated after a temporal delay from the peak bone resorption. Even though the osteoblasts were treated with high-dose corticosteroids, the bone remodelling mechanism was still active. After the initial breakdown

of bony substances by osteoclasts, the coupled osteoblasts would be activated and enter into proliferative phase. Although it is impossible to separate the matrix synthesis from mineralisation phase with the current biochemical markers and technology, an enhanced expression of alkaline phosphatase immediately following the proliferative period, and later, an increased expression of osteocalcin and osteopontin at the onset of mineralisation have been hypothesised.<sup>22</sup> Thus, the significant increase in serum BALP from Day 28–44 and OC from Day 51 onwards may suggest that the living osteoblasts were trying to replenish the previous bone loss and the effect of corticosteroids on bone metabolism could be transient and reversible.<sup>23</sup>

Serum peak CTx and BALP concentrations were positively associated with each other ( $\rho$  0.241) but differ in the time sequence (Fig. 1A,C) representing the coupling of bone resorption followed by bone formation in a normal bone remodelling process.<sup>24</sup> As a biochemical marker of bone formation, the amount of osteocalcin release was thought to represent the osteoblastic activity during the bone mineralisation phase<sup>25</sup> of which newly synthesised proteins were being incorporated into bone matrix where osteocalcin functions to bind calcium.<sup>26</sup> The mineralisation process also requires the presence of BALP that is involved in the breakdown of pyrophosphate. Without BALP, pyrophosphate will then inhibit the deposition of calcium and phosphate ions onto these extra-cellular matrices.<sup>18</sup> Therefore, there was no surprise that the serum concentrations of these two biochemical bone markers (OC and BALP) were positively associated with each other ( $\rho$  0.448) representing the physiological coupling of new bone matrix synthesis and mineralisation phase in a normal bone remodelling process.

The limitation of our study was that we could only retrieve those sera remaining in our SARS Serum Bank to study the biochemical bone markers because some of the samples had been used for the serological or molecular diagnosis of SARS infection as well as other SARS-related research.

In conclusion, the prevalence of osteonecrosis in SARS patients treated with high-dose short-term corticosteroid is about 10%. Both bone markers of resorption and formation are unable to predict its subsequent development. Cumulative doses of >2000 mg of methylprednisolone, >1900 mg of hydrocortisone, >13 340 mg of hydrocortisone equivalent, and >18 days on corticosteroid therapy are significant risk predictors for osteonecrosis, while pulse steroid therapy is not. Biochemical bone markers are useful in studying the pathophysiology of bone metabolism in response to massive doses of corticosteroids.

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