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Original Research Paper

Fresh capillary blood analysis using darkfield microscopy as a tool for screening nutritional deficiencies of iron and cobalamin (vitamin B12): A validity study

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ABSTRACT

Objectives: The practice of fresh capillary blood analysis using darkfield microscopy (FCB-DM), commonly termed 'live blood analysis', is currently used as a point of care screening tool for several health aberrations, including nutritional deficiencies. There is currently a paucity of scientific research into the use of this technique and there is no research to date investigating the use of FCB-DM as a screening tool for nutritional deficiencies. The purpose of this study was to begin the process of validating this technique in screening for nutritional deficiencies of iron and cobalamin.

Methods: FCB-DM screenings were performed on 29 consenting participants who were likely to be deficient in iron or cobalamin. The FCB-DM screenings were photographed to permit a quantitative analysis of cell size and morphology. Each participant provided a sample of venous blood soon after the FCB-DM screening for diagnostic pathology testing. The researcher was blinded to the pathology results until all FCB-DM data analysis was complete. Data from the FCB-DM screenings were correlated with the results of pathology blood tests used for the diagnosis of iron and cobalamin deficiencies. The sensitivity and specificity of FCB-DM parameters for detecting low iron or cobalamin levels were calculated.

Results: Significant correlations were found between serum ferritin and the FCB-DM parameters of annulocytosis, elliptocytosis and microcytosis. Elliptocytosis showed the best performance for test sensitivity and specificity for low iron levels. The FCB-DM parameters of macrocytosis and anisocytosis showed significant correlations with pathology methylmalonic acid, homocysteine and holotranscobalamin II. Anisocytosis was found to have good test sensitivity and specificity for low cobalamin levels.

Conclusion: These findings suggest that the FCB-DM parameter elliptocytosis is a valid marker of low iron levels and that anisocytosis is a valid marker of low cobalamin levels. However further research into all FCB-DM parameters is required to validate their use.

1. Introduction

Fresh capillary blood analysis using darkfield microscopy (FCB-DM) is a technique of analysing fresh, capillary blood cells at point-of-care, using darkfield microscopy. The advantage of using darkfield microscopy is that the FCB-DM practitioner can assess blood cell morphology and dynamics without the need of staining or drying procedures. This technique is widely used by complementary and alternative medicine (CAM) and integrative medicine professionals. According to a workforce study by Bensoussan et al. [1] 11.9% of Australian naturopaths and western herbal medicine practitioners use FCB-DM in clinical practice. Despite its popularity in clinical practice, there is a paucity of research investigating the use of this technique. An extensive review of the literature revealed few studies that have specifically investigated the FCB-DM technique and no studies have been published that addressed the use of FCB-DM in screening for nutritional deficiencies. Therefore the scope of this study was not to provide definitive evidence for the use of this technique, but to explore its strengths and weaknesses, evaluate appropriate research methods for assessing the validity of the technique and flag areas for future research.

Iron deficiency is considered to be the most common nutrient deficiency in both developing and developed countries [2,3]. An estimated 10.6% of Australian women aged <50 years and 9–11% of menstruating women in the US are hypoferremic [3,4]. Iron deficiency (ID) is the result of persistent negative iron balance

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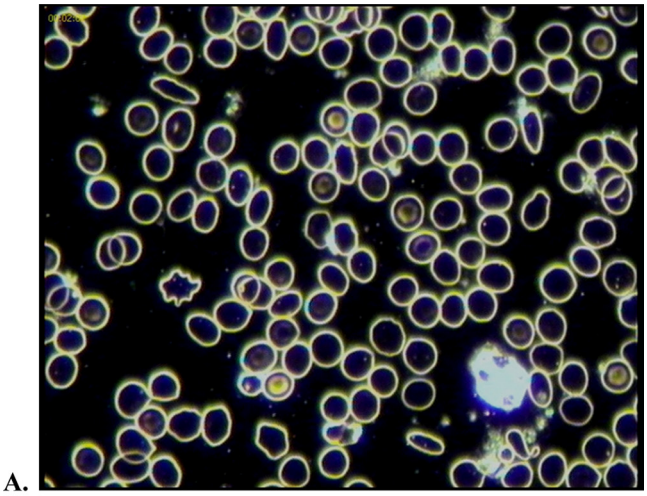
causing the depletion of iron stores. ID will progress to iron deficiency anaemia (IDA) if untreated, characterized by hypochromic microcytosis. A systematic review by Guyatt et al. [5] found serum ferritin levels <40 μg/L to be the most powerful marker of ID and that serum ferritin levels <15 μg/L could unequivocally diagnose IDA.

The population most at risk of developing cobalamin deficiency (CD) is the elderly and is most commonly the result of food-cobalamin malabsorption [6,7]. Estimates of the prevalence of CD in American and British elders generally range from 6 to 20% [7–9]. CD is more common in developing nations, with prevalence rates as high as 80% in India and 40% in Latin America [9]. Methylmalonic acid (MMA) has proven to be a highly sensitive and specific marker of CD [10,11]. Homocysteine is also a highly sensitive test for CD; however, it lacks specificity as levels can increase in conditions other than CD (i.e. folate deficiency).

The changes in blood morphology characteristic of nutritional deficiencies that are assessed in a FCB-DM screening (see Figs. 1 and 2) are very similar to parameters assessed in a typical haematological peripheral blood smear. Microcytes and elliptocytes, or pencil cell formation, are associated with iron deficiency, and macrocytes or macro-ovalocytes and hyper-segmented neutrophils are associated with cobalamin deficiency (see Appendix A for full definitions). Anisocytosis is associated with both iron and cobalamin nutritional deficiencies. The haematological parameter of target cell formation (termed annulocytes in FCB-DM practice) has a different presentation under darkfield conditions, having a solid central disc rather than the typical ring appearance of a target cell. The hypochromia seen in the peripheral blood smear of iron deficient individuals is not perceptible using darkfield microscopy.



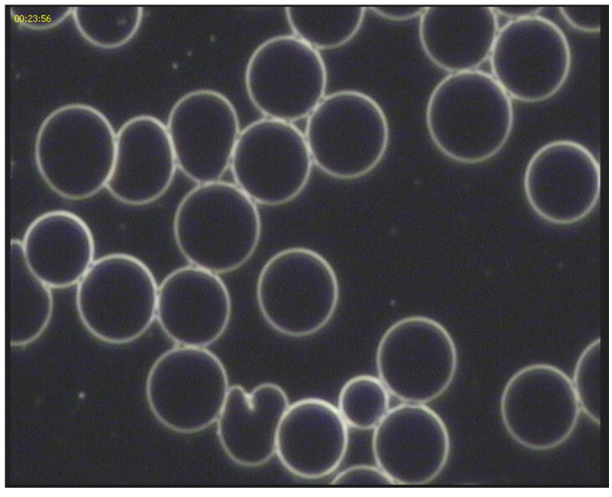
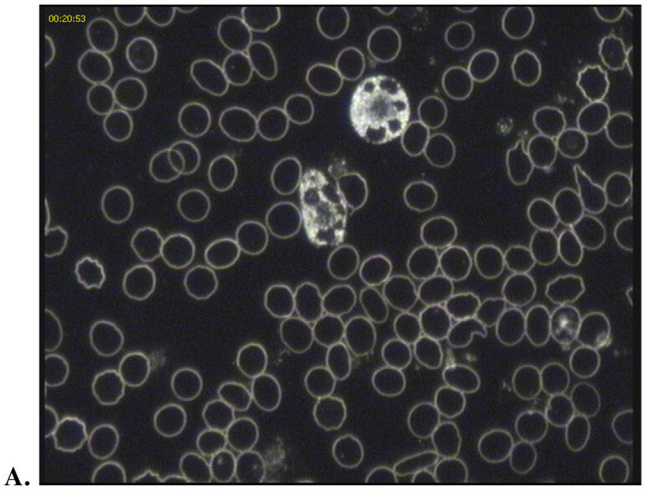


Fig. 1. FCB-DM parameters of iron deficiency.
(A) This photograph shows the blood of an individual diagnosed with IDA. Elliptocytes (long and narrow), annulocyte (solid gold centre) and non-specific poikilocyte (highly crenated membrane). (B) A microcyte viewed at 100× magnification. Photographs taken by author, used with permission.



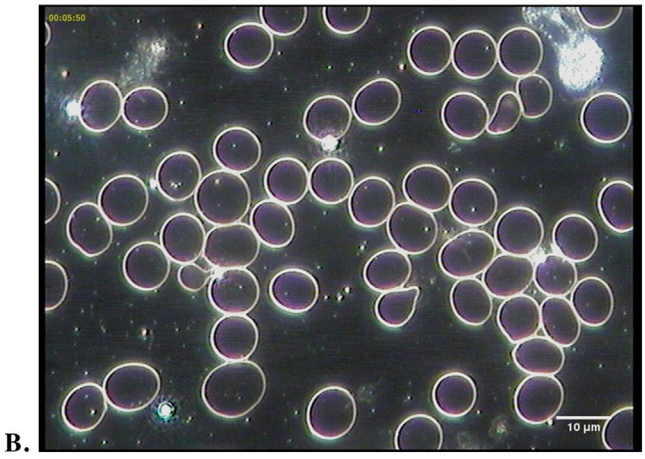


Fig. 2. FCB-DM parameters of cobalamin deficiency.
(A) An example of hypersegmented neutrophils, showing two five-lobed neutrophils. Photo taken by the researcher, used with permission. (B) The two photos, taken from a cobalamin deficient patient, display anisocytosis along with round macrocytes and macro-ovalocytes. Photo courtesy of Wayne Reilly, photo used with permission.

As FCB-DM is used at point-of-care and aimed at early detection of nutritional deficiencies and preventative healthcare [12], this study assessed FCB-DM as a screening tool for both clinical and sub-clinical iron and cobalamin deficiency states.

2. Materials and method

The validity of FCB-DM as a screening tool for nutritional deficiencies of iron and cobalamin was assessed by comparing FCB-DM results with established 'gold standard' diagnostic pathology tests. The researcher was blinded to the results of the pathology tests. Unlike a standard FCB-DM consultation that subjectively grades the severity of parameters, this study assessed FCB-DM parameters quantitatively by measuring red blood cell (RBC) size