Inflammatory bone loss induced by SARS-CoV-2 infection

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INTRODUCTION: Severe acute COVID-19 may be complicated by both pulmonary and extrapulmonary manifestations, such as anosmia, ageusia, diarrhoea, lymphopenia, and multi-organ dysfunction syndrome. More recently, it has been increasingly recognized that some patients may develop long- term complications and persistent symptoms of COVID-19, such as fatigue, headache, dyspnea, anosmia, muscle weakness, low grade fever, and cognitive dysfunction. However, the full spectrum of clinical manifestations in the long-term post-acute sequelae of SARS-CoV-2 infection, or "long COVID", remains incompletely understood. In particular, SARS-CoV-2-associated pathological changes in the skeletal system remain largely unknown. Recently, a multi-center study showed that COVID-19 patients requiring intensive care had significantly lower bone mineral density (BMD) than those who were managed in non-intensive are setting. In this work, we characterize the effects of SARS-CoV-2 infection on bone metabolism and its underlying mechanism during the acute and post-recovery phases in our established golden Syrian hamster model which closely mimics human infection.

METHODS: To study the effects of SARS-CoV-2 infection on bone metabolism, Syrian hamsters were intranasally challenged with SARS-CoV-2 (i.e., Delta variant, strain HKU-001a, GenBank accession number: MT230904) and then their bone tissues were collected serially after the infection (**Figure 1a-b**). The animal experiments were approved by the HKU Committee on the Use of Live Animals in Teaching and Research. Briefly, 6–10-week-old male or female golden Syrian hamsters (*Mesocricetus auratus*) were obtained from the Chinese University of Hong Kong Laboratory Animal Service Center through the HKU Center for Comparative Medicine Research. Each animal was intranasally treated with 10^5 PFU of SARS-CoV-2 in 50 µL of PBS under intraperitoneal ketamine (200 mg/kg) and xylazine (10 mg/kg) anesthesia at 0 dpi. Mock-infected animals were treated with 50 µL of PBS. Their blood, bone, and lung tissues were collected at sacrifice at 4, 30, and/or 60 dpi for µCT, virological, and histopathological analyses.

RESULTS SECTION: In general, SARS-CoV-2 causes significant multifocal loss of bone trabeculae in the long bones and lumbar vertebrae of all infected hamsters. The most prominent disease manifestations were seen at about 4 days post-infection (dpi) and the hamsters generally recovered at about 7 to 10 dpi. The infected hamsters exhibited progressive loss of bone trabeculae at the distal metaphysis of femurs from the acute phase (4 dpi) to the post-recovery phase (30 dpi) and the chronic phase (60 dpi) of infection (**Figure 1c**). On D60, when the hamsters had recovered from the SARS-CoV-2 infection, the bone density was not restored. Instead, a gradual decrease of bone volume fraction, as well as progressive increases of trabecular pattern factor and the specific bone surface was detected. Compared with mock-infected hamsters, a significantly higher number of tartrate-resistant acid phosphatase positive ($TRAP^+$) osteoclasts were found in the bone trabeculae at the distal metaphysis of the femur, the proximal metaphysis of the tibia, and the lumbar vertebrae of SARS-CoV-2-infected hamsters (**Figure 2a-c**). Moreover, we show that the bone loss is associated with SARS-CoV-2-induced cytokine dysregulation, as the circulating pro-inflammatory cytokines not only upregulate osteoclastic differentiation in bone tissues, but also trigger an amplified pro-inflammatory cascade in the skeletal tissues to augment their pro-osteoclastogenesis effect.

DISCUSSION: Musculoskeletal sequelae have been increasingly reported in COVID-19 patients including those who have recovered from the acute phase of the infection. Our findings in this study demonstrated that SARS-CoV-2-induced pathological bone resorption through a pro-inflammatory cascade instead of direct infection in the skeletal tissue. Indeed, the direct infection of SARS-CoV-2 in bone tissue is very unlikely because there is little to no expression of ACE2 or co-expression of TMPRSS2, which are both vital for viral entry, in the bone marrow. However, our study suggests that pathological bone loss may be a neglected complication which warrants more extensive investigations during the long-term follow-up of COVID-19 patients. The benefits of potential prophylactic and therapeutic interventions against pathological bone loss should be further evaluated.

SIGNIFICANCE/CLINICAL RELEVANCE: The findings of this study highlight the need for optimizing clinical protocols for monitoring long-term complications of COVID-19 and finding novel treatment strategies for SARS-CoV-2-induced inflammatory osteopenia/osteoporosis.

IMAGES AND TABLES:

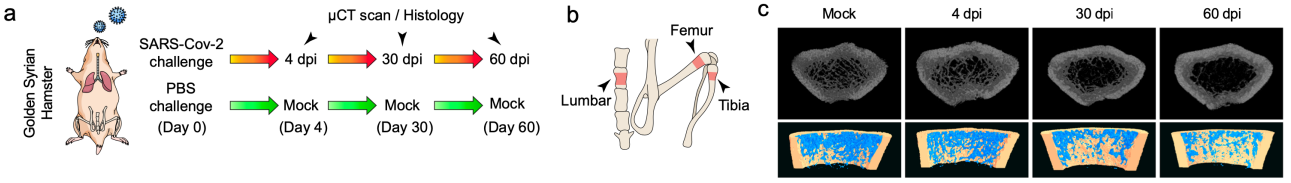

Figure 1. (a) Golden Syrian hamsters were either treated with SARS-CoV-2 or PBS (Mock), followed by a µCT scan and histology analysis at 4, 30, and 60 days post-infection (dpi). (b) The regions of interest for µCT evaluation of bone density included the distal metaphysis of the femur, proximal metaphysis of tibia, and lumbar vertebrae. (c) Representative µCT images showing the reduction in trabecular bone volume in the femurs of the SAR-CoV-2-infected hamsters.

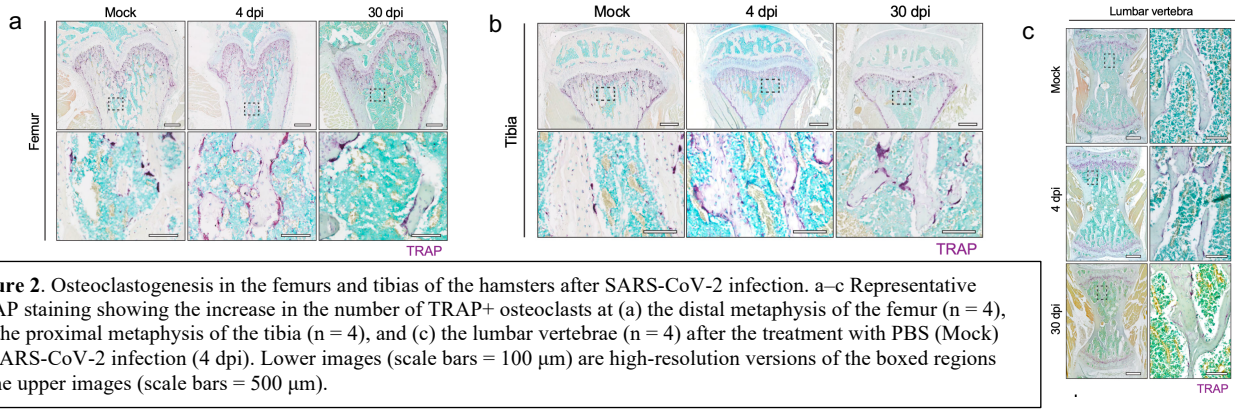

Figure 2. Osteoclastogenesis in the femurs and tibias of the hamsters after SARS-CoV-2 infection. a–c Representative TRAP staining showing the increase in the number of TRAP+ osteoclasts at (a) the distal metaphysis of the femur (n = 4), (b) the proximal metaphysis of the tibia (n = 4), and (c) the lumbar vertebrae (n = 4) after the treatment with PBS (Mock) or SARS-CoV-2 infection (4 dpi). Lower images (scale bars = 100 µm) are high-resolution versions of the boxed regions in the upper images (scale bars = 500 µm).