Research Article

Higher levels of Monocyte Distribution Width as a potential flagging parameter of HIV progression: results of a monocentric observational study

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Keywords
MDW, HIV, Immune System Activation, Risk Prediction

Abstract

Objectives: Monocyte Distribution Width (MDW) is the standard deviation of the mean volume of monocytes and may indicate innate immune activation. We investigated the possible association between MDW values and late HIV diagnosis in consecutive patients.

Methods: We retrospectively enrolled newly diagnosed HIV patients admitted to our clinical center. Demographic and clinical characteristics were analyzed.

Results: A total of 97 patients were enrolled. Of these, 63% were late presenters and 43% fulfilled the criteria for advanced HIV disease. Continuous measures showed a significant inverse correlation between CD4 T-cell count and MDW. Multivariate analysis showed that MDW≥21.1 (OR:7.45, 2.13-30.54), HIV viral load >5 log (10) c/mL (OR:3.62, 1.04-13.30), blood lymphocytes<2 x10^3/µL (OR:14.82, 3.19-111.8) and HIV testing without symptoms (OR:0.21, 0.05-0.82) were independently associated with late presentation. Similarly, adjusted ORs for MDW≥22.5 (OR:4.03, 1.28-13.17), blood lymphocytes<1 x10^3/µL (OR:9.67, 2.19-57.57), age (OR:1.05, 1.00-1.10) and HIV testing without symptoms (OR:0.16, 0.04-0.52) were significantly associated with advanced HIV disease.

Conclusions: Our results suggest that MDW may be a potential flagging parameter of innate immune activation in HIV infection. Continuous measurements of MDW showed a significant inverse correlation with CD4 T-cell count. Patients with increased MDW values were more likely to be diagnosed late.

Introduction

Monocytes and macrophages are pivotal cells of innate immunity, playing a relevant role in HIV transmission and viral spread in the early phases of HIV infection [1-3]. Their recognition as a reservoir for HIV raises interest on investigating the role of specific subsets of monocytes and macrophages [1-5].

HIV patients losing CD4-T lymphocytes during disease progression usually exhibit markers of monocyte activation and a chronic proinflammatory response that is hallmark of poor clinical outcome [5].

Several markers of monocyte activation have been characterized [6-9]. Monocyte Distribution Width (MDW), a measure of the size of monocytes in the bloodstream, has been recently introduced as a tool to support the diagnosis of sepsis in Emergency Departments and Intensive Care Units, based upon its rapid variation upon bloodstream invasion by pathogens and the convenient availability of routine blood tests [10-13].

The aim of the present study was to investigate the possible association between increased values of MDW and late HIV diagnosis, adjusting for known major risk factors in consecutive patients first referred to our unit.

Methods

We conducted a retrospective study, including all consecutive newly diagnosed cases of HIV-infected patients referred for antiviral treatment to the Infectious Diseases Unit of the Pescara General Hospital, Italy, between January 2020 and June 2024. The study plan was approved by the local Health Administrative Board.

We considered two main outcomes: late presentation and advanced HIV disease. The former was defined as a person first diagnosed with HIV with a CD4 T-cell count < 350 cells/μL or with an AIDS-defining event, regardless of the CD4 T-cell count. The latter was defined as a person first diagnosed with HIV with a CD4 T-cell count<200 cells/μL [14]. Symptoms, clinical signs, HIV CDC class and AIDS-defining condition(s) were duly recorded, while MDW measurements were available among routine CBC parameters measured using DxH 900 Analyser (Beckman Coulter Inc., Miami, FL).

In addition to clinical and demographics variables, we considered the way of access to HIV testing as a binary variable (1 vs 0), depending on whether it was performed with or without prior symptoms (taking screening as a reference category).

To aid clinical interpretation, we transformed MDW and HIV viremia into binary variables, by using optimal cut-offs based on the Youden index separately for each outcome, to compute odds ratios (ORs) in univariate and multivariate logistic regression [10, 12, 15]. We did not use the total number of lymphocytes as a continuous explanatory variable, due to its collinearity with the outcome, as both contain CD4 T-cells [14].

The level of association between each categorical risk factor and the outcome was tested using the chi-square test for univariate odds ratios (ORs) [15]. Student's t-test and non-parametric Kruskal–Wallis rank tests were used for normal and non-normal continuous variables, respectively. Multivariate logistic regression was used to compute adjusted ORs and 95%CI, taking into account all risk factors [11, 12].

Backward elimination was used to keep only significant variables in the model, to avoid overfitting due to the low number of cases [16]. Age and gender were tested as potential confounders in all models, before deciding on their exclusion. All models were estimated by allowing at least 10 events per variable.

Table 1: General characteristics of study population.

Variable (Category)	Late Presentation		Advanced Disease		Overall
	No	**Yes**	**No**	**Yes**	
N**	36 (37.1)	61 (62.9)	55 (56.7)	42 (43.3)	97 (100.0)
Age*	40.7 (13.5)	47.2 (13.8)	41.7 (14.0)	48.7 (13.1)	44.7 (14.0)
Gender**					
F	8 (22.2)	14 (23.0)	11 (20.0)	11 (26.2)	22 (22.7)
M	28 (77.8)	47 (77.0)	44 (80.0)	31 (73.8)	75 (77.3)
HIV asymptomatic screening**					
No	14 (38.9)	46 (75.4)	24 (43.6)	36 (85.7)	60 (61.9)
Yes	22 (61.1)	15 (24.6)	31 (56.4)	6 (14.3)	37 (38.1)
Heterosexual factor risk**					
No	22 (61.1)	22 (36.1)	29 (52.7)	15 (35.7)	44 (45.4)
Yes	14 (38.9)	39 (63.9)	26 (47.3)	27 (64.3)	53 (54.6)

Years of diagnosis**					
2020	9 (25.0)	10 (16.4)	11 (20.0)	8 (19.0)	19 (19.6)
2021	3 (8.3)	14 (23.0)	10 (18.2)	7 (16.7)	17 (17.5)
2022	15 (41.7)	8 (13.1)	17 (30.9)	6 (14.3)	23 (23.7)
2023	3 (8.3)	21 (34.4)	7 (12.7)	17 (40.5)	24 (24.7)
2024	6 (16.7)	8 (13.1)	10 (18.2)	4 (9.5)	14 (14.5)
Country**					
Africans	2 (5.6)	3 (4.9)	4 (7.3)	1 (2.4)	5 (5.2)
European	33 (91.7)	49 (80.3)	49 (89.1)	33 (78.6)	82 (84.5)
South Americans	1 (2.8)	9 (14.8)	2 (3.6)	8 (19.0)	10 (10.3)
MDW[1], ***	20.4 (15.0, 34.3)	23.6 (18.0, 45.0)	20.8 (15.0, 34.3)	24.2 (18.0, 45.0)	22.3 (20.4, 24.8)
Lymphocytes, x10^3 cell/µL[2],***	1.9 (0.8, 5.2)	1.1 (0.30, 600)	1.8 (0.8, 5.2)	0.9 (0.3, 600)	1.4 (1.0, 1.9)
Viremia, Log (10), c/mL[3]	4.4 (2.1, 6.3)	5.3 (1.5, 7.0)	4.5 (1.5, 7.0)	5.5 (2.5, 7.0)	5.0 (4.4, 5.6)

* Continuous, mean (SD); ** n (%); ***Continuous, median (IQR)

[1]Monocyte Distribution Width; [2]Total number of peripheral lymphocytes; [3]Viremia: blood HIV RNA

Results

The study included 97 consecutive patients with a confirmed new diagnosis of HIV infection (19 in 2020, 17 in 2021, 23 in 2022, 24 in 2023, 14 in 2024).

The general characteristics of the study population are shown in Table 1.

A percentage of 84.5% was of European nationality, and 77.3% were males. The mean age was 44.7 (s.d.=14.0). A total of N=61 (62.9%) were classified as late presenters, while N=42 (43.3%) matched the criteria of advanced disease.

Continuous measures were significantly different between late and early presenters in terms of higher MDW (23.6 vs 20.4, p<0.001) and HIV-RNA viral loads (5.3 vs.
4.4 Log(10) cp/mL, p<0.001), while lower for peripheral blood lymphocytes (1.1 vs. 1.9 x10^3/µL, p<0.001).

Similarly, for advanced disease, values were significantly higher for MDW (24.2 vs. 20.8, p<0.001), HIV-DNA viral loads (5.5 vs. 4.5 Log(10) c/mL, p<0.001), and lower for peripheral blood lymphocytes (0.9 vs. 1.80 x10^3/µL, p<0.001).

The results of univariate analysis are shown in Table 2.

Among categorical variables, the risk of a late HIV diagnosis was significantly higher for MDW≥21.1 (OR: 10.33, 95%CI: 3.94-27.08, p<0.001), blood lymphocytes <2x10^3/µL (OR: 9.17, 95%CI: 3.16-26.63, p<0.001), and higher HIV viral loads ≥5 Log(10) cp/mL (OR: 11.81, 95%CI: 4.00-34.85, p<0.001).

An almost 80% decreased risk was found for those testing HIV without symptoms (OR: 0.21, 95%CI: 0.09-0.5, p<0.001), while the risk was significantly increased for age (OR: 1.03, 95%CI: 1.00-1.07; p=0.028) and heterosexual risk of transmission (OR: 2.79, 95%CI: 1.19-6.52; p=0.02).

Table 2: Results of univariate analysis.

Variable (Category)	Late Presentation		Advanced Disease	
	O.R. (95%C.I.)*	p>χ2	O.R. (95%C.I.)**	p>χ2
Age, years*	1.03 (1.00-1.07)	0.03	1.04 (1.01-1.07)	0.01
Males (r.c. = females)	0.96 (0.36-2.57)	0.93	0.70 (0.27-1.83)	0.47
HIV asymptomatic screening (r.c. = no)	0.21 (0.09-0.50)	<0.001	0.13 (0.05-0.36)	<0.001
Heterosexual (r.c. = no)	2.79 (1.19-6.52)	0.02	2.01 (0.88-4.58)	0.09
European Nationality (r.c. = no)	0.37 (0.10-1.42)	0.12	0.45 (0.15-1.38)	0.16
Year of diagnosis (r.c. = 2000)				
2021	4.20 (0.90-19.56)	0.05	0.96 (0.26-3.63)	0.96
2022	0.48 (0.14- 1.67)	0.24	0.49 (0.13-1.78)	0.27
2023-2024	2.90 (0.90- 9.35)	0.07	1.70 (0.56-5.17)	0.35

MDW[1]				
≥21.1 (r.c. = <21.1)	10.33 (3.94-27.08)	<0.001		
≥22.5 (r.c. = <22.5)			9.56 (3.26-28.01)	<0.001
Lymphocytes, x10³ cell/µL[2]				
<2 (r.c. = ≥2)	9.17 (3.16-26.63)	<0.001		
<1 (r.c. = ≥1)			13.33 (2.92-60.91)	<0.001
Viremia, Log (10), c/mL[3]				
≥5 (r.c. = <5)	**11.81 (4.00-34.85)**	**<0.001**	**6.67 (2.73-16.31)**	**<0.001**

* Continuous; r.c.: Reference Category

[1]Monocyte Distribution Width; [2]Total number of peripheral lymphocytes; [3]Viremia: blood HIV RNA

No association was found for gender and years of diagnosis.
The scatter plot showed a significant inverse correlation between MDW and CD4-T values (Spearman Rho = - 0.61; p < 0.0001, see Figure 1).
Considering advanced disease as the outcome, the risk was significantly higher for: MDW ≥22.5 (OR: 9.56, 95%CI: 3.26-28.01, p<0.001), HIV viral loads ≥ 5 (OR: 6.67, 95%CI: 2.73-16.31, p<0.001), and blood lymphocytes <1 x10³ µL (OR: 13.33, 95%CI: 2.92-60.91, p<0.001).
An almost 87% decreased risk was found for those testing HIV without symptoms (OR: 0.13, 95%CI: 0.05-0.36, p<0.001).
The results of multivariate logistic regression are shown in Table 3.
For late presentation, six variables were left in the model out of 61 events.
Age and gender were initially not retained in the model by backward elimination. However, the estimates were radically different from those obtained with their inclusion. This indicated that age and gender were confounders of the relation between explanatory variables and late presentation. Consequently, age and gender were included in the final model, even if not significant.
Adjusted ORs were significant for MDW≥21.1 (OR:7.45, 95%CI: 2.13-30.54, P<0.01), HIV viral loads ≥5 log(10) c/mL (OR:3.62, 95%CI: 1.04-13.30, p<0.05), blood lymphocytes <2 x10³/µL (OR:14.82, 95%CI: 3.19-111.8, p<0.01) and HIV testing without symptoms (OR:0.21, 95%CI: 0.05-0.82, p=0.03).
For advanced disease, four variables were left in the model out of 42 events.
The risk was significantly increased for: age (OR:1.05, 95%CI:1.00-1.10, p=0.04), MDW≥22.5 (OR:4.03, 95%CI: 1.28-13.17, p=0.02), blood lymphocytes <1 x10³/µL (OR:9.67, 95%CI: 2.19-57.57, p<0.01). The risk was significantly decreased for HIV screening without symptoms (OR:0.16, 95%CI: 0.04-0.52, p<0.01). In this case, excluding gender did not substantially change the estimated coefficients.

Figure 1: Scatter plot of MDW (X axis) by bloodstream CD4-T cell count (Y axis). Overlap is the least squares regression line with slope equal to the Spearman correlation, along with its 95% confidence intervals.

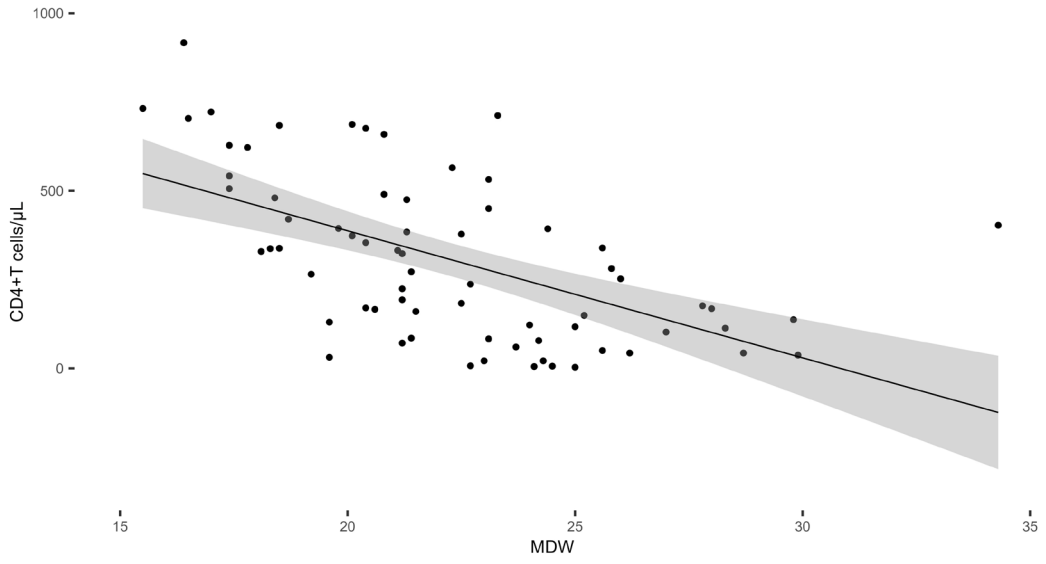

Table 3: Results of multivariate logistic regression analysis.

Variable (Category)	Late Presentation N=97 (Events=61)"		Advanced Disease N=97 (Events=42)	
	O.R. (95%C.I.)	**p**	**O.R. (95%C.I.)**	**p**
Age, years*	1.03 (0.98-1.08)	0.22	1.05 (1.00-1.10)	0.04
Males (r.c. = females)	0.44 (0.09-1.93)	0.29		
HIV asymptomatic screening (r.c. = no)	0.21 (0.05-0.82)	0.03	0.16 (0.04-0.52)	<0.01
MDW[1]				
≥21.1 (r.c. = <21.1)	7.45 (2.13-30.54)	<0.01		
≥22.5 (r.c. = <22.5)			4.03 (1.28-13.17)	0.02
Lymphocytes, x10[3] cell/µL[2]				
<2 (r.c. = ≥2)	14.82 (3.19-111.8)	<0.01		
<1 (r.c. = ≥1)			9.67 (2.19-57.57)	<0.01
Viremia, Log (10) c/mL[3]				
≥5 (r.c. = <5)	3.62 (1.04-13.30)	0.04		

Discussion

Although higher values of MDW are associated with sepsis, limited information is available about changes in viral diseases. This study found that patients with a value of MDW≥21.1 were over seven times more likely with late presentation, independently from age and sex. Similarly, values of MDW≥22.5 were more likely among patients with advanced HIV disease.

As for other European countries, the number of cases presenting late in Abruzzo region remains of concern, despite national programs to achieve earlier diagnosis [17-18].

Our findings confirm that patients presenting late are more frequently males, older, migrants, and exposed through heterosexual contact [19]. However, multivariate regression failed to show any significant association, independently from HIV asymptomatic screening and laboratory parameters e.g. MDW, number of lymphocytes and viremia. Possible explanations are the limited sample size and the high predictive value of biological parameters.

In terms of temporal trends, the scientific literature reports an increase in the percentage of persons with late HIV diagnosis, from 58.2% in 2022 to 60.1% in 2023 [17-18]. However, our study did not confirm a significant increase over four consecutive years.

In terms of progression of HIV over time, several studies reported monocyte activation as a potential correlate [2,5,6,20,21].

A study of flow cytometry showed that HIV patients losing CD4-T cells, both in early and chronic infection, are at higher risk of developing a heightened proinflammatory status [19]. Percentages of blood subsets of intermediate (CD14++CD16+) and non-classical monocytes (CD14+CD16++), which generally increase during inflammatory reactions, were significantly higher in chronic HIV-1-infected cART-naïve patients [22]. Soluble plasma CD14 (sCD14) and CD163 (sCD163), two further biomarkers of monocyte activation, may play a role in the inflammatory response during HIV progression. sCD14 have higher blood concentration in chronically HIV-infected than healthy subjects [23], being inversely associated with CD4-T cell count recovery during ART [20]. Similarly, sCD163 levels were higher compared to healthy subjects, decreasing after the start of ART [24].

The above results support the adoption of MDW as a potential flag of innate immune activation in HIV infection. Higher MDW values may be associated with the pro-inflammatory state of the innate immune system occurring during HIV progression and the subsequent loss of lymphocytes. Therefore, in HIV patients with disease progression, MDW could help identifying bacterial infections related to the immune activation during the loss of CD4+ T cells.

For instance, monocyte indicators may change rapidly in gut-associated lymphoid tissue (GALT) due to CD4+ T-cell depletion and fibrosis, which only improves slowly with antiretroviral therapy. Disruption to the integrity of the gut mucosa and its immunity may result in high plasma levels of bacterial lipopolysaccharide (LPS) and bacterial translocation, leading to an increase in MDW results and the activation of other biomarkers frequently found in this condition, such as HLA-DR and CD69 on monocytes, CD38 on lymphocytes, and CRP [25, 26].

Additionally, MDW may serve as a pro-inflammatory indicator in non-infectious conditions, as in the case of chronic inflammation during antiretroviral therapy (ART) for HIV immunological non responders (INR). However, this represents currently only a research hypothesis that needs to be proven. The cause of chronic inflammation observed in HIV infection during ART has been addressed by many recent studies, due to its role as the underlying cause of increased risk of SNAEs (serious non-AIDS events: CVD, HIV-associated neurocognitive dementia, and aging).

The analysis of MDW values in treated people living with HIV may be a reasonable target of future research, given that monocyte activation related to a heightened proinflammatory status has been described mainly in immunological non-responders (INR), where it was found associated with an increased risk of developing SNAEs [5, 27].
Macrophages take up high amounts of cholesterol-rich low-density lipoprotein (LDL) in vessels [28]. This accumulation leads to macrophage necrosis, leading to the growth of a cholesterol-rich necrotic core and cholesterol accumulation [28,29,30]. Consequently, monocyte indicators such as CD11b and CX3CR1 or blood non-classical monocyte subsets (CD14+CD16+, CD14-CD16+) are associated with subclinical atherosclerosis in HIV patients. However, many studies are ongoing and more results are needed to shed light on this relationship [29].
Additionally, MDW may be investigated in HIV-Associated Neurocognitive Disorders (HAND) and aging. It is widely known that monocytes traversing the blood-brain barrier are a major source of HIV infection in the brain, and that HIV-DNA viral load in monocytes, but not in plasma or in CD4-T cells, is associated with HAND, suggesting that they may contribute to brain injury [31]. However, more studies are needed to support this hypothesis [32].
Finally, similar to CVD risk and HAND, premature immunosenescence is associated with HIV-1 infection, due to the persistent immune activation and chronic inflammation. Evidence suggests that monocytes contribute to pathogenesis. For instance, immunosenescence is associated with a heightened activation of the proinflammatory status of monocytes, with an increase in the blood levels of intermediate or non-classical monocytes [33] and an increased production of monocyte indicators e.g. neopterine, sCD163, and CD11b [34]. Monitoring monocyte activation through MDW may warrant further research in immunological non-responders (INR) to ART, who are known to be at increased risk of developing SNAEs (e.g. cerebral and cardiac acute events, a leading cause of mortality and morbidity in HIV patients) [27].
Relevant limitations of our study are worth to be outlined. Firstly, the observational design is prone to a series of known limitations, including missing data and potential recall bias. Further, the results obtained from a small sample size drawn from a single clinical center need to be validated through additional samples from multiple sites.
Secondly, the automated choice of cut-offs may lead to unstable results, which may not be confirmed by other data collections. In the absence of gold standards, we used the Youden index to find an optimal cut-off for both late presentation and advanced disease.
The cut-off point of MDW=21.1 for late presentation may seem close to normal values, undermining its accuracy for large samples. However, normal MDW values for the general population range between 14 and 18, meaning that values that are substantially higher may be considered appropriate to rule out innate immune activation.
The cut-off value found for MDW in advanced disease is equal to 22. This is consistent with other studies, including the FDA-registered MDW cut-off point for identifying sepsis in the emergency department (ED).
By all means, in this study we did not aim to encourage monitoring MDW to diagnose advanced HIV. Instead, we meant to describe MDW modifications in response to lymphocyte deterioration during HIV infection. Consequently, our results should be only interpreted as exploratory findings that need to be confirmed by future evidence.
Thirdly, we were unable to compare MDW to other biomarkers of monocytes during innate immune activation e.g. sCD14 and sCD163. Since MDW has been only recently introduced in clinical practice, no direct comparisons with sCD14 and sCD163 currently exist. Further studies are needed to address this comparison.
In conclusion, we found that MDW values are inversely correlated with CD4-T cell counts in HIV patients. HIV patients with elevated MDW are more likely to be diagnosed late, suggesting that MDW may be used as a potential flagging parameter of innate immune activation in HIV infection, that can be easily measured using peripheral blood counts.

Ethical approval and Informed consent
The local Health Administrative Board in Pescara reviewed in detail the study plan, set up by the Infectious Diseases Staff in Pescara General Hospital.
Informed consent for participating into the study was not required, because we used archival data, which were anonymized. There was no intervention beyond standard clinical procedures (measurement of blood cell volumes and indices). Moreover, written informed consent for the use of anonymized clinical and laboratory data for institutional research purposes was provided by all patients upon admission. This study was conducted in accordance with the ethical principles for medical research involving human subjects, as reported in the Declaration of Helsinki.

Author contributions
EP, FC, and GP wrote the manuscript; FS and GA retrieved patient data; EP and FC performed the statistical analysis. The corresponding author is responsible for the accuracy of the descriptions. All authors have accepted responsibility for the entire content of this manuscript and approved its submission. All authors declare that they have no conflicts of interest.

Research funding
This research received no funding.

Data availability
The datasets are available from the corresponding author upon reasonable request.

References

1. Simon V, Ho DD, Abdool Karim Q. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. Lancet. 2006;368(9534):489–504. DOI: 10.1016/S0140-6736(06)69157-5. https://pmc.ncbi.nlm.nih.gov/articles/PMC2913538/. (Accessed: 01/04/2025).
2. Campbell JH, Hearps AC, Martin GE, Williams KC, Crowe SM. The importance of monocytes and macrophages in HIV pathogenesis, treatment, and cure. AIDS. 2014;28(15):2175–2187. DOI: 10.1097/QAD.0000000000000408. https://pmc.ncbi.nlm.nih.gov/articles/PMC6331181/ (Accessed: 01/04/2025).
3. Wacleche VS, Tremblay CL, Routy JP, Ancuta P. The Biology of Monocytes and Dendritic Cells: Contribution to HIV Pathogenesis. Viruses. 2018;10(2):65. DOI:10.3390/v10020065. https://pmc.ncbi.nlm.nih.gov/articles/PMC5850372/ (Accessed: 01/04/2025).
4. Prabhu VM, Singh AK, Padwal V, Nagar V, Patil P, Patel V. Monocyte Based Correlates of Immune Activation and Viremia in HIV-Infected Long-Term Non-Progressors. Front Immunol. 2019;10:2849. DOI:10.3389/fimmu.2019.02849. https://pubmed.ncbi.nlm.nih.gov/31867010/ (Accessed: 01/04/2025).
5. Stiksrud B, Aass HCD, Lorvik KB, Ueland T, Trøseid M, Dyrhol-Riise AM. Activated dendritic cells and monocytes in HIV immunological nonresponders: HIV-induced interferon-inducible protein-10 correlates with low future CD4+ recovery. AIDS. 2019;33(7):1117–1129. DOI:10.1097/QAD.0000000000002173. https://journals.lww.com/aidsonline/fulltext/2019/06010/activated_dendritic_cells_and_monocytes_in_hiv.2.aspx (Accessed: 01/04/2025).
6. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019;10:2035. DOI:10.3389/fimmu.2019.02035. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2019.02035/full (Accessed: 01/04/2025).
7. Knudsen AD, Bouazzi R, Afzal S, Gelpi M, Benfield T, Høgh J, et al. Monocyte count and soluble markers of monocyte activation in people living with HIV and uninfected controls. BMC Infect Dis. 2022;22(1):451. DOI: 10.1186/s12879-022-07450-y. https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-022-07450-y (Accessed: 01/04/2025).
8. Kim MJ, Cheng G, Agrawal DK. Cl- channels are expressed in human normal monocytes: a functional role in migration, adhesion and volume change. Clin Exp Immunol. 2004;138(3):453–459. DOI:10.1111/j.1365-2249.2004.02635.x. https://pmc.ncbi.nlm.nih.gov/articles/PMC1809252/ (Accessed: 01/04/2025).
9. Ligi D, Lo Sasso B, Della Franca C, Giglio RV, Agnello L, Ciaccio M, et al. Monocyte distribution width alterations and cytokine storm are modulated by circulating histones. Clin Chem Lab Med. 2023;61(8):1525–1535. DOI:10.1515/cclm-2023-0093. https://www.degruyter.com/document/doi/10.1515/cclm-2023-0093/html (Accessed: 01/04/2025).
10. Polilli E, Sozio F, Frattari A, Persichitti L, Sensi M, Posata R, et al. Comparison of Monocyte Distribution Width (MDW) and Procalcitonin for early recognition of sepsis. PLoS One. 2020;15(1):e0227300. DOI:10.1371/journal.pone.0227300. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0227300 (Accessed: 01/04/2025).
11. Polilli E, Frattari A, Esposito JE, Stanziale A, Giurdanella G, Di Iorio G, et al. Monocyte distribution width (MDW) as a new tool for the prediction of sepsis in critically ill patients: a preliminary investigation in an intensive care unit. BMC Emerg Med. 2021;21(1):147. DOI:10.1186/s12873-021-00521-4. https://bmcemergmed.biomedcentral.com/articles/10.1186/s12873-021-00521-4 (Accessed: 01/04/2025).
12. Polilli E, Di Iorio G, Silveri C, Angelini G, Anelli MC, Esposito JE, et al. Monocyte Distribution Width as a predictor of community acquired sepsis in patients prospectively enrolled at the Emergency Department. BMC Infect Dis. 2022;22(1):849. DOI:10.1186/s12879-022-07803-7.https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-022-07803-7 (Accessed: 01/04/2025).
13. Huang YH, Chen CJ, Shao SC, Li CH, Hsiao CH, Niu KY, et al. Comparison of the Diagnostic Accuracies of Monocyte Distribution Width, Procalcitonin, and C-Reactive Protein for Sepsis: A Systematic Review and Meta-Analysis. Crit Care Med. 2023;51(5):e106–114. DOI:10.1097/CCM.0000000000005820. https://journals.lww.com/ccmjournal/fulltext/2023/05000/comparison_of_the_diagnostic_accuracies_of.19.aspx (Accessed: 01/04/2025).
14. Antinori A, Coenen T, Costagiola D, Dedes N, Ellefson M, Gatell J, et al. Late presentation of HIV infection: a consensus definition. HIV Med. 2011;12(1):61–64. DOI:10.1111/j.1468-1293.2010.00857.x. https://onlinelibrary.wiley.com/doi/10.1111/j.1468-1293.2010.00857.x (Accessed: 01/04/2025).
15. Kleinbaum DG, Klein M. Introduction to Logistic Regression. In: Kleinbaum DG, Klein M, editors. Logistic Regression: A Self-Learning Text [Internet]. New York, NY: Springer; 2010 [cited 2024 Dec 17]. p. 1–39. Available from: https://link.springer.com/chapter/10.1007/978-1-4419-1742-3_1 (Accessed: 26/03/2025).
16. Polilli E, Frattari A, Esposito JE, D'Amato M, Albani A, D'Intino A, Di Iorio G, Carinci F and Parruti G, Reliability of predictive models to support early decision making in the emergency department for patients with

confirmed diagnosis of COVID-19: the Pescara Covid Hospital Score, BMC Health Serv Res. 2022;22(1):1062. Available at: https://bmchealthservres.biomedcentral.com/articles/10.1186/s12913-022-08421-4

17. WHO Regional Office for Europe. European Centre for Disease Prevention and Control. HIV/AIDS Surveillance in Europe 2024–2023 Data. Copenhagen: WHO Regional Office for Europe. 2024. Available online: https://www.ecdc.europa.eu/sites/default/files/documents/HIV_Surveillance_Report_2024.pdf (accessed on 10 October 2025).
18. WHO Regional Office for Europe. European Centre for Disease Prevention and Control. HIV/AIDS Surveillance in Europe 2023–2022 Data. Copenhagen: WHO Regional Office for Europe. 2023. Available online: https://www.ecdc.europa.eu/sites/default/files/documents/HIV-AIDS_surveillance_in_Europe_2023_%28_2022_data_%29_0.pdf (accessed on 10 October 2025).
19. Kostaki EG, Limnaios S, Adamis G, Xylomenos G, Chini M, Mangafas N, Lazanas M, Patrinos S, Metallidis S, Tsachouridou O, Papastamopoulos V, Chatzidimitriou D, Antoniadou A, Papadopoulos A, Protopapas K, Tsiara C, Psichogiou M, Basoulis D, Pilalas D, Paraskeva D, Chrysos G, Paparizos V, Kourkounti S, Sambatakou H, Bolanos V, Sipsas NV, Lada M, Barbounakis E, Kantzilaki E, Panagopoulos P, Petrakis V, Drimis S, Katsarolis I, Lagiou P, Hatzakis A, Magiorkinis G, Skoura L, Paraskevis D. Estimation of the determinants for HIV late presentation using the traditional definition and molecular clock-inferred dates: Evidence that older age, heterosexual risk group and more recent diagnosis are prognostic factors. HIV Med. 2002;23(11):1143-1152. doi: 10.1111/hiv.13415. https://onlinelibrary.wiley.com/doi/10.1111/hiv.13415 (Accessed: 02/10/2025).
20. Lødrup Carlsen KC, Granum B. Soluble CD14: role in atopic disease and recurrent infections, including otitis media. Curr Allergy Asthma Rep. 2007;7(6):436–443. DOI: 10.1007/s11882-007-0067-2. https://link.springer.com/article/10.1007/s11882-007-0067-2 (Accessed: 01/04/2025).
21. Krastinova E, Lecuroux C, Leroy C, Seng R, Cabie A, Rami A, et al. High Soluble CD14 Levels at Primary HIV-1 Infection Predict More Rapid Disease Progression. J Infect Dis. 2015;212(6):909–913. DOI:10.1093/infdis/jiv145. https://academic.oup.com/jid/article-abstract/212/6/909/984625 (Accessed: 01/04/2025).
22. Chen P, Su B, Zhang T, Zhu X, Xia W, Fu Y, et al. Perturbations of Monocyte Subsets and Their Association with T Helper Cell Differentiation in Acute and Chronic HIV-1-Infected Patients. Front Immunol. 2017;8:272. DOI:10.3389/fimmu.2017.00272. https://pmc.ncbi.nlm.nih.gov/articles/PMC5347116/ (Accessed: 01/04/2025).
23. Schnittman SR, Deitchman AN, Beck-Engeser G, Ahn H, York VA, Hartig H, et al. Abnormal Levels of Some Biomarkers of Immune Activation Despite Very Early Treatment of Human Immunodeficiency Virus. J Infect Dis. 2021;223(9):1621–1630. DOI:10.1093/infdis/jiaa580. https://academic.oup.com/jid/article/223/9/1621/5904261 (Accessed: 01/04/2025).
24. Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. J Infect Dis. 2011;204(1):154–163. DOI:10.1093/infdis/jir214. https://academic.oup.com/jid/article-abstract/204/1/154/2192372 (Accessed: 01/04/2025).
25. Ouyang J, Yan J, Zhou X, Isnard S, Harypursat V, Cui H, Routy JP, Chen Y. Relevance of biomarkers indicating gut damage and microbial translocation in people living with HIV. Front Immunol. 2023;14:1173956. DOI: 10.3389/fimmu.2023.1173956. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.1173956/full (Accessed: 02/10/2025).
26. Ruiz-Briseño MDR, De Arcos-Jiménez JC, Ratkovich-González S, Sánchez-Reyes K, González-Hernández LA, Andrade-Villanueva JF, Alvarez-Zavala M. Association of intestinal and systemic inflammatory biomarkers with immune reconstitution in HIV+ patients on ART. J Inflamm (Lond). 2020;17:32. DOI: 10.1186/s12950-020-00262-4. https://journal-inflammation.biomedcentral.com/articles/10.1186/s12950-020-00262-4 (Accessed: 02/10/2025).
27. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. J Leukoc Biol. 2020;107(4):597–612. DOI: 10.1002/JLB.4MR1019-189R. https://academic.oup.com/jleukbio/article-abstract/107/4/597/6884308 (Accessed: 01/04/2025).
28. Kruth HS. Macrophage foam cells and atherosclerosis. Front Biosci. 2001;6:D429-455. DOI: 10.2741/kruth. PMID: 11229875. https://article.imrpress.com/bri/Landmark/articles/pdf/LandmarkA620.pdf (Accessed: 02/10/2025).
29. Westhorpe CLV, Maisa A, Spelman T, Hoy JF, Dewar EM, Karapanagiotidis S, et al. Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. Immunol Cell Biol. 2014;92(2):133–138. DOI: 10.1038/icb.2013.84. https://onlinelibrary.wiley.com/doi/10.1038/icb.2013.84 (Accessed: 01/04/2025).
30. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. Arterioscler Thromb Vasc Biol. 2010;30(7):1282-1292. DOI: 10.1161/ATVBAHA.108.179739. PMID: 20554950. https://www.ahajournals.org/doi/10.1161/ATVBAHA.108.179739?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed (Accessed: 02/10/2025).

31. Bechmann I, Galea I, Perry VH. What is the blood-brain barrier (not)? Trends Immunol. 2007;28(1):5-11. DOI: 10.1016/j.it.2006.11.007. https://linkinghub.elsevier.com/retrieve/pii/S1471-4906(06)00329-2 (Accessed: 02/10/2025).
32. Williams DW, Veenstra M, Gaskill PJ, Morgello S, Calderon TM, Berman JW. Monocytes mediate HIV neuropathogenesis: mechanisms that contribute to HIV associated neurocognitive disorders. Curr HIV Res. 2014;12(2):85-96. DOI: 10.2174/1570162x12666140526. https://jlb.onlinelibrary.wiley.com/doi/full/10.1189/jlb.0811394 (Accessed: 02/10/2025).
33. Neri A, Olivieri G, Pighi C, Amodio D, Cotugno N, Palma P. Monocytes across life span in HIV infection: lights and shadows. Curr Opin HIV AIDS. 2025;20(2):133-144. DOI: 10.1097/COH.0000000000000910. https://journals.lww.com/co-hivandaids/fulltext/2025/03000/monocytes_across_life_span_in_hiv_infection_.5.aspx (Accessed: 02/10/2025).
34. Hearps AC, Martin GE, Angelovich TA, Cheng WJ, Maisa A, Landay AL, Jaworowski A, Crowe SM. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. Aging Cell. 2012;11(5):867-875. DOI: 10.1111/j.1474-9726.2012.00851.x. https://onlinelibrary.wiley.com/doi/10.1111/j.1474-9726.2012.00851.x