The Validity of Screening for Nutritional Deficiencies of Iron and Cobalamin using Fresh Capillary Blood Darkfield Microscopy

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Aim: The purpose of this study was to investigate the validity of the Fresh Capillary Blood Darkfield Microscopy (FCB-DM) technique in screening for nutritional deficiencies of iron and cobalamin. The training and practice of FCB-DM (also termed 'live blood screening') is currently used by clinicians as a point-of-care screening tool for haematology status, including nutritional deficiencies.[1] Despite its popularity in the clinical setting, there is a paucity of scientific research into the use of this technique with no research to date investigating the use of FCB-DM as a screening tool for nutritional deficiencies.

Method: 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. The FCB-DM parameters assessed are listed in Tables 1 and 2 and shown in Figures 1, 2 and 3. 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 Full Blood Count, Iron Studies, Homocysteine (HCY), Methylmalonic Acid (MMA) and Active B_{12}.

Results: The FCB-DM parameter that showed the strongest correlation with serum ferritin was elliptocytosis (Table 1), which was also the only FCB-DM marker to show a significant correlation with TIBC. Elliptocytosis had a sensitivity and specificity of 0.87 and 0.60, respectively, for the detection of low iron (ferritin <15 µg/L, n=8). FCB-DM parameters annulocytosis and microcytosis were also found to have correlations with serum ferritin. HCY was found to significantly correlate with FCB-DM parameter macrocytosis, showing a strong correlation (Table 2). MMA and HCY were both found to correlate with anisocytosis. The FCB-DM mean RBC diameter was calculated from a 500 RBC count for each participant. A strong, significant correlation was found between the pathology MCV and FCB-DM mean RBC diameter (n=29, r =0.577, p<0.01), as illustrated in Figure 4.
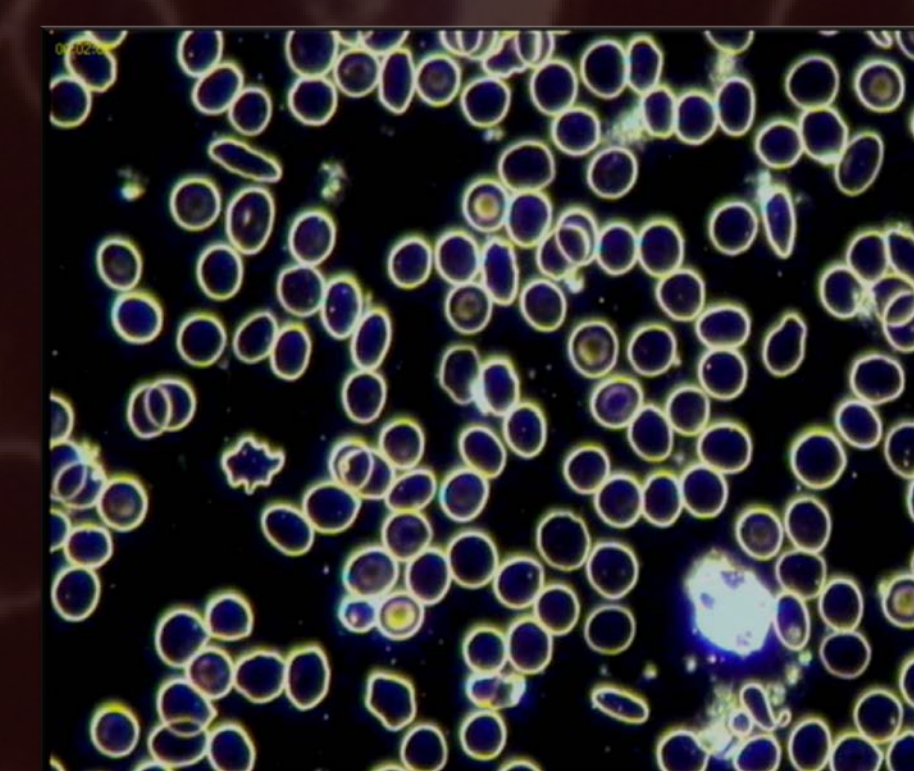

Figure. 1. FCB-DM blood smear demonstrating elliptocytes, annulocytes and microcytes (indicated in descending order by the red arrows)
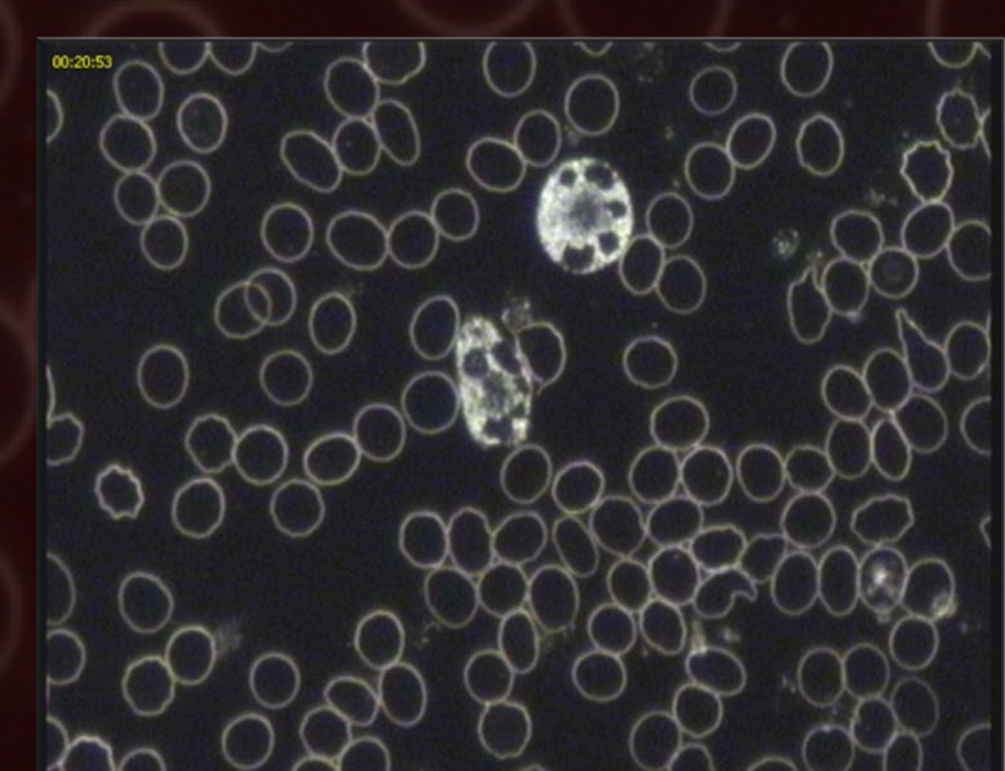

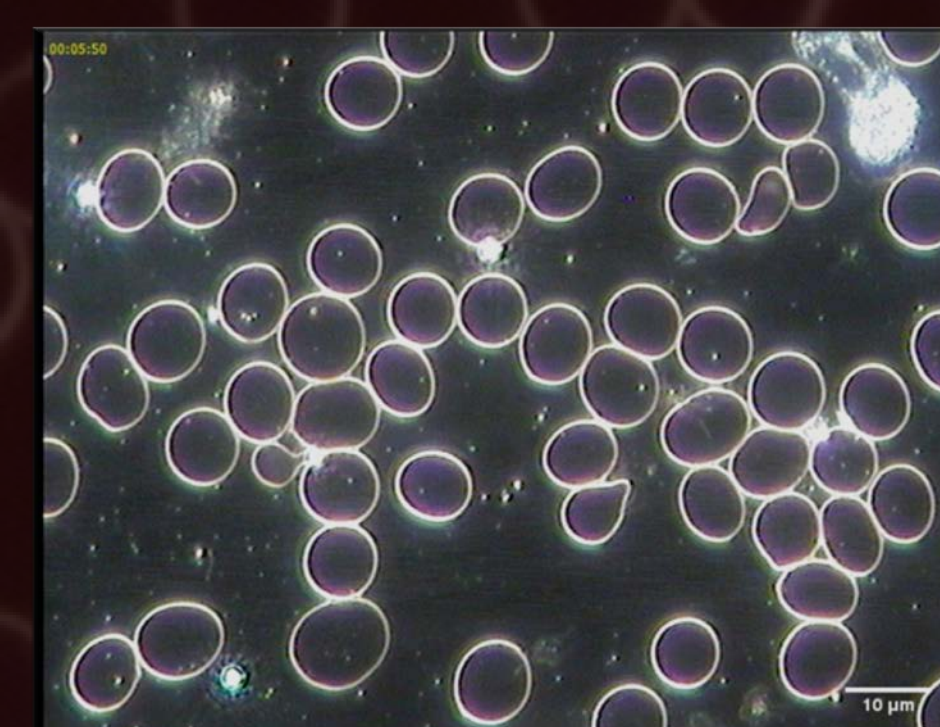Figure. 2. FCB-DM blood smear showing neutrophil hypersegmentation

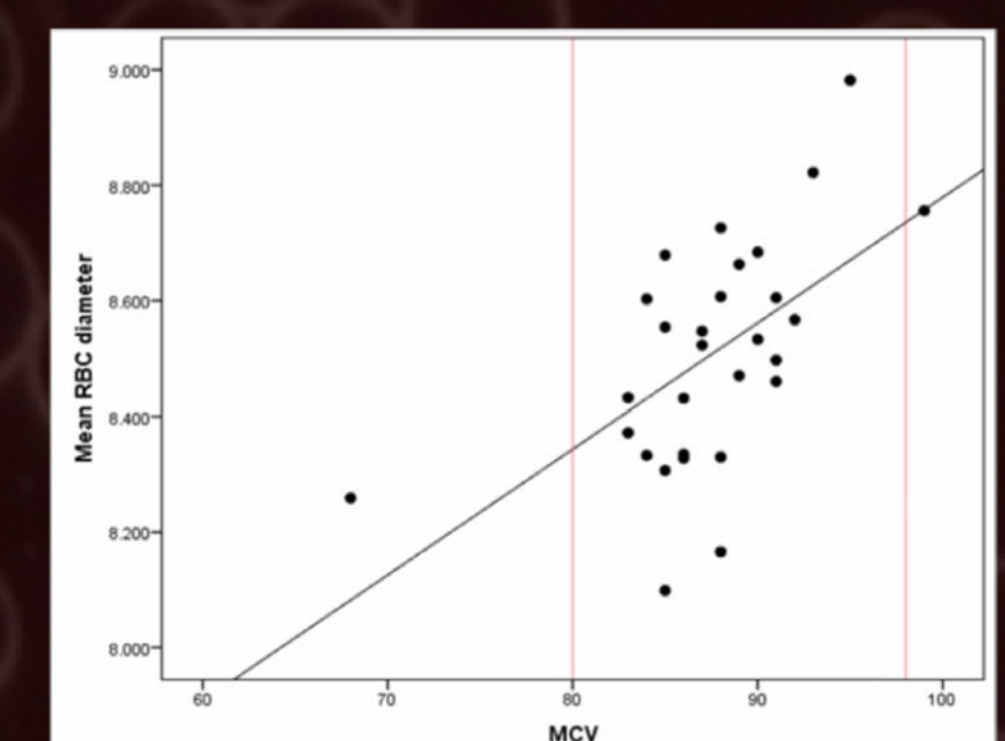Figure. 3. FCB-DM blood smear showing macrocytosis and anisocytosis

Figure 4. Relationship between FCB-DM mean RBC diameter and pathology MCV. The MCV reference range is represented by the red lines (80 – 98 fL).

Table 1. Correlation coefficients for FCB-DM parameters of iron deficiency and pathology markers. Significant correlations marked with an asterisk.

FCB-DM parameter	Serum Ferritin	Haemoglobin	TIBC
Mean RBC diameter	0.557** (p < 0.01)	0.197 (p = 0.162)	-0.218 (p = 0.137)
Microcytes	-0.373* (p < 0.05)	-0.004 (0.493)	0.225 (p = 0.130)
Anisocytosis	0.246 (p = 0.099)	-	-
Elliptocytes	-0.633** (p < 0.01)	-0.303 (p = 0.063)	0.427* (p < 0.05)
Annulocytes	-0.418* (p < 0.05)	-0.029 (p = 0.443)	0.158 (p = 0.215)

Table 2. Correlation coefficients for FCB-DM parameters of cobalamin deficiency and pathology markers. Significant correlations marked with an asterisk.

FCB-DM parameters	MMA	HCY
Mean RBC diameter	-0.004 (p = 0.492)	0.410* (p < 0.05)
Macrocytes	0.228 (p =0.126)	0.530** (p < 0.01)
Anisocytosis	0.434** (p < 0.01)	0.377* (p < 0.05)
Hypersegmented neutrophils	-0.072 (p = 0.355)	-0.208 (p = 0.139)
Macro-ovalocytes	-0.045 (p = 0.408)	-0.285 (p = 0.067)

Conclusions: The results of this study suggest that elliptocytosis is a valid marker of low iron. This supports findings from previous haematological studies of blood morphology that a significant relationship exists between elliptocytosis and low iron according to diagnostic tests[2,3]. Limited support was found for other FCB-DM parameters and further research using a larger sample is required to ascertain their validity. The FCB-DM parameters of hypersegmented neutrophils and oval macrocytes were found to be poor markers of cobalamin deficiency, which agreed with previous studies[1,2], however, may have been due to the small sample of clinically deficient participants (n = 4).

References:
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