nature portfolio

Peer Review File

The clinical manifestations of Sickle Cell Disease in Africa and its association with foetal haemoglobin parameters

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript compared HbF levels, F cells and HbF/F cells ratio with some complications in three countries in Africa viz Ghana, Nigeria and Tanzania in a population of hydroxyurea naïve SCD Patients. The authors observed similar HbF levels among Nigerians and Tanzanians but a significantly higher level among Ghanaians. Similarly, F cells and HbF/F cells ratio were significantly different across the three countries. In addition, HbF was significantly associated with a decreased odds of blood transfusion while F cells were associated with increased odds of blood transfusion and painful crisis.

The study employed automation in the analysis of the HbF levels and the haematological parameters, and flowcytometry in the analysis of the F cells. Though many studies have looked at HbF levels in SCD but fewer studies have analyzed F cells in the African continent.

1. However, the study did not adequately describe the study population since many of the clinics in the subregion are usually classified into adult or paediatric SCD clinics. This may explain why the Ghanaian cohort was mostly paediatrics. Therefore, observed findings especially in the Ghanaian paediatric cohort in this study may therefore be artificial and as a result of selection bias.

2. The observed HbF levels among Nigerians(4.3%) and Tanzanians (4.4%%) is much lower than what is generally reported in the literature whether done 'manually' or by automation. (Nagel RL et al, Blood 1987: 69(4):1026-1030; Kahema SE et al, PLoS One 2024; 19(7):e0286891; Kotila et al, East Afr Medical Journal 2001;78(7):373-375; Adeyemo TA et al Pan African Medical Journal 2014 18:71 doi:10. 11604/pamj.2014.18.71.4239; Akinbami A et al, Annals of Tropical Pathology. 2018; 9:26-31). The discrepancies between the HbF findings in this study and that in the literature should be highlighted in the discussion.

3. Most of what the authors described as clinical manifestations are mostly complications of the disease. It should also be stated in the methodology that the complications/clinical manifestations included past history of these manifestations. Though this was alluded to in the discussion. Specifically, about priapism, it is important to state if this was stuttering priapism or full-blown priapism or any priapism. For those who were recorded in Table 1 as having no pain crisis, it is necessary to be explicit if this meant that they have never had bone pains.

4. The figures require revision: the message in the boxplot (figure 1) is not immediately obvious and the information in the forest plot (figure 3) is better appreciated in prose in the results section.
Too many box and forest plots make assimilation of the message difficult.

5. The discussion was difficult to follow, line 239 states higher HbF and lower Fcells in Nigerians than Tanzanians while the opposite is true as stated in the results. HbF, Fcells and HbF/F cell ratios were analyzed discussed across the different countries and compared with the identified
complications and not between the two age groups as stated in the conclusion.

Reviewer #2

(Remarks to the Author)
This is a well-written, cross-sectional study on clinical and laboratory parameters of primarily children and adolescents with sickle cell disease across Nigeria, Ghana, and Tanzania. It is impressive work, and probably one of few studies using F since this can be a difficult test. I have several questions and comments. Use caution when making conclusions about differences in disease states across entire countries if the samples are not representative and if sample sizes do not adequately support these conclusions. I suggest either explaining further how these studies are representative or softening the definitive nature of the conclusions, especially considering at the end it is stated that this is a pilot study.

Lines 87-89, HbF up to 60% seems extremely high. Is this referring to deletional hereditary persistance of fetal hemoglobin? Which many consider a separate disease from sickle cell disease? Please include a citation for this value

Line 110: Suspect a typo in the dates: Jan to July 2023 or July 2023 to Jan 2024?

Line 110: Are they the only 3 sites in Phase 1 of SickleInAfrica or are they 3 among many?

Lines 116-117: Did the investigators complete the questionnaire based on review of medical records, asking patients/families, or both? This appears to be at least partially answered at the end, in limitations, but should be stated earlier.

Methods (general questions)
- How was this sample size determined?
- How were patients selected/recruited?
- With only ~100 patients per site (out of populations of presumably thousands), it is important to understand how reflective these included patients are of the entire population.
- How did the authors minimize selection bias?
- Were there differences in recruiting between one site or the other?
- Why were only patients older than 5 years old included?
- Were labs drawn at steady state? Or were any patients being seen for acute illnesses?
Please elaborate as these are helpful to put the study findings into context for internal and external validity. The authors touch on this in limitations at the end, but do not discuss strategies to mitigate these limitations.

Results:
Were patients predominantly less than 18 years old due to the natural history of the disease or because they were recruited at pediatric centers? Similarly, are the findings about fewer adults in Ghana reflective of study recruitment or of the natural history of the disease?

- For the WBC count, do the authors have absolute neutrophil count? Are the WBC fluctuations due to ANC differences? Absolute lymphocyte count differences?

Reviewer #3

(Remarks to the Author)
Dear authors,
Your paper entitled as “A cross-sectional study of the clinical manifestations of Sickle Cell Disease in Ghana, Nigeria and Tanzania and its association with foetal haemoglobin parameters” is well written and provide a novel approach of treatments for children/adults with SCD. Here below are my minor comments to improve this article.
Introduction:
Comment 1: Line 69; MUHAS should be written in a long format
Comment 2: Line 75-76; Is there any reason of focusing on three countries out of seven? If there is a specific reason should be explained.
Comment 3: Line 87-88; “…while in SCD, the range is broader and levels are higher (up to 60%)”. Is there a known/established range for SCD?
Comment 4: Line 95; Remove the word ref
Comment 5: Line 103; In a sentence “Such studies provide a more robust platform…” Consider rewording as “This will provide a more robust platform…”

Methodology
Comment 1: Line 109; “July 2023 to January 2023” Is it July 2022 to January 2023?” Please revise
Comment 2: Line 116 to 117; Be specific that the information was self-reported by caregivers of children & adult participants through questionnaires completed by investigators
Comment 3: Line 126-127; It is clear that this paper focused on assessing the level of HbF and F cells in relation to clinical features but you must note that even the sickle cells subtypes such as HbSC, HbSS could be an important modifier/confounder of this associtation. Authors should also think of reporting the sickle cell subtypes present in these three countries unless if they chose to focus on one type i.e., HbSS
Comment 4: Line 145-148; “Red blood cell indexes, platelet count, HbF, F and HbF per F cells were compared between participants from the three countries using the Kruskal-Wallis test, as these variables were not normally distributed”. Add a sentence mentioning the type figures that was used to represent the results from this analysis

Comment 5: Line 148-150; “The association between HbF, F and HbF per F cells with the occurrence of clinical events among sickle cell patients was analysed using age and site-adjusted logistic regression”. Do as comment no 4 above
Comment 6: Line 148-150; “The association between HbF, F and HbF per F cells with the occurrence of clinical events among sickle cell patients was analysed using age and site-adjusted logistic regression” Reword to read as “was analysed using logistic regression adjusted for age and site”. Further, did you only control for age and site? What other factors did you control in this analysis? In addition to age and site, you should think of controlling for Sickle cell subtypes which may also confound your results

Results
Comment 1: In section of “Haematological parameters of the study participants”; Think of adding results regarding the sickle cell subtypes obtained for each country. This is a very important information
Comment 2: Line 205; Change f into capital F
Comment 3: Line 208; “Effect of HbF, F-cell and Hbf per f cell” Change the f into capital F

Discussion
Comment 1: Line 230-231; Did you take any measurements to control for recal bias? This should be explained
Comment 2: Line 236; “The levels of HbF are known to decrease by about 5% per week from birth until 6 months” Does the HbF stay stable after 6 months? You should add information on this
Comment 3: Line 246; Why did you introduce the abbreviation “HU” here? Should be defined at the beginning
Comment 4; Line 250; Put a comma (,) after the word However
Comment 5; Line 261-263; “The statistically significant differences in the countries could also be due to healthcare seeking behaviours and accessibility to care differences in the countries”. Also SCD subtypes can be the cause of the difference but was neither reported nor adjusted despite being tested
Comment 6; Line 278-280; “On the other hand, the higher HbF levels in Ghana might be due to their predominant paediatric sample population and not necessarily a marked variation from what was observed in the other countries” Are you regarding age as a modifier or a confounder here? If being paediatric is considered as a confounder, then controlling for age when assessing the association of HbF and clinical features would perish the significant association we have observed in this study
Comment 7; Line 284; “HUT” This abbreviation was not expanded
Comment 8; Line 292 “Hydroxyurea” Is it HU, HUT or Hyroxyurea? Should be persistence

Conclusion
Comment 1; Line 301-302; “The low levels however provide some significant protection from SCD clinical manifestations which provides opportunity and challenge to use HbF modifying therapies to reduce severity of SCD” Did you mean low levels or high levels?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have responded to most of the queries.
However, the explanation did not remove the possibility of selection bias in the Ghana participants especially since the centre has been involved in newborn screening for about 30 years

Reviewer #2

(Remarks to the Author)
The manuscript is much improved, thank you to the authors for your work

Abstract
- Line 44: "across sites" doesn't seem to need to be in parentheses
- Line 45: Is the parenthetical after hospital setting "(in-patient or outpatient)" necessary?

Background
- Line 88: again, HbF up to 60% in children with sickle cell on hydroxyurea is an extreme outlier. I suggest the authors leave this out unless there is a reason to keep it in.The referenced article states "Average HbF levels in SCD patients between the ages of 1 and 4 years were 23.5% (range 1.5–38.5%)" and "The two HU-treated patients had higher HbF (range 20–24.8%)."
- Line 101: adding the three countries to the end of this sentence is odd. Consider moving these three countries to the following sentence.

Methods
- Are the authors able to add an approximate sickle cell population size of MUHAS?
- Can the authors add a sentence about the availability and indications of hydroxyurea at each site? This is helpful in

understanding if there is something unique (a selection bias) about your hydroxyurea-naive cohort.
- Very excellent work by KAHAS screening and retaining so many patients!

Discussion:
- Line 247: This sentence is not clear, please rephrase "This goes a long way reflecting in the statistically significant differences in all the complications assessed"
- Line 254: you may consider starting the discussion with this paragraph since this is the main point. The statements about males and age, etc can follow later
- Line 257: would you also include HbF and F/F cell in this list of what is statistically significant?
- To strengthen the discussion, consider adding a few sentences with the genetic differences that might impact the different lab parameters (gamma globin mutations, non-deletional HPFH, as well as alpha globin or duffy mutations contributing to the other parameters)
- As a future study, might the SickleInAfrica registry help the rest of us to understand additional genetic modifiers of HbF

Reviewer #3

(Remarks to the Author)
Hi,
I have revised a rebuttal letter and all of my comments has been addressed. I have no further comments

Point-by-point response to the reviewer's comments

Reviewer #1 (Remarks to the Author):

Comments	**Response**	**Page Number (Line number)**
1. However, the study did not adequately describe the study population since many of the clinics in the subregion are usually classified into adult or paediatric SCD clinics. This may explain why the Ghanaian cohort was mostly paediatrics. Therefore, observed findings especially in the Ghanaian paediatric cohort in this study may therefore be artificial and as a result of selection bias.	A description of each of the recruiting sites has been added.	Page 6 & 7 (Line 112-133)
2. The observed HbF levels among Nigerians(4.3%) and Tanzanians (4.4%%) is much lower than what is generally reported in the literature whether done 'manually' or by automation. (Nagel RL et al, Blood 1987: 69(4):1026-1030; Kahema SE et al, PLoS One 2024; 19(7):e0286891; Kotila et al, East Afr Medical Journal 2001;78(7):373-375; Adeyemo TA et al Pan African Medical Journal 2014 18:71 doi:10.11604/pamj.2014.18.71.4239; Akinbami A et al, Annals of Tropical Pathology. 2018; 9:26-31). The discrepancies between the HbF findings in this study and that in the literature should be highlighted in the discussion.	Similarities and differences of HbF findings in this study have been highlighted in the discussion.	Page 15 (Line 269-272)
3. Most of what the authors described as clinical manifestations are mostly complications of the disease. a. It should also be stated in the methodology that the complications/clinical manifestations included past history of these manifestations. Though this was alluded to in the discussion. b. Specifically, about priapism, ***it is important to state if this was stuttering priapism or full-blown priapism or any priapism.*** c. For those who were recorded in Table 1 as having no pain crisis, it is necessary to be explicit if this meant that they have never had bone pains.	a..The methodology already indicates past histories and the period within which they were asked. (line 142-147) b…The question asked was about any type of priapism. c…The pain crisis elicited is those necessitating a visit to the hospital either as outpatient or in-patient. Changes have been made in the methods and the table to reflect this.	a..Page 7(Line 142-147) b…Not applicable c..Page 7(Line 142-143)
4. The figures require revision: the message in the boxplot (figure 1) is not immediately obvious and the information in the forest plot (figure 3) is better appreciated in prose in the results section.	Figure 1 moved to supplementary mateirals	

Comments	Response	Page Number (Line number)
Too many box and forest plots make assimilation of the message difficult.		
5. The discussion was difficult to follow, a. line 239 states higher HbF and lower Fcells in Nigerians than Tanzanians while the opposite is true as stated in the results. b. HbF, Fcells and HbF/F cell ratios were analyzed discussed across the different countries and compared with the identified complications and not between the two age groups as stated in the conclusion.	a…Typographical error corrected to ***"The Nigerian cohort had lower HbF and a higher F cell compared to the Tanzanian cohort, irrespective of their similarities in terms of ratio of children to adults"*** b…HbF, Fcells and HbF/F cell ratios are compared between persons less than 18 and persons 18 years and over and shown in Figure 1 and Supplementary table 1	a…Page 15(Line 273) b. Page 24 (Supplementary Table)

Reviewer #2 (Remarks to the Author):

Comment	Response	Page Number (Line Number)
Use caution when making conclusions about differences in disease states across entire countries if the samples are not representative and if sample sizes do not adequately support these conclusions. I suggest either explaining further how these studies are representative or softening the definitive nature of the conclusions, especially considering at the end it is stated that this is a pilot study.	This pilot study was planned with a 95% power to detect mean difference of 0.36 to aid in proving feasibility in low resource settings and pointing to a probable case for further investigation. The sites are included in the list of countries with the highest proportion of SCD patients and established SCD clinics which provide care to the patients. Definitive nature of conclusions have however been softened.	Page 18 (Line 336)
Lines 87-89, HbF up to 60% seems extremely high. Is this referring to deletional hereditary persistance of fetal hemoglobin? Which many consider a separate disease from sickle cell disease? Please include a citation for this value	The level is reported in studies for persons with SCD on Hydroxyurea. Clarification and citation inserted	Page 5 (Line 88)
Line 110: Suspect a typo in the dates: Jan to July 2023 or July 2023 to Jan 2024?	This is a typo. study was conducted from July 2022 to January 2023	Page 6 (Line 110)
Line 110: Are they the only 3 sites in Phase 1 of SickleInAfrica or are they 3 among many?	The study processes were initiated in Phase 1 of the SickleInAfrica Consortium which was made up of three countries. The sentence has been revised to capture this explanation.	Page 4 (Line 75)
Lines 116-117: Did the investigators complete the questionnaire based on review of medical records, asking patients/families, or both? This appears to be at least partially answered at the end, in limitations, but should be stated earlier.	Investigators completed the questionnaires based on responses from participants or caregivers. Sentence has been revised to clarify this as follows; .. ***"self-reported by caregivers of children or adult participants through a standardised case report form completed by the investigators."***	Page 7 (Line 145-147
Methods (general questions) - How was this sample size determined?	Sample size was estimated by taking into consideration the intended assessment of relationships between nominal and discrete variables using cross-tabulations and Spearman correlation. The significance was ascribed to values less than 0.05. A sample size of 100 in each group has a 95% power to detect a difference between means of 0.36 for HbF parameters (HbF level,	Page 7 (Line 134-138)

Comment	Response	Page Number (Line Number)
	HbF/F cell) with a significance level (alpha) of 0.05 (two-tailed).	
- How were patients selected/recruited?	Patients were randomly selected during their routine clinic visits.	Page 7 (Line 138-141)
- With only ~100 patients per site (out of populations of presumably thousands), it is important to understand how reflective these included patients are of the entire population.	The sample size has a 95% power to detect a difference between means of 0.36 for HbF parameters (HbF level, HbF/F cell) with a significance level of 0.05. The sample also forms 1-2% of registered attendees to clinics in these sites.	Page 7 (Line 134-138)
- How did the authors minimize selection bias?	By simple random sampling during routine clinic visits.	Page 7 (Line 138-141)
- Were there differences in recruiting between one site or the other?	The study employed a standardised protocol and procedures and an enrolment case report form was utilised across the three sites. Differences in recruiting are therefore not expected.	
- Why were only patients older than 5 years old included?	The main goal of the study was to compare HbF parameters i.e HbF%, Fcells and HbF/Fcells across the three populations. It is well established that there is a considerable intraindividual variation of the levels of HbF in children of the age below 5 years.	Page 7 (Line 139-140)
- Were labs drawn at steady state? Or were any patients being seen for acute illnesses?	Labs were drawn at steady state.	Page 7 (Line 141)
Results: Were patients predominantly less than 18 years old due to the natural history of the disease or because they were recruited at pediatric centers? Similarly, are the findings about fewer adults in Ghana reflective of study recruitment or of the natural history of the disease?	The predominantly less than 18-year-olds is due to the natural history of disease without intervention in the past and start of newborn screening activities which have helped in early identification of babies and enrolment in clinics.	
- For the WBC count, do the authors have absolute neutrophil count? Are the WBC fluctuations due to ANC differences? Absolute lymphocyte count differences?	The data on absolute neutrophil count and absolute lymphocyte count were not included in the analysis	

Reviewer #3 (Remarks to the Author):

Comment	Response	Page number (Line number)
Introduction: Comment 1: Line 69; MUHAS should be written in a long format	MUHAS has been written on full Muhimbili University of Health and Allied Sciences	Page 4 (Line 68-69)
Comment 2: Line 75-76; Is there any reason of focusing on three countries out of seven? If there is a specific reason should be explained.	The study processes were initiated in Phase 1 of the SickleInAfrica Consortium which was made up of three countries. The sentence has been revised to capture this explanation.	Page 4 (Line 75)
Comment 3: Line 87-88; "…while in SCD, the range is broader and levels are higher (up to 60%)". b. Is there a known/established range for SCD?	a. Reference to 60% inserted b. No standard has been documented	Page 5 (Line 88)
Comment 4: Line 95; Remove the word ref	Removed	Page 5 (Line 96)
Comment 5: Line 103; In a sentence "Such studies provide a more robust platform…" Consider rewording as "This will provide a more robust platform…"	Revised as suggested	Page 5 (Line 104)
Methodology Comment 1: Line 109; "July 2023 to January 2023" Is it July 2022 to January 2023?" Please revise	This is a typo. study was conducted from July 2022 to January 2023	Page 6 (Line 110)
Comment 2: Line 116 to 117; Be specific that the information was self-reported by caregivers of children & adult participants through questionnaires completed by investigators	Revised as suggested	Page 7 (Line 145-146)
Comment 3: Line 126-127; It is clear that this paper focused on assessing the level of HbF and F cells in relation to clinical features but you must note that even the sickle cells subtypes such as HbSC, HbSS could be an important modifier/confounder of this association. Authors should also think of reporting the sickle cell subtypes present in these three countries unless if they chose to focus on one type i.e., HbSS	The study enrolled patients who were HbSS. Added to study participant description.	Page 7 (Line 139)
Comment 4: Line 145-148; "Red blood cell indexes, platelet count, HbF, F cells, and HbF per F cells were compared between participants from the three countries using the Kruskal-Wallis test, as these variables were not normally distributed". Add a sentence mentioning the type figures that was used to represent the results from this analysis	Sentence revised and phrase ***"and presented as boxplots"*** added to provide the requested information.	Page 9(Line 177-179)

Comment	Response	Page number (Line number)
Comment 5: Line 148-150; "The association between HbF, F cells, and HbF per F cells with the occurrence of clinical events among sickle cell patients was analysed using age and site-adjusted logistic regression". Do as comment no 4 above	Sentence revised and phrase ***"and presented as forest plots"*** added to provide the requested information.	Page 9 (Line 181-182)
Comment 6: Line 148-150; "The association between HbF, F cells, and HbF per F cells with the occurrence of clinical events among sickle cell patients was analysed using age and site-adjusted logistic regression" Reword to read as "was analysed using logistic regression adjusted for age and site". b.. Further, did you only control for age and site? What other factors did you control in this analysis? In addition to age and site, you should think of controlling for Sickle cell subtypes which may also confound your results	Rewording done as requested. …. b. Only age and site were controlled for. Study recruited patients with HbSS and therefore the subtypes would have no effect.	Page 9 (Line 181-182)
Results Comment 1: In section of "Haematological parameters of the study participants"; Think of adding results regarding the sickle cell subtypes obtained for each country. This is a very important information	Study enrolled patients who were SCD-SS as indicated in Study participant description	Page 7 (Line 139)
Comment 2: Line 205; Change f into capital F	Changed as requested	Page 13 (Line 235)
Comment 3: Line 208; "Effect of HbF, F-cell and Hbf per f cell" Change the f into capital F	Changed as requested	Page 13 (Line 238)
Discussion Comment 1: Line 230-231; Did you take any measurements to control for recal bias? This should be explained	No other measurement was taken as this was a self-report eliciting patient determined severity. . Recall bias was controlled by limits to events requiring hospital visits which would be significant and more easily remembered as described in methods.	Page 7(Line 142-146)
Comment 2: Line 236; "The levels of HbF are known to decrease by about 5% per week from birth until 6 months" Does the HbF stay stable after 6 months? You should add information on this	HbF is expected to decline significantly by 6 months without intervention, however the variation continues and stabilises at around 5 years. Information has been added.	Page 14&15 (Line 266-267)

Comment	Response	Page number (Line number)
Comment 3: Line 246; Why did you introduce the abbreviation "HU" here? Should be defined at the beginning	Abbreviation written out in full	Page 15 (Line 280)
Comment 4; Line 250; Put a comma (,) after the word However	Revised as requested	Page 16 (Line 285)
Comment 5; Line 261-263; "The statistically significant differences in the countries could also be due to healthcare seeking behaviours and accessibility to care differences in the countries". Also SCD subtypes can be the cause of the difference but was neither reported nor adjusted despite being tested	Study enrolled patients who were SCD-SS as indicated in Study participant description. However, SCD haplotypes may be different within and across sites.	Page 7 (Line 139)
Comment 6; Line 278-280; "On the other hand, the higher HbF levels in Ghana might be due to their predominant paediatric sample population and not necessarily a marked variation from what was observed in the other countries" Are you regarding age as a modifier or a confounder here? If being paediatric is considered as a confounder, then controlling for age when assessing the association of HbF and clinical features would perish the significant association we have observed in this study	All participants across the three sites were 5 years and above. We did include age as a confounder and controlled for. Categorising by age still showed a statistically significant difference in HbF levels across the countries (supplementary table 1). Sentence has therefore been removed.	Page 17 (Line 313-315)
Comment 7; Line 284; "HUT" This abbreviation was not expanded	Abbreviation written out in full	Page 17 (Line 319)
Comment 8; Line 292 "Hydroxyurea" Is it HU, HUT or Hyroxyurea? Should be persistence	Abbreviations written out in full to clarify the ambiguity	Page 15(Line 280, 287) Page 16 (Line 289, 290,309) Page 17 (Line 319)
Conclusion Comment 1; Line 301-302; "The low levels however provide some significant protection from SCD clinical manifestations which provides opportunity and challenge to use HbF modifying therapies to reduce severity of SCD" Did you mean low levels or high levels?	It is high levels of HbF that seem to provide significant protection. Sentence revised accordingly	Page 18 (Line 336)

Point-by-point response to reviewers' comments

Reviewer #1 (Remarks to the Author):

Comments	**Response**
However, the explanation did not remove the possibility of selection bias in the Ghana participants especially since the centre has been involved in newborn screening for about 30 years	Selection bias added to the limitations of the study Line 346-348

Reviewer #2 (Remarks to the Author):

Comments	**Response**
Line 44: "across sites" doesn't seem to need to be in parentheses	Parentheses removed
- Line 45: Is the parenthetical after hospital setting "(in-patient or outpatient)" necessary?	It is to clarify that both out-patient and admitted patients were included
- Line 88: again, HbF up to 60% in children with sickle cell on hydroxyurea is an extreme outlier. I suggest the authors leave this out unless there is a reason to keep it in.The referenced article states "Average HbF levels in SCD patients between the ages of 1 and 4 years were 23.5% (range 1.5–38.5%)" and "The two HU-treated patients had higher HbF (range 20–24.8%)."	The 60% is in relation to F-cells and not HbF. It is necessary to indicate this for comparison with the f-cell levels in non-SCD patients.
- Line 101: adding the three countries to the end of this sentence is odd. Consider moving these three countries to the following sentence.	Moved to the end of the next sentence Line 102-103
- Are the authors able to add an approximate sickle cell population size of MUHAS?	Sickle Cell Population size of MUHAS is approximately 5,551. Inserted in Line 118
- Can the authors add a sentence about the availability and indications of hydroxyurea at each site? This is helpful in understanding if there is something unique (a selection bias) about your hydroxyurea-naive cohort.	Added Line 118-119 Line 126-128 Line135-137

- Line 247: This sentence is not clear, please rephrase "This goes a long way reflecting in the statistically significant differences in all the complications assessed"	Sentence removed
- Line 254: you may consider starting the discussion with this paragraph since this is the main point. The statements about males and age, etc can follow later	Suggestion accepted. Paragraph moved to Line 243-248
- Line 257: would you also include HbF and F/F cell in this list of what is statistically significant?	Included in line 247
- To strengthen the discussion, consider adding a few sentences with the genetic differences that might impact the different lab parameters (gamma globin mutations, non-deletional HPFH, as well as alpha globin or duffy mutations contributing to the other parameters)	Included in line 304-305
- As a future study, might the SickleInAfrica registry help the rest of us to understand additional genetic modifiers of HbF	The SickleInAfrica registry will help in understanding this and other issues with SCD with the appropriate funding and support