Commentary

Haemoglobin and haematocrit: the threefold conversion is also non valid for assessing anaemia in Plasmodium vivax malaria-endemic settings

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Abstract

It has been recently reported that the standard threefold conversion from haematocrit to haemoglobin underestimates the prevalence of anaemia and low levels of haemoglobin in children living in areas endemic for Plasmodium falciparum malaria. The data presented herein describes the experience in a malaria-endemic zone in northeastern Venezuela (state of Sucre), where a similar bias between haematocrit and haemoglobin in patients with Plasmodium vivax infection was found. In summary, the relationship between haematocrit and haemoglobin needs to be specifically evaluated according to each particular region or epidemiological setting.

Background

Carneiro et al [1] recently reported that the standard threefold conversion from haematocrit to haemoglobin underestimates the prevalence of anaemia and low levels of haemoglobin in children under five years of age in malaria endemic settings.

In agreement with Carneiro et al findings, demonstrating that the usual threefold conversion represents a significant bias when haemoglobin (Hb) is estimated based on haematocrit (Hct) values in children with malaria, the present report describes the experience in a vivax malaria-endemic zone in northeastern Venezuela. It has been a widely accepted assumption that this conversion could be used as an alternative measurement to haemoglobin in malaria studies [2]. This is particularly important in African settings where Plasmodium falciparum malaria is endemic, but the corresponding haemoglobin levels cannot be derived with an acceptable accuracy using the value three as a conversion factor [2]. As a consequence, the relationship between haematocrit and haemoglobin needs to be specifically evaluated according to each particular region or epidemiological setting. In order to illustrate this important issue, the experience in a malaria-endemic zone in northeastern Venezuela (state of Sucre) is described [3,4], showing a similar bias between haematocrit and haemoglobin in patients with Plasmodium vivax infection.
Methods and results
Data from one study that have measured haemoglobin and haematocrit was used to assess the reliability of the standard threefold conversion factor. Finger-prick blood samples were collected for determination of anaemia status from 120 patients with malaria aged 4–89 years-old from a prospective survey carried out between 2000 and 2002 in Carupano, Sucre, Venezuela [5]. In this study, haemoglobin concentration was assessed by haemophtometry and haematocrit was assessed by centrifugation using standard procedures for microhaematocrit tubes and centrifuge (i.e. 10 minutes at a fixed speed of 11,000 rpm).

Statistical methods
As described by Carneiro et al [1], the measurement of observed and estimated haemoglobin using the method of plotting the difference between the measures against the mean of the two measures, as reported by Bland & Altman [6], is compared. Then, a comparison between haemoglobin (g/dl) and haematocrit (%) divided by a factor of three was done to be able to compare the measurements on approximately the same scale ("grams of haemoglobin per dl"). The difference between the haemoglobin and haematocrit/3 measurements (i.e. Hb - Hct/3) and the mean of the two measurements (i.e. (Hb + Hct/3)/2 – now called average to avoid confusion with mean difference), were calculated for each individual. Linear regression analyses were used to define the relationship between the mean difference and the average of the two measures [6], adjusting for a priori covariates of age and sex. The differences between both measures were also tested in terms of means comparison using the Student’s t test for one-sample, for independent-samples and paired-samples.

Haemoglobin measurements were lower than haematocrit/3 in this study in 92.5% (111/120), with a linear trend in the relationship so that more negative differences were seen with increasing haemoglobin levels ($r^2 = 0.1379$, $F = 18.87$, $P < 0.0001$) (Figure 1). Logarithmic transformations of the means and differences as suggested by Bland & Altman [7] did not improve this relationship.

The mean difference between haemoglobin and haematocrit/3 values was -0.6 (± 0.4, range -1.6 to 0.5). The differences between both measures in terms of means comparison for Student’s $t$ tests were also significantly different (Table 1). When the haematocrit was calculated using the haemoglobin (haemoglobin × 3), similar significant ($P < 0.05$) differences were evidenced between the observed and estimated haematocrit. These differences not showed a statistically significant variation in regard to the age neither with the sex ($P > 0.05$).

Then, the relation between observed haematocrit and observed haemoglobin was modelated (Figure 2)(y = 3.1906x - 0.6215). The final regression model gave the

Figure 1
Scatter-plots of difference against average of haemoglobin and haematocrit/3 (A), as well after correction using (haematocrit+0.6215)/3.1906 (B). Scatter-plots of difference against average of haemoglobin and haematocrit/3 for paired measurements, and between average of haemoglobin and (haematocrit+0.6215)/3.1906. The line of best fit (blue) indicates the trend towards greater differences at higher haemoglobin values (significantly lower after correction). Both axes are in "grams of haemoglobin/dl".
following relationship, which represents the line of best agreement between the two measures ($r^2 = 0.9526, F = 2372.0, P < 0.0001$):

\[
\text{Estimated Haemoglobin} = \frac{(\text{Haematocrit} + 0.6215)}{3.1906} \quad (1)
\]

When the estimation of haemoglobin using this model was corrected, the new estimated haemoglobin ($([\text{Haematocrit} + 0.6215]/3.1906$) shown that the haemoglobin measurements were lower than corrected observed haemoglobin in 49.2% (59/120), with a linear trend in the relationship significantly more slight than the original ($r^2 = 0.013, F = 1.55, P = 0.216$) (Figure 1).

**Discussion**

These results showed a consistent bias of haemoglobin measurements to indicate a greater degree of anaemia than haematocrit measurements in the same individuals and populations if the standard threefold conversion is used, as has been reported previously [8], and most recently by Carneiro [1].

The difference between the haemoglobin and centrifuged haematocrit/3 was found to be non-uniform, decreasing with average values of these measures, but conversely not significantly modified by age or sex. These results suggest that the relationship between haemoglobin and centrifuged haematocrit could be independent to the recent exposure to malaria, which is a continuous risk in endemic settings.

Both Hct and Hgb levels could be affected by factors such as the method and equipment used for its determination,
environment or subject’s differences that may cause a spur-
rious change in the measured value and lead to inaccura-
ties [2,9].

These and previous data [1,2] have shown that Hgb levels
cannot be derived from the Hct values with an acceptable
accuracy using the general rule of dividing by 3. The rela-
tionship between Hgb and Hct is not exactly 3 and could
be affected by age, sex, infection status, malarial etiology
and season, among other factors. Due to the lack of agree-
ment, the commonly assumed ‘equivalent’ cutoff points
for anaemia definitions need to be re-evaluated. More
information is needed for different aetiologies of anae-
mia.

Conclusion
The present study demonstrates that the standard three-
fold conversion from haematocrit to haemoglobin under-
estimates the prevalence of anaemia and low levels of
haemoglobin in children and adults in a Plasmodium vivax
malaria endemic settings. In contrast to the results of Car-
neiro et al [1], it was found that the bias was less acute for
more severe anaemia defined by haemoglobin<8 g/dl and
haemoglobin<5 g/dl.

Competing interests
The author(s) declare that they have no competing inter-
ests.

Authors’ contributions
AJRM analysed and interpreted the data, and wrote the
first draft of the paper. CFP designed and co-ordinated the
original studies, contributed to data interpretation and to
drafting and revising the manuscript. ES, MA, MV, CP and
RC were involved in data collection and critically revised
the manuscript. All authors read and approved the final
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