

# Optical phase contrast microscopy in reporting anisopoikilocytosis in stained blood smears; a pilot study

KAC Wickramaratne<sup>1</sup>, SLNA Mannapperuma<sup>2</sup>, DC Wijewickrama<sup>1</sup>

<sup>1</sup>Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

<sup>2</sup>Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.

**Correspondence:** Dr. Chandana Wickramaratne  
e-mail: [kacw@med.ruh.ac.lk](mailto:kacw@med.ruh.ac.lk)  
 <https://orcid.org/0000-0003-4394-2535>  
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## ABSTRACT

**Introduction:** Conventionally, to examine stained blood smears, bright field microscope (BFM) is used. Appreciation of outlines and three-dimensional images of cells is possible with optical phase contrast microscope (OPCM) in wet smears

**Methods:** This study was performed to determine applicability of OPCM in reporting anisopoikilocytosis in stained blood smears. Leishman-stained blood smears were assessed using bright field microscope (BFM) and OPCM and findings related to anisopoikilocytosis were compared with corresponding red cell indices (RI) of complete blood count.

**Results:** There were 25 and 24 samples with normal and abnormal RI respectively. Mean anisopoikilocytosis percentage of both groups reported with OPCM were significantly higher than those of BFM. Anisopoikilocytosis percentage reported with BFM and OPCM were significantly positively correlated. Mean corpuscular volume and microcyte percentage and mean corpuscular haemoglobin and hypochromic cell percentage were significantly negatively correlated ( $p < 0.05$ ).

**Conclusion:** Optical phase contrast microscopy could significantly improve quantification and reporting of anisopoikilocytosis in stained blood smears.

**Keywords:** *Anisopoikilocytosis, blood smears, light microscopy, optical phase contrast microscopy, red cell morphology.*

## Introduction

Conventionally, red cell morphology is assessed using red cell indices in complete blood count report (CBC) and by evaluating stained blood smears with bright field microscope (BFM) (1-5). To ensure uniformity of reports and to minimize the subjective nature of identification and quantification of red blood cells with abnormal morphology, International Council for Standardization in Haematology (ICSH) has proposed objective methods to standardize the use of different terms such as

anisocytosis, poikilocytes, acanthocytes, echinocytes, schistocytes, dacrocytes etc. in blood smear reporting (6, 7). Further, ICSH proposes to grade the quantity of abnormal red cells as percentages of total red cells as mild (less than 10%), moderate (10-20%) and marked (more than 20%) (6).

Traditional reporting of blood smear uses bright field microscope which gives only two-dimensional image. Appreciation of outlines and three-dimensional images of cells is possible with optical

phase contrast microscope (OPCM) in wet smears (8-10). Therefore, use of OPCM to examine stained blood smears would improve the recognition of red blood cells with abnormal shapes. Thus, this study was designed to assess the plausibility of using OPCM in quantification of anisocytosis and poikilocytosis (anisopoikilocytosis) in stained blood smears.

## Methods

Fresh anticoagulated blood samples collected for routine CBC or blood smear reporting over a one month period in a private sector laboratory were included in the study. Inadequate or delayed samples (more than six hours since collection) and samples with clots and haemolysis were excluded from the study. Blood smears were prepared within six hours of collection of blood using standard procedures described previously.

Based on red cell distribution width (RDW) in automated CBC reports, samples were divided into normal (RDW <14%) and abnormal (RDW >14%) groups. From each blood sample, one Leishman-stained blood smear was prepared and reviewed using BFM and OPCM (Olympus CX 43 RF biological microscope BFM & OPCM combined) under 40 x and 100 x magnifications. Number of red cells showing anisocytosis and poikilocytosis per 1000 red cells (approximately in 5 oil immersion fields) were counted and expressed as percentages of means. Counting was performed blinded in duplicate by two investigators independently. Crenated red cells were identified and excluded from calculation.

The red cell indices in automated CBC reports were compared with corresponding anisocytosis (microcytosis and macrocytosis) and poikilocytosis reported by both BFM and OPCM to assess the correlations.

Data were analysed with *IBM SPSS* statistics version 20. Independent t-Test and Pearson correlation were applied to assess the significances and the correlations between the two microscopic methods ( $p < 0.05$  was considered statistically significant).

Ethical approval for the study was obtained from the Ethical Review Committee of Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka.

## Results

There were total of 49 fresh blood samples which fulfilled the inclusion criteria. There were 24 samples with RDW above 14%, thus considered abnormal and the remaining 25 were considered normal. The RDW in the study ranged from 11.9 to 31.4 with a median of 14.

The median of MCV of the study group was 89.2 fL (range 62 - 100.5 fL). According to the population reference interval (11), MCV less than 80 fL were considered microcytic. Over 3% microcytes were found in 9.3% and 11.6% samples by BFM and by OPCM respectively in the samples with MCV >80 fL. Samples with MCV values within the reference interval of MCV for the population showed over 3% macrocytes in 22.5% samples by both microscopic methods. The MCH of the study group ranged from 19.5 pg to 32.7 pg and the median was 29.6 pg. In samples with MCH >27 pg (n=39), {lower limit of the reference interval of MCH for the population (11)}, both microscopic methods detected hypochromia in 20.5% samples.

The mean percentages of anisopoikilocytosis reported in the abnormal group (RDW >14%) by using BFM and OPCM were  $12.58 \pm 7.52\%$  and  $14.19 \pm 6.63\%$  respectively. The mean percentages of anisopoikilocytosis obtained in the normal group (RDW <14%) using BFM was  $4.33 \pm 1.53\%$  and using OPCM was  $5.16 \pm 1.64\%$ . The mean percentages of anisopoikilocytosis of both abnormal and normal groups reported using OPCM were significantly higher than those reported using BFM ( $p < 0.002$ ). Percentages of anisopoikilocytosis reported using BFM and OPCM were significantly positively correlated ( $r = .96, p < 0.01, N = 49$ ) (Figure 1).

When the results of red cell parameters obtained using BFM and OPCM were compared with those obtained by automated method, the percentages of microcytosis and MCV were found to be significantly negatively correlated (BFM:  $r = -.30, p = .035, N = 49$  and OPCM:  $r = -.35, p = .015, N = 49$ ). The percentages of cells with hypochromia obtained by microscopic methods and automated mean corpuscular haemoglobin were also found to be significantly negatively correlated (BFM:  $r = -.36, p = .015, N = 49$  and OPCM:  $r = -.40, p = .006, N = 49$ ).

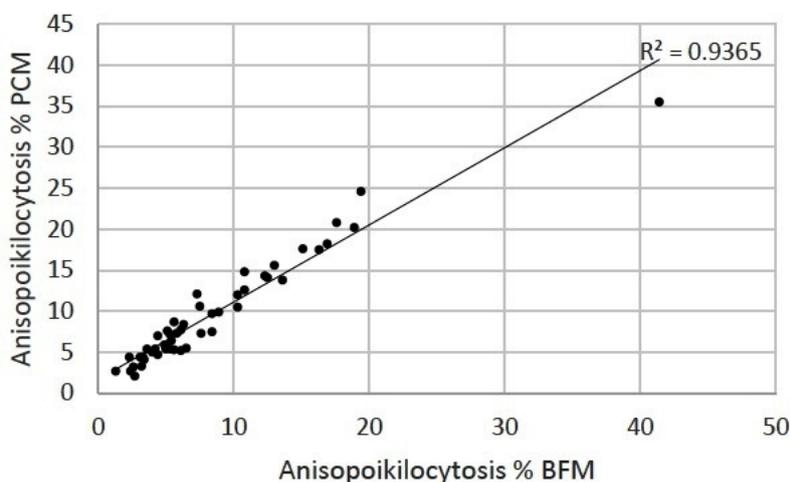
**Discussion**

The traditional BFM is the gold standard of assessment of red cell morphology in stained blood smears. Morphological assessment of red blood cells is strengthened by recent advances in automated CBC techniques. Use of OPCM in wet smear evaluation enables clear identification of cell contours. This helps the detection of abnormal red cell morphologies (dysmorphic red cells) in urine aiding the diagnosis of glomerulopathies accurately. Use of OPCM in assessment of stained smears is not a common practice. We found that the visualization of red cell deformities is comparatively clear and distinct with OPCM (Figure 2) in stained smears.

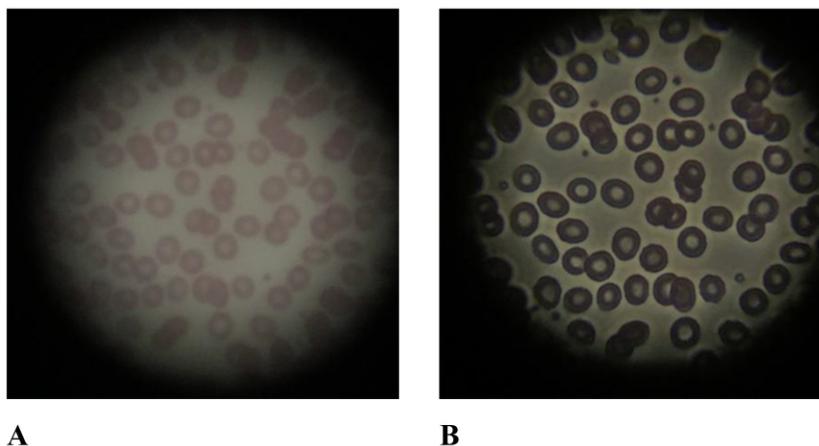
According to literature, false negative rates acceptable in blood smear reporting are considered

either <5% or <3% (12, 13). This stresses the need of lesser false negatives in morphological assessment of cells to assure safety of patients. Findings of this study show higher degree of precision of OPCM in detection of anisocytosis and poikilocytosis in stained smears compared to the traditional method. This observation is strengthened objectively by the finding of higher mean percentages of anisopoikilocytosis with OPCM and better correlation of its findings with red cell indices than with BFM.

In addition, the percentages of microcytosis and hypochromia detected by OPCM showed significantly higher negative correlation with MCV and MCH compared to the findings of BFM.



**Figure1:** Correlation of percentages of anisopoikilocytosis reported with bright field microscopy (BFM) and phase contrast microscopy (PCM) (n=49)



**Figure 2:** Comparison of bright field and phase contrast microscopic appearances of stained blood smears.  
 A: Bright field microscopy 100 x objective  
 B: Phase contrast microscopy 100 x objective

Technologies that improve sensitivity and specificity of assessment of morphology are costly and not freely available (14). Although, combined BFM and OPCM microscopes are now available in laboratories, OPCM is not used for evaluation of stained blood smears due to the dearth of information in literature. Since, OPCM improves detection and quantification anisopoikilocytosis in stained blood smears, OPCM in reviewing of stained blood smear will meaningfully improve objectivity of blood smear reporting.

Not correlating the red blood cell morphological findings of BFM and OPCM with final diagnosis is a limitation of this study.

Further studies are needed to define a grading system of anisopoikilocytosis for OPCM as present grading system is developed only for BFM. In addition, the effects of prolonged storage of stained blood smears on OPCM findings are also should be studied.

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