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A comparison of differential leucocyte counts measured by conventional automated venous haematology and darkfield microscopic examination of fresh capillary blood

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ABSTRACT

Analysis of fresh capillary blood using darkfield microscopy (FCB-DM) is a point of care screening tool, used by healthcare practitioners in Australia. However, the relationship between the outcomes of FCB-DM measures and the automated venous haematology measures has not been determined.

Objectives: The aim of this study was to compare differential white blood cell counts obtained using automated haematology and FCB-DM.

Methods: Data of 125 individuals were collected either retrospectively ($n = 74$) or from participants specifically recruited for the project ($n = 51$). Retrospective data were collected from active files at a naturopathic clinic. Newly recruited participants provided a fasting capillary blood sample for FCB-DM analysis within 1 h of providing a venous blood sample at a commercial laboratory for automated haematologic analysis.

Results: The mean score of neutrophils was found to be higher, and lymphocytes and basophils to be lower, in FCB-DM analysis ($p < 0.05$). A significant and positive Pearson's correlation coefficient was found between automated haematology and FCB-DM in the cell counts for neutrophils ($r = 0.60$, $p < 0.05$) and lymphocytes ($r = 0.63$, $p < 0.05$), and a significant and positive Spearman's correlation was found for monocytes ($r_s = 0.32$, $p < 0.05$) and eosinophils ($r_s = 0.596$, $p < 0.05$). Linear regression analysis was also conducted to assess the relationship between the two techniques. The variance explained by the regression model was large for neutrophil (37%), lymphocyte (39%), and eosinophil (37%) scores.

Conclusion: Despite significant differences in the mean scores of cells counts, significant correlations between the data obtained by the two techniques for neutrophil, lymphocyte, monocyte and eosinophil cells were observed. Given the small amount of blood sample required, FCB-DM would have an advantage in clinical practice, though further research is required to determine the clinical implications of the FCB-DM cell counts.

What is already known about the topic

- Differential white blood cell counts are routinely carried out as part of a full blood count in mainstream healthcare. However, it requires venous blood sampling.
- FCB-DM analysis requires only a small size of blood sample from finger prick and reveals morphology of cells. However, there are no published studies to date regarding the clinical validity of FCB-DM technique in the differential white blood cell count.

What this paper adds

- Investigation into the clinical validity of FCB-DM for measuring differential white blood cell populations in human capillary blood samples.

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- Comparative analysis of differential white blood cell counts obtained from FCB-DM and gold standard haematology analysis.
- Demonstrated significant correlations between FCB-DM and automated haematology differential white cell counts that warrant further research.

1. Objective

The aim of this study was to compare differential white blood cell counts performed using fresh capillary blood analysis using darkfield microscopy (FCB-DM) and automated venous blood haematology.

2. Background

There are at least two different FCB-DM techniques currently practised in Australia that differ in both method and interpretation of findings. The Hemaview™ technique of FCB-DM, which was developed from traditional haematology practices, was assessed in this study. There has been very little research published regarding FCB-DM and its use as a health screening tool. Much of the information about FCB-DM's validity and reliability is anecdotal [1] or based on clinical experience [2–6]. Comparing FCB-DM to the gold standard, automated blood cell analysis, will help clinicians have a better understanding of the validity of the FCB-DM differential white cell counts. This is important in providing safe and effective treatments for patients. The FCB-DM technique requires a drop of blood from a finger prick for analysis. If shown to be valid, reliable and accurate in rigorous clinical trials, the FCB-DM technique could be recommended for many health care applications, considering its less invasive nature in comparison with venepuncture.

Darkfield microscopy (DM) is used to investigate transparent and unstained specimens [7]. Central light rays along the optical axis of the darkfield microscope are blocked out, thus the sample is illuminated with oblique light. The oblique rays cross the specimen and are refracted, producing a black background with objects brightly lit in the foreground [7].

Scientists and clinicians have observed bacteria, human blood and body fluids using DM since its development in the early 1900s [8]. Clinically, DM has been used since the 1970s in dentistry and periodontal care [9–11] to identify bacterial strains in saliva and tissue, enabling treatment to be adjusted to the patient's needs at the time of care [12–15]. DM examination of diarrheal stool specimens is utilised for diagnosis of *Campylobacter enteritis* [16]. DM has also been successfully used to monitor commercial yeast cultures. Wei et al. [17] stated that "the contrast of the images is higher than those taken by a lightfield microscope", allowing for more accurate monitoring. The ease of detection and identification of parasite-infected cells as seen through DM also permits easy malaria diagnosis [18,19].

3. White blood cell counts

Differential white blood cell counts are routinely included in automated haematological full blood count examinations and are utilised clinically in monitoring patients with suspected inflammatory, haematologic, neoplastic or infectious diseases, some cancers and immunological conditions, and in the screening of infants, the elderly and the infirm [20]. Automated venous haematology requires four millilitres of blood for a full blood count that includes a total white cell and differential white cell counts [21,22].

White blood cells, or leukocytes, are nucleated immunologically active cells present in varying concentrations in human blood. They can be classified according to their morphological appearance and immunological function [23]. In conventional venous blood haematology, disorders can be classified as quantitative where leukocytes are present in abnormal numbers, or qualitative where cell abnormalities are observed in a stained blood smear [24]. Capillary blood collection has been developed as an alternative to venepuncture notably in paediatrics and in field medicine, since it requires only a small amount of blood for analysis [25].

4. Methodology

4.1. Design

The data were collected from two separate sources. The first was from a retrospective group (Retrospective) whose data was collected from files at a naturopathic clinic. The collection process involved systematic selection from the 3000 archived clinic files obtained during the period from 2008 to 2012. The data selection was based on patients' suitability for this study defined by the following inclusion and exclusion criteria:

- Each of the patients should have had a complete FCB-DM screening performed by the same FCB-DM practitioner.
- Data should be collected from files of any patient who had an automated differential white cell count and a FCB-DM differential white cell count completed within 14 days of each other.
- Patients who were, at the time of screening, under 18 years of age, pregnant, undergoing chemotherapy, or who were on immunosuppressive agents, non-steroidal anti inflammatory drugs or antibiotics should be excluded.

A total of 172 patients who satisfied the criteria were contacted in writing, and consent was obtained from 72 eligible participants. Two participants contributed two separate sets of FCB-DM and haematology data for the study. The automated haematology analysis was carried out at a number of different commercial haematology laboratories.

The second data set was collected as part of a FCB-DM research trial carried out at the same clinic during 2013 (Recent). In a cohort of 51 participants, fasting venous blood samples were taken and automated differential white blood cell counts were performed at a commercial pathology laboratory (QML Pathology Service), and fasting fingerpick blood samples were obtained within 1 h of venous sampling for FCB-DM differential white blood cell counts.

The average age of the participants was 61 ± 10.5 years for the Retrospective and 45 ± 14.4 years for the Recent, and the average age of the whole cohort was 55 ± 14.2 years. The disparate age ranges were highly significant between the two groups (independent groups t test, $t = 3.34$, $p < 0.001$). The Retrospective group contained 47 (63.5%) women and 27 (36.5%) men, and the Recent group contained 40 (78.4%) women and 11 (21.6%) men.

4.2. Procedures

The Hemaview™ FCB-DM technique, as used in both the Retrospective and Recent groups analysis, requires one drop of capillary blood from a finger prick that was collected onto a glass microscope slide (Menzel-Gläser) and a coverslip (Menzel-Gläser) was carefully placed onto the drop within a few seconds of collection. A second back up slide was prepared from a second drop of blood from the same finger prick but not used in the analysis related to this manuscript. The blood was then examined using either a Zeiss Optiphot (Retrospective) or Zeiss Axiolab (Recent) microscope with a darkfield oil condenser, using Achroplan 10×, 40× objective lens and Immersol 518 N immersion oil.

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