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-high, medium and low 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 monocyctic 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 Fcγlll 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 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 LTNP] 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.

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

| Patient Groups | CD4+T cell count | CD8+T cell count | Plasma Viral Load (copies RNA/mL plasma) |
|---------------|-----------------|-----------------|----------------------------------------|
| BDL          | 595             | 832             | <40 copies RNA with 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

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 follicled 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% Parafornaldehyde) 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.
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 \((\text{BDL vs neg } p<0.00000374 \text{ and Viremic vs neg } p<0.000056)\) (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 \((\text{LTNP vs BDL } p<0.0075102 \text{ and LTNP vs Viremic } p<0.0015809)\). Moreover, the HAART-treated groups (viremic and BDL) were also characterized by the elevated levels of CD14+CD16-high population (33 and 45%, respectively) \((\text{Figure 2, Table 2,3})\), when compared against LTNPs \((\text{BDL vs LTNP } p<0.0000180 \text{ and Viremic vs LTNP } p<0.0009354)\) 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 \((p<0.0048514 \text{ and } p<0.0114045, \text{respectively})\) (Table 3).

![Figure 1](image_url)

**Figure 1:** The representative flow cytometry dot pots showing the gating strategy of discriminating the CD14+CD16-high, CD14+CD16-medium and CD14+CD16-low populations in HIV patients at different stages of plasma viremia compared to the HIV negative healthy donors.
From these data two significant aspects of phenotypic regulation of monocytes are clear that the high levels of CD14+CD16- low and low levels of 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 monocyte 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 naive 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 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).

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 naive 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-medium 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% (LTNP) 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.

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## SUPPLEMENTARY DATA

**CD16high CD16med CD16low Viral load (Log10)**

| Sample Description | CD16high | CD16med | CD16low | Viral Load (Log10) | Notes |
|--------------------|----------|---------|---------|-------------------|-------|
| ANCR_CD14-PE+CD16AF647.fcs | 24.2     | 62.2    | 13.6    | 2.0:107           | 194   | 1642 Viremic |
| BN_-CD14-PE+CD16AF647.fcs | 1.99     | 66      | 31.6    | BDL               | 476   | 571 BDL |
| BERA_-CD14-PE+CD16- AF647.fcs | 34.5     | 63.9    | 1.6     | 4.4:22900         | 320   | 1109 BDL |
| BW-CD14-PE+CD16AF647.fcs | 4.35     | 19.4    | 76.2    | Normal control    |       | Negative |
| BrAI-CD14-PE+CD16- AF647.fcs | 66.6     | 30.8    | 2.53    | BDL<40            | 682   | 946 BDL |
| BRUJ-CD14-PE+CD16- AF647.fcs | 73       | 24.2    | 2.84    | BDL               | 706   | 706 BDL |
| CRBR-Cd14-PE+CD16- AF647.fcs | 1.96     | 67.2    | 26.2    | BDL               | 363   | 1597 BDL |
| DEBR-CD14-PE+CD16- AF647.fcs | 75.3     | 19.8    | 4.81    | BDL<40            | 45    | 566 BDL |
| DJA-CD14-PE+CD16AF647.fcs | 8.67     | 55.9    | 35.5    | 84                | 222   | 1122 Viremic |
| DRJ-CD14-PE+CD16AF647.fcs | 2.32     | 73.9    | 23.7    | BDL               | 515   | 1576 BDL |
| EL-CD14-PE+CD16_AF647.fcs | 40.4     | 53      | 6.62    | 1.9:91 n/a        |       | Viremic |
| GaWa_CD14-PE+CD16- AF647.fcs | 49.6     | 48.3    | 2.04    | BDL               | 435   | 435 BDL |
| GTPCD14-PE+CD16AF647.fcs | 20.3     | 65.1    | 14.6    | 231000            | 7     | 318 Viremic |
| Gar_CD14-PE+CD16- AF647.fcs | 8.72     | 61.9    | 29.4    | BDL<40            | 437   | 494 BDL BDL BDL |
| GS_CD14-PE+CD16- AF647.fcs | 61.6     | 36.2    | 2.19    | BDL               | 338   | 520 |
| Gh G_CD14-PE+CD16- AF647.fcs | 30.7     | 57.6    | 11.7    | BDL               | 368   | 1472 |
| GWJ_CD14-PE+CD16- AF647.fcs | 31.8     | 66.2    | 2.02    | BDL<40           | 4.1:153800 n/a | 1472 |
| H_CD14-PE+CD16-AF647.fcs | 59.6     | 36.1    | 4.28    | BDL               | 615   | 315 BDL |
| HAR_CD14-PE+CD16AF647.fcs | 5.23     | 77.1    | 17.5    | BDL               | 437   | 760 BDL |
| HK_CD14-PE+CD16- AF647.fcs | 43.3     | 53.8    | 2.83    | BDL               | 443   | 586 BDL |
| IAK_CD14-PE+CD16- AF647.fcs | 69.3     | 29.3    | 1.34    | BDL n/a           |       | BDL |
| J_CD14-PE+CD16- AF647.fcs | 4.24     | 7.59    | 87.9    | BDL               |       | BDL |
| JU_Cd14-PE+CD16-AQF647.fcs | 26.2     | 64      | 9.88    | 2.0:104          | 850   | 975 Viremic |
| KA_CD14-PE+CD16AF647.fcs | 84.8     | 14.4    | 0.82    | BDL BDL BDL BDL | 377   | 406 BDL BDL BDL |
| KG_CD14-PE+CD16AF647.fcs | 9.96     | 76.8    | 13.2    | BDL               | 922   | 1920 |
| LEJO_CD14-PE+CD16AF647.fcs | 79.1     | 19.8    | 1.04    | BDL               | 592   | 444 |
| MS_CD14-PE+CD16-AF647.fcs | 54.6     | 44.5    | 0.92    | 2.1:121           | 597   | 760 Viremic |
| MR_CD14-PE+CD16-AF647.fcs | 50.9     | 47.4    | 1.64    | 2.1:120           | 597   | 688 Viremic |
| MP_CD14-PE+CD16-AF647.fcs | 63.8     | 35.5    | 0.693   | BDL n/a           |       | BDL |
| mOJA_CD14-PE+CD16AF647.fcs | 55.9     | 40.9    | 3.13    | BDL               | 608   | 496 BDL |
| MOM_CD14-PE+CD16AF647.fcs | 16.7     | 76.6    | 6.64    | BDL<40            | 1078  | 868 BDL |
We have divided the groups in into 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 Logarithmic 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 CD4 count (380-1390) normal range for CD8+ T cell count (200-690).

| Group | Viral Load (Log10) | CD4 count | CD8 count | Group | Viral Load (new) |
|-------|-------------------|-----------|-----------|-------|-----------------|
| BDL   | 4.2:16300         | 271       | 1747LNTP  |
| Viremic | 1200LNTP         | 660       | 1378Viremic |
| LNTP  | n/a               | BDL       | n/a       |
| Negative | n/a              | BDL       | BDL      |

| Group | Viral Load (Log10) | CD4 count | CD8 count | Group | Viral Load (new) |
|-------|-------------------|-----------|-----------|-------|-----------------|
| BDL   | 4.2:16300         | 271       | 1747LNTP  |
| Viremic | 1200LNTP         | 660       | 1378Viremic |
| LNTP  | n/a               | BDL       | n/a       |
| Negative | n/a              | BDL       | BDL      |

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.
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| 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+CD16AF647.fcs | 30.7 | 57.6 | 11.7 | 3.2:1690 | 368 | 1472 | BDL | 40 |
| 31: G_14-PE+CD16AF647.fcs | 9.96 | 76.8 | 13.2 | BDL | 922 | 1920 | BDL | 40 |
| 35: HA_CD14-PE+CD16AF647.fcs | 5.23 | 77.1 | 17.5 | BDL | 592 | 444 | BDL | 40 |
| 37: Harris_CD14-PE+CD16AF647.fcs | 63.8 | 35.5 | 0.693 | BDL | n/a | n/a | BDL | 40 |
| 39: HK_CD14-PE+CD16-AF647.fcs | 16.7 | 76.6 | 6.64 | BDL<40 | 1078 | 868 | BDL | 40 |
| 41: iAK_CD14-PE+CD16-AF647.fcs | 52.7 | 46.3 | 1.01 | BDL<40 | 450 | 868 | BDL | 40 |
| 47: KA_CD14-PE+CD16AF647.fcs | 79.1 | 19.8 | 1.04 | BDL | 592 | 444 | BDL | 40 |
| 49: KJ_CD14-PE+CD16AF647.fcs | 75.2 | 22.6 | 2.18 | BDL | 907 | 734 | BDL | 40 |
| 51: lu70108_CD14-PE+CD16AF647.fcs | 26.2 | 64 | 9.88 | 2.0:104 | 850 | 975 | BDL | 104 |
| 59: mP_CD14-PE+CD16-AF647.fcs | 34.5 | 63.9 | 1.6 | 4.4:22900 | 320 | 1109 | BDL | 22900 |
| 77: S_D_CD14-PE+CD16AF647.fcs | 20.3 | 65.1 | 14.6 | 231000 | 7 | 318 | BDL | 231000 |
| 81: spJo_CD14-PE+CD16AF647.fcs | 23.4 | 62.2 | 14.4 | 4.5:33600 | 156 | 1672 | BDL | 33600 |
| 97: YU_CD14-PE+CD16AF647.fcs | 24.2 | 62.2 | 13.6 | 2.0:107 | 194 | 1642 | Viremic | 107 |
| 101: 15893plilo_CD14-PE+CD16-AF647.fcs | 31.8 | 66.2 | 2.02 | 4.1:13800 | n/a | n/a | BDL | 13800 |
| 1: ANCR_CD14-PE+CD16AF647.fcs | 24.2 | 62.2 | 13.6 | 2.0:107 | 194 | 1642 | Viremic | 107 |
| 45: JU_CD14-PE+CD16-AQF647.fcs | 20.3 | 65.1 | 14.6 | 231000 | 7 | 318 | BDL | 231000 |
| 53: LE(2)_CD14-PE+CD16AF647.fcs | 20.3 | 65.1 | 14.6 | 231000 | 7 | 318 | BDL | 231000 |
| Sample ID          | CD14-PE+CD16-AF647 (%) | CD16-AF647 (%) | CD14-PE+CD16-AF647/CD16-AF647 | Viremic | LNTP |
|-------------------|------------------------|----------------|-------------------------------|---------|------|
| 55: MS_CD14-PE+CD16-AF647.fcs | 54.6                   | 44.5           | 0.92                          | 2.1:121 | 597  | Viremic | 121 |
| 57: mR_CD14-PE+CD16-AF647.fcs | 50.9                   | 47.4           | 1.64                          | 2.1:120 | 597  | Viremic | 120 |
| 67: ok_CD14-PE+CD16AF647.fcs | 42.9                   | 52.8           | 4.29                          | 1.6:40  | 520  | Viremic | 40  |
| 87: TK_CD14-PE+CD16-AF647.fcs | 14.4                   | 75.2           | 10.4                          | 2.3:192 | n/a  | Viremic | 192 |
| 95: YF_CD14-PE+CD16AF647.fcs | 56.2                   | 42             | 1.82                          | 2.1:121 | 235  | Viremic | 121 |
| 99: YY_CD14-PE+CD16-AF647.fcs | 43.4                   | 50.6           | 6                             | 3.5:2820| 487  | Viremic | 2820|
| 69: PH_CD14-PE+CD16AF647.fcs | 6.34                   | 39.2           | 54.4                          | BDL     | 660  | Viremic | 120 |
| 79: SD_CD14-PE+CD16AF647.fcs | 0.226                  | 39             | 60.3                          | BDL     | 669  | LNTP   | 40  |
| 1: C122_Double+.fcs | 4.9                    | 17.1           | 78                            |         | 664  | LNTP   | 40  |
| 3: C13_Double+.fcs | 1.14                   | 12.5           | 86.3                          |         | 760  | LNTP   | 40  |
| 5: C53_Double+.fcs | 5.48                   | 33             | 61.5                          |         | 854  | LNTP   | 40  |
| 7: HIPE_Double+.fcs | 4.59                   | 36             | 59.4                          |         | 710  | LNTP   | 40  |
| 9: S24_Double+.fcs | 7.8                    | 42.7           | 49.5                          |         | 590  | 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   |
| Viral  | 33.705   | 57.500  | 8.806   |
| LNTF  | 4.356    | 31.357  | 64.20E  |
| Negative | 4.903     | 14.773  | 95.70E  |

### P value

|        | CD16high | CD16med | CD16low |
|--------|----------|---------|---------|
| BDL vs Neg | 0.000    | 0.000   | 0.000   |
| Viral vs Neg | 0.000    | 0.000   | 0.000   |
| LNTF vs Neg | 0.457    | 0.015   | 0.633   |
| BDL vs Viral | 0.1075   | 0.0182  | 0.0034  |
| BDL vs LNTF | 0.0000   | 0.0177  | 0.0000  |
| Viral vs LNTF | 0.3000   | 0.0005  | 0.0000  |

### SD

|        | CD16high | CD16med | CD16low |
|--------|----------|---------|---------|
| BDL    | 28.965   | 22.054  | 9.210   |
| Viral  | 14.930   | 9.519   | 9.188   |
| LNTF   | 2.733    | 11.774  | 13.144  |
| Negative | 1.672     | 6.419   | 7.588   |

### Viral load vs CD4 count

|        | Mean CD4 count | Viral Load |
|--------|----------------|------------|
| BDL    | 40             | 47         |
| Viral  | 21793          | 58         |
| LNTF   | 40             | 39         |

### CD16 vs CD4 correlation

|        | CD16low | CD4 count | CD16med | CD16high |
|--------|---------|-----------|---------|----------|
| BDL    | 6       | 595       | 47      | 45       |
| Viral  | 9       | 380       | 53      | 34       |
| LNTF   | 64      | 701       | 31      | 4        |

### Viral load vs CD16 correlation

|        | CD16low | Viral load | CD16med | CD16high |
|--------|---------|------------|---------|----------|
| BDL    | 8       | 40         | 47      | 43       |
| Viral  | 9       | 21793      | 58      | 34       |
| LNTF   | 64      | 40         | 31      | 4        |

### CD16low vs CD4 count correlation

- 0.745438677

### CD16med vs CD16high correlation

- 0.049587122
- 0.5552841717

### Viral load vs CD16 correlation

- 0.48961321
- 0.798017619
- 0.258026659