Author's response to reviews

Title: Microcytosis and possible early iron deficiency in paediatric in-patients: a retrospective audit

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Author's response to reviews: see over
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Dear Sir/Madam,

I am pleased to re-submit our paper, ‘Microcytosis and Early Iron Deficiency In Paediatric In-Patients: A Retrospective Audit,’ to your journal, having addressed the issues that were flagged up by your reviewers.

We were overall pleased with the reviewers’ responses. In particular, we noted Reviewer 3’s comments on the importance of the paper, and Reviewer 1’s compliments regarding the readability and structure of the paper.

Below we have addressed each reviewer’s comments, organised by section:

1) Abstract

We have addressed the comments made by Reviewer 1 in regards to the abstract, and have added details regarding the educational presentation and the fact that the 85% of patients referred to had ‘potentially explicable’ or ‘inexplicable’ results.

2) Background

We have added a whole new paragraph regarding the reliability of MCV in screening for iron deficiency, which includes details regarding the pre-analytical factors that may interfere with MCV measurement. Although it is not an ideal marker for diagnosing iron deficiency (especially at a very early stage), we chose it since it was easily available for every inpatient that had blood tests, whereas other tests for iron deficiency (such as ferritin levels) were very infrequently performed in these patients. There is also good awareness amongst clinicians about the classification of anaemias by MCV, making it easier to highlight the importance of a low MCV during our quality improvement step (the educational presentation). The mean corpuscular haemoglobin (MCH) is no better in screening for iron deficiency that the MCV, since both decrease in parallel in evolving iron-deficiency anaemia, and we have mentioned and referenced this fact in the second paragraph. We were unable to find any data regarding the proportion of patients with a low MCV that will go on to develop frank iron-deficiency...
anaemia; however, we did find other studies that found the positive predictive value of a low MCV result for iron deficiency to be between 69% and 83% (PMID 3965599 and 2318292). Neither of these studies was in a paediatric population, so we have decided not to cite them in the present version of the text. Assuming that none of these patients with microcytosis and iron deficiency had treatment for their iron deficiency or self-corrected their iron intake, we would expect the vast majority of these patients to go on to develop frank iron-deficiency anaemia.

We noted Reviewer 3’s concerns regarding the accuracy of using only MCV to diagnose iron deficiency when there are other conditions causing microcytosis; however, the red cell distribution width (RDW) can also be used to help separate iron-deficiency anaemia from other conditions, and can be easily added to any pre-existing full blood count tests to aid this. It was also recommended as one of the indices that could be requested on any patient with a low MCV during our presentation. We have therefore added a sentence pertinent to this at the end of the second paragraph. Other tests can also be performed prior to discharge or on an outpatient basis to further differentiate iron deficiency from other conditions (e.g. ferritin levels, haemoglobin electrophoresis for thalassaemia), and we have added a sentence to reinforce this fact at the end of the fourth paragraph, in place of the sentence deleted at the request of Reviewer 3. Although we take note of this reviewer’s assertion that clinicians can refer to the reference ranges for ferritin in the context of acute inflammation, knowledge of these is poor and their use fraught with difficulties; we therefore would prefer that ferritin levels are included in the battery of tests performed as an outpatient once the acute illness has resolved. Our aim in this audit was always to use MCV as an indicator for those who need further testing to differentiate the cause of their microcytosis (which could include iron deficiency), rather than as a diagnostic end in itself. Only when a patient presents with clinically obvious iron deficiency (i.e. significantly decreased Hb and MCV with raised RDW and correlating factors in the history and examination and a low likelihood of thalassaemia) might it be prudent to treat first before performing further tests.

In the first paragraph, we have also cited figures for iron deficiency for different age groups from a US study. This is in response to a query from Reviewer 1; we felt that it was more appropriate to mention this study along with the general information about iron-deficiency anaemia in the Background section rather than in the Discussion section.

3) Methods

We have clarified the exclusion criteria used for results in the text for the second paragraph. The hospital lab only uses the adult range for MCV; therefore our first step was to retrieve every FBC result from the computer system that had a MCV of 82 fL or less for patients between 1 month and 16 years old. We then applied the age-specific MCV ranges to those results we had retrieved to identify patients with a low MCV. Since this first step was used to facilitate data retrieval, rather than used as the exclusion criteria, we have deleted any mention of this step from the Methods section to make it clear that the age-specific MCV ranges were used as the main exclusion criteria.
With reference to the third paragraph, Reviewer 3 has expressed her concerns that our classification of results will result in the inclusion of microcytic results that may be unrelated to iron deficiency. However, we have always made it clear that detection of a low MCV is only the first step in trying to work out the cause for it, whether it is iron deficiency or some other cause. Our audit’s focus therefore was on identification of a low MCV *prior* to later definitive investigation for its cause. If any further tests were later performed to investigate the cause, we made note of them in our audit; however, our results subsequently showed that only a handful of patients had any further investigations for their microcytosis. To go back and subject those patients we had identified with a low MCV to further tests for identifying iron deficiency would have been far beyond the scope of an audit or quality improvement study, where one’s focus should be to look at things that were not done ideally in the past in order to make suggestions for further improvement in the future before re-auditing. Therefore, for those patients that did not have further tests, there will be the possibility that their microcytosis may not be due to iron deficiency. This is perfectly reflected in our title, where we have included the phrase ‘possible early iron deficiency’ i.e. iron deficiency that may or may not be present.

We have addressed Reviewer 2’s comments and have provided more details as to how we assigned patients to the subjective categories. We have also made it clear in the text that the assignments were made by one investigator (DNS) for the first audit data, and a second investigator (SK) for the second audit data. The reason for this was that DNS was no longer working at the hospital where the study was performed when the time came to collect the second audit data, and so this task was delegated by him to one of the other doctors working there at the time (SK). It would therefore be inappropriate to perform correlation studies or inter-rater reliability studies, since each investigator looked at different sets of data. However, steps were taken to ensure that SK used exactly the same data retrieval criteria that DNS had used previously (which we have added mention of in the text), and we hope that this has minimised any bias that may be present from having a second person involved in data collection.

Regarding Reviewer 1’s queries regarding whether any investigators were amongst the clinicians being assessed, DNS was amongst one of those clinicians involved in patient care during the first audit period. However, the audit proposal and data collection were only formulated and performed respectively in May 2006 i.e. *after* the audited period. We therefore do not believe that his involvement would have resulted in bias, since he had no knowledge that FBC result documentation and follow-up were to be audited during the period where he was involved with patient care between February and April 2006. None of the investigators were involved in patient care during the second audit period. We have added a statement to make absolutely clear that all clinical decisions affecting the patients had already been made and acted upon prior to commencement of the retrospective study.

With regards to other requested changes, we have clarified in the text which laboratory abnormalities we looked for documentation of (Hb and MCV) in the third paragraph, and have modified the description of the statistical test described in the seventh paragraph and have referenced it appropriately. We have also, in the second paragraph, provided further
details regarding the content of the education presentation that was given following the results of the first audit. In the first paragraph, we have mentioned the total number of clinicians that were involved with inpatient care over the time course of the two audits, in response to a query from Reviewer 2.

We noted two of the reviewers’ concerns regarding the necessity for ethics committee approval. Clinical audit, when performed as an internal process involving no new tests or interventions on patients, does not normally require formal ethics committee approval. We sought guidance from the Norfolk Research Ethics Committee and hospital Research Governance Committee, and both of these committees also confirmed that this study did not require formal ethics committee review and approval. We have added documentation of this fact to the text, and have sent a copy of the letter we received from them to the BMC editorial administrator (Ms. Roxane Rajabi).

We have been through Andrew Worster’s checklist that was suggested by Reviewer 2. Obviously, since our medical record review and data retrieval steps have long since been completed, there is some limitation as to how much further we can minimise any biases that could have been introduced during our medical record review stage. Relying on what other doctors have written with regards to a patient’s diagnosis will always result in a certain level of subjective bias when later using these diagnoses to help classify patient’s MCV results, in the absence of any objective evidence from other tests. We did, however, try to ensure that the data abstraction step was kept as consistent and unbiased as possible between myself and SK by ensuring that she used the same criteria that I had used for recording patient results’ documentation and evidence of treatment/follow-up, and for the classification of MCV results by explicable.

4) Results

All of our percentages have now been rounded to two significant figures; our statistician agrees that this better reflects the precision of our results. The p values were also recalculated to reflect this fact and were also rounded to two significant figures; all are the same as before except the p value for the number of patients with a low MCV (recalculated as 0.080). We have combined the results from both audits into one flow diagram (Figure 1), as requested by Reviewer 2, and have included overall numbers/proportions from both audits. We believe that this method of displaying our results is easier to follow than displaying them in a table, as suggested by Reviewer 3.

Regarding Reviewer 1’s request as to whether any individuals featured in both audit periods, we have identified two individuals with low MCVs in the first audit period that were also noted to have low MCVs in the second audit period; both of them were categorised in similar ways across both audit periods as well (one was ‘potentially explicable’; the other was ‘explicable’). As this was such a small number, we do not feel that there is any merit in excluding these patients from the second audit analysis. Due to the method in which we obtained our data, we are unable to say for certain whether those with normal MCVs in the first audit period had low MCVs in the second audit period, or whether the converse was true.
This is because we did not obtain the details of patients that had a MCV in the adult normal range (greater than 82), since these patients were not the focus of our audit. For similar reasons, we are unable to say for certain if any of the four treated patients in the first audit period subsequently had normal MCVs during the second audit period.

We have taken on board Reviewer 1’s comments regarding the total proportions with low MCV that had treatment and/or follow-up, and have made the necessary textual alterations.

As requested by Reviewer 2, we have listed the sex distribution and age distribution for both sets of our results, shown in Tables 2 and 3 for the first and second audit periods respectively. Unfortunately, as none of the authors are currently working at the hospital where the audit was performed, we were unable to retrieve the list of diagnoses for each category in each audit period, since this data was kept at the hospital site with the hospital notes. For similar reasons, we were unable to obtain details regarding any further lab testing that the low MCV patients had in the subsequent year. Also, no dietary screen is performed at the hospital, and we did not record any particular details of a patients’ dietary history beyond the fact that they had an appropriate dietician review as part of their microcytosis work-up and treatment.

Although we have the figure for the total number of paediatric admission for the first audit period (877), we do not have a corresponding figure for the second audit period, and we were unable to obtain this figure prior to the resubmission deadline. We have therefore omitted mention of these figures from this version of the manuscript.

As suggested by Reviewer 2, we have included a scatter plot of MCV results versus age, with the age-adjusted MCV limit highlighted for ease of comparison (Figure 2). We hope this will give our readers some indication of the spread of our data, and we have included a comment regarding this in the first paragraph of the Discussion section.

Lastly, we have ensured that all of the tables and figures related to the results are referred to in the main body of the text.

5) Discussion

We have made some major revisions to the fourth paragraph, at the request of Reviewers 1 and 2. As well as condensing the paragraph, we have added a new sentence mentioning the weakness of the educational presentation when used in isolation to change departmental practice without accompanying printed guidelines.

We have responded to Reviewer 3’s request for details of a guideline and have detailed one such guideline in a new paragraph, which was the one that was proposed during our educational presentation between the two audits.

With regards to the second paragraph, we felt it was unwise to blindly speculate in the paper on the number needed to screen to prevent cognitive impairment, when the proportion of patients with low MCV results that then progress to frank iron-deficiency anaemia and cognitive problems is currently unknown. We did however add a brief mention that other
conditions such as thalassaemia may be picked up by this type of screening, as suggested by Reviewer 2.

6) Conclusions

We have made the textual alteration requested by Reviewer 3, and have moved the discussion regarding changes in lab practice etc. to the Discussion section, as requested by Reviewer 2.

Finally, we have addressed all of the paper’s style considerations for your journal, and have corrected any outstanding typography, spelling and grammatical errors where they were still present. We have opted to use UK spellings for terms such as ‘anaemia’; however, we are happy for the spellings to be altered to the US versions if this fits better with your journal’s house style.

We hope that the above points considered in conjunction with our revisions have enhanced the merit of publishing our paper in the journal. We look forward to your response.

Many thanks.

Yours sincerely,

Dr. Deepak Subramanian