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CD14+CD16-Low Monocyte Subset Predicts Non-Progressive HIV Disease: Evidence of A New Prognostic and Diagnostic Biomarker

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Abstract

Monocytes are phenotypically pliable, which allows them to play several significant immunological roles in combating HIV infection. Monocytes can be subcategorized into subsets based on the expression of CD14 and CD16 antigens. Although the CD4+ T cell counts have been shown to predict HIV viremia, the actual predictive value of these monocyte subsets at different stages of plasma viremia is not known. We derived *ex-vivo* monocytes from HIV+ patients with detectable and below detectable plasma viremia, HIV+ Long-Term Non-Progressors (LTNP) and HIV negative individuals. We subdivided monocytes into CD14+/ CD16-low, medium and high populations and visualized the phenotypic changes in expression of both CD14 and CD16 antigens in HIV+ patients at different stages of HIV disease.

The expression of surface markers on monocytes (CD14+/CD16) was measured from the EDTA blood of 50 HIV+ individuals [14 viremic and 29 Below Detectable Level (BDL) whilst on HAART, 7 therapy naïve, aviremic LTNP's] and 6 HIV-negative donors using the FACSCanto (6-color) flow cytometer. Percentage of CD16/CD14+ sub-populations were measured on FACSCantoA with FACSDiva (v 6.1.2) software and analysed by FlowJo software (v10.0.7), respectively.

By categorizing monocyte population into CD14+, CD16 high, medium and low, we could clearly discriminate between viremic and aviremic HIV patients. There was considerable elevation of CD16-low population (80%) in HIV-negative individuals and LTNPS (57%), as opposed to 9% in HAART-treated group. Noteworthy was the CD16-low population failed to recover despite complete viral control during HAART therapy suggesting their definitive role as indicators of viremic control as seen with their marked prominence in LTNPs. In contrast, the HAART-treated group showed elevated CD16-high populations (34%), as opposed to relatively low percentages in the viremic group (3%). The robust maintenance and elevation of CD16-low populations and substantial low levels of CD16-high populations distinctively in HIV-negative and non-progressing HIV+ individuals correlated with the natural control of HIV in LTNPs. This feature of CD16-low monocytic population can be exploited as a biomarker in predicting plasma viremia and the strength of the immune system.

Keywords: CD16+ Monocytes; Monocyte; Non-Progressive HIV Disease; HAART; HIV

Introduction

The phenotypic pliability and the differentiation ability of monocytes empower this cell type to play a crucial role in HIV pathogenesis through cellular differentiation, phagocytosis, and antigen presentation. Compared to T cells and macrophages, monocytes are much less permissive to HIV infection [1,2], although all these cells express HIV receptor CD4 and co-receptors CCR5 and/or CXCR4. In spite of less than 1% of circulating monocytes directly infected *in vivo*, infectious virus can be isolated from circulating monocytes in untreated patients and HAART responders [2,3], which could become a dominant source of plasma virus in HAART responders in whom HIV replication in activated T cells is blocked [4]. In addition, monocytes represent an important cellular reservoir by harboring and trafficking HIV into various tissue compartments through differentiating into tissue macrophages or dendritic cells, which enable productive HIV replication [4]. Furthermore, undifferentiated monocytic precursor cells, such as CD34+ progenitor cells, may be infected with HIV and pass on the virus to progeny monocytes and keep on renewing the viral pool in peripheral blood monocytes [5,6]. Monocyte subpopulations exist with differing levels of maturation and functions. Monocytes that express CD14, the LPS receptor, and CD16, the Fcylll receptor, are a mature population of cells that are highly susceptible to HIV.

Based on these sub-populations, monocytes can be subcategorized into subsets based on the surface expression of CD14 and CD16 antigens. CD14+CD16+ monocytes are present in significantly greater numbers in HIV-infected people, despite viral suppression, in contrast to individuals without HIV, but the modulation of such populations, as we have visualized by sub-categorizing, has never been looked into at various stages of HIV disease. Although the CD4+ T cell counts have been shown to predict HIV viremia, the actual predictive value of surface antigen changes on monocytes, particularly CD16 at different stages of plasma viremia has also not been evaluated. In this study, we have derived *ex vivo* monocytes from HIV positive patients with detectable (84-231,000 copies of HIV RNA/ml plasma) and Below Detectable Levels (BDL) of plasma viremia (<40 copies of HIV RNA/ml plasma), HIV negative individuals and therapy naïve HIV+ Long-Term Non-Progressors (LTNP) who have been infected with HIV for >25 years, remained therapy naïve and have maintained below detectable levels of plasma viremia throughout the course of infection. Since CD14+CD16+ monocytes represent an important heterogeneous cell population that is often targeted, particularly for HIV-1 entry, we evaluated the effects of HIV infection and distinct subsets of ex-vivo-derived CD16+ monocytes. We subdivided monocytes into CD14+CD16-high, CD14+CD16-medium and CD14+CD16-low populations and visualized the phenotypic changes in expression of CD16 antigen in CD14+ monocytes in HIV+ patients during different stages of plasma viremia.

Methods and Materials

Derivation and Processing of *Ex-Vivo* Monocytes from HIV Patients

The human Peripheral Blood Mononuclear Cells (PBMCs) were obtained from the EDTA blood of 50 HIV positive individuals [14 viremic patients; 29 BDL on Highly Active Antiretroviral Therapy (HAART) and 7 therapies naïve, aviremic LTNPs] and 4 HIV negative donors (See Table 1 and supplementary File 1 for Patient clinical details and raw Excel data). The work was cleared by the human Ethics Committee of the Sydney West Western Area Health Research Committee, Westmead Hospital, Sydney, NSW. Australia. All blood samples were collected strictly after individual informed written consent.

Patient Groups	CD4+Tcell count	CD8+T cell count	Plasma Viral Load (copies RNA/mL plasma)
BDL	595	832	<40copiesRNAwhileon treatment
Viremic	380	987	Variable
LNTP	665	846	<40 copies RNA with no treatment
*Individual plasma viral loads and cell counts for all the patients are shown in			

Table 1: Clinical profile showing cell counts and plasma viremia levels of patient groups.

Immuno-Staining and Flow Cytometry Analysis on Human Peripheral Blood Mononuclear Cells Samples

A two-color antibody panel was used to identify the CD14 and CD16 antigen expression on monocytes. Cells were ficolled and stained with CD14-PE (BD Biosciences, Australia) and CD16-Alex Fluor 647 (BD Biosciences, Australia) for 20 min at 40C. Following washing with PBS, cells were fixed with (2% Paraformaldehyde) for 10 Min at room temperature, washed and then re suspended in PBS before flow cytometry. Flow cytometry was performed on a Canto A cytometer (BD Biosciences, Australia) using DIVA 6.1.2 software (BD Biosciences). Monocyte population was first identified by FSC and SSC dot plot. Following gating on the CD14 positive population, based on the fluorescent intensity, CD16 expression on CD14 positive monocytes was divided into three groups, CD16-low, CD16-med and CD16-high. The same gating strategy was used across all samples. Percentages of three CD16 populations were analyzed by FlowJo software (v10.0.7; Treestar, USA).

Statistical Analysis

Results are expressed as mean ± standard error. Differences among groups were measured using Student's t test with one-tailed distribution and two sample equal variance test, P<0.05 was considered significant. The relationship of CD16 with CD4 count and viral load was determined by the correlation coefficient with the formula shown below.

$$Correl(X,Y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

Results

By categorizing monocyte population into CD14+CD16-high, CD14+CD16-medium and CD14+CD16-low, we could discriminate between viremic and aviremic HIV patients (Figure 1). There was considerable elevation of CD14+CD16-low population (80%) in HIV-negative individuals and 57% in LTNPS ($p<0.0114$), as opposed to 8% in HAART-treated viremic and aviremic (BDL) groups (BDL vs neg $P<0.0000374$ and Viremic vs neg $P<0.0000056$) (Figure 2, Table 3). Notable was that the CD14+CD16-low population never recovered despite complete viral control during HAART in the BDL group, suggesting their low levels as definite indicators of quality of monocyte in BDL and viremic groups when compared against their elevated levels in the LTNPs, who control the plasma virus naturally (LTNP vs BDL $p<0.0075102$ and LTNP vs Viremic $p<0.0015809$). Moreover, the HAART-treated groups (viremic and BDL) were also characterized by the elevated levels of CD14+CD16-high population (33 and 45%, respectively) (Figure 2, Table 2,3), when compared against LTNPs (BDL vs LTNP $p<0.0000180$ and Viremic vs LTNP $p<0.0009354$) and negative controls, which showed low levels of CD14+CD16-high populations. Although LTNPs were comparable in the expression of CD14+CD16-high populations with HIV negative donors ($p<0.6796$), they could be segregated from LTNPs based on CD14+CD16-medium and CD14+CD16-low populations ($p<0.0048514$ and $p<0.0114045$, respectively) (Table 3).

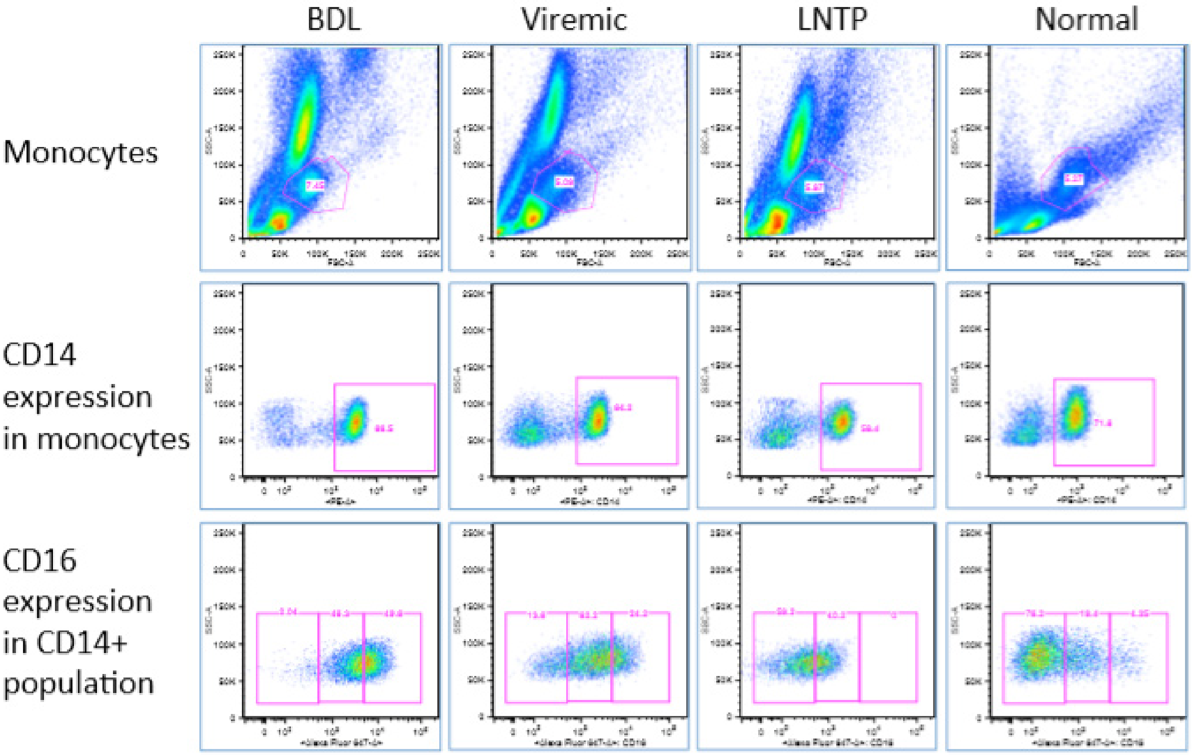

Figure 1: The representative flow cytometry dot pots showing the gating strategy of discriminating the CD14+CD16high, CD14+CD16-medium and CD14+CD16-low populations in HIV patients at different stages of plasma viremia compared to the HIV negative healthy donors.

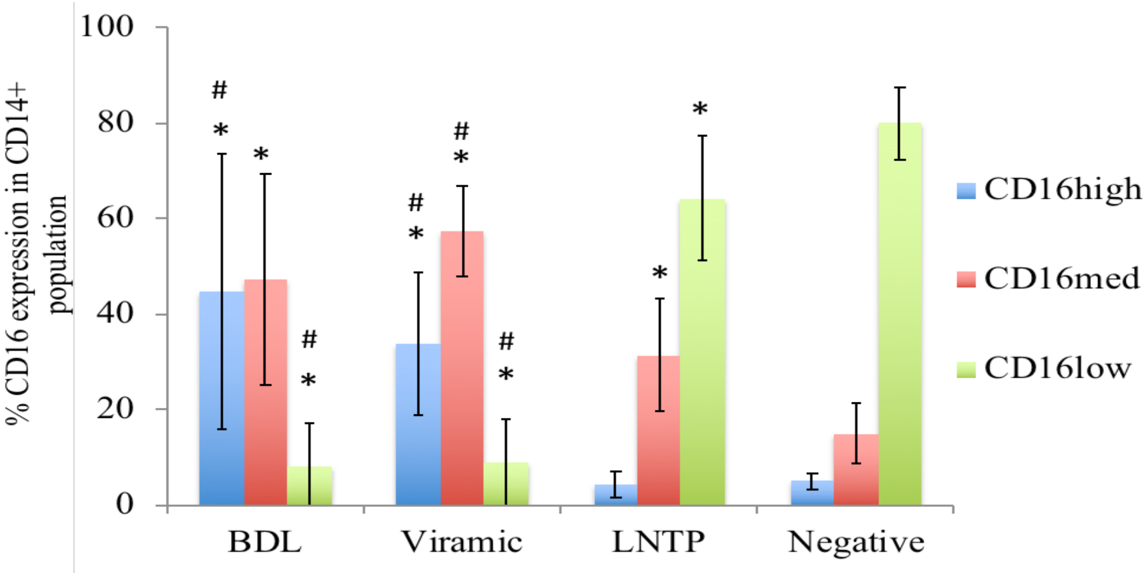

Figure 2: Histogram showing comparative analysis of CD14+CD16-high (blue), CD14+CD16- medium (brown) and CD14+CD16-low (green) populations in HIV patients at different stages of viremia compared to the normal donors. * Indicates P value <0.05 compared individual CD16 populations in HIV patients to the negative controls accordingly. # Indicates P value <0.05 compared individual CD16 populations in BDL and Viremic patients to the LTNP patients accordingly.

Patient Group	**CD16high**	**CD16med**	**CD16low**
BDL	44.73 (1.99-84.8)	47.17 (14.4-84.3)	8.05 (0.55-31.6)
Viremic	33.71 (8.67-56.2)	57.50 (42-75.2)	8.81 (0.92-35.5)
LNTP	4.35 (0.226-7.8)	31.36 (12.5-42.7)	64.2 (49.5-86.3)
Negative	4.97 (3.82-7.45)	14.97 (7.59-21.2)	79.90 (71.2-87.9)
*Please refer to Figure 1 for data analysis			

Table 2: Mean percentages of CD16 high, Medium and Low monocyte populations in different HIV groups and Negative healthy group.

	CD16high	CD16med	CD16low
BDL vs Neg	0.00000005	0.00001252	0.00003744
Viremic vsNeg	0.0000057	0.0000125	0.0000056
LNTP vs Neg	0.6796310	0.0048514	0.0114045
BDL vs LNTP	0.0000180	0.0588265	0.0075102
Viremis vs LNTP	0.0009354	0.0000066	0.0015809

Table 3: Inter-group statistical comparison of CD16 high, medium and low monocytes populations between different disease groups.

From these data two significant aspects of phenotypic regulation of monocytes are clear that the high levels of CD14+CD16- low and low levels CD14+CD16-high of populations characterize LTNPs and HIV-negative individuals implying that although the LTNPS are closer to HIV negative individuals when compared against the viremic and BDL groups, they could be segregated from each other by both CD14+CD16-medium and -low populations. Secondly, that the CD14+CD16-low and - high populations of monocytes were strong and reliable indicators of plasma viremia, immune deterioration and the quality of monocytes during viremia. It also raises the possibility that this phenotypic modulation in monocyte may also be linked to the deterioration in quality of monocytes, which even fails to recover despite HAART as apparent in BDL HIV patients on HAART.

We also evaluated, if this modulation in monocytic subset was linked to CD4+T cell counts, but we obtained inverse relationship between monocytic deterioration and CD4+T cells counts. The correlation between CD16-med vs CD4 count was 0.31176704 for the BDL group, while in Viremic group the CD16-high vs CD4 count showed p value of 0.42996958, with R2 values for both groups at 0.0972; 0.18487, respectively, suggesting that monocytic deterioration has less bearing on overall CD4+ T cell counts (supplementary file 1).

Discussion

Quality of monocyte subsets and their modulation *in vivo* plays a vital role in guiding immune responses during HIV infection [7]. The data shown in our study not only highlights the significance of CD16+ monocytes in HIV infection but also demonstrates the vital dynamics of these monocyte subsets exhibited in viral suppression and disease progression. This novel way of visualizing the trichotomy between low, medium and high subsets of CD14+CD16+ monocytes from HIV- and HIV+ groups has allowed us for the first time in elucidating not only the immunologic relationship they hold with different stages of HIV disease, but also their association with the natural control of HIV disease in therapy naïve Elite Controllers (EC)- a phenomenon not shown previously. We believe that this could offer new insights into the roles of innate immunity in HIV pathogenesis, underpinning their role as new biomarkers in HIV disease diagnosis and prognosis.

There is a significant rise in the proportions of non-classical monocytes in HIV-1 disease [8,9]. This heterogeneous subset which represents a minor sub-population of monocytes in healthy individuals, increases in peripheral blood and may represent up to 40% of total circulating monocytes during HIV infection and in patients with AIDS [9]. An important goal in clinical manifestation and diagnosis of HIV infection is to find laboratory parameters to monitor the disease progression. By further diversifying the established classification of non-classical and intermediate monocytes population into three subsets based of on CD16+ antigen, we sub categorized them into low, medium and high clusters. Through this way of visualizing, we could clearly discriminate between viremic and aviremic HIV patients (Figure 1 and Table 3). There was considerable and statistically significant elevation of CD14+CD16-low population (80%) in HIV-negative individuals in comparison to 57% in the LTNPs ($p<0.0114$). In contrast, it was only 8% in HAART-treated viremic and aviremic groups (BDL vs neg $P<0.0000374$ and Viremic vs Negatives $P<0.0000056$) (Figure 2 and Table 2,3).

Notably, the CD14+CD16-low population never recovered despite complete viral control during HAART in the BDL group, suggesting their low levels as definite indicators of quality of monocyte in the BDL and viremic groups when compared against their high levels in the LTNPs, who control the plasma virus naturally (LTNP vs BDL $p<0.0075102$ and LTNP vs Viremic $p<0.0015809$). And secondly, this also highlights the fact that even in the face of complete control of viremia during HAART, the CD14+CD16-low population failed to recover, suggesting HAART has no bearing on the recovery of this population, which may be one of the underlying reasons for partial immune restoration during HAART.

The robust maintenance and elevation of CD16-low populations and substantially low levels of CD16-high populations distinctively in HIV-negative and non-progressing HIV+ individuals correlated with the natural control of HIV in the LTNPs, thereby demonstrating the ability of this subset in predicting the strength of the immune system at different stages of HIV disease and their possible role in innate immunity. Further comparing CD16-low population at complete control of plasma viremia under HAART as opposed to its natural control in LTNPs, it appears that the quality of immune cells and the overall strength of the immune system is vital for this cell subset, and even a little virus compromises their quality. This fact emerges from the comparison of LTNPs with HIV-negative individuals, where the difference becomes apparent despite the two groups sharing closeness. This further suggests that LTNPs may maintain therapy naïve and virus-free status, the overall numbers of CD16-low population in the blood can serve not only as excellent indicators of even very low and below detectable levels viremic states as seen in case of LTNPs, but also in stratifying individuals based on the strength of their immune system.

Further to this, the HAART-treated groups (viremic and BDL) were also characterized by elevated levels of CD14+CD16-high populations (45% and 33% respectively) (Figure 2 and Table 2), when compared against LTNPs (BDL vs LTNP $p<0.0000180$ and Viremic vs LTNP $p<0.0009354$) and negative controls, who displayed low levels of CD14+CD16-high populations. Although LTNPs were comparable in the expression of CD14+CD16-high populations with HIV negative donors ($p<0.6796$), the HIV- donors could only be segregated from the LTNPs based on CD14+CD16-medium and CD14+CD16-low populations ($p<0.0048514$ and $p<0.0114045$, respectively) (Figure 2 and Table 3), suggesting a clear demarcation between HIV- and HIV+ individuals, raising a possibility of subliminal infection in LTNPs which is under a tight natural control.

Furthermore, HIV infectivity correlated with elevated CD16-mediium monocytes population, underscoring the distinction between HIV positive patients and negative individuals, which was highlighted to a measurable extent by the expression of CD16-medium population relatively pronounced in the HIV+ group 47% (BDL) and 58% (Viremic), as opposed to 39% (LNTP) and 15% (healthy donors)- the HIV negative group, implying the functional relevance of CD16-low and CD16-med monocytic populations in discriminating LTNPs from the negative donors. Thus, for the maintenance of the LTNP status, it was the high levels of CD16-low and low-levels of CD14_CD16-high appeared essential, which essentially coincides with the levels HIV- healthy individuals.

Theiblemont et al., (1995) [9] suggested that IN HIV Infection the expansion of CD14 low CD16 high monocyte subset, which produce high amount of TNF-alpha and IL-1 alpha may participate in the immune dysfunction observed during HIV infection. Thus their elevated levels in patients with viremia, is consistent with our data. Also consistent is their low levels in healthy individuals, in addition to LTNPs-which Thieblemont et al., did not show. The CD14lowCD16high circulating monocytes co-express MAX.1, p150, 95 and HLA- DR, which are typical of tissue macrophage markers. These cells also express higher levels of Intracellular Interleukin (IL)-1 alpha and Tumor Necrosis Factor (TNF)-alpha than the CD14highCD16low monocyte population from the same patients, which could form the biological basis of natural viremia control in LTNPs as seen our study.

Conclusions

The robust maintenance and elevation of CD14+CD16-low populations and low levels of CD14+CD16-high populations uniquely in HIV-negative and non-progressing HIV+ individuals correlate with natural control of HIV in LTNPs and is able to predict viremia, strength of the immune system and quality of both monocytes and T cells. LTNPs and HIV-negative individuals could be segregated based on CD14+CD16-medium populations, and despite the elevation of this population in the BDL and viremic groups, they significantly differed from LTNPs ($p<0.0000066$), suggesting possible differences in the quality of these monocytes in the LTNP group. These data may allow the development of new diagnostic and prognostic tools for the prediction of HIV disease staging in HIV patients.

Financial and Competing Interest Disclosure

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SUPPLEMENTARY DATA

CD16high CD16med CD16low Viral load (Log10)						
1: ANCR_CD14-PE+CD16AF647.fcs	24.2	62.2	13.6	2.0:107	194	1642Viremic
3: BN-_CD14-PE+CD16AF647.fcs	1.99	66	31.6	BDL	476	571BDL
5: BERA-_CD14-PE+CD16-AF647.fcs	34.5	63.9	1.6	4.4:22900	320	1109Viremic
7: BW-CD14-PE+CD16AF647.fcs	4.35	19.4	76.2	Normal control		Negative
9: BrAl-CD14-PE+CD16-AF647.fcs	66.6	30.8	2.53	BDL<40	682	946BDL
11: BRJU-CD14-PE+CD16-AF647.fcs	73	24.2	2.84	BDL	706	706BDL
13: CRBR-Cd14-PE+CD16-AF647.fcs	7.06	66.7	26.2	BDL	363	1597BDL
15: DEBR-CD14-PE+CD16AF647.fcs	75.3	19.8	4.81	BDL<40	45	566BDL
17: DIJA-CD14-PE+CD16AF647.fcs	8.67	55.9	35.5	84	222	1112Viremic
19: DRJ-CD14-PE+CD16AF647.fcs	2.32	73.9	23.7	BDL	515	1576BDL
21: EL-CD14-PE+CD16_AF647.fcs	40.4	53	6.62	1.9:91 n/a	n/a	Viremic
23: GaWa_CD14-PE+CD16-AF647.fcs	49.6	48.3	2.04	BDL	435	435BDL
25: GTPCD14-PE+CD16AF647.fcs	20.3	65.1	14.6	231000	7	318Viremic
27: Gar_CD14-PE+CD16-AF647.fcs	8.72	61.9	29.4	BDL BDL<40 3.2:1690	437	494BDL BDL BDL
29: GS_CD14-PE+CD16-AF647.fcs	61.6	36.2	2.19		338	520
31: Gh G_CD14-PE+CD16-AF647.fcs	30.7	57.6	11.7		368	1472
33: GWJ_CD14-PE+CD16AF647.fcs	31.8	66.2	2.02	4.1:13800 n/a	n/a	Viremic
35: H_CD14-PE+CD16-AF647.fcs	59.6	36.1	4.28	BDL	615	315BDL
37: HAR_CD14-PE+CD16AF647.fcs	5.23	77.1	17.5	BDL	437	760BDL
39: HK_CD14-PE+CD16-AF647.fcs	43.3	53.8	2.83	BDL	443	586BDL
41: IAK_CD14-PE+CD16-AF647.fcs	69.3	29.3	1.34	BDL n/a	n/a	BDL
43: J_CD14-PE+CD16-AF647.fcs	4.24	7.59	87.9			Negative
45: JU_Cd14-PE+CD16-AQF647.fcs	26.2	64	9.88	2.0:104	850	975Viremic
47: KA_CD14-PE+CD16AF647.fcs	84.8	14.4	0.82	BDL BDL BDL	377	406BDL BDL BDL
48. KJ_CD14-PE+CD16AF647.fcs 51: la wu 700108_CD14-PE+CD16AF647.fcs	9.96	76.8	13.2		922	1920
LW 700108_CD14-PE+CD16AF647.fcs	79.1	19.8	1.04		592	444
53: LEJO_CD14-PE+CD16AF647.fcs	23.4	62.2	14.4	4.5:33600	156	1672Viremic
55: MS_CD14-PE+CD16-AF647.fcs	54.6	44.5	0.92	2.1:121	597	760Viremic
57: MR_CD14-PE+CD16-AF647.fcs	50.9	47.4	1.64	2.1:120	597	688Viremic
59: MP_CD14-PE+CD16-AF647.fcs	63.8	35.5	0.693	BDL n/a	n/a	BDL
61: mOJA_CD14-PE+CD16-AF647.fcs	55.9	40.9	3.13	BDL	608	496BDL
63: MOM_CD14-PE+CD16AF647.fcs	16.7	76.6	6.64	BDL<40	1078	868BDL

65: NTTH_CD14-PE+CD16AF647.fcs	0	40.2	59.2	4.2:16300	271	1747LNTP
67: OK_CD14-PE+CD16AF647.fcs	42.9	52.8	4.29	1.6:40	520	1378Viremic
69: PH_CD14-PE+CD16AF647.fcs	6.34	39.2	54.4	BDL	660	1200LNTP
71: PT_CD14-PE+CD16-AF647.fcs	83.6	15.8	0.547	BDL n/a	n/a	BDL
73: PG_CD14-PE+CD16AF647.fcs	7.45	21.2	71.2	normal control		Negative
75: SR_CD14-PE+CD16AF647.fcs	83.3	15.8	0.904	BDL n/a	n/a	BDL
77: SD_CD14-PE+Cd16-AF647.fcs	24.9	70.7	4.36	BDL	1131	485BDL
79: SD_CD14-PE+CD16AF647.fcs	0.226	39	60.3	BDL	669	491BDL
81: SP_CD14-PE+CD16AF647.fcs	75.2	22.6	2.18	BDL	907	734BDL
83: Th_CD14-PE+CD16-AF647.fcs	52.7	46.3	1.01	BDL<40	450	868BDL
85: TO_CD14-PE+CD16AF647.fcs	49.4	47.4	3.28	BDL<40 n/a	n/a	BDL
87: TY_CD14-PE+CD16-AF647.fcs	14.4	75.2	10.4	2.3:192 n/a	n/a	Viremic
89: ULE_CD14-PE+CD16AF647.fcs	39.8	56.4	3.79 no record			
91: VJ_CD14-PE+CD16-AF647.fcs	18.8	70.5	10.6	BDL	934	759BDL
93: VC_CD14-PE+CD16AF647.fcs	3.82	11.7	84.3	Normal control		Negative
95: YE_CD14-PE+CD16AF647.fcs	56.2	42	1.82	2.1:121	235	706 Viremic
97: YK_CD14-PE+CD16AF647.fcs	3.7	84.3	11.9	BDL n/a	n/a	BDL
99: YY_CD14-PE+CD16-AF647.fcs	43.4	50.6	6	3.5:2820	487	502 Viremic
101: 15893_CD14-PE+CD16-AF647.fcs	40.9	48.8	10.3	BDL	834	1607 BDL
1: C122_Double +.fcs	4.9	17.1	78	40	664	920 LTNP
3: C13_Double +.fcs	1.14	12.5	86.3	40	760	1110 LTNP
5: C53_Double +.fcs	5.48	33	61.5	40	854	900 LTNP
7: HIPE_Double +.fcs	4.59	36	59.4	40	710	1900 LTNP
9: S24_Double +.fcs	7.8	42.7	49.5	40	590	1720 LTNP

We have divided the groups in to 4 groups as color coded 1. BDL, 2. Viremic ,3. LNTP (Long Term Non Progressors) and 4. Negative.

The spread sheet includes the viral load number as a Logrithmic scale (log10) for each patient. The CD4/CD8 count was also included for each patient. N:B... those highlighted in blue are those that did not have the CD4/CD8 stats available hence (n/a) highlighted in red is that of subjects with high CD16 low percentage. normal range for CD4count (380-1390) normal range for CD8+ T cell count (200-690).

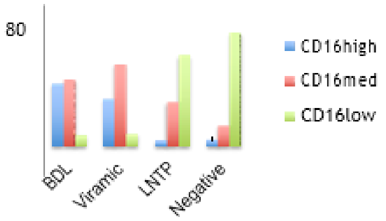
	CD16high	CD16med	CD16low	Viral load (Log10)	CD4 count	CD8count	Group	Viral Load (new)
3: Bn_CD14-PE+CD16AF647.fcs	1.99	66	31.6	BDL	476	571	BDL	40
9: BrAl_CD14-PE+CD16-AF647.fcs	66.6	30.8	2.53	BDL<40	682	946	BDL	40

11: BRJU_CD14-PE+CD16-AF647.fcs	73	24.2	2.84	BDL	706	706	BDL	40
13: CRBR_Cd14-PE+CD16-AF647.fcs	7.06	66.7	26.2	BDL	363	1597	BDL	40
15: DEBR_CD14-PE+CD16AF647.fcs	75.3	19.8	4.81	BDL<40	45	566	BDL	40
19: DMR_CD14-PE+CD16AF647.fcs	2.32	73.9	23.7	BDL	515	1576	BDL	40
23: GaWa_CD14-PE+CD16-AF647.fcs	49.6	48.3	2.04	BDL	435	435	BDL	40
27: GA_CD14-PE+CD16-AF647.fcs	8.72	61.9	29.4	BDL	437	494	BDL	40
29: GS_CD14-PE+CD16-AF647.fcs	61.6	36.2	2.19	BDL<40	338	520	BDL	40
31: GG_CD14-PE+CD16-AF647.fcs	30.7	57.6	11.7	3.2:1690	368	1472	BDL	40
35: HA_CD14-PE+CD16-AF647.fcs	59.6	36.1	4.28	BDL	615	315	BDL	40
37: Harris_CD14-PE+CD16AF647.fcs	5.23	77.1	17.5	BDL	437	760	BDL	40
39: HK_CD14-PE+CD16-AF647.fcs	43.3	53.8	2.83	BDL	443	586	BDL	40
41: iAK_CD14-PE+CD16-AF647.fcs	69.3	29.3	1.34	BDL	n/a	n/a	BDL	40
47: KA_CD14-PE+CD16AF647.fcs	84.8	14.4	0.82	BDL	377	406	BDL	40
49:KJ_CD14-PE+CD16AF647.fcs	9.96	76.8	13.2	BDL	922	1920	BDL	40
51: lu 700108_CD14-PE+CD16AF647.fcs	79.1	19.8	1.04	BDL	592	444	BDL	40
59: m P_CD14-PE+CD16-AF647.fcs	63.8	35.5	0.693	BDL	n/a	n/a	BDL	40
61: mJ_CD14-PE+CD16-AF647.fcs	55.9	40.9	3.13	BDL	608	496	BDL	40
63: MI_CD14-PE+CD16AF647.fcs	16.7	76.6	6.64	BDL<40	1078	868	BDL	40
71: pO T_CD14-PE+CD16-AF647.fcs	83.6	15.8	0.547	BDL	n/a	n/a	BDL	40
75: SAR_CD14-PE+CD16AF647.fcs	83.3	15.8	0.904	BDL	n/a	n/a	BDL	40
77: S D_CD14-PE+Cd16-AF647.fcs	24.9	70.7	4.36	BDL	1131	485	BDL	40
81: spjo_CD14-PE+CD16AF647.fcs	75.2	22.6	2.18	BDL	907	734	BDL	40
83: Th_CD14-PE+CD16-AF647.fcs	52.7	46.3	1.01	BDL<40	450	868	BDL	40
85: TO_CD14-PE+CD16AF647.fcs	49.4	47.4	3.28	BDL<40	n/a	n/a	BDL	40
91: VAR_CD14-PE+CD16-AF647.fcs	18.8	70.5	10.6	BDL	934	759	BDL	40
97: YU_CD14-PE+CD16AF647.fcs	3.7	84.3	11.9	BDL	n/a	n/a	BDL	40
101: 15893 pl lo_CD14-PE+CD16-AF647.fcs	40.9	48.8	10.3	BDL	834	1607	BDL	40
1: ANCR_CD14-PE+CD16AF647.fcs	24.2	62.2	13.6	2.0:107	194	1642	Viremic	107
5: BeRa_CD14-PE+CD16-AF647.fcs	34.5	63.9	1.6	4.4:22900	320	1109	Viremic	22900
17: DIJA_CD14-PE+CD16AF647.fcs	8.67	55.9	35.5	84	222	1112	Viremic	84
21:EP_CD14-PE+CD16_AF647.fcs	40.4	53	6.62	1.9:91	n/a	n/a	Viremic	91
25: GTP_CD14-PE+CD16AF647.fcs	20.3	65.1	14.6	231000	7	318	Viremic	231000
33: GWJ_CD14-PE+CD16AF647.fcs	31.8	66.2	2.02	4.1:13800	n/a	n/a	Viremic	13800
45: JU_Cd14-PE+CD16-AQF647.fcs	26.2	64	9.88	2.0:104	850	975	Viremic	104
53: LE(2)_CD14-PE+CD16AF647.fcs	23.4	62.2	14.4	4.5:33600	156	1672	Viremic	33600

55: MS_CD14-PE+CD16-AF647.fcs	54.6	44.5	0.92	2.1:121	597	760	Viremic	121
57: mR_CD14-PE+CD16-AF647.fcs	50.9	47.4	1.64	2.1:120	597	688	Viremic	120
67: ok_CD14-PE+CD16AF647.fcs	42.9	52.8	4.29	1.6:40	520	1378	Viremic	40
87: TK_CD14-PE+CD16-AF647.fcs	14.4	75.2	10.4	2.3:192	n/a	n/a	Viremic	192
95: YF_CD14-PE+CD16AF647.fcs	56.2	42	1.82	2.1:121	235	706	Viremic	121
99: YY_CD14-PE+CD16-AF647.fcs	43.4	50.6	6	3.5:2820	487	502	Viremic	2820
69: PH_CD14-PE+CD16AF647.fcs	6.34	39.2	54.4	BDL	660	1200	LNTP	40
79: SD_CD14-PE+CD16AF647.fcs	0.226	39	60.3	BDL	669	491	LNTP	40
1: C122_Double +.fcs	4.9	17.1	78		664	920	LNTP	40
3: C13_Double +.fcs	1.14	12.5	86.3		760	1110	LNTP	40
5: C53_Double +.fcs	5.48	33	61.5		854	900	LNTP	40
7: HIPE_Double +.fcs	4.59	36	59.4		710	1900	LNTP	40
9: S24_Double +.fcs	7.8	42.7	49.5		590	1720	LNTP	40
7: BW_CD14-PE+CD16AF647.fcs	4.35	19.4	76.2	Normal control			Negative	
43: JO_CD14-PE+CD16-AF647.fcs	4.24	7.59	87.9				Negative	
73: PG 2 retake_CD14-PE+CD16AF647.fcs	7.45	21.2	71.2	normal control			Negative	
93:VC_CD14-PE+CD16AF647.fcs	3.82	11.7	84.3	Normal control			Negative	

Citation: Matlho K, Wang XM, Conceicao V, Perera SS, Wang B, et al. (2018) CD14+CD16-Low Monocyte Subset Predicts Non-Progressive HIV Disease: Evidence of A New Prognostic and Diagnostic Biomarker. Biomark Applic BMAP-128. DOI: 10.29011/2576-9588. 100028

Mean	CD16high	CD16med	CD16low
BDL	44.727	47.169	8.054
Viramic	33.705	57.500	8.806
LNTP	4.354	31.357	64.200
Negative	[illegible]	[illegible]	[illegible]



P value	CD16high	CD16med	CD16low
BDL vs Neg	0.000	0.000	0.000
Viramic vs Neg	0.000	0.000	0.000
LNTP vs Neg	0.657	0.015	0.033
BDL vs viremic	0.1075	0.0382	0.8034
BDL vs LNTP	0.0000	0.0177	0.0000
Viremic vs LNTP	0.0000	0.0005	0.0000

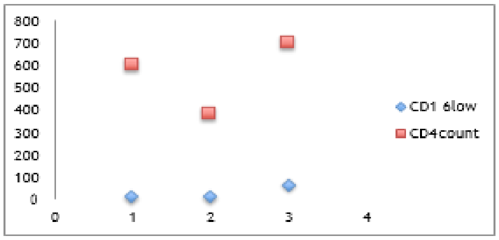
SD	CD16high	CD16med	CD16low
BDL	28.965	22.054	9.210
Viramic	14.930	9.519	9.188
LNTP	2.733	11.774	13.144
Negative	1.672	6.419	7.588

	Viral load	CD16med
BDL	40	47
Viramic	21793	58
LNTP	40	39

Mean	CD4count	CD8count	Viral Load
BDL	595	832	<20
Viramic	380	987	
LNTP	701	1177	
Negative			

CD16 vs CD4 correlation

	CD16low	CD4count	CD16med	CD16high
BDL	8	595	47	45
Viramic	9	380	58	34
LNTP	64	701	31	4
	CD16low vs CD4		CD16med vs CD	CD16high vs CD4
correlation	0.745438877		-0.948987122	-0.552841717



Viral load vs CD16 correlation

	CD16low	Viral load	CD16med	CD16high
BDL	8	40	47	45
Viramic	9	21793	58	34
LNTP	64	40	31	4
	CD16low vs Viral load		CD16med vs vir	CD16high vs vira. loa
correlation	-0.48981021		0.796017619	0.256026659