Author’s response to reviews

Title: A PILOT STUDY ON THE USEFULNESS OF PERIPHERAL BLOOD FLOW CYTOMETRY FOR THE DIAGNOSIS OF LOWER RISK MYELODYSPLASTIC SYNDROMES: THE "MDS THERMOMETER"

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Author’s response to reviews:

BHEM-D-17-00045: Revised version 1
Dear Editor,

The manuscript was revised following reviewer' recommendations (please see the answers to the reviewer' questions) and all changes made are flagged in green in the revised version.

Changes can be summarized as follows:

• Background: More detailed information about the MDS classification and prognostic groups is provided (as suggested by Reviewer 1)

• Methods: This section was reduced by about 600 words (as suggested by Reviewer 2)

• Abbreviations: new abbreviations were added or modified; the list of abbreviations was updated.

• References: Websites were removed from the text and mentioned as references; in addition, 2 new references were added; reference numbers were updated and references were formatted according to the Journal's standard. (as suggested by Reviewer 1)

• Tables: Most tables were simplified and reformatted (as suggested by Reviewer 1). Namely, data in Tables is no longer presented as mean standard deviation and median and range values (please see Tables 3, 5 and 7, and also Tables S1, S2 and S3), but only as median and range values, as most variables did not have a Normal distribution. In addition, in Table S1 only data at the diagnosis is now provided (data obtained at the time of the study is now provided in the text as well as in Table S1 footnotes).

• Others:

  - The FAB classification is no longer mentioned (as suggested by Reviewer 2). In addition, the nomenclature adopted in the last WHO classification (2016) is being used in the revised version of the manuscript.

  - Comments on the fact that control and patient groups were not paired for age were added, and additional statistical analysis was performed in the control group in order to assure that differences obtained were not age-related.
Finally, some grammar and typographical errors were corrected.

Hoping to have satisfactorily answered all the questions and that our work now deserves the approval for publication in BMC Hematology,

Yours Sincerely

Margarida Lima

BHEM-D-17-00045: Revised version 1

Dear Reviewers

Thanks the constructive comments and for helping us to improve our work. Please find bellow the answers to your questions. All changes made are flagged in green in the revised manuscript.

Best regards

Margarida Lima, corresponding author

REVIEWER 1 - KAREN CORDOVIL, M.E.

Firstly, I would like to congratulate the authors for their work. The present study is rather large but objective. The theme is original and interesting, as it brings a new methodology that can improve the treatment of people with the low-risk myelodysplastic syndrome. It is up to me to make comments to clarify and make the most useful article for the entire scientific community interested in the subject.
1. Abstract

1.1. Authors should place FSC and SSC in full, followed by acronyms.

Done.

2. Background

2.1. Second paragraph (line 4-6): The third sentence is confusing. Authors should improve the explanation and categorization of MDS types.

More detailed information about the MDS classification and categorization into prognostic groups is now provided, as suggested.

3. Methods

3.1. At the end of the first paragraph, remove the word "had" (this is repeated in the text).

Removed.

3.2. Second paragraph: controls were matched according to gender. OK. But why did not they match the ages, too? Would placing a control containing adults be a bias to compare with their sample composed only of the elderly?
Healthy controls and MDS patients were not paired for age due to the difficulties in finding blood donors older than 60 years, and we agree that it could be argued that differences between groups may be age-related. However, the fact that no differences were found for the analyzed parameters when younger (<55 years-old, n=7) and older (≥ 55 years-old, n=7) healthy volunteers were compared, except for a higher fraction of CD16+ monocytes in the last group (in accordance to previous publications – reference 60 added), strongly argue against this possibility.

These aspects are now mentioned in the revised manuscript – Results: Flow cytometry studies (Peripheral blood neutrophils and Peripheral blood monocytes); Discussion – CD16+ monocytes; study limitations.

3.3. Page 9: Flow cytometry set-up and calibration. The site "HTTP://euroflow.org" is unnecessary in the text. Put in the references (available in ....) and flag in the text.

The Euroflow Website was removed from the text and is now mentioned as a reference as suggested (reference 41, added). Reference numbers were updated.

3.4. Page 12: End of the second paragraph. The website is unnecessary in the text. Put in the references (available in ....) and flag in the text.

The Website was removed from the text, because we feel unnecessary to mention it (references 43 and 44 are enough to document the idea).

3.5. Where are the ethical issues of the study? Please put in the text.

Ethical issues were already mentioned in the section DECLARATIONS.
4. Results

4.1. Table 1: Authors should improve the presentation of data on the gender. It isn't good.

4.2. The data present in the table should be clear.

I suppose that you are referring to Table S1, and not to Table 1. Table S1 is provided as supplementary information because it is exhaustive and contains all the clinical and laboratorial characteristics of the study population. This table was simplified in the revised version of the manuscript, as mentioned below.


Thanks for the reference.

Most tables were simplified and reformatted, as suggested. Namely, data are no longer presented as mean standard deviation and median and range values (please see Tables 3, 5 and 7, and also Tables S1, S2 and S3), but only as median and range values, as most variables did not have a Normal distribution. In addition, in Table S1 only data at the diagnosis is now provided (data obtained at the time of the study is provided in the text as well as in Table S1 footnotes of the revised manuscript).

7. References

7.1. Review reference 11
Reference 11 (now reference 12) - the year of publication was corrected.

7.2. Some references with large numbers of authors are written extensively (12), others with et al (21, 34). Authors should place the references according to the journal's standard.

The references were corrected and formatted according to the journal's standard.

REVIEWER 2 - ALAULDEEN MUDHAFAR ZUBAIR, M.D.

Dear author,

Thank you for this high quality research. However, you need to make the following corrections:

1) The paper is very long (56 pages) and very difficult to finish. It should be made much shorter especially that there are unnecessary details in the methods. No need for so much technical details.

The Methods section was reduced by about 600 words.

2) Why did you use two classification systems for MDS especially that the FAB system is very old. What is the benefit of this dual classification? It is better to rely on the recent WHO system because RAEB has changed to RAEB1 and RAEB2 while RAEBt has been changed to AML by definition (blast percentage >20%).
The FAB classification is no longer mentioned, and it was removed from the text and from Table 3, as suggested. In addition, the nomenclature adopted in the last WHO classification (2016) is being used in the revised manuscript, both in the text and in Table 3. A new reference was added (reference 5) and reference numbers were updated.