ORIGINAL ARTICLE

The reticulocyte haemoglobin equivalent (RET_He) and laboratory screening for iron deficiency

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ABSTRACT

Background: The reticulocyte haemoglobin equivalent (RET_He) parameter provides the haemoglobin (Hb) concentration of peripheral blood reticulocytes on Sysmex Haematology analysers. In the iron deficient (ID) state, reticulocytes and mature red cells have a lower Hb concentration due to the reduced bioavailability of iron. Reticulocyte haemoglobin parameters have been available on cell counting analysers for years. Recently there has been renewed interest surrounding the suitability of the RET_He as a laboratory screening test for ID. This work presents the findings of a study in which the RET_He was evaluated for its use as a marker of ID.

Aim: To establish a reference range for the RET_He and to assess its performance as a laboratory screening test for patient iron deficiency.

Methods: One hundred and seventy-eight patient samples submitted for iron studies to Labcare Pathology and Taranaki MedLab were retested to provide a CBC with a RET_He measurement using the Sysmex XN-2000 (Sysmex, Japan) analyser. All samples had previously been tested for ferritin, serum iron, transferrin and saturation. The results for the RET_He were stratified against iron studies, the CBC results and patient clinical details. A reference range for the RET_He was established using 66 samples from selected patients at Taranaki Base Hospital.

Results: A working cutoff value for ID for the RET_He was established as <26 picograms (pg) of Hb per reticulocyte. Values at or below this cutoff were mainly from patients with either iron deficiency, anaemia of chronic disease or known haemoglobinopathy patients. The reference range for the RET_He with a 95% confidence interval was 30.3-35.0 pg.

Discussion: This study showed the potential for the use of the RET_He as a simple laboratory screening test for iron deficiency. With a cutoff of <26pg, the RET_He correlated well for red cell microcytosis but was not specific for iron deficiency. Used as a screening test for ID, RET_He results of <26pg could signal the need for iron studies with results above this cutoff acting as a negative predictor of ID in anaemic patients. Used in this way the RET_He could better guide the use of iron studies for patients in whom ID may be suspected.

Keywords: Reticulocyte, RET_He, iron deficiency, anaemia, iron studies.

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INTRODUCTION

Iron deficiency (ID) and iron deficiency anaemia (IDA) affects approximately 2 billion people globally making it the most common of the nutritional deficiencies (1,2). Iron is essential for normal biological function and untreated IDA has been associated with developmental delays in the young (2). Iron deficiency can be difficult to diagnose using traditional biochemical markers of iron metabolism and the presence of the morphologically characteristic hypochromic microcytic red blood cells (RBC) in the blood film are typically only apparent once iron deficient erythropoiesis is advanced (3).

The complete blood count (CBC) is the most frequently ordered of all laboratory tests (1) and so expansion of the clinical utility of results produced as part of the CBC could be beneficial for patient diagnosis and management. The reticulocyte haemoglobin equivalent (RET_He) is a red cell parameter available on the XN-2000 and other Sysmex haematology analysers and provides a measure of the bioavailability of iron during erythropoiesis (4,5). Reticulocytes have a short life-span of 1-2 days in the peripheral blood before full maturation, and during iron deficient erythropoiesis, reticulocytes have reduced levels of haemoglobin production. The RET_He parameter has the potential for greater clinical use as an adjunct to current biochemical-based assays for ID. It has previously been proposed as a laboratory tool to distinguish between IDA and anaemia of chronic disease (ACD) with both aetiologies producing morphologically similar hypochromic microcytic RBC populations (1). ACD results from the inability of erythropoiesis to utilise body iron while IDA results from the lack of body iron.

The aim of this study was to establish a reference range for the RET_He at Taranaki Base Hospital and to assess its clinical value as a tool for the identification of ID.

MATERIALS AND METHODS

The study utilised samples submitted to LabCare Pathology and Taranaki MedLab for iron studies over a period of eight weeks. Results were collated twice daily from the laboratory information systems and EDTA anticoagulated blood samples were run on the XN-2000 (Sysmex, Japan) using the RET channel analysis feature. Testing was performed twice daily to ensure samples were less than six hours old to reduce any effects of sample ageing. A total of 178 patient samples were included in the study and all laboratory testing results and patient clinical details were collated in Microsoft Excel.

In the RET channel, blood cells are exposed to a surfactant reagent that lightly perforates the membrane of the RBC, WBC and platelet populations. In the machine the blood sample, surfactant and a fluorescent dye (Fluorocell RET) are incubated

together for a short period, allowing the dye to penetrate the cells (1). The stained cells are then cycled through a flow cell and past a beam of high intensity laser light. Reticulocytes containing RNA, fluoresce producing forward and side scattered light that is captured by light-detectors producing results that are presented graphically in the form of a 2D-scatterplot. Total cell numbers are counted with the forward light-scatter providing cell size and side-scattered light indicating the presence of cytoplasmic nucleic acid (DNA/RNA). In the RBC population the degree of fluorescence is proportional to the cytoplasmic RNA and provides the reticulocyte population. Results are presented as picograms (pg) of Hb per reticulocyte (1).

The biochemistry analyser used for iron studies at LabCare Pathology was the Cobas 6000 (Roche Diagnostics, Germany) with the iron panel providing results for serum ferritin, serum iron, serum transferrin and transferrin saturation. Ferritin was measured by electrochemiluminescent immunoassay, iron by colorimetric assay and transferrin using an immunoturbidimetric assay (6-8).

A RET_He reference range was constructed from the results of 66 EDTA anticoagulated peripheral blood samples. The samples were selected from patients presenting to Taranaki Base Hospital with a normal CBC and medical conditions unlikely to impact on their iron status or reticulocyte parameters.

The statistical software program MedCalc®, was used to provide the reference range with a 95% confidence interval and an online Clinical Calculator software package (9) used to calculate sensitivity, specificity, positive and negative predictive values for selected RET_He cutoff values.

RESULTS

In the RET channel of the Sysmex XN-2000 analyser, normal and ID samples vary in the scatterplots they produce as a result of the differences in the RBC and reticulocyte populations. In Figures 1 and 2 the reticulocyte population (pink/red) is presented along with the mature RBC population in blue. Figure 2 shows the presence of microcytic RBC & reticulocyte populations lower on the Y axis of the scatterplot in contrast to the iron replete example in Figure 1. The microcytic RBCs in Figure 2 are the result of iron deficient erythropoiesis caused by reduced cellular Hb levels.

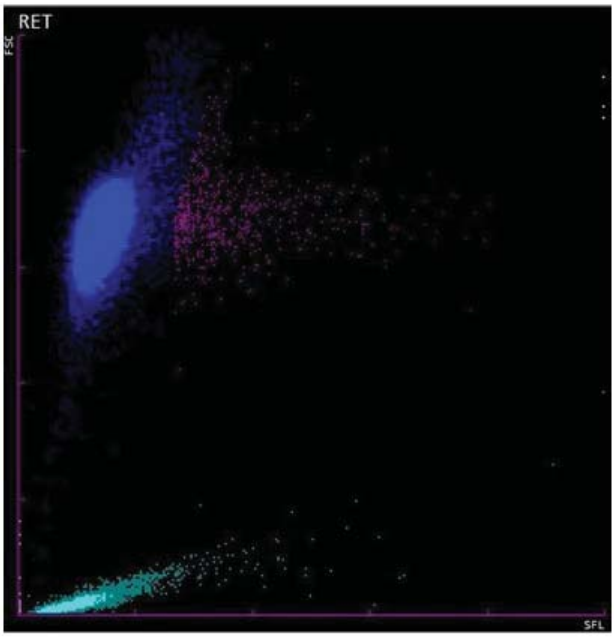

Figure 1. RET scatter gram - normal iron.

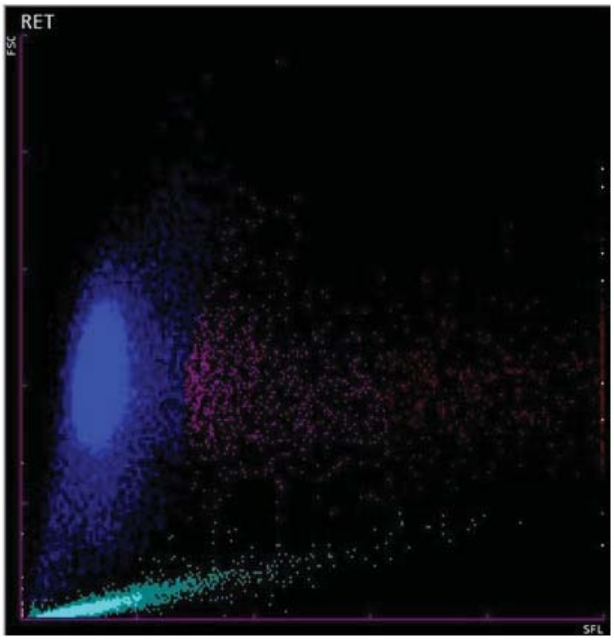

Figure 2. RET scatter gram - iron level deficiency.

Participants had an average age of 44 with a male to female ratio of 1:2. The RET_He results were stratified against the iron studies, CBC results and relevant clinical information. This divided patients into normal, ID/ACD, IDA and haemoglobinopathy clinical groupings. The diagnostic cutoff values for ID were those used at LabCare Pathology with ferritin <20 ug/L and saturation <16%. Using the World Health Organisation (WHO) criteria, patients were classified as anaemic if the Hb was <120g/L for non-pregnant females and <130g/L for males (9). A C-reactive protein assay (ref range <5 mg/L) was used to establish cause when clinical details were not available for some apparent ID patients.

A summary of the results of the laboratory testing divided participants into four groups and is presented in Figure 3.

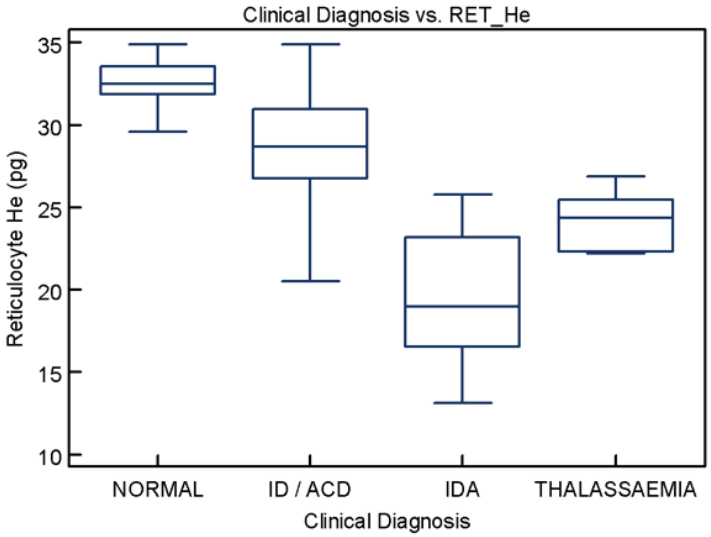

Figure 3. Clinical diagnoses vs RET_He results.

The data used to establish rates of True and False positives for the patient groups is presented in Tables 1 and 2. A RET_He cutoff of <26 pg favoured the detection of more true positives (21) but also more false positives (10). The cutoff of <25pg detected less true positives (19) but also had less false positives (7).

Table 1. True and false positives using a <25pg cut-off for the RET_He.

RET_He	True positives	False positives	Totals
< 25pg	19	7	26
> 25pg	2	150	152
Totals	21	157	178

Table 2. True and false positives using a <26pg cut-off for the RET_He.

RET_He	True positives	False positives	Totals
< 26pg	21	10	31
> 26pg	0	147	147
Totals	21	157	178

Sensitivity, specificity and positive and negative predictive values for the RET_He were calculated for the two cutoff options <25pg and <26pg rf. Table 3 (10). Results showed that a cutoff of <26pg had a small advantage for the detection of ID.

Table 3. Summary of sensitivity, specificity, positive (PPV) & negative predictive values (NPV) for two cut-off values.

RET_He	Sensitivity	Specificity	PPV	NPV
< 25pg	0.905	0.955	0.730	0.987
< 26pg	1	0.936	0.677	1

The results for the RET_He from 66 patient samples were used to calculate a reference range with a 95% confidence interval of 30.3-35.0 pg with a mean of 32.7 pg. The coefficient of skewness (-0.034) showed the data set was normally distributed (Figure 4b) with support from a low coefficient of Kurtosis or Z score of (-0.335) (Figure 4a).

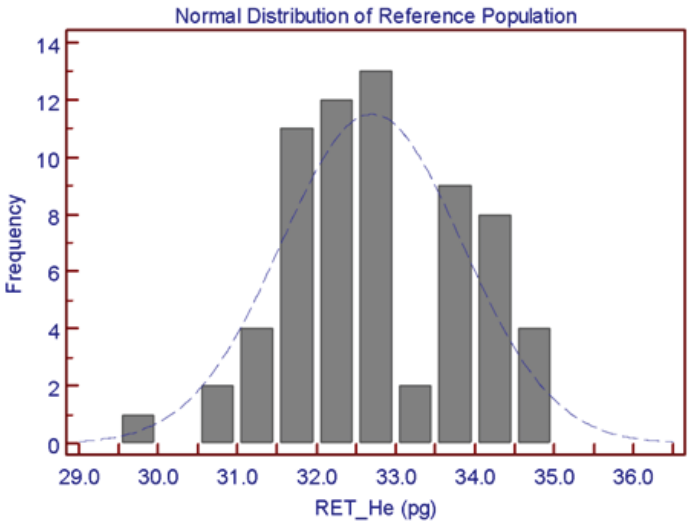

Figure 4a. Z score and population distribution for the RET_He reference range.

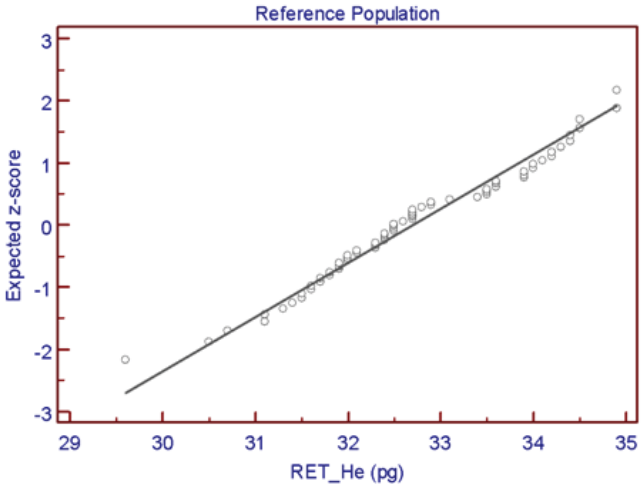

Figure 4b. Population distribution for the RET_He reference range.

DISCUSSION

The gold standard for the assessment of body iron is a bone marrow biopsy but it is an invasive procedure rarely used in the diagnosis of ID related disorders (11). Instead the quantitation of iron is traditionally performed using biochemistry-based assays. Ferritin is the long term storage form of iron and in the plasma it reflects total body iron stores. Its use as a marker for ID is complicated, as together with transferrin, both are also acute phase reactants that are elevated in infection, chronic disorders and other inflammatory states. As a result, the diagnosis of ID using iron studies is not always straight forward.

This study correlated patient clinical information against the results of iron studies, CBC data and the RET_He parameter. Results indicated that a RET_He cutoff of <26pg was able to identify ID patient groups and best supported the use of the RET_He as a screening test for iron deficiency. The RET_He uses the mean cell volume (MCV) and so results can be affected when there is microcytosis unrelated to IDA, such as, in double RBC populations, in cases of RBC aggregation and when there is hyper or hyponatraemia (12). To aid interpretation, the RET_He results should be considered together with the results of the red cell distribution width (RDW).

In this study the RET_He failed to provide a clear division for the ID and ACD groups affecting the overall specificity of the parameter. Given this, the follow-up of patients with a RET_He less than or close to the 26pg cutoff should include iron studies (13).

The RET_He reference range for the population in this study was 30.3-35.0 pg with a 95% confidence interval and is comparable to the range of 28.9-36.3 pg developed by LabPlus in New Zealand for a demographically similar population group (14). That study recommended a RET_He cutoff value for ID of <25pg slightly lower than the cutoff of <26pg in this work. A possible limitation of this study could have been the participant group selected for the reference range. Made up of selected inpatients at Taranaki Base Hospital instead of healthy members of the public, the reference range data could have been skewed. This does not appear to have been the case with other researchers producing reference ranges comparable to this study (2,5,11,12).

Laboratory cost containment has always been an important consideration in laboratory testing. A cost advantage for the RET_He as a screen for ID may be significant as compared to traditional iron studies. In this study iron study costs were estimated to be approx 1.5 times that of the CBC + RET. A closer consideration of the cost/benefit of the RET_He over the use of traditional iron studies as a screen for ID may or may not support the findings of this study.

CONCLUSIONS

This study highlighted the clinical potential of the RET_He. Its use as part of a screening algorithm together with Hb, MCV and RDW, could better guide laboratory recommendations for iron studies, reducing costs when iron studies are not warranted. The study showed that the RET_He with a cutoff of <26pg was highly sensitive but not specific for the detection of ID. Its future clinical utility could be as a screening test for ID but also as a negative predictor of ID when the RET_He results fall within the reference range in anaemic patients. Its utility as an early marker for ID has been previously reported and has been confirmed with the demographic investigated in this study. The future acceptance of the value of the RET_He by clinical staff may be hampered by a lack of awareness of its potential for patient diagnosis and treatment. The education of clinical staff could start by reporting the parameter in anaemic patients with results below the RET_He cutoff, triggering a comment about additional laboratory testing to rule out possible ID .

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