

Reviewer A

In this observational study, 4 cohorts were compared for the incidence of PCR-positive COVID-19 and development/evolution of fever; one cohort, Group A (nasopharyngeal carcinoma (NPC) undergoing radiotherapy) was exposed to saline 'douching' [saline nasal irrigation (SNI)], the 3 others weren't [Group B: Head-neck cancer treated with radiotherapy; Group C: others NPC not receiving radiotherapy; and Group D: Health Care Workers (HCWs)]. The results are interesting to publish from an explorative point of view; based on the cohort comparisons, and despite NPC patients may be at high risk for SARS-CoV-2 infection from a pathophysiological perspective, benefits were observed with SNI on the incidence and duration of fever.

Yet, the manuscript can be improved for readers who are interested in effects of saline nasal irrigation (SNI), but not familiar with use of SNI in NPC. NPC is a common condition in Asian countries, like China, but rare in Europe and United States. In the latter continents, SNI is not mentioned in treatment guidelines, nor on local internet sites that give information on NPC. Hence, the rationale of SNI in NPC (apparently established in China, but not in Western countries) and details about the technique(s) applied and benefits obtained are at best covered. Also the design and comparability of cohorts can be better explained or addressed (or/and shortcomings be discussed accordingly).

Major points to address in the manuscript:

1. Study design: please address under Methods:

Comment 1: Were the cohorts explicitly asked to use, respectively NOT to use saline, and/or was this checked retroactively, and were cases selected accordingly? (e.g. HCWs performing SNI excluded?)

-> With other words: Is it a retrospective analysis of cases, of which cohort A was instructed to use SNI in the frame of NPC treatment (with their consent asked/given afterwards for the analysis), or was it a prospective study for incidence of COVID-19 in different cohorts (patients given instructions, and having given informed consent at start)? [To note: Prospective study = continuously identifies incident cases (subjects getting COVID-19) during the period that the cohorts is followed; Retrospective study = identify the diseased subjects afterwards by interview or written records] Or was it a

mixed method? E.g. NPC prospectively as instructed to perform SNI, Group D (health care workers) retrospectively – But then what about Group B and C?

Reply 1: Thank you for pointing this out. This was a retrospective study formulated based on findings in our clinical diagnostic and therapeutic work. Saline nasal irrigation, employed as an adjuvant treatment for patients underwent NPC radiotherapy in our hospital, was routinely applied. The effectiveness of patient irrigation is assessed through nasopharyngoscopy during treatment. In contrast, participants in the other groups were confirmed not to have undergone nasal irrigation during follow-up.

Changes in the text: we have modified our text as advise (see the page 4-5, line 129-146)

Comment 2: Target groups/patients:

Describe briefly the patient groups and COVID-19 work-up for the reader, not familiar with treatment of NPC and SNI for NPC, and how /when patients were diagnosed with COVID-19 under Methods:

-> “Outpatients” on ambulatory radiotherapy? (If so, at which usual frequency(ies) and duration(s) - comparable for groups B and C? group D = health care workers (HCWs) at risk of being exposed frequently to the fast-circulating omicron variants in the hospital over the whole study period.

Reply 2: Thank you for your valuable comments. There were not “Outpatients” on ambulatory radiotherapy in our hospital. All patients underwent radiotherapy once daily from Monday to Friday.

Changes in the text: we have modified our text as advise (see the page 4-5, line 118-124, line 129-139)

Comment 3:-> When+frequency+method of testing for COVID-19 by PCR? Routine at each admission, or only /and when developing symptoms? Self-sampling or where? Oropharyngeal, saliva, nasopharyngeal NAAT? Same or various tests used over the time frame of the study?

Reply 3: Thank you for your valuable comments. All patients underwent routine COVID-19 testing each admission and when developing symptoms. The healthcare personnel underwent COVID-19 testing daily. The testing was conducted by our hospital's specialized sampling personnel using nasopharyngeal swabs.

Changes in the text: we have modified our text as advise (see the page 4, line 118-124)

Comment 4: -> PCR-positive patients post-testing: were they kept in the hospital? Of

further treated as outpatients in each of the groups? RT was stopped and they were given standard of care; which was? While continuing or not SNI?

Reply 4: Thank you for your valuable comments. All the participants infected with COVID-19 were required to temporarily suspend ongoing anti-tumor treatment and received the standard treatment for COVID-19. Conventional treatment included physical cooling and traditional Chinese medicine (TCM) prescriptions such as Lianhua Qingwen granules, which have been recommended by the Chinese National Health Commission to treat COVID-19. Patients were kept in the hospital. And patients in group A continuing SNI after infected with COVID-19.

Changes in the text: we have modified our text as advise (see the page 4-5, line 126-129, line 143-144)

Comment 5: Main instructions for use of SNI and/or COVID-19 prophylaxis: Briefly describe the reason(s) why NPC patients are/were instructed for doing the SNI procedure (in Intro or Method):

- Was SNI specifically instructed at start of the patient's management, as potentially reducing risks for COVID-19? (so likely evoking more diligent compliance than when just used for hygiene or comfort)

Reply 5: Thank you for your valuable comments. As part of routine care measures during radiotherapy for NPC at our hospital, patients are regularly guided by healthcare personnel to perform SNI. This study is a retrospective analysis conducted based on interesting phenomena observed during diagnosis and treatment. Patients were not informed of relevant information regarding nasal irrigation reducing the risk of COVID-19 as part of this study.

Changes in the text: -

Comment 6: - Or was SNI rather instructed as "usual nasal care" for NPC with RT, and for what reason e.g. to remove crusting, soothing, to reduce post-irradiation rhinosinusitis and/or to enhance survival (add references)

Reply 6: Thank you for your valuable comments. SNI can flush secretions and necrotic materials from the nasal cavity, achieving the goal of moist and clean conditions. Additionally, it can reduce the incidence of adverse reactions such as nasal adhesions and sinusitis after radiotherapy, thereby improving the quality of life for patients with nasopharyngeal cancer. (reference1: Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.)(reference2: Dalton RM, Mullin AE, Amorim MJ, et al. Temperature sensitive influenza A virus genome replication results from low thermal stability of polymerase-cRNA complexes. Virol J 2006;3:58.)

Changes in the text: we have modified our text as advise (see the page 3, line 78-81)

Comment 7:- Did the cohorts get other advice as to manage/avoid COVID-19, and if so, which instructions in each (or all) of the cohort(s)? What about other measures to avoid transmission? E.g. mask wear? (+ change of mask wear?), distancing, hand hygiene)

Reply 7: Thank you for your valuable comments. According to the COVID-19 prevention and control measures in our country, all patients have received two doses of COVID-19 vaccination at least, while healthcare personnel have received three doses of the COVID-19 vaccine. Participants were required to wear masks when going out and change them daily. They were also instructed to maintain physical distancing, reduce entry into crowded places such as supermarkets, and practice hand hygiene after going out. However, in the context of a retrospective study, these measures were not explicitly communicated during the study.

Changes in the text:-

Comment 8:- To note: to act prophylactically in patients at risk, SNI should at best also be performed immediately after a hospital visit or after any potential exposure at risk: was this instructed? (e.g. guidelines Society of Hospital Hygiene. See instructions under Mouth rinse/gargling & Nasal cavity (rinse/irrigation), point 4.2 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9285112/pdf/HIC-17-13.pdf>. Please discuss if not instructed or applied.

Reply 8: Thank you for your valuable comments. As mentioned above, being a retrospective exploratory study, participants were not received any information regarding the correlation between nasal irrigation and COVID-19 infection.

Changes in the text:-

Comment 9: SNI intervention: please provide under Methods briefly details (or give a reference where the technique is described): the method mentions ‘douche’, while the discussion refers to “nasal” and further “nasal and pharyngeal” irrigation and hypertonic saline: so please specify: whether nasally or also combined with gargling; recommended volume (range); saline concentration(s); preparation (self-made? temperature?) and dispensing mode (e.g. spray? pressurizing flask? special technique?) used for NPC. At last, specify also whether/how compliance was monitored.

Reply 9: Thank you for your valuable comments. Specific procedures were as follows: The patients used self-provided nasal irrigation squeeze bottles while sitting with the head slightly tilted forward. Approximately 500ml of normal saline at about 37°C was injected into the squeeze bottles. The output end of the squeeze bottles was inserted into the anterior nasal vestibule, closing the nostrils. Then, the squeeze bottles were gently and uniformly pressed. The patients were instructed to breathe through the

mouth. Nasal irrigation was performed alternately on the left and right sides of the nasal cavities. Additionally, they underwent nasopharyngoscopy every 14 days to assess the nasal irrigation situation.

Changes in the text: we have modified our text as advise (see the page 4-5, line 129-141)

Comment 10: a. Severe COVID-19: The information on the outcome of fever is interesting and it is acceptable to limit the observations on fever to mild-to-moderate COVID-19 - with its limitations, as presented in the article. Yet, if referring to prophylaxis, at best, also add numbers (proportions) of patients in each cohort developing severe COVID-19 and % hospitalizations, as well as co-morbidities and vaccination status. If this information is not available, please state the reasons why, under Methods or Discussion. (e.g. Admitted to different unit? Or not included, simply because with the Omicron variant, little or no NPC patients developed severe disease?). In this respect it may be worthy to address NPC undergoing RT as a risk factor for COVID-19: are there any clinical data suggesting NCP is a risk factor? At least from a pathophysiological perspective, NPC undergoing RT may possibly represent a risk for getting infected with SARS-CoV-2 for at least 2 reasons: (1) Furin expression is often enhanced in malignancies and can be increased after RT (e.g. Braun & Sauter 2019, doi: 10.1002/cti.2.1073; Lee: DOI: 10.21873/anticanres.11081). Vice versa, furin presence may lead to enhanced priming of the SARS-CoV-2 spike (S) and cell-cell fusion: SARS-CoV-2 is characterized by enhanced infectivity and invasiveness due to its furin cleavage site (FCS) in the S protein (e.g. Frolova 2022 doi: 10.1128/jvi.00753-22; Zhong 2020: <https://doi.org/10.3389/fmed.2020.580796>). (2) In addition, RT of the nasopharyngeal area leads to direct damage and loss of ciliated cells, impaired ciliary activity/mucociliary clearance (doi: 10.3389/fonc.2022.1010131). Both conditions may thus create a nasal environment prone to infection with SARS-CoV-2.

Reply 10: Thank you for your valuable comments. Due to the Omicron variant and the pre-treatment assessment for anti-tumor therapy, all participants have not developed severe COVID-19 according to the “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 9). Simultaneously, due to experimental constraints, the correlation between NPC and COVID-19 was not explored, which is one of the limitations of this study.

Changes in the text: we have modified our text as advise (see the page 5, line 149-152; page 11, line 382-384)

Comment 11. Vaccination: If no data on vaccination, it may be useful to specify the local policy: was rollout of vaccination and boosters implemented locally (which

vaccine(s) used?); was vaccination recommended by the hospital in NPC when diagnosed with cancer and/or scheduled for RT? Can it be assumed to be comparable across the cohorts? This may give at least some insights on the likelihood for potential interaction with vaccination.

Reply 11: Thank you for your valuable comments. In line with our country's COVID-19 prevention and control policy, there is no difference in the number of vaccine doses administered to participants in each group. However, the lack of information on the vaccine product formulation is one of the shortcomings of this study. Considering the policy, we can assume its comparability across different cohorts.

Changes in the text: we have modified our text as advise (see the page 19-20, Table 1; page10-11, line 375-382)

Comment 12: c. Relevance to other populations? When referring to unsuccessful prophylaxis in the Discussion, please link the outcome to the group of NPC patients applying twice daily SNI. It is possible to discuss outcome with regard to other studies. The findings with twice daily NSI in PC patients may not necessarily reflect outcomes in other groups (e.g. prophylaxis data in HCWs with SNI adjunctive to mask wear (e.g. Cao 2022 & Chuayruksa 2023)]. Combined nose and throat irrigation (gargling) has recently also been shown to reduce risk of hospitalization, albeit large randomized clinical trials are still missing and unlikely to come due to come in the next future.

Reply 12: Thank you for your valuable comments. The potential of nasal irrigation to improve viral shedding time and certain symptoms after COVID-19 infection has been reported in other populations, and this information is detailed in the discussion. Nevertheless, there is ongoing debate about its efficacy in certain experiments; this study intends to complement by investigating its practical value in self-care.

Changes in the text: we have modified our text as advise (see the page 11, line 384-389)

d. Suggest to integrate relevant sources, if judged appropriate, such as:

- Cao J, et al. doi: 10.5582/bst.2022.01261.
- Espinoza S, et al. <https://doi.org/10.1016/j.anai.2023.08.245>
- Huijghebaert S, et al. doi:10.3389/fpubh.2023.1161881
- Chuayruksa et al. Oral rinse, nasal irrigation, and risk factor of COVID-19 screening. *Journal of International Dental and Medical Research* 2023;16 (3):1227 – 1231. https://www.jidmr.com/journal/wp-content/uploads/2023/09/46-D23_2820_Wuttiphong_Phakdeekul_Indonesia.pdf [See prophylactic impact of SNI use among HCWs in Table 2 (qPCR); SNI may however lead to false positives with As-Ab testing).

Specific comments:

Comment 13: Line 103: Wording “had severe COVID-19 according”: is it meant “developed” severe COVID-19? ... as line 99 excludes active COVID-19 infection, so automatically also excludes severe COVID-19. Or were such cases excluded if developing severe COVID-19 over the observation period of the study? Please adapt accordingly to the exact study design. In the latter data were not taken into account in Table 2, it is hard to conclude on (lack of) a prophylactic effect (see also suggestion a).

Reply 13: Thank you for pointing this out. It has been revised in the text.

Changes in the text: we have modified our text as advise (see the page 3, line 105-106)

Comment 14: Lines 116-124: see major points.

Line 117: Wording: should it read: “All patients in Group A “becoming” infected with COVID-19 during the study (?) - According to line 99, active COVID-19 infection at start of study was excluded.

Reply 14: Thank you for pointing this out. It has been revised in the text.

Changes in the text: we have modified our text as advise (see the page 4, line 124-125)

Comment 15: Line 119: “The participants in Group A underwent ... “: suggests SNI treatment was administered by someone else: was it administered by HCWs? Or is it meant: “performed” SNI...? The same question is valid for the other groups: ‘did not undergo’, do you mean, ‘did not perform’ and/or: were they explicitly asked to abstain from SNI? (cfr Major points 2- 3)

Reply 15: Thank you for pointing this out. It has been modified in the text that the remaining participants in each group were confirmed not to have undergone nasal irrigation during follow-up.

Changes in the text: we have modified our text as advise (see the page 4-5, line 129-143; line 145-146)

Comment 16: Line 164: .. in Group D: should it read “became” infected – rather than ‘were’ infected? Using the wording “were” infected can also suggest that they were already infected at start of study, in which case they should have been excluded according to line 99.

Reply 16: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 4, line 124-125)

Comment 17: Line 194: ref. Chalageri refers to ‘gargling’. Please elaborate with more sources on SNI (e.g. for duration of symptoms, see e.g. Siregar doi: <https://doi.org/10.3889/oamjms.2022.9013> and/or consult Huijghebaert: doi: 10.3389/fpubh.2023.1161881)

Reply 17: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 7, line 234-236; reference 12,13)

Comment 18: Line 194: ‘Controversy’ relates to 2 studies, yet the 2 referenced studies had a majority of patients being asymptomatic and only a limited number of patients developed (predominantly) mild symptoms. So, the patient samples may have been too small (and symptom severity too mild), so the study being insufficiently powered to allow for a detection of significant differences in duration or symptom severity. This can be overcome in the cohort study, as covered in the article.

Suggestion: If also applicable to NPC patients it may be acknowledge here, the higher asymptomatic status and milder, fluctuating symptoms with Omicron infection, affecting variably throat and nose. This may make it overall more difficult to detect significant improvement of symptoms in clinical studies,

Reply 18: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 11, line 382-385)

Comment 19: Line 196: SNI becomes here labeled as “nasal pharyngeal irrigation”, a term also further throughout the text – Please make sure to use a consistent denomination and abbreviation. Was there maybe anything done extra in the irrigation procedure to better cover the pharynx?

Reply 19: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 7, line 239; page 8 line 266)

Comment 20: Line 203: COVID-19 infection rates did not differ “statistically significantly” between... Was SNI (which appears to be often used traditionally in China) excluded in the other groups (cfr line 119)? If not, this should be acknowledged as a shortcoming of the study, thereby emphasizing the explorative objective of the study.

Reply 20: Thank you for pointing this out. Participants in the other groups were confirmed not to have undergone nasal irrigation during follow-up.

Changes in the text: we have modified our text as advise (see the page 5, line 145-146)

Comment 21: Line 214: At best, the statement on SNI is limited to the observation with the exact SNI procedure in NPC patients on RT: “Based on these findings it is apparent that while.. irrigation ‘twice daily’ cannot prevent infection ‘in NPC patients undergoing RT’, it may play a role in mitigating the

Reply 21: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 263-264)

Comment 22: Line 216-17: “We observed SNI did not affect body temperature during fever.” This is a confusing statement. The data you present overall show that a lower proportion of PCR-positive NPC patients routinely using NSI twice daily developed fever (Table 2), while if developing fever, this fever (at peak temperature) was comparable to, or lower than, observed in the other cohorts, and moreover lasted shorter, so resolving significantly faster, than in the other cohorts. So, albeit the body’s Reply to fever has been proposed by some as being a protective mechanism of the body’s immune Reply against the viral challenge (as you suggest by references from Lines 217-231), the data obtained with SNI do not support such mechanism.

Line 216-231: Suggestion to rephrase/reduce or rather fully delete this section on the proposition of fever as a protective mechanism, and on the concept that reducing fever with antipyretics imposes a risk for prolonging infection, which would not be shared by SNI. Most of the sources are based on experimental models, external changes in (environmental) temperature, or clinical observations that are not representative of the human anti-fever Reply, or that can be explained by actions of antipyretics other than their effect on fever. Due to the many data presented, the point is missed that this would be different with SNI, so the paragraph overall weakens the rationale for SNI use to reduce fever, as documented in the study.

Moreover, current recommendation of the WHO for mild COVID-19 is that can be treated by self-care at home and proposes the use of anti-fever medication. So, the arguments for the proposition that antipyretics adversely affect infectious outcome are likely too weak to be considered as relevant to treatment of COVID-19. In contrast, the outcome on fever in the NPC patients is interesting in regard of the proposition for management by WHO to reduce fever as part of self-care (see living guidelines 2023), as SNI not only reduced presence and duration of fever, but as has been shown in other studies to reduce other oronasal symptoms and viral load.

Line 221: ... fever temperature through ‘antipyretic’ medication?? + specify with what physical intervention is meant? Or rather delete this information overall as already suggested.

Line 223-224: As mentioned under line 216-231, the arguments are weak to prove or

claim that “SNI does not compromise the body’s intrinsic resistance to virus but reduces symptom duration through other mechanisms”.

Reply 22: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 266)

Comment 23: 228-233: You refer to “age, disease conditions and lifestyle habits” that may have differed between the cohorts. This is correct. Yet these parameters, as well as the ‘scarcity of medical resources’ are (inappropriately?) linked to the reason why HCWs contracted more frequently SARS-CoV-2. Please explain if relevant. Other factors that may be acknowledged include: (1) the fast-spreading nature of omicron (despite mask wear and often lower protection with vaccination against omicron variants), (2) the continuous exposure of HCWs over the complete study period to potentially omicron-infected patients in the hospital: with Omicron infection, patients are frequently asymptomatic, yet can carry high viral loads (e.g. Liu 2023). Why HCWs developed significantly higher fever, cannot be deduced from the study observations, yet mental and physical exhaustion of HCWs, as documented during the pandemic (for references, see PubMed) deserves mentioning (line 231-232).

Reply 23: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 269-273)

Comment 24: Line 220; Ref. 14. Graham: this study with Rhinovirus may be worthy to mention but is better placed in relation to the discussion in lines 238-241: in contrast to the trend toward longer duration of viral shedding observed with antipyretics in Rhinovirus infection, SNI has been shown to reduce viral load and shorten viral shedding both in common cold and in SARS-CoV-2 infection, as you explained under lines 241-246.

Reply 24: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 283-285)

Comment 25: Line 273-: hypertonic: specify concentration(s) used in the study in this regard. Both isotonic and hypertonic solutions seem to work (Huijghebaert 2023, Espinoza 2023, Liu 2023). The considerations pro or against hypertonic solution may be relevant for chronic conditions; upon repeated applications throughout the day for acute infection, isotonic solutions for SNI and gargling may possibly be better tolerated.

Reply 25: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 10, line

340-345)

Comment 26: Table 2: If feasible, it would be relevant if % with severe COVID-19 is also integrated, as well as % hospitalized, albeit it is recognized that this percentage may differ between the groups by differences in nature of the disease state and associated co-morbidities.

Reply: Thank you for your valuable comments. As all included participants did not develop severe COVID-19 infections, it is not possible to supplement this portion of the data.

Changes in the text:-

Reviewer B

GENERAL FEEDBACK

This retrospective study examined the effectiveness of nasal irrigation with hypertonic saline solutions to improve COVID-19 symptoms of 518 study participants at Fujian Cancer Hospital from December 7, 2022, to January 7, 2023. Participants were broken down into:

- patients were broken down as follows:
 - Group A (N=147) : Nasopharyngeal carcinoma (NPC) patients treated with RT;
 - Group B (N=30): head and neck cancer (HNC) patients treated with radiotherapy (RT);
 - Group C (N=191): NPC patients treated without RT.
 - Group D (N= 51): healthcare workers (HCWs) directly involved in daily clinical care during the study period

Only participants in Group A received saline nasal irrigation twice daily. All participants were measured daily axillary temperature by a mercury thermometer. Confirmed feverish participants (temperature > 37.5) were monitored at least twice a day for body temperature.

Four study endpoints were assessed:

- rate of COVID-19 infection;
- rate of developing fever after COVID-19 infection
- highest body temperature in feverish study participants
- duration of the fever;

Main findings:

- All HCWs tested positive for SARS-CoV-2, against 81% cancer patients
- The overall fever rate of the patients infected with the virus was 50.1%, against 98% HCWs

- COVID-19 infection rate in Group A was lower (77.6%) than other groups, though but the latter difference was not significant.
- The fever rate after COVID-19 infection in Group A was significantly lower (37.7%) than those of Groups B (61.5%) and C (54.8%).
- The highest fever temperature recorded in Group A was significantly lower than Group D

SPECIFIC COMMENTS

Comment 1: Line 115-132: What saline formulation was administered... hypertonic or isotonic?

Reply 1: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 133-139)

Comment 2: Lines 67-71: Saline irrigations are featured for anti-inflammatory properties and are used also after major otolaryngological surgery. The authors should mention the different composition of saline solutions, depending on concentration of NaCl. The cidal activity increases with saline concentration, being higher for hypertonic saline solution (sweater). Some recent clinical studies assessed the impact of intra-nasal administration of seawater to reduce viral shedding time (VST) of SARS-CoV-2 [recommended citations: PMID: 36432693; PMID: 37691090]. The cidal property of sweater has large spectrum cidal capacity, as it was effective also against common cold [PMID: 30705369].

Reply 2: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 10, line 340-345)

Comment 3: Methods: the study design (in particular, the partition of study groups) is not clear. The number of study participants in each study arm should be mentioned in methods as well as results. The authors only reported that total oncologic patients were 468 and group D was made of 51 HCWS. How many were in groups A, B and C? These number can only be derived from Table 2...

Reply 3: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 4, line 108-112)

Comment 3: Sample size calculation was considered before recruiting study subjects?

Reply 3: Thank you for pointing this out. As a retrospective study, there was no strict sample size calculation.

Changes in the text:-

Comment 4: Lines 88-104: Exclusion and inclusions criteria apply only to cancer patients (groups A, B and C) or also to Group A? it is unclear from methods.

Reply 4: Thank you for pointing this out; the exclusion criteria only apply to Groups A, B, and C.

Changes in the text: we have modified our text as advise (see the page 17, Figure 1)

Comment 5: Lines 129-131: “We conducted follow-up data collection from February 1, 2023, to March 1, 2023”... it was previously mentioned that participants were recruited from December 7, 2022, to January 7, 2023... please clarify the study timeline.

Reply 5: Thank you for pointing this out; to avoid ambiguity, it has been revised in the text.

Changes in the text: we have modified our text as advise (see the page 5, line 156-157)

Comment 6: Methods: viral shedding time (time since COVID-19 diagnosis through first negative swab test) was not considered? This will likely have had major impact on fever duration.

Reply 6: Thank you for pointing this out; as a retrospective study and due to limitations in medical resources, the timing of COVID-19 negative diagnoses was not addressed in this study.

Changes in the text: we have modified our text as advise (see the page 11, line 379-381)

Comment 7: Line 163-168: The overall fever rate of patients infected with the virus was 50.1%, while that of the healthcare group was 98.0%. Rather than contrasting the rate of infection between HCWs and all cancer patients, it would be more interesting to compare the rate of infection between patients treated with saline solution (Group A) against other groups.

Reply 7: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 6, line 204-207)

Comment 8: Line 175-179: descriptive measures (mean \pm SD), median (IQR), range of fever by sub-groups should be reported in results section. This important

information was never reported.

Reply 8: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 6, line 188-190; page 7 line 217-220)

Comment 9: Lines 192-94: again, there are other relevant clinical studies supporting the use of hypertonic saline solutions to reduce viral shedding time from COVID-19 patients affected by mild to moderate disease [PMID: 36432693; PMID: 37691090, PMID: 36839483]. One important point to mention is that saline seems effective in immune-competent patients affected by mild to moderate COVID-19 [PMID: 36432693; PMID: 37691090], whereas in high risk patients vaccination may have stronger effect size to reduce VST [PMID: 37242504; PMID: 38005858].

Reply 9: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we added some data (see the table 1)

Comment 10: Line 209-211: Different baseline characteristics of patients may explain the findings of this study. Only information on age and sex of patients was included in the analysis. Vaccination status or comorbidities other than otolaryngological cancers were not accounted for in the analysis.

Reply: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we added some data (see the table 1)

Comment 11: Line 243- 257: intra-nasal administration of seawater was reportedly effective against SARS-CoV-2 within 5 days since COVID-19 diagnosis, vanishing afterwards [references: PMID: 36432693; PMID: 37691090]. This may be related with overproduction of HOCl. The microbial activity of chloride may not be mediated only by over production of hypochlorous acid. In addition to over production of HOCl, chloride reportedly also induced membrane depolarization on infected cells in vitro [PMID: 36432693].

Reply 11: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 10, line 347-349)

Comment 12: Lines 267-272: Administration of saline solutions by spray (rather than irrigation) may more practical for care homes residents, avoiding ab ingestis [PMID: 36839483; PMID: 36432693].

Reply 12: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 9, line 334-335)

Comment 13: Line 273- 290: Combining sweater with compounds as N-chlorotaurine (NCT) or thiocyanate anion (OSCN⁻) has been proposed as a strategy to mitigate the irritative effect of hypochlorous acid. [PMID: 36839483; PMID: 36432693].

Reply 13: Thank you for your valuable suggestion. As stated in the text, further research is needed to determine the optimal approach for nasal irrigation with saline combined with medication to improve COVID-19 symptoms.

Changes in the text:-

Reviewer C

Significance of saline nasal irrigation for COVID-19 infection: Observations and reflections from nursing care of nasopharyngeal carcinoma

Comment 1: This seems to be an interesting study however lacks clarity it was a retrospective observational study.

Reply 1: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 10, line 372)

Comment 2: Literature search has not been done adequately. Saline nasal irrigation is a regular treatment method in India for various disease conditions pertaining to nose and recently it has been successfully employed to treat Post Covid -19 mucormycosis. Following references can be of much help to authors for broadening their view point and to build their arguments-

- Rastogi S, Rastogi R. Use of Saline Nasal Irrigation (Jala Neti) in SARS-CoV-2 Infection and its Complications Like Mucormycosis Needs to be Given a Serious Consideration. *Indian J Otolaryngol Head Neck Surg.* 2022 Oct;74 (Suppl 2):3534-3535. doi: 10.1007/s12070-022-03149-3. Epub 2022 Sep 9. PMID: 36105433; PMCID: PMC9462610.
- Rastogi S, Verma A. Jala neti as primary intervention in suspected rhino-orbito-cerebral mucormycosis helps improving the recovery: A case report. *J Ayurveda Integr Med.* 2022 Apr-Jun; 13(2):100516. doi: 10.1016/j.jaim.2021.08.009. Epub 2021 Nov 1. PMID: 34736856; PMCID: PMC8560025.
- Rastogi S, Rastogi R. Nasal Irrigation as a Complementary Strategy in Preventing COVID-associated Mucormycosis: Standardizing the Technique can have Far-reaching Implications. *The Journal of Contemporary Dental Practice.* 2023 Sep 13; 24(8):507-8.

Reply 2: Thank you for your valuable suggestion. The therapeutic potential of nasal irrigation for COVID-19 has been studied and proven. This paper primarily focuses on the value of nasal irrigation in improving symptoms and its role as a self-care measure.

Changes in the text:-

Comment 3: Keeping the viral load clearance possibility by saline nasal lavage, it was suggested that saline nasal irrigation can be adopted as the first home based measure once the symptoms of Covid -19 or mucor mycosis are observed.

The paper do not discuss about the technique adopted for doing the saline nasal irrigation. In recent studies, it was suggested that rather than doing though irrigation pot, if it done through saline squeezable bottle, the possible complications of the procedure can be avoided.

Reply 3: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 4-5, line 129-142)

Comment 4: In Indian traditional medicine viz. Ayurveda and Yoga, saline nasal irrigation is called as jalaneti and it has a long history of its clinical use in India. This would be sensible to refer to this historical aspect of SNI also.

Reply 4: Thank you for your valuable suggestion. However, we believe that the nasal irrigation practices used in modern times may differ from traditional jalaneti in India. Recent research validating the application value of nasal irrigation may be more appropriate as evidence.

Changes in the text: -

Reviewer D

Comment 1: This prospective (not retrospective, informed consent is noted line 327) cohort study evaluated the correlation of nasal irrigation with infection, fever, and fever duration with COVID-19 in patients with nasopharyngeal carcinoma undergoing treatment and their caregivers. 468 patients and 50 (51?) healthcare workers without COVID were grouped by those receiving nasal irrigation with treatment or not and type of cancer. If COVID was diagnosed, one group then received BID irrigation and treatment was stopped. Incidence and duration, but not height of fever, was lower in those with saline irrigation after diagnosis. Rate of infection, fever, and symptoms were worse in the healthcare workers than patients.

Major: This well-written study and excellent discussion is difficult to interpret due to

lack of protocol standards and vaccine status. While some clarity can be added, the vaccine status and numbers of possible patients versus those enrolled must be presented or the analysis is more difficult to interpret. What made you test for COVID if the patients were afebrile – were patients tested every day? Were Healthcare workers tested daily? What was the standard for PPE (protective measures for the healthcare workers during NI if it was actually administered rather than self-care)? How often were the patients receiving saline NI as part of their NPC prior to catching COVID and in what quantity?

Reply 1: Thank you for pointing this out. Our imprecise writing may have led to your misunderstanding. This is a retrospective study. The lack of information on the vaccine product formulation is one of the shortcomings of this study. Nevertheless, based on our country's COVID-19 prevention and control policies, as well as the strategy for free vaccine administration, the vaccination status among groups can be assumed to be consistent. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 4-5, line 118-143) and added some data (see Table 1)

Comment 2: Need a consort diagram with lost to followup and how many were treated at this time at this hospital, or excluded from analysis. The issue of severe COVID as an exclusion criteria needs more explanation (see below)

Minor: The background in the abstract is long relating to COVID, short relating to the fact that NI is part of the protocol for RT in NPC/HNC.

Include data collection duration (3/2023 – 4/2023) in abstract to give context of the circulating COVID variant.

Reply 2: Thank you for pointing this out. The article has been revised to avoid ambiguity.

Changes in the text: we have modified our text as advise (see the Figure 1; page 5 line 156)

Comment 3: How do HNC and NPC patients differ such that they would constitute a different group?

Reply 3: Thank you for pointing this out. NPC patients routinely receive nasal irrigation during radiotherapy, while HNC patients undergoing radiotherapy do not require nasal irrigation. This information has been added to the text.

Changes in the text: we have modified our text as advise (see the page 4-5, line 118-143)

Comment 4: I'd prefer to have the statistical significance reported with the results in the abstract.

Reply 4: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 2, line 44-47)

Comment 5: Line 68: space after refs.

Reply 5: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 2, line 66)

Comment 6: Line 91: The line and the tables state 51 healthcare participants, the abstract says 50.

Reply 6: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 1, line 37)

Comment 7: Line 118: What was the standard treatment for COVID-19 at your institution? Did you ask if any healthcare workers routinely practiced nasal irrigation? Is this ever a part of hygiene in your region, as it is in Thailand/Laos? Assuming not, state this if the healthcare workers were not asked if they use irrigation.

Reply 7: Thank you for pointing this out. Our hospital conventional treatment included physical cooling and traditional Chinese medicine (TCM) prescriptions, which have been recommended by the Chinese National Health Commission to treat COVID-19. The participants in the other groups were confirmed to have not undergone did not undergo saline nasal irrigation during subsequent follow-ups. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 4-5, line 126-142)

Comment 8: Line: 190 Discussion is misspelled.

Reply 8: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 7, line 233)

Comment 9: Line 194: A relevant study found that NI reduced the incidence of COVID in hospital workers <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8719325/> – worth mentioning, as saline may render the microbiome of the sinuses resistant to the disease.

Reply 9: Thank you for pointing this out. The potential related mechanisms mentioned in this literature have been elaborated on in the subsequent sections.

Changes in the text:-

Comment 10: Line 204: How often were the patients receiving saline NI and in what quantity?

Reply 10: Thank you for pointing this out. Patients undergo nasal irrigation twice daily, and this has been specified in the text.

Changes in the text: we have modified our text as advise (see the page 5, line 143-144)

Comment 11: Line 208: If you allude to the disease type differences, you need to qualify and quantify them earlier for readers not familiar with severity or disease type implications.

Reply 11: Thank you for pointing this out. We added some data in the text.

Changes in the text: we added some data (see the page 6, line 191-198; Table 1)

Comment 12: Line 214: It is not “apparent” – this statement is too definitive based on the information given in the study design. Instead, “In our study, [biweekly? Daily?] Nasal irrigation as part of a radiation therapy treatment did not prevent COVID-19 infection.”

Reply 12: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 269)

Comment 13: Line 217: This is interesting and the fact that temperatures were taken around the irrigation should be mentioned in the methods and results before introducing the concept in the discussion.

Reply 13: Thank you for pointing this out; based on the comments from Review A, this argument may not be rigorous enough, and it has been trimmed in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 265-266)

Comment 14: Line 227: “Self-care” is frequently mentioned but it is not described in the methods whether or not saline irrigation is self-administered in this study or administered by the healthcare workers. Other studies have been concerned about transmission of COVID because of saline irrigation or nebulization, so the process of administration and the PPE (protective measures for the healthcare workers during NI if it was actually administered rather than self-care) should be made explicit in the methods.

Reply 14: Thank you for pointing this out. NSI as a self-care measure for NPC patients, is applied during and after radiotherapy in our hospital. However, as a retrospective study, we may lack the implementation of protective measures for healthcare workers.

Changes in the text:-

Comment 14: Line 229: Again, “It is evident that” is too broad – “In our study, clinical doctors...” because other studies have not found the same thing.

Reply: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 8, line 270)

Comment 15: Line 235+: Nice history, biologic mechanism review and references. Line 299 – Oh, dear – this is really important information, and you do note that complete medical records are available. You need to find the vaccination status out and report it otherwise the results are difficult to interpret. Surely the treating doctors were vaccinated? Also, report that it was the Sinovax or what vaccine was used.

Reply 15: Thank you for pointing this out. Although the number of vaccinated patients has been supplemented, information about the vaccine formulation is still missing, which is one of the limitations of this study. However, based on our country's free vaccine administration strategy, we can reasonably assume that there is no difference in vaccine administration among patient groups. This has been added to the text.

Changes in the text: we have modified our text as advise (see the page 10-11, line 375-382) and added some data (see Table 1)

Comment 16: 300- Again, if they were excluded if they HAD covid, then at what point with severe covid were they removed from the study? This isn't an exclusion criteria but an off-study criteria, but the intention-to-treat analysis should still include them.

Reply 16: Thank you for pointing this out. Due to the Omicron variant infected and the pre-treatment assessment for anti-tumor therapy, all participants have not developed severe COVID-19.

Changes in the text: we have modified our text as advise (see the page 5, line 149-152)

Comment 17: Reference 17 is now in print.

Reply 17: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 13, line 471)

Comment 18: Figures should include the number of febrile patients since it differed so much between groups. I assume Figure 1 only included those who were febrile, while figure 2 included all who tested positive for COVID but with 0 days? Either way, clarify the numbers of subjects in the denominator/categories that these figures represent.

Reply 18: Thank you for pointing this out. It has been modified in the text.

Changes in the text: we have modified our text as advise (see the page 16-17, line 634-637; page 22, line 641-644)

Comment 19: Major:

The amount and frequency of NI as part of RT therapy is unclear. It is initially unclear whether both Groups A and B were receiving NI with and as part of their RT, or if only NPC gets NI with RT, while HNC never does. then if they got covid, treatment was stopped and only group A (NPC) received BID NI thereafter. If this is correct, then the frequency of RT and NI should be reported for those unfamiliar with the regimen. Was it daily/weekly/monthly for all patients who received NI, or did it differ?

Exclusion criteria are both “active COVID-19 infection or history of past COVID-19” and “severe COVID-19”. Unclear why both are needed. If patients developed severe COVID-19 during the trial and then NI was stopped, this shouldn’t be an exclusion criteria but instead an outcome.

Reply 19: Thank you for pointing this out. As mentioned above, corresponding modifications have been made.

Changes in the text:-