Assessing the stability and suitability of haematology parameters for diagnosing and monitoring iron deficiency

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Abstract

**Introduction:** Iron deficiency is a common condition which has traditionally been evaluated using biochemical parameters as measures of iron status. Each of these has their flaws and two haematological parameters, percentage of hypochromic mature red cells (%HYPO) and reticulocyte haemoglobin content (CHr), have recently been recommended in national guidance for the routine determination of iron status. This study used sample stability as a means of identifying the most appropriate parameter for a high throughput medical laboratory from a logistics perspective.

**Methods:** 15 peripheral blood samples stored at both cold and ambient temperatures were tested over a 24 hour time period for both parameters, to assess stability.

**Results:** %HYPO was found to be highly unstable at room temperature, showing large increases in results from baseline over the 24 hour period. By comparison, CHr was more stable and showed only minimal result fluctuation.

**Conclusion:** This study shows that poor sample stability makes %HYPO unsuitable for evaluation of iron status in community patients, where the majority of iron deficient cases are identified, treated and monitored. CHr has far superior stability and should, therefore, be the preferred test from a laboratory logistics perspective.

**Keywords:** Reticulocyte, hypochromic, iron, anaemia, stability.

Introduction

Iron deficiency is a common laboratory finding and can be associated with numerous medical conditions, in addition to dietary insufficiency. It represents the most common cause of anaemia worldwide and is acknowledged as a significant cause of morbidity and mortality (World Health Organisation, 2001). Accurate investigation of iron status is essential, not only with regards to diagnosis, but also for evaluating response to treatment.

The gold standard for measuring iron status is assessment of bone marrow iron stores using Perl’s stain (Cook & Skikne, 1989). However, such an invasive test in all cases of suspected iron deficiency would be excessive and unnecessary, in addition to incurring a high cost per test. As such, iron status has traditionally been measured using biochemical parameters, primarily ferritin and percentage transferrin saturation (%TSAT)(Cook, 2005). These tests are easily automated and have a low cost, making them ideal for
high throughput testing. Whilst this strategy has been undeniably valuable in diagnosing and monitoring iron deficiency, neither test is without its limitations; %TSAT is subject to diurnal variation (Dale et al, 2002) whilst ferritin, an acute phase protein, is raised in infection and inflammation (Elin et al, 1977; Hulthén et al 1998). Diagnosis of iron deficiency in patients presenting with a microcytic, hypochromic anaemia in the presence of other comorbidities therefore remains challenging.

In light of a continuing need for a robust means of measuring iron status, two new haematological parameters have been included in National Institute for Health and Clinical Excellence (NICE) and British Committee for Standards in Haematology (BCSH) guidelines, as suitable alternatives for biochemical measurement of body iron (NICE NG8, 2015; BCSH Thomas et al 2013). These are percentage hypochromic mature red cells (%HYPO) and reticulocyte haemoglobin content (CHr). %HYPO measures the percentage of mature red cells with a cellular haemoglobin content of less than 28pg, using the principle that iron restricted erythropoiesis would result in an increased proportion of hypochromic red cells, causing a raised %HYPO result. CHr can be used to assess iron via determination of the haemoglobin content of peripheral blood reticulocytes. This will be reduced in the absence of iron, and so CHr results will decrease in iron deficiency. Both parameters are affected by thalassemia (Skarmoutsou, 2003) but as this is the only comorbidity which will influence results, these parameters may fill the need for robust iron status measurement.

%HYPO is recommended as the test of choice in both guidelines but a 6 hour analysis window restricts the use of the test to samples collected in close proximity to testing facilities. Community monitoring of iron deficiency is a strategy used to combat overbooked hospital outpatient clinics but unfortunately, peripheral blood samples collected from the community are unlikely to be analysed within this 6 hour analysis window. Consequently, %HYPO is unsuitable for the vast majority of patients requiring outpatient evaluation of iron status. No analysis window is stated for CHr in either guideline.

This study had two aims. Firstly, to determine if it was possible to prolong the analysis window of %HYPO to support its availability to community clinicians and secondly, to determine the analysis window for CHr. By repeat testing samples over a 24 hour period and comparing results to baseline we determined sample stability and identified the most suitable parameter to implement in a high
throughput medical laboratory for community monitoring of iron status.

Methods and Materials

10mls of venous blood was collected into two K-EDTA anticoagulated tubes from 15 healthy volunteers (7 male, 8 female; age 22-58 years). Participants were assumed to be iron replete based on normal MCV, MCH and haemoglobin. One primary tube was kept at ambient temperature (22±2°C) while the other was separated into 9 aliquots and stored at 4±2°C. CHr and %HYPO results were recorded at the time of sample collection, and then at 2, 4, 6, 7, 8, 9, 12 and 24 hour time points. A fresh aliquot of chilled blood was used at each time point to prevent fluctuations in sample temperature. All sample analyses were carried out on a single Siemens ADVIA 2120 haematology analyser to prevent inter-analyser variation.

Results

Variations in %HYPO and CHr were calculated as the mean percentage deviation from the 0 hour baseline value and data are expressed as mean variation ± standard error of the mean (SEM). At ambient temperature (22±2°C) there was significant variation in the %HYPO over time. We observed a 48.8% (±10.8%) increase at 2 hours; 126% (±17.8%) increase at 6 hours and an 1131% (±202.7%) increase at 24 hours (Figure 1). When samples were stored at 4±2°C, %HYPO was more stable over time: a -15.7% (±5.3%) decrease at 2 hours; 1.7% (±8.6%) increase at 6 hours and a -13.1% (±14.3%) decrease at 24 hours (Figure 1).

By contrast, CHr was consistently stable over time at both temperatures. At ambient temperature (22±2°C) we observed a -1.0% (±0.63%) decrease at 2 hours; -1.6% (±0.63%) decrease at 6 hours and a -2.2% (±0.81%) decrease at 24 hours (Figure 1). Similarly when samples were stored at 4±2°C, CHr varied minimally over time: a 0.0% (±0.57%) change at 2 hours; -0.9% (±0.56%) decrease at 6 hours and a 1.6% (±1.00%) increase at 24 hours (Figure 1).

Discussion

The use of %HYPO and CHr as a means of assessing iron status has been extensively investigated, although the majority of studies have been concerned with determining the correlation between these two new parameters and traditional biochemical ones. The analytical integrity of both parameters has been confirmed by their inclusion in national guidance, but there is still a question as to which is the most suitable with regards to laboratory logistics.
Iron deficiency is a common clinical finding, with a UK incidence of 21% of female teenagers aged 11-18, 18% of women aged 16-64 and 2-5% in adult males and post-menopausal women (Zimmermann & Hurrell, 2007). It is also estimated that approximately 7-9% of children aged 1-3 are iron deficient (USA, Baker & Greer, 2010). Due to its high incidence and relatively benign nature, it is routinely monitored in the community, with samples being taken at GP practices and then transported to local laboratories for analysis. Therefore, when deciding upon the most appropriate parameter for medical laboratory testing, the validated analysis should be a central consideration when deciding on the preferred test.

Whilst both NICE and BCSH guidelines recommend %HYPO as the preferred test, our study demonstrates that it is the least suitable parameter for monitoring patients within the community. While %HYPO is more stable at 4±2°C, samples taken by hospital clinicians, community nurses and at GP practices will be stored at room temperature following phlebotomy, during transport to the laboratory and whilst being analysed. Therefore, investigating sample stability at cool temperatures is purely an academic exercise as it could not be implemented for routinely collected samples.

When assessing sample stability at ambient temperature it is clear that %HYPO significantly increases, even within the 6 hour analysis window recommended in national guidelines. At 6 hours, there is on average a 126% (±17.8%) increase in %HYPO results from baseline, and an average increase of 1131% (±202.7%) at 24 hours, with significant variation between samples. This is likely due to the effects of *ex vivo* red cell swelling and indicates that even within the stated analysis window, there is a significant increase in %HYPO which could lead to over-diagnosis of iron deficiency or be interpreted as a falsely poor response to treatment.

By contrast, there was minimal fluctuation in CHr (±2.0% over 24 hours), with little variation between samples, regardless of sample temperature. At ambient temperatures there is a mild reduction in values across all time points but given the small degree of deviation from 0 hours, this is highly unlikely to impact on clinical decision making.

**Conclusions**

Despite %HYPO being recommended as the test of choice in national guidance, its inherent instability means it is not suitable for routine assessment of iron status at central laboratory sites. A short analysis window means it cannot be offered to community clinicians, which is a significant shortcoming as this is where the majority of iron
deficiency patients are investigated and monitored. CHr was found to be stable for 24 hours, allowing sufficient time for sample collection, transport and analysis. Therefore, whilst NICE and BCSH guidelines may recommend %HYPO as the test of choice based upon sensitivity and specificity, this study suggests that CHr is the most appropriate parameter for routine assessment of iron status from a logistics perspective.

Whilst it must be acknowledged that the sample size in this study is small, the results show a high degree of correlation. Regardless, further work with a larger sample size would be of benefit. Additionally, the investigation of this hypothesis using other haematology analysers will identify whether the above findings are applicable to all laboratories, regardless of the chosen platform.

**Figure Legends**

**Figure 1** Variation in %HYPO and CHr results over 24 hour period. Error bars represent SEM.

**References**


Author Contributions

AS, LB and JM designed the research. AS performed the research and analysed data. AS and JB wrote the paper.