Sensitivity and specificity of the mean corpuscular volume and CD4/CD8 ratio in discriminating between rifampicin resistant and rifampicin sensitive tuberculosis

Joseph Baruch Baluku \textsuperscript{a,b,g,*}, Joseph Musaazi \textsuperscript{c}, Rose Mulwana \textsuperscript{a}, Derrick Bengo \textsuperscript{d}, Christine Sekaggya Wiltshire \textsuperscript{c,e}, Irene Andia-Biraro \textsuperscript{e,f}

\textsuperscript{a} Mulago National Referral Hospital, Pulmonology Division, PO Box 7051 Kampala, Uganda
\textsuperscript{b} Makerere University Lung Institute, PO Box 7749 Kampala, Uganda
\textsuperscript{c} Makerere University College of Health Sciences, Infectious Disease Institute, PO Box 7072 Kampala, Uganda
\textsuperscript{d} Mulago Hospital, Department of Clinical Hematology, PO Box 7051 Kampala, Uganda
\textsuperscript{e} Makerere University College of Health Sciences, Department of Internal Medicine, PO Box 7072 Kampala, Uganda
\textsuperscript{f} Makerere University, Uganda Virus Research Institute Center of Excellence in Training Programme on Infections and Immunity (MUII-PLUS), PO Box 49 Entebbe, Uganda
\textsuperscript{g} Mildmay Uganda, P.O Box 24985 Kampala, Uganda

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\textbf{ABSTRACT}

Background: There is need for simple, cost effective and widely available point of care tests for low level health facilities in developing countries to screen for drug resistant tuberculosis (TB) after bacteriological confirmation of TB by smear microscopy. We evaluated the sensitivity and specificity of the mean corpuscular volume (MCV) and CD4/CD8 ratio in discriminating between rifampicin resistant (RR-TB) and rifampicin sensitive (RS-TB) tuberculosis.

Methods: We performed a secondary analysis of data from a cross sectional study that enrolled adult participants with bacteriologically confirmed pulmonary TB at a national tuberculosis treatment center in Uganda. Blood samples were tested for CD4 and CD8 cell counts, HIV serology and a full hemogram. Rifampicin sensitivity and the bacillary load grade were determined by Xpert MTB/RIF\textsuperscript{®}. Fifty-five participants that had RR-TB (cases) were matched with 110 participants that had RS-TB (controls) for age, sex and HIV status in a ratio of 1:2 respectively. Sensitivity (Se), specificity (Sp), area under curve (AUC) analysis and determination of optimal cut-offs were performed using receiver operating characteristic curves.

Results: Cases differed from controls with respect to residence ($p = 0.031$), bacillary load grade ($p < 0.010$) and MCV ($p = 0.021$). The Se, Sp and AUC of the MCV (cut-off of $> 74.6$ femtolitres (fl)) were 88.9%, 34% and 0.607 ($p = 0.021$) respectively for RR-TB. Among HIV positive participants, the respective Se, Sp and AUC of the MCV for RR-TB (cut-off of $> 72.5$ fl) were 97.2%, 22.2% and 0.608 ($p = 0.061$). The respective Se, Sp and AUC of the CD4/CD8 ratio (cut-off of $> 0.40$) were 67.3%, 50.0% and 0.559 ($p = 0.199$) on the overall and 54.1%, 71.6% and 0.628 ($p = 0.024$) among the HIV positive participants for RR-TB.

Conclusion: The MCV had a high sensitivity but very low specificity for RR-TB. The CD4/CD8 ratio had a low sensitivity and specificity for RR-TB among HIV positive individuals. The utility of either test is low due to low diagnostic accuracy.

1. Introduction

Drug resistant tuberculosis (DR-TB) is an emerging global threat to tuberculosis (TB) control and over 500,000 TB cases were estimated to be drug resistant in 2018 [1]. Notwithstanding, the World Health Organisation global TB report of 2019 indicates that $< 40\%$ of these cases were notified. There are delays in DR-TB diagnosis and treatment initiation in resource limited settings due to low access to drug...
susceptibility testing in low level health care facilities [2-5]. Patients that experience delays in diagnosis and treatment initiation are more likely to have poor treatment outcomes and high attrition [6-7]. Although introduction of rifampicin resistance testing via molecular methods reduces diagnosis turn-around-time and treatment initiation delays, only 35% of newly diagnosed TB patients in Africa get a rifampicin drug susceptibility test performed [8-9]. Further, in low income countries that have diagnostic sites with Xpert MTB/RIF® machines for rifampicin resistance testing, this service is generally underutilised and high costs are associated with the service in low level health care facilities [10-12]. Moreover, scaling up Xpert MTB/RIF® testing would need significant infrastructural and human resource investment that may not be afforded by resource limited countries in the short term [13]. While using non-laboratory technicians in rural areas to operate the Xpert MTB/RIF® machines is feasible, the cost-effectiveness of this intervention needs to be evaluated [14]. There is therefore a need for simple, cost effective and widely available point of care tests for low level health facilities in developing countries to screen for DR-TB, after TB confirmation by microscopy, to facilitate early referral.

The mean corpuscular volume (MCV) correlates with levels of exhaled nitric oxide [15], a marker of pulmonary inflammation that promotes increased T-helper 2 (Th2) immune responses [16]. Studies have demonstrated a local and systemic anti-inflammatory Th2 response in DR – TB that significantly differs from that observed in drug sensitive tuberculosis (DS – TB) [17-20]. However, the relationship between the MCV and drug resistant TB is unknown. Bench-top portable hemoanalyzers are available and can be used to measure red cell indices in low income settings should there be a role of the MCV and other red cell indices in the diagnosis of RR – TB [21].

The CD4/CD8 ratio has also been shown to be significantly different in patients with DR-TB when compared to patients with DS-TB [22-24]. Moreover, CD4 and CD8 testing is widely available, cost effective, acceptable and could be adapted to the existing HIV diagnosis cascade available in resource limited settings [25].

The objective of this study was to evaluate the sensitivity and specificity of the MCV and CD4/CD8 ratio in discriminating between rifampicin resistant TB (RR-TB) and rifampicin sensitive TB (RS-TB). The null hypothesis was that at an optimal cut-off, the area under the curve (AUC) of the receiver operating characteristic (ROC) curves for the aforementioned parameters would be = 0.5.

2. Materials and methods

2.1. Study population and setting

We performed a secondary analysis of data from a cross sectional study at a national tuberculosis treatment center in Uganda conducted between August 2017 and March 2018 [26]. Participants in the primary study were 18 years and older with bacteriologically confirmed TB. Blood samples were tested for HIV serology, CD4 and CD8 T-cell counts and a full hemogram. HIV testing was performed using an immunochromatographic rapid test (Alere Determine™ HIV-1/2) and a positive test was confirmed by sequential testing with another immunochromatographic test (Chembio HIV 1/2 STAT-PAK™) following the Uganda national HIV testing algorithm [27]. Determination of the CD4 and CD8 T-cell counts was performed by flow cytometry using a flow cytometer (BD FACSCalibur™) at Makerere Joint AIDS Program laboratory. Parameters of the hemogram were determined by a hemoanalyser (Sysmex® Automated hematology analyser XN series – XN 1000) at Mulago Hospital hematology laboratory. Bacillary load grade was determined by cycle threshold (Ct) values of a nucleic acid amplification test (Xpert MTB/RIF®) as follows: very low (Ct > 28), low (Ct 22-28), medium (Ct 16-22) and high (Ct < 16) [28]. In the current analysis, we included 55 participants with bacteriologically confirmed TB and rifampicin resistance reported by Xpert MTB/RIF® (Cepheid, USA) as cases. Cases were matched for age (+/- 3 years), sex and HIV status with 110 participants with bacteriologically confirmed RS-TB (controls) in a ratio of 1:2 (Cases: Controls). The effect of age, sex and HIV status on the MCV and CD4/CD8 ratio among TB patients necessitated this matching [29-30]. Eight participants with malaria infection were excluded due to the effects of malaria on the MCV and CD4/CD8 ratio [31-32].

2.2. Study measurements

Demographic and clinical characteristics, hemogram parameters, CD4 and CD8 T-cell counts, HIV serology status and bacillary load grade
were extracted from the primary study dataset. The study outcomes were the optimal cut offs and corresponding specificity, sensitivity and AUC for the MCV and CD4/CD8 ratio. We further performed a sub-group analysis for the sensitivity, specificity and AUC of the MCV and CD4/CD8 ratio among the HIV positive individuals. This is because of the variability of the MCV and CD4 counts among HIV/TB co-infected in individuals who constituted 67% of the study population [33–35].

2.3. Statistical analysis

Data was analysed with STATA 14.2 (StataCorp, College Station, TX, USA) and MedCalc (MedCalc Software, Ostend, Belgium) was used for ROC curve analysis. Participants’ characteristics were described using frequencies and percentages and compared between cases and controls using McNemar test for categorical variables. Continuous variables were summarised as medians with corresponding interquartile ranges and compared between cases and controls using signed-ranks test. We evaluated correlation between the MCV and the variables that were significantly different between cases and controls (bacillary load and residence type) using Spearman’s correlation coefficient. Further, we fitted variables that were significantly different between cases and controls in a conditional logistic regression model for factors associated with RR-TB and controlled for the haemoglobin level and body mass index (a proxy of nutritional status). For the sensitivity and specificity analysis, having RR-TB was considered the positive comparator while having RS-TB was the negative comparator. The point estimate on the ROC curve whose sensitivity and specificity gave the maximal Youden’s index was considered to be the optimal cut-off and its corresponding sensitivity, specificity and AUC is reported [36]. The MCV and CD4/CD8 ratio were considered to have discriminating ability between RR-TB and RS-TB if the AUC was significantly different from the null value of 0.5 (null: AUC = 0.5). Statistical significance was set at p ≤ 0.05 at the 95% confidence interval. Confidence intervals for sensitivity and specificity were obtained using bootstrap method.

2.4. Ethical approval and consent to participate

Study participants provided written informed consent to participate in the primary study that included consent for secondary analyses. The study was approved by the Department of Internal Medicine Scientific Review Committee (SRC) and the School of Medicine Research and Ethics Committee of Makerere University College of Health Sciences.

3. Results

From the primary study dataset, 363 participants were screened for eligibility for this analysis. Fig. 1 shows the study flow.

Table 1

| Characteristic                        | Cases (N = 55) | Control (N = 110) | p-value |
|---------------------------------------|---------------|------------------|---------|
| Sex                                   |               |                  |         |
| Male                                  | 29 (52.7)     | 58 (52.7)        | 0.999   |
| Female                                | 26 (47.3)     | 52 (47.3)        |         |
| BMI < 18.5 (kilograms/meters²)        | 26 (47.3)     | 52 (47.3)        | 1.000   |
| Age in years, median (IQR)            | 34 (27 – 39)  | 33 (27 – 39)     | 0.906   |
| HIV status                            |               |                  |         |
| HIV positive                          | 37 (67.3)     | 74 (67.3)        | 0.999   |
| HIV negative                          | 18 (32.7)     | 36 (32.7)        |         |
| CD4 counts (cells/mL), median (IQR)   | 387 (146 – 613)| 281 (136 – 502) | 0.110   |
| CD4/CD8 ratio, median (IQR)           | 0.59 (0.28 – 1.52) | 0.41 (0.21 – 0.449) | 0.572   |

ART use

| Current ART usage                     | 23 (41.8)     | 41 (37.3)        | 0.572   |
| No ART usage                          | 32 (58.2)     | 69 (62.7)        |         |
| ART regimen¹                          | 3 (13.0)      | 9 (9.8)          | 0.508   |
| AZT/3TC/EFV                           | 3 (13.0)      | 9 (9.8)          |         |
| TDF/3TC/EFV                           | 13 (56.5)     | 29 (29.7)        |         |
| TDF/FTC/EFV                           | 4 (17.4)      | 7 (17.01)        |         |
| Other ART regimen                     | 3 (8.7)       | 1 (2.4)          |         |

Bacillary load grade

| Very low                               | 22/51 (43.1)  | 16/103 (15.5)   | <0.001* |
| Low                                    | 14/51 (27.5)  | 26/103 (25.2)   |         |
| Medium                                 | 9/51 (17.7)   | 43/103 (41.8)   |         |
| High                                   | 6/51 (11.7)   | 18/103 (17.5)   |         |
| Residence                              |               |                  |         |
| Rural                                  | 27 (49.1)     | 35 (31.8)       | 0.031*  |
| Urban                                  | 28 (50.9)     | 75 (68.2)       |         |
| History of TB treatment                | 11 (20.0)     | 21 (19.1)       | 0.889   |
| Ever smoked in last 6 months           | 13 (23.6)     | 26 (23.6)       | 0.999   |
| Ever used alcohol in last 6 months     | 27 (49.1)     | 57 (51.8)       | 0.741   |

ART- Antiretroviral therapy, AZT - Zidovudine, FTC - Emtricitabine, 3TC – Lamivudine, EFV- Efavirenz, BMI – Body mass index, IQR – Interquartile range.

*p-value was considered to be statistically significant at p < 0.05.

Table 2

| Hemogram parameter | Cases Median (IQR) | Control Median (IQR) | p-value |
|--------------------|-------------------|----------------------|---------|
| MCV (fl)           | 82.65 (76.5 – 93.80) | 81.55 (71.50 – 88.20) | 0.021*  |
| MPV (fl)           | 9.50 (8.90 – 10.20)  | 9.40 (8.60 – 10.10)  | 0.271   |
| MCH (pg)           | 27.15 (24.10 – 30.80)| 26.20 (24.10 – 29.40)| 0.184   |
| PDW (fl)           | 9.80 (9.90 – 10.90)  | 9.60 (8.60 – 12.20)  | 0.951   |
| White cell count   | 5.55 (4.38 – 7.12)   | 5.82 (4.28 – 7.30)   | 0.141   |
| Neutrophil count   | 3.06 (1.74 – 4.98)   | 3.81 (2.55 – 5.36)   | 0.567   |
| Lymphocyte count   | 1.27 (0.95 – 1.68)   | 1.22 (0.68 – 1.56)   | 0.261   |
| Monocyte count     | 0.44 (0.38 – 0.65)   | 0.45 (0.34 – 0.71)   | 0.516   |
| Eosinophil count   | 0.05 (0.01 – 0.21)   | 0.05 (0.01 – 0.11)   | 0.100   |
| Basophil count     | 0.03 (0.01 – 0.04)   | 0.02 (0.01 – 0.05)   | 0.705   |
| RBC count†         | 4.44 (3.52 – 5.10)   | 4.38 (3.45 – 5.28)   | 0.829   |
| Platelet count     | 304 (240 – 387)      | 296 (211 – 413)      | 0.697   |
| Hematocrit (%)     | 37.20 (32.7 – 42.0)  | 35.10 (28.6 – 41.6)  | 0.739   |
| Hemoglobin (g/dl)  | 12.35 (9.90 – 14.10) | 11.65 (9.40 – 13.50)| 0.265   |
| PMR                 | 621 (416 – 900)      | 624 (473 – 1017)     | 0.962   |
| NLR                 | 2.43 (1.67 – 5.34)   | 3.06 (2.38 – 5.52)   | 0.536   |
| PLR                 | 243 (154 – 459)      | 265 (198 – 402)      | 0.522   |
| LMR                 | 2.97 (1.63 – 3.91)   | 2.47 (1.56 – 3.21)   | 0.127   |
| WNR                 | 1.63 (1.29 – 1.95)   | 1.47 (1.31 – 1.65)   | 0.091   |

*p-value was considered to be statistically significant at p < 0.05.

Table 3

| Characteristic | Adjusted odds ratio (95% confidence interval) | p-value |
|----------------|----------------------------------------------|---------|
| Mean Corpuscular volume | 1.03 (0.99 – 1.08) | 0.132   |
| Volume          |                                              |         |
| Bacillary load  |                                              |         |
| Very low/low    | 7.47 (2.3 – 24.08) | 0.001*  |
| Medium/High     |                                              |         |
| Residence       |                                              |         |
| Rural           | 2.97 (0.99 – 8.90) | 0.052   |
| Body mass index | 1.07 (0.94 – 1.22) | 0.287   |
| Haemoglobin level | 0.93 (0.76 – 1.12) | 0.437   |

*p-value was considered to be statistically significant at p < 0.05.
3.2. Sensitivity, specificity and AUC of the MCV and CD4/CD8 ratio

At a cut-off of > 74.6 femtolitres (fl), the sensitivity, specificity and AUC of the MCV was 88.9%, 34% and 0.607 (p = 0.021) respectively. Among the HIV positive participants, the sensitivity, specificity and AUC of the MCV (cut-off of > 72.5 fl) was 97.2%, 22.2% and 0.608 (p = 0.061). Similarly, the sensitivity, specificity and AUC of the CD4/CD8 ratio at a cut-off of > 0.40 was 67.3%, 50.0% and 0.559 (p = 0.199) respectively. At the same cut off, the CD4/CD8 ratio had a respective sensitivity, specificity and AUC of 54.1%, 71.6% and 0.628 (p = 0.024) among the HIV positive participants. The sensitivity, specificity and AUC of other hemogram parameters are shown in Table 4. The ROC curves for the MCV and CD4/CD8 ratio on the overall and among the HIV positive participants are shown in Fig. 2, Fig. 3, Fig. 4 and Fig. 5 in the appendix.

4. Discussion

In this study we evaluated the sensitivity and specificity of the MCV and CD4/CD8 ratio in discriminating between RR-TB and RS-TB. The MCV demonstrated a high sensitivity (88.9%) for RR-TB that seemed to increase among HIV positive participants (97.2%). However, the specificity was very low. Therefore, the diagnostic value of the MCV is low due to low diagnostic accuracy. If bacteriologically confirmed TB patients with an MCV > 74.6 fl are prioritised for referral for drug susceptibility testing, the low specificity of the MCV implies that TB patients may falsely be classified as having RR – TB. This may increase the rate of unnecessary referral for DST. Moreover, the optimal cut-off, at which we found a high sensitivity, is too low compared to the median MCV among patients with RR – TB and RS – TB. This further demonstrates the low utility of the test. Additionally, lower health facilities would still need infrastructural and technical competence to perform haematological tests for MCV and may therefore not be cost effective as well.

It was apparent from our analyses that the observed significant difference in the median MCV between the cases and controls was confounded by bacillary load and rural residence. Being a marker of pulmonary inflammation, the MCV could be affected by the bacillary load in the lungs; with higher bacillary loads correlating with microcytosis (low MCV) [15] . Our results suggest this negative correlation, exceptibility testing, the low specificity of the MCV implies that TB patients with an MCV < 74.6 fl may be missed. This may increase the rate of unnecessary referral for DST. Moreover, the optimal cut-off, at which we found a high sensitivity, is too low compared to the median MCV among patients with RR – TB and RS – TB. This further demonstrates the low utility of the test. Additionally, lower health facilities would still need infrastructural and technical competence to perform haematological tests for MCV and may therefore not be cost effective as well.

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3.1. Characteristics of study participants

The median age of study participants was 34 (interquartile range (IQR): 27–39) years and 52.7% (87/165) were males. Of the study participants, 111 (67.3%) were HIV positive. Cases and controls differed in regards to residence (p = 0.031), bacillary load grade (p < 0.001) and the MCV (p = 0.021). The other characteristics and hemogram parameters of study participants are shown in Table 1 and Table 2 respectively. There was weak correlation between the MCV with bacillary load (r = -0.264, p = 0.001) and residence (r = -0.159, p = 0.044) as well as between residence with bacillary load (r = 0.162, p = 0.045). At conditional logistic regression analysis, only very low/bacillary load (adjusted odds ratio = 7.47 95% confidence interval (2.32 – 24.08), p = 0.001) was associated with RR – TB (Table 3).
Changes in the CD4/CD8 ratio among HIV positive individuals have been linked to several pulmonary conditions: lung cancer [43], emphysema [44] and COPD [45]. Also, the CD4/CD8 ratio of < 0.7 predicts HIV infection with a sensitivity of 100% and specificity of 94% among tuberculosis patients [46]. It is unknown whether the predictive value of the CD4/CD8 ratio is different between RR-TB and RS-TB patients co-infected with HIV. Our results suggest that the CD4/CD8 ratio is able to discriminate between RR-TB and RS-TB among HIV positive individuals albeit with a low sensitivity. This limits its utility as a screening test.

A key limitation of our study is the small sample size that could have affected our ability to observe statistical significance in the diagnostic performance of other hemogram parameters. Also, the MCV measurement is affected by hyperlipemia, hyperglycaemia, uremia and other biochemical factors which we did not evaluate for [47].

5. Conclusion

The MCV at a cut-off of 74.6 fl had a high sensitivity for RR-TB but very low specificity. Its utility as a screening test for RR-TB among bacteriologically confirmed TB patients is therefore likely to be low. The CD4/CD8 ratio had a low sensitivity and specificity for RR-TB as well. There is still need for simple and cost effective tests that can be used to screen for DR – TB after confirming TB, by say microscopy, in settings where TB DST is unavailable. A key limitation of our study is the small sample size that could have affected our ability to observe statistical significance in the diagnostic performance of other hemogram parameters. Also, the MCV measurement is affected by hyperlipemia, hyperglycaemia, uremia and other biochemical factors which we did not evaluate for [47].

Ethical Statement

Study participants provided written informed consent to participate in the primary study that included consent for secondary analyses. The study was approved by the Department of Internal Medicine Scientific Review Committee (SRC) and the School of Medicine Research and Ethics Committee of Makerere University College of Health Sciences.

CRediT authorship contribution statement

Joseph Baruch Baluku: Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft, Writing - review & editing. Joseph Musaazi: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - review & editing. Rose Mulwana: Data curation, Project administration, Writing - review & editing. Derrick Bengo: Data curation, Methodology, Writing - review & editing. Christine Sekaggya Wiltshire: Methodology, Supervision, Validation, Writing - review & editing. Irene Andia-Biraro: Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix: Receiver operating characteristic curves for the MCV and CD4/CD8 ratio

Fig. 2. ROC curve for sensitivity Versus 1-Specificity of MCV as a biomarker in predicting RR-TB on the overall.

Fig. 3. ROC curve for sensitivity Versus 1-Specificity of MCV as a biomarker in predicting RR-TB among HIV positive individuals.

Fig. 4. ROC curve for sensitivity Versus 1-Specificity of CD4/CD8 ratio in predicting RR-TB on the over all.
Fig. 5. ROC curve for sensitivity Versus 1-Specificity of CD4/CD8 ratio as a biomarker in predicting RR-TB among HIV positive Participants.

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