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Finger-prick blood samples can be used interchangeably with venous samples for CD4 cell counting indicating their potential for use in CD4 rapid tests

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Abstract

The objective of this study was to investigate the utility of finger-prick blood samples for CD4 counting. We estimated agreement between CD4 counts in paired finger-prick and venous samples from 110 HIV-infected Malawian adults. Bias was 6.6 cells/ul (limits of agreement –50.7 and 63.7 cells/ul) for absolute counts and 0.71% (limits of agreement –2.07% and 3.48%) for %CD4/lymphocyte suggesting that finger-prick blood samples can be used interchangeably with venous samples for CD4 counting.

There is currently a rapid scaling-up of antiretroviral therapy (ART) in resource-poor settings, especially in Africa. WHO clinical staging is usually employed to assess whether an individual should start ART in such settings [1], but clinical staging does not always correspond to the degree of immunosuppression [2] and patient management could be improved by increased availability of CD4+ lymphocyte counts (CD4 counts) [3]. The main reasons why CD4 counts are not widely used are the high cost of flow-cytometry, the ‘gold standard’ method of CD4 counting, and a lack of skilled laboratory staff [4,5]. To overcome these problems, a number of simplified non-flow cytometric CD4 counting methods have been developed [6-9]. There have been problems with the accuracy of such methods [10], but they have the potential to make affordable CD4 counts available for use by staff with minimal training away from major medical centres.

Rapid tests for CD4 counts are currently under development [11] and could increase the availability of CD4 counting. Such tests could be performed on finger-prick blood samples, enabling them to be conducted where trained phlebotomists are not available or when difficulties are encountered with venous sampling. Finger-prick blood samples are commonly used in resource-poor settings for rapid HIV tests and malaria parasite slides and so may be more acceptable than venesection.

Studies comparing CD4 counts in finger-prick and venous blood are currently lacking. Previous studies of full blood counts in healthy adults using haematological analysers have

found mean leucocyte counts which are 7.82% [12] and 8.34% [13] higher in finger-prick blood samples compared with venous blood. Differences were greatest among the large leucocyte subpopulations (principally neutrophils) and least among small leucocytes (principally lymphocytes).

We therefore conducted a study in HIV-infected adults to estimate the agreement between results obtained using finger-prick and venous blood for absolute CD4 counts and CD4 counts as a percentage of total lymphocyte count (%CD4/lymphocyte). The study was conducted in the ART clinic, Queen Elizabeth Central Hospital, Blantyre, Malawi. Following informed consent, simultaneous venous and finger-prick blood samples were taken by three study nurses, experienced in finger-prick and venous blood sampling, from 111 consecutive HIV-infected Malawian adults during September and October 2006. There were no exclusion criteria.

Following informed consent, blood samples were taken simultaneously from the pad of the middle-finger and an antecubital vein of the opposite arm. Finger-prick samples were taken using BD Genie Lancets (2.0 mm × 1.5 mm) according to the manufacturer's recommended procedure [14]. This produced a free flow of blood with minimal squeezing of the finger. Venous sampling was performed using a 21G needle and syringe. Finger-prick and venous samples were collected in separate BD Microtainer EDTA capillary blood tubes (250 µl per tube). The first drop of finger-prick blood was discarded. One set of samples was rejected, because the finger-prick sample clotted.

Paired blood samples were tested in parallel for CD4 counts within 6 hours of collection using Multitest CD3/8/45/4 kits with TruCount tubes (TruCount) and full blood counts on a HMX haematological analyser (Coulter). Full blood counts were not obtained on two samples because of insufficient blood. No other data are missing. TruCount is an established flow cytometric CD4 counting assay that generates both an absolute CD4 count, %CD4/lymphocyte and total lymphocyte count and has excellent repeatability and quality assurance scores [15]. Data were acquired on a FACSCalibur flow cytometer (Becton Dickinson) and analysed using MultiSet software, blinded to the paired result for each sample. The main outcome measure was agreement between finger-prick and venous samples assessed by estimating bias and limits of agreement (bias ±1.96sd) with 95% confidence intervals as described by Bland and Altman [16].

The median age of the 110 participants with analysable samples was 33 years (range 20 to 64). Sixty-nine% were female, 5% were pregnant 26% were taking ART, 14% were on treatment for current bacterial, fungal or TB infection and 15% were febrile (axillary temperature >37.5°C). At the time of sampling or when started on ART, 21% of participants had WHO clinical stage I HIV/AIDS, 31% stage II, 30% stage III and 18% stage IV. No participants complained about either blood sampling procedure and no adverse events were reported. Median CD4 counts on venous blood samples were 277 cells/ul (range 2 to 1121) for absolute counts and 13.28% (0.16% to 38.31%) for %CD4/lymphocyte.

Agreement between paired CD4 counts was good with little bias and narrow limits of agreement (Figure). Finger-prick CD4 values were higher than venous CD4 counts by an average of 6.6 cells/ul (95%CI 1.0,12.0), with limits of agreement -50.7 cells/ul (95%CI -60.3,-41.2) and 63.7 cells/ul (95%CI 54.2,73.3). Total lymphocyte counts (by TruCount) were higher in finger-prick blood with a bias of 183 cells/ul resulting in higher venous compared with finger-prick %CD4/lymphocyte values with a bias of 0.71% (95%CI 0.44,0.97) and limits of agreement -2.07% (95%CI -2.53,-1.61) and 3.48% (95%CI 3.02,3.95). Total leucocyte counts were also higher in finger-prick blood compared with venous blood, with a mean difference 949 cells/ul.

When expressed as a percentage of the mean value for each cell type count, the biases for the CD4+ lymphocyte, total lymphocyte and total leucocyte counts are 2.2%, 8.2% and 15.2% respectively. As previously observed [12,13] these figures suggest that the difference between leucocyte counts in venous and finger-prick blood samples is largely due to large leucocytes rather than lymphocytes.

The study was conducted under optimum blood sampling conditions. Use of lancets with smaller blades might necessitate excessive finger squeezing to produce a blood sample, leading to dilution of the sample with tissue fluid. However, rapid diagnostic tests are likely to require less than the 250 ul blood collected from each finger-prick in this study so this may not be a major issue.

These data indicate that provided careful sampling technique is followed, finger prick blood samples could be used in place of venous blood samples in HIV-infected African adults for both absolute CD4 counts and %CD4/lymphocyte values. This potentially increases the accessibility for CD4 counting in resource-poor settings, especially once rapid tests become widely available.

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Figure.

Comparison of CD4 counts determined for finger-prick blood and venous blood samples from 110 HIV-infected patients. Solid horizontal lines depict bias and upper and lower limits of agreement. Dashed lines denote 95% CIs for these values. Mean counts are the average of the finger-prick and venous CD4 counts for each individual. **A:** absolute CD4 counts, **B:** %CD4/lymphocyte values.