A non-specific biomarker of disease activity in HIV/AIDS patients from resource-limited environments

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Abstract:

Background: A general non-specific marker of disease activity that could alert the clinician and prompt further investigation would be of value in patients with HIV/AIDS, especially in resource limited environments.

Objective: To investigate the potential of neopterin as non-specific biomarker in patients with advanced HIV/AIDS.

Methods: Cross-sectional study in 105 HIV positive patients (75 on highly active antiretroviral treatment (HAART). Neopterin was assessed by enzyme linked immune-absorbent assay and cytokines by flow cytometry.

Results: Neopterin levels were significantly higher (p<0.001) for the total patient than for the control group. Significant correlations between neopterin and plasma indicators of inflammation showed neopterin to be a good indicator of active inflammatory status and of the effect of HAART on the immune system. Neopterin was superior to C-reactive protein and to individual cytokines as indicator of immune deficiency. Increased neopterin levels were associated with a decline in albumin, haemoglobin and the albumin/globulin ratio, and with increases in red cell distribution width.

Conclusions: Plasma neopterin is a good non-specific biomarker of disease activity in HIV/AIDS patients. It is a good indicator of inflammatory activity, perpetuation of inflammation-associated co-morbidities, degree of immune deficiency and has predictive value for underlying disease, and for monitoring the HAART response.

Keywords: neopterin; biomarkers; immune activation; HIV/AIDS

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Introduction

The search for a suitable biomarker in HIV/AIDS is ongoing. Evermore markers are being evaluated as indicators of immune suppression and disease progression and to better understand the immunopathogenesis of the disease [1,2]. In contrast to the need for biomarkers for specific purposes, a general non-specific marker of disease that could alert the clinician to further investigate the patient would be of value, especially in resource limited environments. Such a tool should be relatively inexpensive and facilities for its determination readily available.

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In the present study the suitability of neopterin as a potential non-specific marker of disease activity is examined. Neopterin, a catabolic product of the purine nucleotide guanosine triphosphate, is produced from guanosine 5'-triphosphate (GTP) that is cleaved by GTP- cyclohydrolase 1 to 7,8-dihydroneopterin triphosphate, followed by conversion of 7,8-dihydroneopterin triphosphate to neopterin and 7,8-dihydroneopterin under the influence of phosphatases[3]. GTP-cyclohydrolase 1 is stimulated, predominantly, by T-helper cell type-1 derived interferon-γ but co-stimulation by tumour necrosis factor alpha may contribute[3].

The value of neopterin as non-specific indicator was examined in terms of its potential as indicator of inflammatory status, as indicator of immune deficiency or dysfunction, the effects of anti-retroviral treatment and as indicator of TB co-infection. In these assessments the efficacy of neopterin was validated by comparison with immune-related factors such as the acute phase protein C-reactive protein (CRP), CD4 counts, as well as pro- and anti-inflammatory cytokines. In addition, neopterin levels were compared to that of a number of factors routinely measured for diagnostic purposes and elsewhere described as biomarkers.

Methods

Study population and ethics statement

This was a cross-sectional, non-intervention study comprising of 105 HIV positive adult patients recruited from the Kalafong secondary hospital in Pretoria, South Africa, and a control group of 60 donors from the South African National Blood Service who tested HIV negative and were therefore considered HIV uninfected. A total of 75 patients were on antiretroviral therapy (HAART group) and 30 patients were HAART-naïve. Ethical clearance, in accordance with the declaration of Helsinki, the National Health Act and the policy of the University, was received from the Faculty of Health Sciences Research and Ethics Committee of the University of Pretoria (Number: 107/2008). The committee also approved the documentation used for obtaining either written or verbal voluntary informed consent prior to the study. Participants who could not read or write were informed by a clinician about the nature and purpose of the study before verbal consent was obtained. Verbal consent was documented under the relevant section on the consent form which was acknowledged and signed by the clinician and investigator.

Cytokine and neopterin assays

Plasma neopterin was measured by commercial enzyme linked immune-absorbent assay (Immuno-Biological Laboratories Inc., USA). The cytokines IL-2, IL-4, IL-6, IL-10, TNF and IFN-γ were measured in plasma by cytometric bead array (CBA) kit protocol (BD Biosciences, San Jose, CA, USA) using flow cytometry. The CBA cytokines were analysed on a FACS Array Bioanalyzer using FCAP FCS Filter and FCAP Array software (BD Biosciences, USA). Routine blood variables were analysed by the National Health Laboratory Service (NHLS) at Kalafong.

Statistical analyses

The data was first tested for normality, followed by log transformation as the raw data was not normally distributed. Transformed data were analysed using one way analysis of variance (ANOVA) for comparison between groups. Pairwise comparisons were undertaken by Tukey HSD and Scheffe methods. Spearman rank correlations were computed to determine associations between group variables. Area under the ROC curve (AUROC) values were determined using logistic regression and CD4 cut-offs of less than 200 cells/μl. Analyses were performed at a significance level of p<0.05 using STATA statistical analysis software (version 12.1).

Results

The suitability of neopterin as a general non-specific marker of disease activity in HIV/AIDS patients was examined in a group of 105 HIV/AIDS (HIV-1) patients with a mean viral load of 2.75 ± 1.36 log_{10} copies/ml and a CD4 count of 257.97 ± 193.06 cells/μl. The demographic information of the study subjects can be seen in Table 1.

Table 1 Demographic information for the patient and control groups

	HAART	HAART-naïve	Controls
n	75	30	60
Females	48 (64%)	18 (60%)	38 (63%)
Age (years)	37.86±8.86	37.13±10.24	31.18±8.09
BMI (kg/m^2)	23.83±6.31	20.96±3.62	21.96±4.81
Average months on HAART	15.86±16.49	-	-
TB co-infection at baseline	14 (19%)	10 (33%)	-

CRP, cytokines and CD4 counts were employed in the appraisal of neopterin as non-specific biomarker of the inflammatory status, immune deficiency, the effects of anti-retroviral treatment and TB co-infection. The cytokines included the pro-inflammatory cytokines IL-2, IL-6, TNF and IFN-γ, as well as the anti-inflammatory cytokines IL-4 and IL-10. A comparison between the total patient group, the group on HAART, the HAART-naïve and the control group levels of immunological factors can be seen in Table 2.

Table 2 Comparison of immunological and other variables between the controls and patient groups

Variable	Controls	Total Patients	HAART	HAART-Naïve	p-value			
	C	T	H	N	T vs. C	H vs. C	N vs. C	H vs. N
IL-2 (pg/ml)	9.14±2.26	20.06±8.31	18.95±8.41	22.74±7.53	<0.0001	0.0003	<0.0001	0.076
IL-4 (pg/ml)	8.07±2.01	11.96±4.01	11.65±4.16	12.70±3.40	<0.0001	<0.0001	<0.0001	0.198
IL-6 (pg/ml)	0.69±1.62	11.16±14.95	9.56±12.54	15.04±19.34	0.0001	0.035	0.001	0.010*
IL-10 (pg/ml)	1.45±1.32	14.61±12.53	12.44±12.38	19.82±11.51	<0.0001	<0.0001	<0.0001	0.026*
TNF (pg/ml)	1.71±1.78	5.74±3.68	5.65±3.89	5.95±3.19	<0.0001	0.0002	<0.0001	0.473
IFN-γ (pg/ml)	24.85±2.96	44.00 ± 22.55	41.43±14.14	53.68±34.39	<0.0001	0.0003	<0.0001	0.017*
NPT (nmol/l)	8.23±5.71	45.57±41.82	34.51±35.70	66.63±40.73	<0.0001	<0.0001	<0.0001	0.0001*
NPT/IL-4	1.04±0.75	3.81±3.74	3.04±3.34	5.63±4.05	0.001	0.02	0.0001	0.002*
IL-2/IL-4	1.15±0.26	1.68±0.76	1.59±0.64	1.90±0.97	<0.0001	0.001	0.0007	0.058
IL-6/IL-4	0.06±0.14	0.86±1.17	0.74±1.05	1.16±1.39	<0.0001	0.001	0.0065	0.102
IFN-γ/IL-4	3.25±1.02	3.97±3.01	3.59±1.26	4.87±5.12	0.030	0.019	0.009	0.050
CRP (mg/l)	-	25.93±51.23	22.88±36.13	34.08±75.78	-	-	-	0.839
CD4 (cells/µl)	-	257.97±193.06	296.21±195.50	170.05±167.26	-	-	-	0.003*
VL (log_{10} copies/ml)	-	2.75±1.36	2.48±1.12	3.57±1.71	-	-	-	0.014*
Albumin (g/l)	-	33.40±7.55	34.94±6.67	29.50±8.37	-	-	-	0.027*
A/G ratio	-	0.68±0.25	0.73±0.24	0.53±0.21	-	-	-	0.004*
Haemoglobin (g/dl)	-	11.83±2.36	12.16±2.36	10.97±2.05	-	-	-	0.125
RDW (%)	-	17.45±4.35	17.52±4.50	17.28±4.01	-	-	-	0.334

Data expressed as mean±SD; IL = Interleukin; TNF = tumor necrosis factor; IFN = interferon; NPT = neopterin; VL = HIV viral load; A/G = albumin to globulin ratio; RDW = red cell distribution width
*p<0.05 for HAART vs. HAART-naïve

The validity of neopterin as indicator of immune deficiency was tested against CD4 counts, CRP and cytokine levels. The results can be seen in Table 3.

Table 3 Correlations of neopterin with CD4 counts, CRP, IL-6, albumin, A/G ratio, haemoglobin and red cell distribution width

	Total Patients		HAART		HAART-Naïve	
Neopterin with:	Rho	p-value	Rho	p-value	Rho	p-value
CD4	-0.484	0.0001*	-0.43	0.001*	-0.503	0.02*
IL-6	0.371	0.0010*	0.490	0.00001*	0.128	0.510
CRP	0.355	0.0006*	0.610	0.00001*	0.180	0.597
IFN-γ	0.301	0.002*	0.277	0.017*	0.216	0.260
Albumin	-0.547	0.0001*	-0.447	0.0002*	-0.457	0.014*
A/G ratio	-0.489	0.00001*	-0.423	0.0004*	-0.373	0.061
Haemoglobin	-0.597	0.00001*	-0.555	0.00001*	-0.33	0.093
RDW	0.342	0.001*	0.472	0.0001*	0.112	0.577

*Spearman Rho rank correlation statistically significant, p<0.05. IL = interleukin; CRP = C-reactive protein; IFN = interferon; A/G ratio = albumin/globulin ratio; RDW = red cell distribution width

The power of discrimination, in terms of area under the ROC curve (AUROC), of neopterin, CRP and IL-6 was also tested for the total patient group in relation to CD4 counts of less than 200 using logistic regression. The discriminatory power of neopterin (AUROC = 0.803) was found to be higher than that for CRP (AUROC = 0.658), and for IL-6 (AUROC = 0.753).

Twenty four of the total HIV patient group were confirmed as having tuberculosis (TB) co-infection (sputa smears). Neopterin (p=0.008) and CRP (p=0.004) levels were both significantly higher in the HIV/TB positive group (neopterin: 65.73±48.94 nmol/l; CRP: 47.35±57.12 mg/l) than in the HIV/TB negative group (neopterin: 37.37±34.48 nmol/l; CRP: 18.99±47.56 mg/l).
The discriminatory power for neopterin (AUROC = 0.898) was higher than that for CRP (AUROC=0.6252) for the TB-co-infection group. Likewise neopterin (AUROC = 0.7367) showed to be a better discriminator than CRP (AUROC = 0.5945) for the TB-negative patients.
Substances routinely measured in the clinic and previously described as biomarkers, i.e., albumin, the albumin/globulin (A/G) ratio, haemoglobin and red cell distribution width (RDW) were evaluated as indicators of immune deficiency by comparing their levels to CD4 counts. The results can be seen in Table 4.

Table 4 Correlations for CD4 with blood variables of the different groups

	Total Patients		HAART		Naïve	
CD4 with:	Rho	p-value	Rho	p-value	Rho	p-value
IL-6	-0.431	0.0001*	-0.553	0.009*	-0.285	0.193
CRP	-0.328	0.007*	-0.410	0.005*	-0.250	0.287
Albumin	0.491	0.00001*	0.497	0.0004*	0.142	0.551
A/G ratio	0.486	0.00001*	0.505	0.0003*	0.113	0.636
Haemoglobin	0.420	0.0003*	0.392	0.004*	0.256	0.276
RDW	-0.370	0.010*	-0.470	0.0004*	0.017	0.942

*Spearman Rho rank correlation statistically significant, p<0.05. IL = interleukin; CRP = C-reactive protein; A/G ratio = albumin/globulin ratio; RDW = red cell distribution width

Logistic regression results in a comparison between neopterin, albumin, the AG/ratio, haemoglobin and RDW as indicators of immune deficiency were: neopterin (AUROC = 0.803), albumin (AUROC = 0.487), the A/G ratio (AUROC = 0.504), haemoglobin (AUROC = 0.334) and RDW (AUROC = 0.589).

Discussion

The value of neopterin as non-specific indicator was examined in terms of its potential as indicator of inflammatory status, the effects of anti-retroviral treatment, as indicator of immune deficiency or dysfunction and as indicator of TB co-infection. The validity of neopterin as non-specific biomarker in HIV/AIDS was compared to that of CRP, cytokines and to a number of substances routinely measured in the clinic.

Neopterin as indicator of inflammatory (cellular immunity) status in HIV AIDS

Advanced HIV/AIDS is characterised by chronic immune activation and a concomitant immune deficiency. Although the levels of pro-inflammatory, as well as anti-inflammatory, cytokines may be raised[5], advanced HIV/AIDS is predominantly associated with increased inflammatory activity[6,7]. The inflammatory process is implicated as a major contributor to the pathogenesis of many physical disorders, and is likely to play a role in the cardiovascular, renal, liver, metabolic, haematological and skeletal abnormalities, as well as the premature systemic aging associated with HIV infection[6,7]. Increased inflammatory activity is similarly linked to neuropsychological impairments[8], including that found in HIV/AIDS [9,10].

Neopterin levels are generally considered as an indication of both macrophage function and cell mediated immunity. It is said that when cell-mediated immunity dominates, circulating neopterin levels are usually high and when humoral immunity dominates, neopterin levels are low[3-11]. Abundant evidence exists for neopterin levels to be increased in disturbances marked by inflammatory activity and raised neopterin levels and increases with disease progression, have previously been described[12]. However, other substances, in particular CRP and IL-6, have also been described as markers of inflammation.

In the present study the value of neopterin as general marker of the inflammatory status was examined by comparing neopterin levels to that of two other recognised biomarkers of inflammation, i.e., CRP and IL-6[6,11-13]. As seen in Table 2, the mean neopterin levels were significantly higher than that of the control group for both the HAART-naïve ($p<0.001$) and the HAART ($p<0.001$) groups. IL-6 levels were also significantly higher than that of the control group for the HAART naïve ($p=0.001$) and the HAART groups ($p=0.035$). CRP and IL-6 levels correlated positively with that of neopterin for the total patient group (CRP: $r=0.355$, $p=0.0006$; IL-6: $r=0.371$, $p=0.001$) and for the HAART group (CRP: $r=0.61$, $p<0.00001$; IL-6: $r=0.49$, $p<0.0001$) (Table 3). These results supported the notion of neopterin as the better marker of inflammatory activity and are in agreement with the concept of advanced HIV/AIDS as a condition with increased inflammatory activity [6,7].

However, although higher pro-inflammatory activity was confirmed in the patients, higher than control levels ($p<0.05$) were, as reported elsewhere[5], also seen in the levels of the anti-inflammatory cytokines (Table 2). Nevertheless, when the pro- versus anti-inflammatory activity was investigated it was found that the ratio neopterin/IL-4 was indeed higher in the HAART-naïve ($p=0.0001$), as well as in the HAART patients ($p=0.02$), than in the controls. This imbalance with a shift towards inflammation in HIV/AIDS was confirmed by the ratios of the individual pro-inflammatory cytokines to that of the anti-inflammatory cytokine IL-4. Significantly higher than control ratios were seen for IL-2/IL4 (HAART: $p=0.001$; HAART-naïve: $p=0.0007$), IL-6/IL4 (HAART: $p=0.001$; HAART-naïve: $p=0.0065$), as well as for IFN/IL4 (HAART: $p=0.019$; HAART-naïve: $p=0.009$).

Pro-inflammatory dominance with cell-mediated immunity in ascendancy was thus shown in the group on anti-retroviral therapy, as well as in the group not yet on anti-retroviral therapy. Such inflammatory activity, demonstrated here by neopterin levels and confirmed by cytokine results, is implicated in the pathogenesis of HIV/AIDS[6,7].

Effect of HAART on the pro-inflammatory/anti-inflammatory (cellular/humoral immune) status

The mean neopterin level in the HAART group was 48% lower ($p=0.0001$) than that of the HAART-naïve group, the mean IL-6 level was 43% lower ($p=0.01$), the mean IFN-γ was 22.8% lower ($p=0.017$), the mean IL-2 was 16.7% lower ($p=0.076$) and the CRP level, although not statistically significant, was 33% lower. However, the HAART group still showed significantly higher inflammatory activity than the control group. In contrast, the levels of the anti-inflammatory cytokine, IL-4, were not significantly different between the HAART and HAART-naïve groups (Table 2). These results, which demonstrated a down-regulation of neopterin and therefore in inflammatory activity upon treatment with highly active antiretroviral treatment are in agreement with that of Amirayan-Chevillard et al[14]. Amirayan-Chevillard *et al* further showed that neopterin levels again increase with cessation of antiretroviral treatment[14], a finding that implies neopterin to be a useful marker of the efficacy of HAART and perhaps for the assessment of patient compliance.

As an imbalance between pro- and anti-inflammatory activity, in favour of pro-inflammatory, has previously been implicated in the pathogenesis of HIV/AIDS[7], it was of interest to see to what extent this imbalance was corrected by antiretroviral treatment. The ratio between neopterin and the main anti-inflammatory cytokine IL-4 for HAART was significantly lower ($p=0.002$) than that for the HAART-naïve patients – pointing to suppression of cellular (pro-inflammatory) relative to humoral (anti-inflammatory) activity by antiretroviral treatment. However, the ratio in the HAART group was still higher ($p=0.02$) than in the control group, confirming the persistence of a shift towards pro-inflammatory activity despite anti-retroviral treatment (Table 2). Although the ratios of all the individual pro-inflammatory cytokines to the level of the anti-inflammatory cytokine IL-4 (IL-2/IL-4, IL-6/IL-4, IFN-γ /IL-4) were also lower in the HAART than in the HAART-naïve group, none were significantly lower.

This persistent, albeit downgraded, presence of inflammatory, and imbalance between pro- and anti-inflammatory activity, despite effective antiretroviral therapy, has previously been reported and is described as a major contributor to the perpetuation of non-AIDS defining co-morbidities and premature systemic aging in patients on HAART[7]. Among the best known contributors to such persistence are said to be clinical or subclinical infections, gastrointestinal microbial translocation, infectious, as well as non-infectious, HIV virions and thymic dysfunction[7].

Neopterin as indicator of immune deficiency

Neopterin has previously been shown as a measure of the degree of immune deficiency in HIV/AIDS patients[2,15,16]. In the present study the potential of neopterin as indicator of immune deficiency was again investigated by comparing neopterin levels to that of CD4 counts. Results with neopterin were then compared to that with CRP and cytokines.

Negative correlations were found between neopterin levels and CD4 counts for the total patient group ($r=-0.484$, $p=0.0001$), the HAART group ($r=-0.43$, $p=0.001$) and the HAART-naïve group ($r=-0.503$, $p=0.02$) (Table 3). 93.3% of the HAART-naïve patients and 73.3% of the HAART patients had higher than normal neopterin levels. These results, which suggest neopterin as an indicator of immune deficiency, are in line with that of previous publications [2,15,16].

Neopterin was subsequently compared to CRP and cytokines as immune deficiency indicators. CRP and one of the cytokines investigated, that is, IL-6, showed significant negative correlations with CD4 counts for the total patient group (CRP: $r=-0.328$, $p=0.007$; IL-6: $r=0.431$, $p=0.0001$) and the HAART group (CRP: $r=-0.410$, $p=0.005$; IL-6: $r=-0.553$, $p=0.009$), but not for the HAART-naïve group (Table 4). In contrast to neopterin, only 49.3% of the HAART-naïve patients and 46.7% of the HAART patients had higher than normal CRP reference levels. 83.3% of the HAART-naïve patients and 65.3% of the HAART patients had higher than normal IL-6 levels.

Logistic regression showed the discriminatory power of neopterin (AUROC = 0.803) to be higher than that for CRP (AUROC = 0.658), and for IL-6 (AUROC = 0.753). Neopterin was therefore shown to be superior to CRP and to individual cytokines as indicator of immune deficiency.

Neopterin and HIV/AIDS with TB-co infection

Neopterin levels are known to be significantly higher in individuals with active tuberculosis than in patients with inactive tuberculosis or controls[17]. It would thus seem feasible to expect that HIV/AIDS patients with TB-co-infection would have higher levels of neopterin than HIV patients without TB-co-infection. The ability of neopterin to discriminate between HIV patients with and without active TB was therefore investigated and compared to that of CRP and cytokines.

Neopterin ($p=0.008$) and CRP ($p=0.004$) levels were both significantly higher in the 24 HIV/TB positive than in the HIV/TB negative patients. This is in agreement with previous reports[9]. Logistic regression analysis showed the discriminatory power for neopterin (AUROC = 0.898) to be higher than that for CRP (AUROC=0.6252). Neopterin therefore seems to be the better indicator of TB-co-infection in patients with HIV/AIDS.

Previous indications that cytokines such as IFN-γ, TNF-alpha, IL-6, IL-10 and the IFN/IL-10 ratio may be of diagnostic/prognostic value in TB[19-24] were not substantiated by the results of the present study. In contrast to our expectations that the balance between pro- and anti-inflammatory activity would be more unfavourable with TB-co-infection, the difference for the neopterin/IL-4 ratio between patients with and without TB co-infection ($p=0.059$) was non-significant when judged at a 0.05 level of significance.

Advantage of neopterin above that of the measurement of individual cytokines

Locally produced cytokines could bind to their target tissues or be neutralized by soluble receptors[25]. Cytokine release may therefore not be accurately reflected by circulating levels. The circulating level of neopterin, on the other hand, is largely a product of the balance between synthesis and renal excretion[15]. It therefore speaks for itself that neopterin could very well be a better biomarker than individual cytokines. In addition, because of the pleiotropic nature of cytokines as well as the multiple interactions and co-operations between cytokines, the measurement of one specific cytokine may not reflect its contribution to inflammation and other immune processes. Neopterin is said to reflect the multiple cooperations between immunocompetent cells[26].

Comparison of neopterin levels to that of circulating substances and ratios elsewhere described as biomarkers in HIV/AIDS patients

Albumin concentration, the albumin/globulin (A/G) ratio, red cell distribution width (RDW) and haemoglobin (Hb) concentration are routinely measured during clinical investigations. Yet they have elsewhere all been described as biomarkers of disease progression and/or immune deficiency, in general, as well as in HIV/AIDS [27-32].

Previous associations shown between immune deficiency (decreased CD4 counts) with albumin, A/G ratios, haemoglobin and RDW, respectively[27-32], were supported by results on the total patient and HAART groups of the present study (Table 4). Positive correlations were found between albumin and the CD4 counts for the total patient group (r=0.491, p=0.00001) and the group on HAART (r=0.497, p=0.0004). Positive correlations were also found between the A/G ratio and the CD4 counts for the total patient group (r=0.486, p=0.00001) and the group on HAART (r=0.505, p=0.0003). Haemoglobin concentration correlated positively with the CD4 count for the total patient group (r=0.42, p=0.0003) and for the HAART group (r=0.392, p=0.004). RDW correlated negatively with the CD4 count for the total patient group (r=-0.37, p=0.010) and the group on HAART (r=-0.47, p=0.0004).

It is known that the levels of albumin, A/G ratios, haemoglobin and RDW are all adversely influenced by inflammation. With chronic inflammation the levels of albumin, a negative acute-phase protein, decreases as a result of lower synthesis, an increase in fractional metabolic rate, appetite suppression and through an increase in microvascular albumin leakage[33,34]. Chronic inflammatory conditions can influence haemoglobin levels, RDW and other red blood cell parameters in various ways, most notably processes involved in the anaemia of chronic disease[35]. The association between RDW and inflammation is so strong that RDW has been described as indicative of inflammation[36].

In the present study negative correlations between neopterin and albumin concentrations were observed for the total patient group (r=-0.547, p=00001), the group on HAART (r=-0.447, p=0.0002) and the HAART-naïve group (r=-0.475, p=0.014). As for albumin, the A/G ratio declined with increases in neopterin as seen in the negative correlations for the total patient group (r=-0.489, p=0.00001), the HAART group (r=-0.423, p=0.0004) and the HAART-naïve group (r=-0.373, p=0.061). Negative correlation were also found between the haemoglobin and neopterin levels for the total patient group (r=-0.597, p=0.00001) and for the HAART group (r=-0.555, p=0.00001), while for the HAART-naïve group only a weak negative correlation was found (r=-0.33, p=0.093). Significant positive correlations were found between RDW and neopterin for the total patient group (r=0.342, p=0.001) and for the HAART group (r=0.472, p=0.0001). In line with previous publications, the present study thus demonstrated a decline in the levels of albumin, the A/G ratio and haemoglobin concentrations, and an increased RDW, with increases in inflammatory activity, as reflected by neopterin levels.

Although albumin, the A/G ratio, haemoglobin and RDW were seen to have a positive association with the degree of immune deficiency, neopterin (AUROC = 0.803) was shown to be a better indicator of immune deficiency than albumin (AUROC = 0.487), the A/G ratio (AUROC = 0.504), haemoglobin (AUROC = 0.334) and the RDW (AUROC = 0.589).

Additional reasons why neopterin could be a good non-specific biomarker in HIV/AIDS patients

In addition to evidence of neopterin as a good non-specific marker of disease, as shown in our study, various other phenomena support this assumption. The value of neopterin as indicator of disease progression in HIV has been shown by several laboratories[16], and in an earlier paper on HIV/AIDS patients of African ethnicity we showed neopterin to be superior to CRP and procalcitonin as indicator of disease progression[2]. Another connection between neopterin levels and disease is suggested by the correlation between neopterin and IFN-γ (Table 3). IFN-γ is the major factor responsible for a shift in tryptophan metabolism towards the kynurenine pathway[37]. Abnormalities in tryptophan metabolism can have a widespread influence on both physical and psychological well-being. In the present study significant positive correlations were found between neopterin levels and the levels of IFN-γ (total patient group: r=0.301, p=0.002; HAART: r=0.277, p=0.017). A further potential link between neopterin levels and disease activity in HIV/AIDS lies in the production of reactive oxygen species (ROS). Excessive ROS is known to contribute to the pathogenesis of HIV/AIDS and correlations have been shown to exist between neopterin levels, increased production of ROS and decreased circulating antioxidants[38-41]. Other associations, not to be discussed here exist, such as that between neopterin and upregulation of the expression of the proto-oncogene c-fos[42], the nuclear factor-κB[41], and the iNOS gene[43]. However, the primary association is probably that between neopterin and inflammation.

Conclusions

This study in HIV/AIDS patients showed neopterin to be a better indicator of the inflammatory status, immune deficiency, TB-co-infection and the effects of HAART than CRP or cytokines. Changes in plasma neopterin levels as an indication of the efficacy of HAART or of patient compliance, should be investigated further. In line with the effects of inflammation on various systems, neopterin levels reflect the negative effects of the disease on levels of albumin, haemoglobin, the albumin/globulin ratio and the red cell distribution width.

It is our contention that neopterin levels represent a good non-specific indicator, not only of inflammatory activity, but of the general health status. This statement is made in view of its associations with inflammation, the effects of HAART, immune deficiency, TB-co-infection, the levels of several plasma proteins and, as previously reported, disease progression. The statement is further supported by previously supported diagnostic and prognostic associations between plasma neopterin levels and disorders other than HIV/AIDS. These include viral, intracellular bacterial and parasitic infections, burns, cancer, cardiovascular disease, neurodegeneration, graft versus host disease, autoimmune disorders and a variety of oral afflictions[12]. However, in view of the effect of inflammation on all physiological systems, the primary importance of neopterin probably lies in its reflection of the degree of inflammation, a major contributor to the pathogenesis of HIV/AIDS and a process involved in a wide variety of pathological processes. The skills required for the analysis of neopterin fall within that of most trained laboratory analysts and the costs are below that of the more specialized techniques. Neopterin therefore offers a relatively inexpensive non-specific biomarker in resource limited environments to alert the clinician to investigate further..

Conflict of interest

None

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