PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Establishing the reference interval for pulse oxygen saturation in neonates at high altitudes: protocol for a multicentre, open, cross-sectional study
AUTHORS	Wang, Bo; Liu, Chongde; Yao, Yanli; Lu, Zhihui; Yu, Rong; CaiRen, Zhuoma; Wang, Zhixiu; Liu, Runwu; Wu, Yazhen; Yu, Zhangbin

VERSION 1 – REVIEW

REVIEWER	Simba, Justus Jomo Kenyatta University of Agriculture and Technology, Child Health and Paediatrics
REVIEW RETURNED	31-Jan-2022

GENERAL COMMENTS	I am privileged to have reviewed this study protocol, I have a few comments that require clarifications: 1. Page 3 line 19-20, is the date filling meant to be data filling? 2. Unclear why the study is referred both as 'prospective' and 'cross-sectional'. From the study protocol there will only be one off reading for each neonate with no follow up data collected, making it a cross-sectional design in my view as much as the data will have to be collected over a long duration but for each individual, there are no repeat measurement thus unclear why authors include 'prospective'. 3. Page 5 line 45-47, 'currently recruiting' yet in line 28-29 study will start in February 2022, kindly clarify. 4. Page 8 line 34-37, 'the operator shall not be notified of the inspection' yet a video will be taken for verification, how will the video be taken without the individual noticing? 5. Few editorial issues noted eg, page 13 line 12, 'funding supported by' there is a misplaced s in that line, check through out document, for example might be clearer to write 10 seconds rather than 10 s. in a sentence that has used hours rather than hr. for uniformity. 6. Sampling technique is unclear especially because one hospital has close to 10,000 deliveries per year yet the sample size per site is a minimum of 503. If consecutive method is adopted in all the site, does sampling stop once the minimum per site has been attained? Thank you

REVIEWER	Maina, Jackson KEMRI/ Wellcome Trust Research Programme, Health Systems Unit
REVIEW RETURNED	31-Jan-2022

GENERAL COMMENTS	This is well written protocol and looking forward to the results of this project. 1.The only clarification i needed to find out from Table 1 page 5. I note that one hospitals is at an altitude of more than 4000m. Seeing this marked difference in the altitude, i propose you conduct a subgroup analysis for this cohort and see if the normal ranges will change significantly with the different altitudes. 2. Any reason why there will be no repeat SPO2 measures for the study populations during the duration these newborns are in hospital? E.g. To compare the awake and asleep SPO2 values since the authors mentioned this as a area that needs to be studied more ?

VERSION 1 – AUTHOR RESPONSE

Responses to the comments of Reviewer #1:
1. Page 3 line 19-20, is the date filling meant to be data filling?

Response: Thank you for the reminder, and it was indeed our oversight. What we mean here is "data filling". We have made changes.

2. Unclear why the study is referred both as 'prospective' and 'cross-sectional'. From the study protocol there will only be one off reading for each neonate with no follow up data collected, making it a cross-sectional design in my view as much as the data will have to be collected over a long duration but for each individual, there are no repeat measurement thus unclear why authors include 'prospective'.

Response: Thanks for your comments. By reviewing the literature, we were surprised by the finding that we deeply thought that the correct view turned out to be wrong, and that we ignored the important feature of follow-up by focusing only on the temporal properties of prospective studies. We appreciate you for this suggestion, as it not only corrects the mistakes in our article but also increases our knowledge. We have made changes to the corresponding sections of the article.

3. Page 5 line 45-47, 'currently recruiting' yet in line 28-29 study will start in February 2022, kindly clarify.

Response: Thank you for your reminder. Our words do cause ambiguity. We originally meant that this study is currently open and more eligible hospitals are being recruited instead of subjects. This study began in February 2022. Therefore, we have made the following revision: This study is an open study. We welcome additional eligible hospitals to join this study.

4. Page 8 line 34-37, 'the operator shall not be notified of the inspection' yet a video will be taken for verification, how will the video be taken without the individual noticing?

Response: Thank you very much for your reminder. We really have no way to take video verification without the operator's knowledge, which is our negligence. After our discussion, we decided to remove this step. The corresponding part of the article has been revised as follows: Each hospital will designate at least one high-level physician as the quality control person, who will conduct on-site operational inspections of the measuring personnel at least once a week in their respective hospitals. If the inspection finds that the operator was unqualified, the operator will be trained again.

5. Few editorial issues noted eg, page 13 line 12, 'funding supported by' there is a misplaced s in that line, check through out document, for example might be clearer to write 10 seconds rather than 10 s. in a sentence that has used hours rather than hr. for uniformity.

Response: Thank you so much for such a meticulous review. We have re-examined the full text and unified the expression of the unit. Please refer to the re-uploaded document for the revisions.

6. Sampling technique is unclear especially because one hospital has close to 10,000 deliveries per year yet the sample size per site is a minimum of 503. If consecutive method is adopted in all the site, does sampling stop once the minimum per site has been attained?

Response: Thank you for your comments. Our previous investigation revealed that in this area with an altitude of 2,808m, it is expected to take one year to collect the sample of the minimum size that meets our requirements, so we chose time as the node. However, we have overlooked one point, that is, other regions are expected to meet this requirement early. Your opinion is a very good reminder for us in the high-altitude areas where medical resources are very scarce. To stop after reaching the sample size requirement can not only meet the needs of our study but also save a lot of manpower and physical resources. Thanks again for your comments. We have made changes in the corresponding sections of the article. Please refer to the re-uploaded document for the revisions.

Responses to the comments of Reviewer #2：
1. The only clarification i needed to find out from Table 1 page 5. I note that one hospitals is at an altitude of more than 4000m. Seeing this marked difference in the altitude, i propose you conduct a subgroup analysis for this cohort and see if the normal ranges will change significantly with the different altitudes.

Response: Thanks for your comments. Our original design was flawed, and subgroup analysis was indeed necessary. We have made changes in the section of statistical analysis, and please refer to the re-uploaded document for the revisions. Thanks again for this constructive suggestion.

2. Any reason why there will be no repeat SpO_2 measures for the study populations during the duration these newborns are in hospital? E.g. To compare the awake and asleep SpO_2 values since the authors mentioned this as a area that needs to be studied more ?

Response: Thanks for your comments. We decided against including multiple measurements after 24 hours of birth in our protocol. This is because, i) Within 24 hours of birth, fetal circulation gradually changes to neonatal circulation.[1] In theory, during this period, the SpO_2 value should gradually increase and eventually reach a steady state; and, ii) a large multicenter study of 41,097 measurements at altitudes ranging from 0 to 2,500 m showed no differences in multiple SpO_2 measurements obtained from 24 hours after birth until discharge.[2] Therefore, our protocol focuses on personnel training and quality control to ensure that each SpO_2 measurement is accurate, rather than obtaining multiple measurements. It is possible that our statement is not clear enough to dispel your doubt. Therefore, we have revised this part accordingly.

Thank you very much for reviewing our article so carefully. The measurements in this study require the neonates to be awake and quiet. It will not consider measurements obtained while the infants are asleep. This is because neonates are more prone to apnea or hypoventilation during sleep, resulting in transient periods of hypoxemia, due to immature control of breathing, and higher compliance of the upper respiratory airway and chest resulting in lower respiratory reserve.[3] While these transient periods of neonatal hypoxemia might be physiological phenomena, the value of SpO_2 measured in this case might be inaccurate, which is why our protocol excludes sleep periods. Of course, we

considered whether there are differences in SpO_2 between the two different states of wakefulness and sleep in neonates is a question worthy of further study, but we did not make attempt to explore this further in this study. This is because multiple measurements or continuous monitoring may be required to measure accurate data during sleep. It is thus difficult to carry out large-scale deveopment, and this will further burden the high-altitude areas where medical resources are scarce. This may be the main reason why most of the current studies have chosen to perform the measurement in the awake state.[4-7] Therefore, we did not include a subgroup of the sleep state. However, we are already planning another study, which is to conduct a small sample study in a unit with relatively sufficient medical resources. Of course, we also found that we were negligent. The 29th reference we quoted in the original text to explain the problem is very inappropriate because it not only fails to explain our behavior very well, but also will cause serious doubts to readers including you, so we have revised this part of the discussion. Thanks again for your opinion.

REFERENCES

1 Morgan MC, Maina B, Waiyego M, et al. Oxygen saturation ranges for healthy newborns within 24 hours at 1800 m. Arch Dis Child Fetal Neonatal Ed 2017;102:F266-8. doi: 10.1136/archdischild-2016-311813.

2 Guo F, Tang S, Guo T, et al. Revised threshold values for neonatal oxygen saturation at mild and moderate altitudes. Acta Paediatr 2020;109:321-6 PubMed . doi:10.1111/apa.14962.

3 Daftary AS, Jalou HE, Shively L, et al. Polysomnography Reference Values in Healthy Newborns. J Clin Sleep Med 2019;15:437-43 PubMed . doi: 10.5664/jcsm.7670.

4 Salas AA. Pulse oximetry values in healthy term newborns at high altitude. Ann Trop Paediatr 2008;28:275-8 PubMed . doi: 10.1179/146532808X375431.

5 Gonzáles GF, Salirrosas A. Arterial oxygen saturation in healthy newborns delivered at term in Cerro de Pasco (4340m) and Lima (150 m). Reprod Biol Endocrinol 2005;3:46. doi: 10.1186/1477-7827-3-46.

6 Niermeyer S, Yang P, Shanmina, et al. Arterial oxygen saturation in Tibetan and Han infants born in Lhasa, Tibet. N Engl J Med 1995;333:1248-52 PubMed . doi: 10.1056/NEJM199511093331903.

7 Niermeyer S, Shaffer EM, Thilo E, et al. Arterial oxygenation and pulmonary arterial pressure in healthy neonates and infants at high altitude. J Pediatr 1993;123:767-72 PubMed . doi: 10.1016/s0022-3476(05)80857-1.

VERSION 2 – REVIEW

REVIEWER	Simba, Justus Jomo Kenyatta University of Agriculture and Technology, Child Health and Paediatrics
REVIEW RETURNED	21-Mar-2022

GENERAL COMMENTS	I am satisfied with the corrections made.

REVIEWER	Maina, Jackson KEMRI/ Wellcome Trust Research Programme, Health Systems Unit
REVIEW RETURNED	09-Mar-2022

GENERAL COMMENTS	I want to thank the authors for addressing all the issues that were raised from the previous review.

	I am happy with the changes and have no additional comments. Wishing the team the very best as they carry out the study