RESPONSES TO THE REVIEWER

We are most grateful for the critical comments and helpful suggestions made by reviewers, and applaud the reviewer's careful scrutiny of our manuscript. We have responded point-by-point to the comments raised by the reviewers, as described below.

REVIEWER COMMENTS:

Reviewer 1:

This article is well written, points to a therapeutic possibility for osteoporosis in chronic hepatitis. The sample is small, but it opens up the possibility of larger studies to better assess the risk and benefit of long-term use of the medication. Denosumab appears to be better tolerated than bisphosphonates and appears safe. Answers that may come from larger studies and with longer follow-up.

Responses: We are deeply grateful for the reviewer's encouraging comments. We fully agree to the reviewer's comments. We believe that our study opens up new possibilities for osteoporosis treatment in CLD patients. In the future, we are willing to conduct a large-scale, randomized controlled study to conclude the long-term effects and safety of denosumab in CLD patients.

Reviewer 2:

In the manuscript, Saeki C et al. were devoted to evaluate the effect of denosumab in chromic liver disease (CLD), which had already been verified to increase bone mineral density (BMD) and decrease the risk of osteoporotic fracture in general population. According to the parameters, such as BMD, serum TRACP-5b and P1NP levels, they gave the conclusions that denosumab treatment was safety and increased BMD, suppressed bone turnover, and improved bone quality marker levels in CLD patients with osteoporosis, irrespective of differences in baseline characteristics. Honestly, the results is not surprised, but the logic is straight, and data are kind of solid. I personally support the publication of this manuscript in the world journal of gastroenterology. However, there still exist some doubts:

(1). The main idea of this paper aimed to investigate the effect and safety of denosumab in CLD patients, but denosumab was used to cure osteoporosis, while the osteoporosis is a common complication in patients with CLD. The necessity or connection need to specifically address to stand as the background if there do exist correlations.

Responses: We greatly appreciate the reviewer's critical remarks. Osteoporosis is a common complication that causes fragility fractures and reduces health-related quality of life in CLD patients. Therefore, effective treatment of osteoporosis is essential in CLD patients. Recent reports demonstrated that denosumab improved BMD and decreased the risk of osteoporotic fracture risk in the general population. In addition, denosumab treatment improved health-related quality of life in patients with osteoporosis. Therefore, denosumab has been the focus of public attention as an attractive treatment for osteoporosis. However, it is uncertain whether denosumab treatment similarly improves BMD in CLD patients. Accordingly, we aimed to investigate the effects and safety of denosumab in CLD patients with osteoporosis. We clearly stated these situations in the manuscript.

(2). As the authors mentioned in the manuscript, the sample size was a drawback as it displayed.

Responses: We are deeply grateful for the reviewer's critical comments. As described in the original manuscript, the sample size was not large enough to evaluate the effects of denosumab. In the present study, we screened 405 CLD patients for osteoporosis and diagnosed osteoporosis in 138 patients. However, 78 patients met the exclusion criteria. Therefore, only 60 patients were finally included in the analysis, as indicated in Figure 1. However, there have been no reports that analyzed dozens of CLD patients receiving denosumab as the first treatment for osteoporosis. In the future, a large-scale, multicenter study is needed to confirm the effects of denosumab in CLD patients.

Reviewer 3:

This is an interesting study, aiming to evaluate the effect of denosumab in patients with CLD and osteoporosis, however, the study has several major issues that must be improved to better sustain the conclusions stated by the authors:

(1) Design of the study: this is a quasi-experimental study, not including a proper control study, rendering the main findings difficult to fully sustain. Even though an improvement in BMD and markers of bone turnover and quality was seen, there is no a fair point of comparison, as had been done with a different analysis. The same is true for occurrence of adverse events.

Responses: We greatly appreciate the reviewer's critical remarks. We fully agree to the reviewer's comments. Certainly, this was not a randomized control study to evaluate the efficacy and safety of denosumab versus placebo or other medications such as

bisphosphonate (BP). Therefore, we could not specify the characteristic profiles of denosumab treatment in CLD patients with osteoporosis. Previous report has demonstrated that 12-month denosumab treatment was superior to placebo or BP treatment in the general population (Ref. 19, Saag KG, et al. Lancet Diabetes Endocrinol 2018). However, there are few reports evaluating the effects and safety of denosumab in CLD patients. Therefore, we believe that this study opens up new possibilities for osteoporosis treatment in CLD patients. In the future, a large-scale, long-term, randomized controlled study is needed to confirm our findings. Accordingly, we have changed the manuscript type "prospective study" to "retrospective study".

(2) Description of patients with CLD is rather poorly addressed throughout the manuscript. None of the patients with autoimmune CLD were receiving steroids during the study? Were these autoimmune liver diseases only PBC and PSC, or was AIH also included? The basic characteristics of patients with cirrhosis are not shown (i.e. MELD and Child-Pugh scores).

Responses: We are deeply grateful for the reviewer's critical comments. In the present study, patients with autoimmune liver disease consisted of 19 PBC and 1AIH. None of the autoimmune CLD patients had been receiving steroids, as described in the Materials and Methods section. Accordingly, we replaced the term "autoimmune" with "PBC" and assigned one AIH patient to the "others" category in revised Table 1. As suggested by the reviewer, we added the baseline characteristics of patients with cirrhosis (Child-Pugh class/score and MELD score) to revised Table 1. The median Child-Pugh and MELD sore were 5 (5–6) and 3.0 (0.5–6.0), respectively.

(3) Regarding safety of the drug, there are no biochemical tests at follow up, which would be the most appropriate given that the effect of the drug in chronic liver diseases (specially in cirrhosis), has been scarcely studied.

Responses: We are deeply grateful for the reviewer's critical comments. The safety of denosumab in CLD patients has not yet been elucidated so far. Therefore, time-course changes in the levels of biochemical tests during denosumab treatment are important information. We added the results of biochemical tests during the follow-up period in revised Table S5. No patients experienced exacerbation of chronic liver disease or development of renal dysfunction during denosumab treatment. The median ALP level of 283 U/L at baseline gradually decreased by 33.2% at 3 months and thereafter remained at a similar level, which possibly reflects the suppressed bone turnover

(Iwamoto J. et al. Clin Rheumatol 2016).

(4) Due to the design of the study, it is difficult to reach those conclusions, mainly because there is no comparison group nor a sample size estimation.

Responses: We quite agree to the reviewer's comment. We considered the need for the control group, but before that we desired to evaluate and confirm the effect and safety of denosumab in CLD patients, including those with advanced disease stage. Although the sample size was small, we screened 405 CLD patients for osteoporosis and diagnosed osteoporosis in 138 patients. As shown in Figure 1, we excluded subjects who met the exclusion criteria and eventually evaluated only 60 patients. In the future, a large-scale, multicenter, randomized controlled study is needed to confirm the efficacy and safety of denosumab in CLD patients.

(5) How many patients were diagnosed as postmenopausal osteoporosis? And how many had CLD-related osteoporosis? There is no description of this point, and together with the more detailed characteristics of CLD (and specially cirrhosis) is extremely important in order to provide to the reader with sufficient information to implement this intervention in clinical practice.

Responses: We are deeply grateful for the reviewer's critical comments. In the present study, all female patients were in the postmenopausal state. Menopause causes a drastic decline in estrogen, leading to a decrease in bone mass (Manolagas SC, et al. Nat Rev Endocrinol 2013). Meanwhile, CLD contributes to loss of bone mass, which is known as secondary osteoporosis. However, it is difficult to distinguish secondary osteoporosis clearly from primary osteoporosis in postmenopausal CLD patients. We added the information about the prevalence of menopause in revised Table 1.

Science Editor:

(1) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Responses: As instructed by the science editor, we attached the original figures (file format, Microsoft PowerPoint).

(2) I found the authors did not write the "article highlight" section. Please write the "article highlights"

section at the end of the main text.

Responses: As instructed by the science editor, we wrote the "article highlights" section at the end of the main text.

(3) please don't include any *, #, †, §, ‡, ¥, @….in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as aP < 0.05, bP < 0.01 (P > 0.05 usually does not need to be denoted). If there are other series of P values, cP < 0.05 and dP < 0.01 are used, and a third series of P values is expressed as eP < 0.05 and fP < 0.01.

Responses: As instructed by the science editor, we replaced the symbol " * " with the alphabet " a " in Figure 2 and 4. Statistical significance is expressed as "a" for P < 0.001 and "b" for P < 0.05 in Table S5.

(4) I have changed the manuscript type "prospective study" to "retrospective study".

Responses: As instructed by the science editor, we have changed the manuscript type "prospective study" to "retrospective study" and mentioned that 'this was a retrospective study' in the method section.

REVIEWER COMMENTS: Reviewer 1 (Reviewer's code: 04105237): Thank you for the responses. Additional comments: 1) Please specify others in the footnote in Table 1 (which specific disease had the patients, v.gr. AIH=x, etc.) Responses: We are deeply grateful for the reviewer's critical comments. As suggested by the reviewer, we specified "others" in the footnote in revised Table 1. We attached revised Table1 in the revised manuscript. Othrers (alcoholic liver disease, n = 4; autoimmune hepatitis, n = 1; nonalcoholic steatohepatitis, n = 1). 2) Please use a current calculator to obtain the median MELD score (the numbers provided are too low). Responses: As suggested by the reviewer, we used a current calculator to analyze the MELD score. In the present study, most of LC patients were classified as Child-Pugh A (compensated LC) and liver function was preserved. Accordingly, the median MELD score was too low. Since other reviewer suggested that we should add the basic characteristics of patients with cirrhosis (i.e. MELD and Child-Pugh scores), we indicated these information in revised Table 1. We consider that information about MELD score may be unnecessary. 3) Please check for typos. In the abstract in the conclusion it should be safe, instead of safety. Responses: As instructed by the reviewer, we have changed the term "safety" to "safe" in the conclusions and core tip. Reviewer 2 (Reviewer's code: 03700188): 1 Title. The title reflects the main subject. 2 Abstract. The abstract summarizes and reflect the work described in the manuscript 3 Key words. The key words reflect the focus of the manuscript 4 Background. The manuscript was adequately described 5 Methods. The manuscript described methods well, but I had a doubt about statistic. Why did the authors use the Mann-Whiney test for all continuous variables? 6 Results. The research objectives were achieved by the experiments used in this study and they showed that the drug is save and it helps improve the bone disease. 7 Discussion. The manuscript interpreted the findings adequately and appropriately and highlighted the key points logically. 8 Illustrations and tables. They were of good quality and illustrative 9 Biostatistics. I only had one doubt about continuous variables. 10 Units. It met the requirements of use of SI units. 11 References. The manuscript cited appropriately the latest, important and authoritative references in the introduction and discussion sections (12 of them had 3 years). The author self-cited once. 12 Quality of manuscript organization and presentation. The manuscript was well written. Responses: We are deeply grateful for the reviewer's encouraging comments. We used the Mann-Whitney U test (non-parametric test) to compare continuous variables between the two groups in Figure 3, because the percentage values among these groups were independent. As suggested by the reviewer, we used the SI units in Table 1 and Table S5. Which unit should I revise concretely?