AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 35 NUMBER 2 JUNE 2006

ISSN 1116—4077

Quality control of the total lymphocyte count parameter obtained from routine haematology analyzers, and its relevance in HIV management.

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Summary

Lymphocyte subsets/CD4 T Helper cell enumeration in HIV care and treatment in resource constrained settings can be difficult to ascertain as a result of the lack of the necessary instrumentation, capacity and infrastructure. However, it is imperative to gain such information for patient monitoring in HIV. The Total Lymphocyte Count (TLC) is useful as a surrogate marker for CD4 count as recommended by the World Health Organisation (WHO) and to calculate CD4% for paediatric use. This study therefore sets out to determine and compare the accuracy of the total lymphocyte counts obtained from three haematology analysers designated A, B and C, that are in regular use for routine haematological parameters at the main referral hospital in Barbados, West Indies. The TLC of 263 HIV treatment naïve individuals attending the HIV Reference Unit in Barbados were enumerated on the three haematology analysers. The 'lymphosum' (Sum of lymphocyte subsets: T-helper cell, T-cytotoxic cells, B lymphocytes and Natural killer cells) should be equal to the TLC, and is derived by immunophenotypic analysis on a 4-colour flowcytometer. Machine C had the highest positive correlation between the TLC and the lymphosum with and R^2 of 0.9031 compared to machine A with an R^2 values of 0.7119 and Machine B with R^2 values of 0.637. These results show that there can be dramatic inaccuracies when using routine haematology analysers for both routine use, as a surrogate marker of CD4 or for derivation of CD4% in HIV management. It further demonstrates that all haematology analyzers require some form of Quality control. The possible lack of accuracy of the TLC by haematology analysers should be taken into consideration when following the recommendations of the WHO in resource poor settings or using it as a denominator for calculating CD4%.

Keywords: *Flowcytometry, total lymphocyte count, CD4, haematology analyzer*

Résumé

L'énumeration des sous-type de lymphocytes durant les soins du VIH et le traitement en locations de resources limités peut etre difficile a évaluer du au manque des appariels appropriés, la capacité et les infrastructures. Cependant il est impératif documenté telle information pour les soins de ces patients. L'estimation des lymphocytes totale et est utile comme un markeur par taux des cellules CD4 recommendé par l'OMS et calculer le pourcentage des CD4 pour l'utilisation pédiatrique. Cette étude était faite pour determiner et comparaitre la précision de l'estimation totale des cellules lymphocytaires et les paramétres hématologiques sur 3 machines d'analyse hématologique A,B et C dans l'hopital de Barbados. La differentiation des lymphocytes totale(T et B) sur 263 patients VIH visitant l'unité de reference des patients VIH a Barbados étaient énumerés sur les 3 machines et l'analyse du immunophénotype par un cytometre a quatre couleurs. La machine C avait une correlation plus positive entre l'estimation totale des lymphocytes et le lymphosum(R^2=0.9031) compare a la achine A(R^2=0.7190)et la machine B(R^2=0.637). Ces résultats montrent que il peut avoir des imprécisions dramatique de mesures durant l'usage routiniere des analysateurs hématologiques necessitant des controles permanent de qualité. Le markeur CD4 pourrait etre utilisé durant le ménagement du VIH tout en réduisant les dérivations de pourcentage des CD4. Le manque de précise dans l'estimation des lymphocytes totale par ces appariels doivent etre considerés suivant les recommendations de L'OMS en pays sous-developpés ou den utilisant un dénomiteur commun pour calculer les pourcentage des cellules CD4.

Introduction

The determination of absolute CD4 T Helper cell counts and quantification of plasma HIV RNA levels are predominantly used to monitor disease progression and make clinical decisions in HIV-infected persons. However treatment and management strategies in HIV disease still poses a challenge in developing nations due to high costs of therapy and monitoring of immunological and virological parameters.

Many regions in resource constrained locations in the developing world are not able to afford to implement these high cost technologies in order to effectively manage persons with this disease.

Consequently there is an increasing need to provide a 'salvage' service in situations where enumeration of CD4 counts by expensive techniques are not economically feasible. For this reason various parameters have been instituted as substitutes for a CD4 count by the World Health Organization (WHO) suggesting using a total lym

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phocyte count (TLC) as a surrogate marker for the CD4 count, implying that a TLC obtained on a haematology analyzer of less than 1200 is an indication of a CD4 count of less than 200 [1,2,3,4].

To generate a CD4% count which is the preferred parameter for paediatric cases [5], will increase the cost of flowcytometry protocols. Therefore there are some platforms and kits that can only generate an absolute CD4 counts. In this scenario the absolute CD4 count is divided by the TLC and multiplied by 100 to get a CD4% value. The TLC is derived from a second instrument and therefore an additional source of error.

[CD4% =CD4Absolute count divided by the TLC and multiplied by 100]

In laboratories worldwide, determination of the TLC is a routine test determined using automated haematology analysers, which rely on physical properties of white blood cells such as size and complexity to differentiate between the subtypes to derive a differential count. Using flowcytometry the same parameters can be determined using monoclonal markers, in addition to physical properties to generate more accurate and specific enumeration of the subsets of the white blood cell count. By this technique a derivative called the lymphosum is the sum total of all the lymphocyte subsets, comprised of the T helper cell, T suppressor cells, B lymphocytes and Natural killer cells using CD45, CD3, CD4, CD8, CD19 and CD56 monoclonals to distinguish and enumerate. Two lymphosums can be derived from these monoclonals. Lymphosum 1 derived from the sum of: CD4/CD8/CD56/CD19 and lymphosum 2 which is derived from the sum of CD3/CD56/CD19. Lymphosum which will capture the small proportion of gamma delta T-cells that would be missed in lymphosum 1. In this study the latter lymphosum was used as it is a more accurate measure of TLC.

The TLC derived from a routine haematology analyzer should have a good correlation with the lymphosum derived from a flowcytometer if the analyzer is measuring white cells differentials accurately, and should be a prerequisite quality control step before the TLC can be utilized as a surrogate marker particularly in resource poor settings where routine haematology analyzers may be old or may have been refurbished and under serviced.

This study has evaluates the accuracy of three makes of haematology analyzers used in routine laboratories to obtain a TLC by comparison with the lymphosum derived by flowcytometry which is considered to be the gold standard. If results from haematology analysers are going to be used to make important clinical decisions, the clinician must be assured that the results obtained will be as accurate and precise as possible [6,7].

Materials and methods

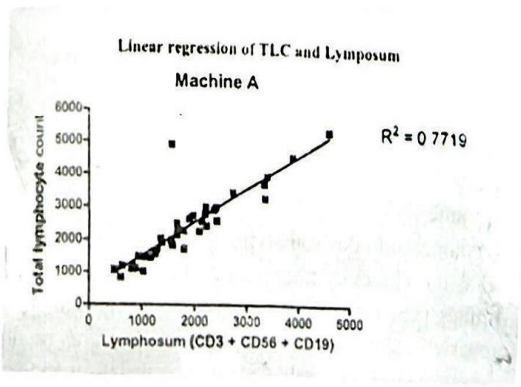
EDTA anticoagulated samples from 263 treatment naïve HIV positive individuals attending a dedicated HIV referral centre in Barbados were randomly selected from routine batches for lymphocyte subset determinations. The samples were prepared following the manufacturer's protocol using a commercial mixture of monoclonal antibodies (mAbs) CD45-FITC, CD4-PE, CD8-ECD, CD3-PC5, CD56-RD1 and CD19-ECD (Beckman Coulter Inc., Florida, USA). The flowcytometry laboratory has a routine quality assurance arrangement with Health Canada international.

Method: 100µL of whole blood was mixed with 10µL of mAbs and incubated for 10mins after which the cells were lysed with 500µL of Optilyse-C solution and incubated for another 10mins. 500µL of PBS solution was added and the samples were then stored at 4°C and tested within 24 hrs on the Beckman Coulter Epics XL flowcytometer. Flow-Set™ and Flow-Check™ fluorospheres were used as quality control measures to adjust the voltages and align the lasers on the machine.

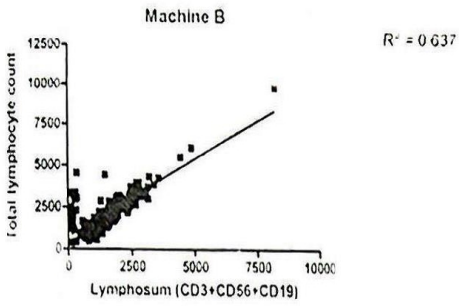
The Tetra-one software was used to determine the percentages and absolute counts of the lymphocyte subsets. Total lymphocyte counts were assessed on the same day of collection on three haematology analysers that are in routine use in the main referral hospital laboratory. The models of the machines have not been disclosed, however the machines were referred to as Machine A (15 years old), B (8 years old) and C (4 months old). The lymphosums were categorized as Lymphosum 1 and 2 where lymphosum 1 is the sum of CD4, CD8, CD56, and CD19 and lymphosum 2 is the sum of CD3, CD56 and CD19.

Results

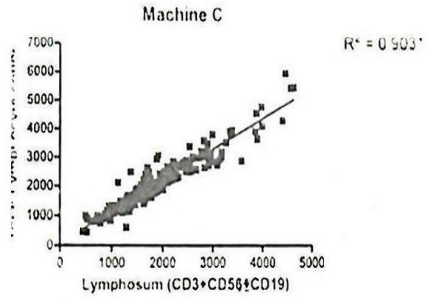
The highest correlations between the TLC and the lymphosum were seen in Machine C with an R^2 values 0.9031 followed by Machine A with an R^2 values of 0.7719 and Machine B had the poorest and unacceptable R^2 correlation of 0.637. (See graphs showing coefficient of correlation.)



Machine A



Machine B



Machine C

Discussion

The use of a TLC as a surrogate marker is based on the fact at TLC is the sum of all lymphocytes comprised of T-cells (CD3), B-cells (CD19) and natural killer cells (CD56), and can therefore be implemented in lieu of the enumeration of lymphocyte subsets by flowcytometry [1]. However, when using TLC as a surrogate marker for CD4 or as a denominator for calculating CD4%, it is essential to determine the accuracy of the haematology analyzer to be used. It is a well-accepted fact that with age the accuracy of laboratory equipment may begin to decline and results may not be as reliable. Although machine C which was almost brand new gave a better relationship of the parameters than machine A and B, both of the older machines did not have a significant difference between them. Therefore a TLC should only be used as a substitute for the lymphocyte subsets in setting where the analysis of these subsets by flowcytometry is not available. This is rather a disappointing outcome and demonstrates that distinguishing between white blood cells based on physical properties instead of surface cluster designated (CD) markers is fraught with error [6].

Although flowcytometry may be considered as the optimum standard for lymphocyte subset evaluation this method also has its own limitations that can range from pipetting errors, laser beam decay to the lack of maintenance of the machine [7].

Therefore it is wise to note that whichever system is used, quality control checks must be incorporated into routine laboratory practices to ensure that the test results from a haematology analyser or a flowcytometer are accurate and precise. In the developing world, machines may be old, refurbished or poorly maintained, which makes the need for such quality control checks to be embarked upon before clinical decision are made based on derivative assumptions.

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Accepted: 22/02/06