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RESEARCH

The use of readily available biomarkers to predict CD4 cell counts in HIV-infected individuals

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Background: The use of readily available biochemical investigations to predict the CD4 cell count in HIV-infected patients may provide clinicians with insight regarding disease severity at first contact. The aims of the study were to determine the relationship of calculated globulin and white cell count (WCC) with CD4 cell count.
Methods: Data were collected prospectively from ambulatory HIV-infected, anti-retro viral therapy (ART) naive patients at the HIV clinic of King Edward Hospital, Durban, between 2010 and 2012.
Results: The mean age of the participants was 39 ± 9.53 years and 70% were female. Median calculated globulin and WCC was 49 g/l and 4.74 × 10^9 cells/l respectively, whilst the CD4 cell count was 244 cells/mm^3. A significant positive correlation was demonstrated between CD4 cell count and WCC ($r = 0.25$, $p < 0.001$). WCC and albumin were identified as potential surrogate markers for CD4 count ≤ 200 cells/mm^3. Combination of WCC with either albumin or globulin predicts a CD4 count of less than 200 cells/ mm^3 with moderate accuracy.
Conclusion: The use of combined biomarkers may influence initiation of *Pneumocystis jiroveci* pneumonia prophylaxis in resource-limited settings. Further evaluation is warranted to assess the role of these markers in disease progression and ART.

Keywords: albumin, calculated globulin, CD4 cell count, HIV infection, White cell count

Introduction

The prevalence of human immunodeficiency virus (HIV) infection in the South African population was 12.2%; this corresponds to approximately 5.26 million infected people. An estimated 850 000 new HIV infections occurred in individuals between the ages of 15 and 49 in the year 2013.[1]

Various strategies have been developed to combat the HIV pandemic including early initiation of anti-retroviral therapy (ART) as well as prompt recognition of ART failure. Common HIV staging methods use the CD4 cell count or the presence of specific HIV-associated conditions to determine advanced disease and the need for ART. Limitations to the use of CD4 staging include the several-day delays in obtaining the result and the cost incurred. Ideally, the CD4 cell count result should be available at the time of post-test counselling.[2] Particularly in resource-limited settings, the use of alternative readily available biochemical tests to predict the CD4 cell count may improve patient enrolment in ART programmes and prevent patient loss to follow-up after obtaining their HIV test result.

Persistent HIV replication is associated with increased immune activation, which manifests itself in the B cell compartment as hypergammaglobunemia, polyclonal B cell activation, induction of terminal difference of B cells, increased levels of antibodies and increased frequency of B cell malignancy.[3] Increased globulin levels have been described in HIV-infected patients; however, data correlating calculated globulin levels to viral load and CD4 counts are lacking.[4,5] Calculated globulin can be readily determined from a routine liver function test by calculating the difference between total protein and albumin. The wide spectrum of haematological derangements in HIV-infected individuals includes both lymphopenia and leucopenia, while lymphopenia has been demonstrated in several studies to be an unreliable substitute marker for CD4 cell count and there is a paucity of studies that has investigated the total white cell count (WCC) as a suitable marker surrogate.[6–10]

The aims of the study were to determine the relationship of the calculated globulin measurement and WCC with CD4 cell count in HIV-infected individuals. We also sought to determine the predictive value of these biochemical tests individually and in combination for a specific threshold of CD4.

Methods

We collected data prospectively from HIV-1-infected patients at King Edward Hospital, Durban, KwaZulu-Natal. The study had a cross-sectional design; data for ambulatory HIV-infected patients referred to the HIV clinic between January 2010 and January 2012 were recorded. Males and females, 18 years and older who were ART naive, were recruited. Written informed consent was obtained from all participants. Patient demographic data, CD4 cell count, WCC, total protein and albumin were extracted and analysed.

Analysis was performed in Stata 13™ (StataCorp, College Station, TX, USA). Chi-squared tests were used to test the relationship between categorical variables and CD4 cell count category. The Wilcoxon rank-sum test was used to test whether the distribution of calculated globulin, WCC, albumin and total protein differed between the CD4 count groups (CD4 cell count ≤ 200 and > 200). Unadjusted logistic regression was used to assess the predictive value of each marker separately and adjusted logistic

regression was used to ascertain whether the markers were independent predictors of low CD4 cell count. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of each marker were calculated at predetermined cut-offs. The overall diagnostic accuracy of each marker was assessed by the area under the receiver operator curve (ROC). The optimal linear combination of the markers was found using estimated parameters from the logistic regression model. Ethical permission was obtained from the UKZN Biomedical Research Ethics Committee; BE312/12.

Results

A total of 172 HIV-positive, ART naive patients were included in the analysis, equally distributed between the CD4 cell count ≤ 200 cells/mm^3 and CD4 cell count > 200 cells/mm^3 groups. The mean age was 39 years and approximately 70% of the patients were female. The two CD4 groups were comparable in terms of age and gender (*p*-value 0.40 and 0.06 respectively). The median albumin and white cell count were significantly lower in patients with CD4 count ≤ 200 cells/mm^3 compared with those with CD4 > 200 cells/mm^3. The globulin level was higher in the low CD4 cell count group; however, this result was borderline statistically significant. The mean WCC was significantly higher in the low CD4 cell count group (4.8 × 10^9 cells/l versus 4.5 × 10^{9}cells/l) (see Table 1).

In multivariate logistic regression, WCC and albumin were independent predictors of a CD4 cell count of 200 cells/mm^3 and less, after adjusting for age, gender and globulin (Table 2).

Following the results found in Table 1, which were reaffirmed by the multivariate logistic regression model, white cell count and albumin were identified as potential surrogate markers for a CD4 count ≤ 200 cells/mm^3. Significant positive correlation was observed between white cell count and CD4 count ($r = 0.25$, *p*-value < 0.001), whilst a weak negative correlation exists with calculated globulin level (*p*-value 0.13).

The white cell count displayed moderate diagnostic accuracy, evidenced by the ROC, where it displayed an area under the curve (AUC) of 0.69 (Figure 1). The sensitivity, specificity, negative predictive value and positive predictive value associated with white cell count cut-offs between 3.75 × 10^9 cells/l and 5.75 × 10^9 cells/l were analysed. A cut-off of 4.25 ×10^9 cells/l and less confers a specificity of 80% and a sensitivity of 56%. Higher cut-offs conferred a higher sensitivity at the sacrifice of specificity. The overall diagnostic accuracy of globulin was poor (ROC area under the curve = 0.59). A globulin cut-off of more than 50 g/l resulted in a sensitivity of 50% and specificity of 61%.

Due to the finding that albumin was identified as a potential surrogate marker by data analysis and multivariate logistic

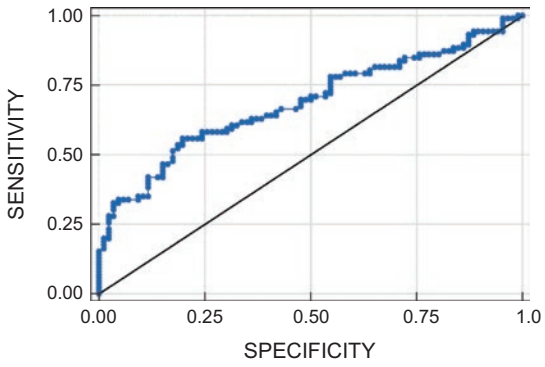

Figure 1: Receiver operator curve of white cell count

regression, further detailed analysis was performed. Albumin had also performed poorly as the sole marker of a CD4 cell count of 200 cells/mm^3 and less, evident by ROC area under the curve of 0.60. In spite of the adequate specificities achieved at cut-offs of less than 32 g/l, 33 g/l and 34 g/l, the sensitivity at each of these cut-offs was lower than 45%. Hence, at least 55% of patients requiring fast-tracked ART will erroneously be diagnosed as ineligible for treatment (i.e. diagnosed as having CD4 > 200 as opposed to ≤ 200).

Combining white cell count and albumin resulted in a slightly higher ROC, AUC compared with WCC alone (0.72 vs. 0.69). The ROC curve of the combined markers is presented in Figure 2. This optimal linear combination of the markers was WCC + 0.30 albumin, which enabled us to achieve a slightly higher sensitivity at fixed specificity compared with WCC alone. For ease of interpretation and clinical usage, the sensitivity and specificity for specific combinations are presented (Table 3). Combining WCC with

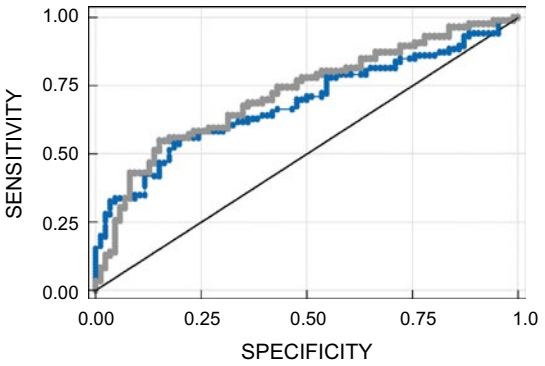

Figure 2: Receiver operator curve of the combined surrogate markers

Table 1: Demographics and the investigated variables between the two CD4 threshold cohorts

Factor	Total	CD4 ≤ 200 cells/mm^3	CD4 > 200 cells/mm^3	*p*-value
n	172	86	86	
% male	51 (29.6%)	28 (33%)	23 (27%)	0.404
	Mean (SD)			
Age (years)	38.6 (9.53)	40.0 (9.8)	37 (9.1)	0.060
	Median (IQR)			
Globulin	49 (42–56)	50.5 (43–60)	47 (42–55)	0.051
White cell count (x10^9 cells/l)	4.74 (3.72–6.16)	4.09 (3.13–5.46)	5.01 (4.41–6.4)	< 0.001
Albumin (g/l)	36 (33–39)	35 (32–38)	37 (34–39)	0.018
Total protein (g/l)	86 (79–91)	86 (80–92)	84.5 (79–90)	0.368

Table 2: Multivariate analysis of the predictors of a CD4 cell count ≤ 200

Factor	Univariate		Multivariate	
	Odds ratio	*p*-value	Odds ratio	*p*-value
Age (per 5-year increase)	1.17 (0.99, 1.37)	0.061	1.11 (0.94, 1.32)	0.224
Gender (Female vs. male)	0.76 (0.39, 1.46)	0.404	0.79 (0.39–1.63)	0.531
Log viral load (HIV RNA)	1.54 (1.06, 2.24)	0.024		
WCC	0.72 (0.60, 0.87)	0.001	0.69 (0.57, 0.84)	< 0.001
Globulin (per 5-unit increase)	1.14 (0.99, 1.31)	0.05	1.04 (0.88–1.23)	0.606
Total protein	1.02 (0.99, 1.05)	0.264		
Albumin (per 5-unit increase)	0.68 (0.49, 0.94)	0.019	0.61 (0.40, 0.93)	0.021

Table 3: Combination of markers at specific cut-offs

Combination	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
WCC ≤ 4.25 & albumin ≤ 34	76.7	57.0	64.1	71.0
WCC ≤ 4 & albumin ≤ 34	73.3	59.3	64.3	68.9
WCC ≤ 4.25 & globulin > 53	81.4	55.8	64.8	75

either globulin or albumin results in higher sensitivity at a lower sacrifice of specificity compared with WCC alone.

Discussion

Leukopenia has been suggested to correlate with severity of HIV disease and was prevalent in 88% of patients with AIDS at presentation.[7] WCC was found to have a significant positive correlation with CD4 cell count in this study, which is in keeping with the recent study by Vanker *et al*., which showed the CD4 cell count's correlation with WCC had a Spearman rho rank correlation of 0.484.[11] Calculated globulin had a negative correlation with CD4 cell count; however, both calculated globulin and WCC were found to be unreliable when used in isolation to predict CD4 cell counts of 200 cells/mm^3 and lower. There is a paucity of comparative studies evaluating the use of calculated globulin in HIV. In contrast to the investigated biomarker's individual performance as a surrogate, when used in combination there appears to be an increased predictive value. Albumin was shown to be a more robust tool than calculated globulin for predicting low CD4 cell count status. This is not surprising as a previous study supported its value as a useful surrogate for predicting severity of HIV infection; however, its practical application was not demonstrated.[12]

A reliable surrogate for the CD4 cell count in HIV-infected individuals from easily available biomarkers may arm the clinician with more insight into a patient's immune status and disease activity thereby potentially improving management during the initial assessment. It is apparent that there is a substantial loss of patient follow-up between HIV testing and ART initiation.[13] This may be prevented by making CD4 cell count results immediately available after HIV testing through point-of-care testing facilities.[14–16] This concept has been supported by a meta-analysis study, in which 15 sub-Saharan studies were reviewed and it was concluded that point-of-care CD4 testing increased patient retention in care, which may result in an upscale of ART. This was supported by the finding that the time from CD4 testing to receiving the result was reduced by 17 days.[14] Jani *et al*. have shown that through CD4 point-of-care testing, loss of patient follow-up decreased from 57% to 21% in the pre-ART patient, with an increase in ART initiation from 12% to 22%.[16] Furthermore Mamsallah *et al*. demonstrated that patients who received their results immediately were 2.1 times more likely to enrol for ART than patients who had to return for their results.[17] Therefore a reliable surrogate for CD4 cell count, which is available within hours, may become a valuable substitute for a point-of-care CD4 cell count test, having the potential ability to both decrease the number of HIV-infected patients lost to follow-up and increase the number of patients who initiate ART.

HIV-infected individuals with depleted CD4 cells are at risk for increased opportunistic infection and therefore the latest South African guidelines advocate fast tracking of therapy for patients with CD4 cell counts less than 200 cells/mm^3.[18–20] According to this study there is a 65% chance of predicting a CD4 cell count less than 200 if the white cell count together with albumin or calculated globulin thresholds is met. Patients with depleted CD4 cell counts less than 200 cells/mm^3 are more likely to develop *Pneumocystis jiroveci* pneumonia (PJP) amongst various other opportunistic infections.[21] The Centres for Disease Control and Prevention (CDC) advocates primary prophylaxis with co-trimoxazole for adults whose CD4 cell count is less than 200 cells/mm^3.[22] The majority of patients with CD4 counts of < 200 cells/mm^3 who were treated with PJP had a mortality of between 20% and 40%; this is further increased to 60% in PJP patients who are intubated (Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents. Available from http://www.cdc.gov/mmwr).[22] A study evaluating the effect of co-trimoxazole for PJP prophylaxis in Uganda demonstrated that the number needed to treat (prophylaxis) per life saved was 2.6 in individuals with a CD4 cell count below 200 cells/mm^3 compared with 8.3 for all participants in the study.[23] Therefore despite the risk of misclassification of the immune status using this study's methods, when weighted against long waiting periods for measured CD4 count results in resource-limited settings, primary prophylaxis with co-trimoxazole may be advocated using this method due to the high mortality rate associated with PJP. Whilst early initiation of ART is imperative, as a practical indicator of ART initiation further prospective studies are required before the use of this method can be advocated.

A tool that has shown promise to reflect both the CD4 status and disease progression is neopterin. It may assist future studies that seek a more robust surrogate, pending its availability and cost.[24,25] The study had several limitations. First, our study was a sin-

gle-centre cross-sectional observational study. Second, patients were not screened for liver disease, multiple myeloma and other causes that may have contributed to a heightened globulin fraction. All patients recruited were ambulatory and from an outpatient setting, therefore the suggested method of predicting immune status cannot be used in an acute admission, as the biomarkers investigated would be expected to fluctuate considerably with acute illness.

Conclusion

White cell count, calculated globulin and albumin have limited value as a sole surrogate marker for CD4 status, when used in an ambulatory ART naive HIV-infected patient. However, the combined use of these biomarkers may provide the clinician with an additional tool to assess the patient's immune status at outpatient consultation at low cost.

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