Reviewer’s report

Title: Tanzanian malignant lymphomas: WHO classification, presentation, ploidy, proliferation and HIV/EBV association

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Reviewer: Claudiu V Cotta

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The descriptive study conducted by Mwakigonja et al. incorporates a large number of cases from Tanzania. A subset of the cases have been studied using immunohistochemical, molecular and flow cytometric techniques. It is unclear whether all or some of the case have been incorporated in previous studies (references 4 and 13).

Below are is the assessment of the submitted article following the suggested guidelines:

1. “Is the question posed by the authors well defined?” The question posed by the authors is relatively clearly defined.

2. “Are the methods appropriate and well described?” Most of the methods used are well described. However, there are several issues that have not been addressed: Which WHO classification has been used (2001 or 2008)? Were the cases reviewed by one or multiple pathologists? Were there any discrepancies/disagreements between pathologists? Changes in diagnoses due to changes in classification? Cases with unclear diagnosis?

3. “Are the data sound?” Most of the data is sound. Specific concerns are addressed below.

4. “Does the manuscript adhere to the relevant standards for reporting and data deposition?” The MNH policies are not stated, but there is a statement mentioning that the samples have been processed in accordance with the MNH policies and that the personal identifiers have been deleted.

5. “Are the discussion and conclusions well balanced and adequately supported by the data?” Most of the data are reported in a relatively intuitive fashion. There are multiple problems with the text references to the tables incorporating the data (many references to table 3 probably should read table 4, etc.). Also, there are references to figures 7-11, probably images included in figures 5 and 6. The discussion of the data partially reflects the data presented in the text, tables and figures, although often the references are wrong. As a descriptive study, the article does not have a clearly defined conclusion.

6. “Are limitations of the work clearly stated?” Some of the limitations of the work are stated (absence of clinical information, insufficient sample for further testing, etc.), while other limitations are not clear. Was there variability in fixation during the 10 year period? Were studies for MYC translocations performed? DLBCL
categories not included in the WHO classification are mentioned (GCB/ABC), but
types of LBCL included in the WHO are not mentioned (example: primary
mediastinal large B-cell lymphoma, the two gray zone lymphomas). Also,
“DLBCL” with follicular remnants are mentioned: are some of these cases
follicular lymphomas grade 3B? The Ki-67 positivity in cases of Burkitt lymphoma
seems to be below the range mentioned in the WHO 2008.
7. “Do the authors clearly acknowledge any work upon which they are building,
both published and unpublished?” There are many references, including one in
German and one in Chinese. Clear identification of the contribution of each
author and acknowledgment of collaborators is included at the end of the
document.
8. “Do the title and abstract accurately convey what has been found?” The title
and the abstract accurately reflect the goals and the findings of the study.
9. “Is the writing acceptable?” The writing could be improved. Throughout the
manuscript there are many numeric inconsistencies. For example in flow
cytometry results the diagnoses for the 60 cases analyzed mention only 59
cases. Sometimes periods are missing (example: Abstract, Methods, before
Available sera), there are rare typographical errors (example: expression of
proteins), or abbreviations are not initially fully spelled (example: PEL/BCBL).
Obsolete terms (Hodgkin’s, Burkitt’s) can be easily corrected.

A more detailed criticism of the paper:
This study may be of local or regional interest. However, there is ambiguity
whether the cases have already been included in several studies already
published by some of the authors (and thus not adding significant information).
The WHO classification is mentioned in the title of the article, but is not clear
whether it is the 2001 or the 2008 version of the document. This is important, as
a significant number of the cases discussed fall into the Burkitt lymphoma and
diffuse large B-cell lymphoma categories and these categories have undergone
changes. Information such as inter-observer variability and changes in diagnosis
due to changes in classification (from REAL to WHO) should be included, if
available. There is abundant use of percentages and statistical tests, often
unnecessary, definitely obscuring the points of the article.
Changes that, in this reviewer’s opinion, would improve the article:
1. There is a discrepancy between the abstract and the body of the article: How
many cases have actually been stained? 150 (per abstract) or 174 (per results)?
If of the 174 cases 16 proved not to be lymphomas, how many of the 281
biopsies may not be lymphomas? Is the information included in table 1 accurate
in these circumstances (the non-lymphoma cases do not seem to have been
removed from the statistics)?
3. Abstract: Follicular remnants. Some of these cases could be diagnosed as
follicular lymphoma. WHO (2001 and 2008) indicate that areas with follicular
architecture should be quantified and mentioned in the report.
4. Abstract: The statement that EBV infection (probably what is meant is positivity for EBER) is more frequent in GCB cases is not supported by the data: 2 cases with ABC phenotype are listed in table 6 as EBER nd, so the data should indicate that 5/13 cases were positive (not 5/15), which is not significantly different from 5/12 for GCB.

5. Abstract: Burkitt’s lymphoma should be changed to Burkitt lymphoma.

6. Abstract: Conclusions: EBV infection-do the authors mean EBER positivity?


8. Background: Capital P in partly due...

9. Background: Some ML subtypes can be recognized by numerical chromosomal abnormalities (aneuploidy) including complex mostly hyperdiploid karyotypes? The numerical chromosomal abnormalities can be identified, but the subtypes of lymphomas cannot be identified based on this feature (this is what the references actually state).

10. Background: Thus, the expression of – please correct expression.

11. Methods: Immunohistochemistry: Stating the immunophenotype of the tumors classified as GCB or ABC would provide further clarification.

12. Methods: Immunohistochemistry: incubation with ABC? The abbreviation is used with a different meaning in the article. Complete spelling of the name of the reagent is necessary.


14. Results: ML frequency and general demography: Again, were the cases with no lymphoma eliminated from the statistics? Maybe the entire demographic and presentation analysis should be limited to the cases with confirmed diagnosis.

15. Clinical presentation: Please spell what PEB/BCBL stands for.

16. Histology and immunohistochemistry: This entire section has wrong table references. In most cases table 3 should be 4 and table 4 should be 5.

17. Histology and immunohistochemistry: 24 cases are HL (CD30+)? Table 5 suggests that 2/24 cases were NLPHL, a disease with malignant cells usually negative for CD30. Clarifications necessary. Also, pop-corn cells have been described in NLPHL, not in classical HL (legend figure 3). More clarifications needed (and adjustment of terminology).

18. Flow cytometry results: the 60 selected ML biopsies are only 59 (the cases listed by diagnosis add up only to 59)?

19. The references to figures 6-11 have to be corrected (only 6 figures included in the article).

20. It is very difficult to understand what the author is describing as there is a constant shift from absolute numbers to percentages, from one category to another, with many unnecessary statistical tests. Probably this is why there are some numerical inconsistencies: 9 cases are listed as hyperdiploid, but in the
next line they are only 8 (7 DLBCL and 1 TCL). Then the author states that 12.5% (n=3) of cases showed triploidy. The assumption is that 12.5% of the aneuploid cases? Clarifications needed.

21. Cell proliferation by Ki-67: the mean of positive cells is very low for Burkitt lymphoma (80%). This is lower than what the WHO classification describes.

22. ML association with HIV: Are cases of non-lymphoma included in the statistics? As there are only 35 patients, 2-3 missed diagnoses could impact the statistics.

23. ML association with HIV: Figure 12?

24. ML association with HIV: most of the HIV seroreactive (n=7/9) ML showed high …. Probably stating that most of the seroreactive cases stained for Ki-67 is more accurate (as stated before, 35 of the patients were seroreactive). Clarification needed.

25. Epstein-Barr virus: Again, the statement regarding the correlation with GCB is not based on the data (if the data presented in table 6 is accurate).

26. Figure 3b,f (legend). The term of popcorn cell is usually used for L&H cells in NLPHL.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.