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Evaluation of mean corpuscular volume among anemic people with HIV in North America following ART initiation

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Abstract

Background Anemia is common and associated with increased morbidity among people with HIV (PWH). Classification of anemia using the mean corpuscular volume (MCV) can help investigate the underlying causative factors of anemia. We characterize anemia using MCV among PWH receiving antiretroviral therapy (ART), and identify the risk factors for normocytic, macrocytic, and microcytic anemias.

Methods Including PWH with anemia (hemoglobin measure < 12.9 g/dL among men and < 11.9 g/dL among women) in the NA-ACCORD from 01/01/2007 to 12/31/2017, we estimated the annual distribution of normocytic (80–100 femtolitre (fL)), macrocytic (> 100 fL) or microcytic (< 80 fL) anemia based on the lowest hemoglobin within each year. Poisson regression models with robust variance and general estimating equations were used to estimate crude and adjusted prevalence ratios and 95% confidence intervals for risk factors for macrocytic (vs. normocytic) and microcytic (vs. normocytic) anemia stratified by sex.

Results Among 37,984 hemoglobin measurements that identified anemia in 14,590 PWH, 27,909 (74%) were normocytic, 4257 (11%) were microcytic, and 5818 (15%) were macrocytic. Of the anemic PWH included over the study period, 1910 (13%) experienced at least one measure of microcytic anemia and 3208 (22%) at least one measure of macrocytic anemia. Normocytic anemia was most common among both males and females, followed by microcytic among females and macrocytic among males. Over time, the proportion of anemic PWH who have macrocytosis decreased while microcytosis increased. Macrocytic (vs. normocytic) anemia is associated with increasing age and comorbidities. With increasing age, microcytic anemia decreased among females but not males. A greater proportion of PWH with normocytic anemia had CD4 counts $\leq$ 200 cells/mm^3 and had recently initiated ART.

Conclusion In anemic PWH, normocytic anemia was most common. Over time macrocytic anemia decreased, and microcytic anemia increased irrespective of sex. Normocytic anemia is often due to chronic disease and may explain the greater risk for normocytic anemia among those with lower CD4 counts or recent ART initiation. Identified risk factors for type-specific anemias including sex, age, comorbidities, and HIV factors, can help inform targeted investigation into the underlying causes.

Keywords Anemia, HIV, NA-ACCORD, Mean corpuscular volume, Hemoglobin

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Introduction

Despite improvements in care for people with HIV (PWH), approximately half will experience anemia which is associated with increased morbidity and mortality [1, 2]. The pathogenesis of anemia in PWH can be both heterogenous and multifactorial [3]. The mean corpuscular volume (MCV) documents the average size of red blood cells (RBC) and is used to classify anemia into normocytic (average sized), macrocytic (large) and microcytic (small) [4]. Classifying anemia by MCV provides context into potential underlying mechanisms and directs the subsequent investigations of anemia [5]. MCV type-specific anemia has also been associated with different mortality risks, with macrocytic anemia linked with higher mortality than normocytic or microcytic anemia [6–8]. Therefore, as ART has improved mortality among PWH, it has also likely altered the risk factors of anemia, necessitating a contemporary evaluation of MCV type-specific anemia.

Normocytic anemia typically results from decreased production of RBC (chronic disease, inflammation), increased destruction or loss of RBC (hemolysis, post-hemorrhagic), increased plasma volume (pregnancy, hypervolemia) and may be multifactorial [9]. Normocytic anemia can also occur if there is a mixture of conditions contributing to both microcytic or macrocytic anemia [9]. Macrocytosis results from impaired DNA synthesis and RBC nuclear maturation abnormalities, which can be from nutrient deficiencies (vitamin B12, folate), bone marrow disorders, hypothyroidism, liver disease, and medications [10]. Microcytosis typically results from a decreased production of hemoglobin with the most common cause being iron deficiency [11]. Genetic blood disorders such as thalassemia also leads to microcytosis [11, 12].

Prior studies of the general population demonstrate that MCV type-specific anemias differ by both age and sex. Microcytosis due to iron deficiency from menstruation is more common among younger females compared with males; however, differences in prevalence and risk factors by sex decrease following menopause [13, 14]. Macrocytosis is known to increase with age among both males and females [10]. The prevalence of normocytic, macrocytic, and microcytic anemias and relative risk factors remain largely unexplored among PWH, especially by sex and age.

The objective of this study was to characterize trends in prevalence and identify risk factors of normocytic, macrocytic, and microcytic anemias among anemic PWH using ART, by sex. Our findings will identify if there are sex-based differences in contributing factors of anemia and inform targets for prioritization of investigations into underlying mechanisms for type-specific anemias among PWH who have initiated ART.

Methods

Study population

The North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) is a consortium of longitudinal HIV cohort studies of adults followed in the United States and Canada, and is the North American region of the International epidemiology Databases to Evaluate AIDS (IeDEA) initiative of the National Institute of Allergy and Infectious Diseases [15]. Participants must have successfully linked into HIV care, defined as attending $\geq$ 2 HIV care visits within 12 months to be included in the NA-ACCORD. NA-ACCORD is designed to be representative of PWH in care in the United States and Canada [16]. Cohorts submit data annually to the Data Management Core (DMC, University of Washington, Seattle WA), where data undergo quality checks and transfer to the Epidemiology/Biostatistics Core (EBC, Johns Hopkins University, Baltimore MD).

The source population for our nested study were PWH ($\geq$18 years) participating in 13 NA-ACCORD-contributing clinical cohorts contributing data on hemoglobin and MCV measures. Individual-level selection criteria included those who were observed in care from 01/01/2007– 31/12/2017 (study period) and had initiated ART prior to hemoglobin measurement.

Anemia and mean corpuscular volume (MCV) ascertainment

Anemia was defined as a hemoglobin measure $\leq$ 12.9 g/dL among men and $\leq$ 11.9 g/dL among women [17] and then categorized into a type using the MCV measurement. If there were multiple hemoglobin measures per year the lowest was used for categorization based on World Health Organization (WHO) guidelines into mild (hemoglobin 11.0–12.9 g/dL male, 11.0–11.9 g/dL female), moderate (8.0–10.9 g/dL) and severe (<8.0 g/dL) anemia [17]. MCV associated with each hemoglobin measure was categorized as normocytic (80–100 femtolitre (fL)), macrocytic (>100 fL), or microcytic (<80 fL) [4] per each year participants were observed in the study. Therefore, PWH could contribute data to various anemia severity and MCV categories over the study period.

Covariates of interest

Sex was defined as sex at birth (male or female). Due to a small population size and the lack of hemoglobin thresholds to define anemia for intersexed individuals, they were not included. HIV acquisition risk group was determined using a mutually exclusive hierarchy

including: (1) injection drug use (IDU); (2) men who have sex with men (MSM); (3) heterosexual sexual contact; (4) other or unknown risk. Comorbidity ascertainment was time-varying and assessed at time of hemoglobin measure (i.e., ever observed prior to each hemoglobin measure) using NA-ACCORD operationalized definitions for hypertension (HTN), diabetes mellitus (DM) and hypercholesterolemia (Supplemental Table 1). Chronic kidney disease (CKD) was defined as having estimated glomerular filtration rate (eGFR) using the CKD-EPI study equation < 60 mL/min/1.73 m^2 (serum creatinine) [18]. Hepatitis B (HBV) and Hepatitis C (HCV) infection were parameterized as ever/never status. Non-AIDS defining cancer (NADC) diagnosis was ascertained using a standardized abstraction protocol [19] (Supplemental Table 1).

The closest CD4 count, HIV RNA, and body mass index (BMI measure) ± 6 months to hemoglobin measurement was used. CD4 count was categorized as ≤ 200 cells/mm^3 and > 200 cells/mm^3 and HIV RNA was categorized as detectable > 200 copies/mL and undetectable ≤ 200 copies/mL. ART use was identified within ± 30 days to the hemoglobin measure. Concurrent medications (zidovudine [ZDV], ribavirin, or interferon) were defined as use anytime during the month of hemoglobin measure. Missing values for smoking, CD4 count, viral load, and BMI were imputed using multiple imputation by chained equations which assumes data are missing at random. CD4 count, viral load and BMI were imputed as continuous variables and then categorized. All covariates were selected a priori based on clinical knowledge and review of literature.

Statistical analysis

To minimize the risk of falsely assuming complete event ascertainment from electronic health records, we used an "observation window" [20] approach. Study entry was defined as the date the cohort began observing patients, anemia observation window opened, patient enrollment into the NA-ACCORD, ART initiation, or January 1, 2007, whichever came last. Study exit was defined as the earliest date the cohort stopped observing patients, anemia observation window closed, patient was lost-to-follow-up (defined as their last CD4 or HIV RNA measurement prior to a gap of > 18 months), died, or December 31, 2017.

We estimated among PWH with anemia the annual proportion stratified by sex of normocytic, macrocytic, and microcytic anemia. Poisson regression models with robust variance and general estimating equations (GEE) (to account for correlation in repeated measures from an individual) were used to approximate log binomial models and estimate crude (PR) and adjusted prevalence ratios (aPR) and 95% confidence intervals for risk factors for macrocytic (vs. normocytic) and microcytic (vs. normocytic) anemia measured as time-updated variables and stratified by sex. Adjusted models included cohort, anemia severity, year of hemoglobin measure, age, race/ethnicity, BMI, HIV acquisition risk, DM, HTN, hypercholesterolemia, CKD, NADC, AIDS-defining condition, HCV and HBV infection, smoking, low CD4 count (≤ 200 cells/mm^3), unsuppressed HIV RNA (> 200 copies/mL), ART use and duration following initiation, ZDV, and ribavirin/interferon use. All analysis were stratified by sex. As zidovudine (ZDV) is known to cause macrocytosis [21], we conducted a subgroup analysis removing all observations that were taken while on ZDV (within the same month). This subgroup analysis was done to identify trends in prevalence and risk factors for macrocytosis independent of the known association with ZDV use. Two-tailed p-values < 0.05 guided statistical interpretation. All analyses were performed using Stata version 16.0 (College Station, TX).

Results

Characteristics of the study population

Within 156,899 hemoglobin measurements from 35,414 PWH in the NA-ACCORD, 37,984 (24%) signaled anemia from 14,590 individual PWH (Supplemental Fig. 1), 27,909 (74%) were normocytic with a median MCV of 91 fL (interquartile range [IQR] 86–95 fL), 5,818 (15%) were macrocytic with a median MCV of 106 fL (IQR 103–111 fL), and 4,257 (11%) were microcytic with a median MCV of 75 fL (IQR 71–78 fL) (Table 1). A total of 95,486 person-years were observed with a median follow-up time of 7.0 years (IQR 3.7–10.0 years). The median hemoglobin measurement of those with normocytic anemia was 11.5 g/dL (IQR 10.3–12.3 g/dL), macrocytic anemia was 11.4 g/dL (IQR 10.1–12.2 g/dL) and microcytic anemia was 10.4 g/dL (IQR 8.8–11.6 g/dL).

The median age of females was younger than males among all anemia types. Among both males and females, those with macrocytic anemia had the highest median age and those with microcytic anemia the lowest median age (Table 1, Supplemental Fig. 3). A greater proportion of PWH with macrocytic anemia (vs. microcytic or normocytic anemia) had HCV, CKD, hypercholesterolemia, HTN, NADC, and reported ever smoking. PWH with normocytic anemia had the greatest proportion with recent ART initiation (within the last 6-months) and the lowest median CD4 count at the time of hemoglobin measure (males: 372 cells/mm^3, females: 477 cells/mm^3) (Table 1).

Table 1 Demographic and clinical factors of MCV observations among PWH stratified by sex with normocytic, microcytic, and macrocytic anemia in the NA-ACCORD, 2007–2017

	Total MCV Observations N = 37,984	Normocytic anemia N = 27,909		Microcytic anemia N = 4,257		Macrocytic anemia N = 5,818	
		Males (n = 19,463)	Females (n = 8,446)	Males (n = 2,171)	Female (n = 2,086)	Males (n = 4,496)	Females (n = 1,322)
MCV (median, IQR)	91 (85–97)	91 (87–95)	89 (85–94)	75 (71–78)	75 (71–78)	106 (103–112)	105 (102–110)
Anemia severity							
Mild anemia	22,768 (60%)	13,360 (69%)	4262 (50%)	1200 (55%)	470 (23%)	2865 (64%)	611 (46%)
Moderate anemia	11,998 (32%)	4706 (24%)	3501 (41%)	692 (32%)	1257 (60%)	1272 (28%)	570 (43%)
Severe anemia	3218 (8%)	1397 (7%)	683 (8%)	279 (13%)	359 (17%)	359 (8%)	141 (11%)
Year of hemoglobin measurement							
2007–2010	14,672 (39%)	7230 (37%)	3184 (38%)	723 (33%)	722 (35%)	2155 (48%)	658 (50%)
2011–2013	11,628 (31%)	5989 (31%)	2660 (31%)	689 (32%)	636 (30%)	1279 (28%)	375 (28%)
2014–2017	11,684 (31%)	6244 (32%)	2602 (31%)	759 (35%)	728 (35%)	1062 (24%)	289 (22%)
Demographics							
Age at hemoglobin measurement (years; median, IQR)	48 (41–56)	49 (42–56)	45 (38–53)	48 (39–56)	42 (35–48)	52 (46–60)	49 (42–58)
Race/ethnicity							
Non-Hispanic white	12,240 (32%)	7513 (39%)	1359 (16%)	552 (25%)	197 (9%)	2336 (52%)	283 (21%)
Non-Hispanic black	20,466 (54%)	9045 (46%)	6202 (73%)	1240 (57%)	1643 (79%)	1485 (33%)	851 (64%)
Asian	712 (2%)	335 (2%)	94 (1%)	132 (6%)	28 (1%)	81 (2%)	42 (3%)
Hispanic	3537 (9%)	2038 (10%)	560 (7%)	181 (8%)	169 (8%)	492 (11%)	97 (7%)
Other/unknown	1029 (3%)	532 (3%)	231 (3%)	66 (3%)	49 (2%)	102 (2%)	49 (4%)
BMI (kg/m^2; median, IQR)	25 (22–30)	25 (22–28)	28 (23–34)	25 (22–29)	28 (24–35)	24 (21–27)	26 (22–32)
HIV acquisition risk							
MSM	15,584 (41%)	11,652 (60%)	-	1225 (56%)	-	2,702 (60%)	-
IDU	5921 (16%)	3247 (17%)	1207 (14%)	325 (15%)	153 (7%)	779 (17%)	210 (16%)
Heterosexual	14,419 (38%)	3610 (19%)	6732 (80%)	507 (23%)	1819 (87%)	751 (17%)	1000 (76%)
Other/unknown	2060 (5%)	954 (5%)	507 (6%)	114 (5%)	114 (5%)	264 (6%)	112 (8%)
Comorbidities at hemoglobin measurement							
*Hepatitis B infection	2315 (6%)	1379 (7%)	318 (4%)	160 (7%)	89 (4%)	312 (7%)	57 (4%)
Hepatitis C infection	8486 (22%)	4538 (23%)	1621 (19%)	448 (21%)	263 (13%)	1252 (28%)	364 (28%)
CKD eGFR < 60 mL/min	6729 (18%)	3357 (17%)	1417 (17%)	201 (9%)	129 (6%)	1222 (27%)	403 (30%)
*Diabetes mellitus	6331 (17%)	3287 (17%)	1330 (16%)	316 (15%)	326 (16%)	849 (19%)	223 (17%)
Hypercholesterolemia	9010 (24%)	4275 (22%)	2093 (25%)	422 (19%)	434 (21%)	1366 (30%)	420 (32%)
Hypertension	16,736 (44%)	8520 (44%)	3653 (43%)	883 (41%)	730 (35%)	2268 (50%)	682 (52%)
Non-AIDS defining malignancy	3839 (10%)	2153 (11%)	532 (6%)	240 (11%)	65 (3%)	711 (16%)	138 (10%)

Table 1 (continued)

	Total MCV Observations N = 37,984	Normocytic anemia N = 27,909		Microcytic anemia N = 4,257		Macrocytic anemia N = 5,818	
		Males (n = 19,463)	Females (n = 8,446)	Males (n = 2,171)	Female (n = 2,086)	Males (n = 4,496)	Females (n = 1,322)
Clinical AIDS diagnosis	13,117 (35%)	7202 (37%)	2501 (30%)	648 (30%)	532 (26%)	1760 (39%)	474 (36%)
Reported ever smoking							
No	10,441 (27%)	4699 (24%)	2977 (35%)	578 (27%)	859 (41%)	969 (22%)	359 (27%)
Yes	24,703 (65%)	13,474 (69%)	4702 (56%)	1441 (66%)	959 (46%)	3260 (73%)	867 (66%)
Missing	2840 (7%)	1290 (7%)	767 (9%)	152 (7%)	268 (13%)	267 (6%)	96 (7%)
HIV Co-factors at hemoglobin measure							
Viral load							
> 200 copies/mL	9825 (26%)	5504 (28%)	2379 (28%)	594 (27%)	588 (28%)	566 (13%)	194 (15%)
≤ 200 copies/mL	26,544 (70%)	13,158 (68%)	5682 (67%)	1454 (67%)	1362 (65%)	3803 (85%)	1085 (82%)
Missing	1615 (4%)	801 (4%)	385 (5%)	123 (6%)	136 (7%)	127 (3%)	43 (3%)
CD4 count (median, IQR)	408 (210–635)	372 (185–590)	477 (245–708)	394 (199–632)	486 (282–731)	400 (231–611)	494 (277–765)
ART Class + NRTI							
NNRTI	10,117 (27%)	5317 (27%)	2041 (24%)	674 (31%)	528 (25%)	1272 (28%)	285 (22%)
PI	13,955 (37%)	6665 (34%)	3470 (41%)	702 (32%)	835 (40%)	1,675 (7%)	608 (46%)
INSTI	4954 (13%)	2665 (14%)	1068 (13%)	314 (14%)	290 (14%)	491 (11%)	126 (10%)
Dual	6665 (18%)	3667 (19%)	1334 (16%)	336 (15%)	290 (14%)	795 (18%)	243 (18%)
Other/unknown	1356 (4%)	668 (3%)	313 (4%)	66 (3%)	63 (3%)	201 (4%)	45 (3%)
No-ART	937 (2%)	481 (2%)	220 (3%)	79 (4%)	80 (4%)	62 (1%)	15 (1%)
Time since ART initiation							
> 1 year	32,676 (86%)	16,412 (84%)	7250 (86%)	1867 (86%)	1832 (88%)	4155 (92%)	1160 (88%)
6–12 months	1747 (5%)	927 (5%)	393 (5%)	113 (5%)	116 (6%)	137 (3%)	61 (5%)
0–6 months	3561 (9%)	2124 (11%)	803 (10%)	191 (9%)	138 (7%)	204 (5%)	101 (8%)
Concurrent medications							
Zidovudine	4333 (11%)	1131 (6%)	715 (8%)	114 (5%)	121 (6%)	1692 (38%)	560 (42%)
Ribavirin	422 (1%)	219 (1%)	35 (0%)	4 (0%)	1 (0%)	145 (3%)	18 (1%)
Interferon	348 (1%)	181 (1%)	32 (0%)	2 (0%)	2 (0%)	119 (3%)	12 (1%)

Two intersex PWH were identified but excluded from analysis due to lack of standardized hemoglobin categorization for anemia, however both did not meet criteria for anemia with either male or female WHO criteria

P values < 0.001 unless specified with *

Trends in normocytic, macrocytic and microcytic *Anemia* among PWH

Normocytic anemia was predominant among both males (74%) and females (71%) (Fig. 1A and B). Among males, the proportion with normocytic anemia increased with time (p-value for trend 0.008), however did not change among females (p-value for trend 0.223). Among males, the average proportion of macrocytic anemia was 17% and decreased over time (p-value for trend < 0.001) whereas microcytic anemia increased from 2007 (8%) to 2017 (13%) (p-value for trend < 0.001). Among females, the average proportion of macrocytic anemia was 11% and decreased over time (p-value for trend < 0.001), whereas microcytic anemia increased from 2007 (16%) to 2017 (25%) (p-value for trend < 0.001). In a subgroup analysis removing hemoglobin measurements that were taken while on ZDV, the average proportion with macrocytosis was lower—12% in males and 7% in females and still decreased over time among both males and females. (Supplemental Fig. 2).

Risk factors for macrocytic compared to normocytic *anemia* by sex

In adjusted analyses, each 10-year increase in age among males and females was associated with an approximate

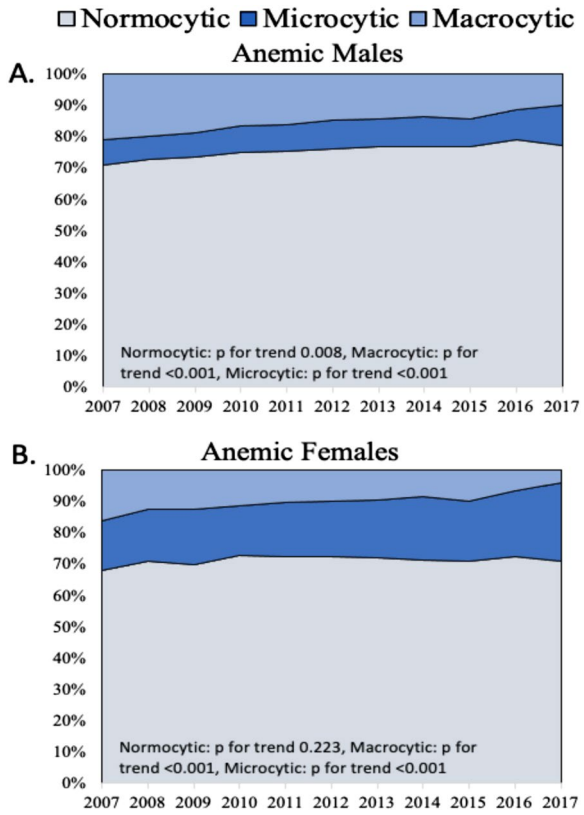

Fig. 1 Annual distribution of Normocytic, Microcytic and Macrocytic anemia among PWH and anemia in the NA-ACCORD from 2007 to 2017 among **A** male PWH, and **B** female PWH

20% increase in the prevalence of macrocytic (vs. normocytic) anemia (Table 2). After dichotomizing age as ≤ vs. > 50 years, older males (aged > 50 years) had a 24% (aPR = 1.24 [95% CI 1.15–1.33]) and older females had a 33% (aPR = 1.33 [95% CI 1.15–1.55]) increase in the prevalence of macrocytic (vs. normocytic) anemia compared with those of the same sex ≤ 50 years. Non-Hispanic Black PWH were less likely to have macrocytic (vs. normocytic) anemia compared with non-Hispanic White PWH. Non-Hispanic Asian females had an increased prevalence of macrocytic (vs. normocytic) anemia compared with non-Hispanic White females. The prevalence of macrocytic (vs. normocytic) anemia was higher among both males and females with HCV coinfection, CKD, and who had ever smoked (Table 2).

Risk factors for microcytic compared to normocytic *anemia* by sex

In adjusted analyses, the prevalence of microcytic (vs. normocytic) anemia decreased by 12% per 10-year increase in age among females but did not significantly change among males (Table 3). Older males had a non-significant (aPR = 1.05 [95% CI 0.88–1.24]) increased risk and older females had a 15% (aPR = 0.85 [95% CI 0.73–1.00]) decreased risk of microcytic (vs. normocytic) anemia compared with males and females ≤ 50 years. Severe anemia was more likely to be microcytic (vs. normocytic) anemia compared to mild anemia (males aPR = 2.31 [95% CI 1.86–2.87] females aPR = 3.25 [95% CI 2.80–3.78]) (Table 3, Fig. 2A). Irrespective of sex, non-Hispanic Black (vs. non-Hispanic White) PWH were more likely to have microcytic anemia (Table 3).

HIV disease factors associated with MCV

Macrocytic (vs. normocytic) anemia was less common among PWH with a detectable HIV viral load (> 200 copies/mL) compared with those with virologic suppression (Fig. 2B, Table 2).

Among males with a CD4 count ≤ 200 cells/mm^3 or initiation of ART within the last 6 months there was a decreased prevalence of macrocytic (vs. normocytic) anemia (Fig. 2C). Among females with a CD4 count ≤ 200 cells/mm^3 or initiation of ART within the last 6 months vs. ≥ 1 year, there was a decreased prevalence of microcytic (vs. normocytic) anemia.

Discussion

Anemia is a complex and multifactorial condition. Identifying the mechanism(s) of anemia is challenging in PWH due to additional factors such as chronic inflammation, antiretroviral therapies, comorbidities, and HIV itself. As anemia often requires intensive and costly investigations, MCV can be utilized as an initial step towards identifying the underlying causative factors. The proportion of normocytic, macrocytic, and microcytic anemia changed over time for both males and females and is likely related to improvements in HIV care. This paper adds to the medical knowledge of anemia among PWH by identifying risk factors for type-specific anemias by MCV including age, race/ethnicity, comorbidities, and HIV disease characteristics, which vary by sex. These factors should be considered when targeting investigations into the mechanisms of anemia for PWH.

Few studies have evaluated MCV among PWH, particularly in the current ART era. In 2014, Borges and colleagues evaluated 313 PWH and found that the most prevalent type of anemia was macrocytic (69%), which was likely due a large proportion of PWH taking ZDV (60%) [22]. We identified normocytic was the most prevalent type of anemia among PWH and was associated with recent initiation on ART and having lower CD4 counts. Normocytic anemia is often a result of chronic disease and may be related to ongoing inflammation [23]. Prior studies have documented a significant decline in anemia of chronic disease and microcytic anemia following initiation of ART with an

Table 2 Crude (cPR) and adjusted (aPR) prevalence ratios of risk factors associated with Macrocytic anemia compared Normocytic anemia (2007–2017), stratified by sex

Characteristic	Crude prevalence ratio males N = 23,958 in 10,146 Males with HIV)		Adjusted prevalence ratio Males		Crude prevalence ratio females (N = 9767 in 3570 Females with HIV)		Adjusted prevalence ratio Females	
	cPR	95% CI	aPR	95% CI	cPR	95% CI	aPR	95% CI
Anemia severity								
Mild anemia	Ref	Ref	Ref		Ref		Ref	
Moderate anemia	**1.09**	**1.02–1.16**	**1.10**	**1.04–1.16**	1.09	0.98–1.22	1.00	0.90–1.10
Severe anemia	0.89	0.78–1.02	0.97	0.85–1.09	1.19	0.99–1.45	1.16	0.96–1.41
Age (per 10-year increase) at hemoglobin measure	**1.28**	**1.24–1.32**	**1.20**	**1.16–1.24**	**1.21**	**1.14–1.30**	**1.23**	**1.16–1.31**
Race and ethnicity								
Non-Hispanic white	Ref	Ref	Ref		Ref		Ref	
Non-Hispanic black	**0.60**	**0.55–0.66**	**0.66**	**0.61–0.73**	**0.65**	**0.55–0.77**	**0.80**	**0.68–0.94**
Non-Hispanic Asian	0.86	0.65–1.14	0.99	0.76–1.29	**1.55**	**1.03–2.33**	**1.47**	**1.05–2.07**
Hispanic	**0.80**	**0.71–0.91**	1.04	0.93–1.17	0.82	0.62–1.10	1.12	0.88–1.43
Other/unknown	**0.78**	**0.62–0.99**	0.89	0.72–1.09	0.86	0.56–1.33	1.09	0.74–1.61
Comorbidities at hemoglobin measure								
Hepatitis C infection	**1.28**	**1.17–1.40**	**1.14**	**1.04–1.25**	**1.47**	**1.24–1.74**	**1.26**	**1.04–1.52**
Chronic kidney disease	**1.26**	**1.13–1.42**	**1.18**	**1.08–1.30**	**1.76**	**1.48–2.09**	**1.40**	**1.16–1.68**
Diabetes	0.98	0.87–1.09	**0.85**	**0.75–0.95**	0.97	0.78–1.19	0.89	0.71–1.12
Hypercholesterolemia	**1.42**	**1.29–1.56**	1.04	0.94–1.16	**1.19**	**1.00–1.42**	0.99	0.83–1.19
Hypertension	**1.24**	**1.14–1.35**	1.07	0.97–1.18	**1.18**	**1.01–1.38**	1.03	0.85–1.25
Non-AIDS defining Cancer	**1.29**	**1.15–1.44**	**1.19**	**1.08–1.31**	1.25	0.95–1.66	1.22	0.97–1.53
History of clinical AIDS Diagnosis	1.07	0.99–1.16	**1.08**	**1.01–1.16**	1.13	0.96–1.33	1.11	0.96–1.30
*Ever smoking	**1.22**	**1.10–1.35**	**1.12**	**1.03–1.23**	**1.42**	**1.21–1.67**	**1.29**	**1.11–1.50**
HIV Co-factors at hemoglobin measure								
*Detectable Viral Load (> 200 copies/mL)	**0.54**	**0.49–0.59**	**0.60**	**0.54–0.66**	**0.54**	**0.46–0.64**	**0.54**	**0.46–0.64**
*Low CD4 count ($\leq$ 200 cells/mm^3)	**0.76**	**0.70–0.83**	**0.88**	**0.81–0.95**	**0.76**	**0.63–0.91**	0.85	0.71–1.02
Not on ART at hemoglobin measure	**0.76**	**0.63–0.92**	0.93	0.74–1.17	**0.59**	**0.43–0.81**	0.74	0.52–1.06
Time on ART								
> 1 year	Ref	Ref	Ref		Ref		Ref	
6 months-1 year	0.84	0.74–0.95	0.97	0.85–1.12	1.10	0.89–1.37	1.18	0.94–1.47
0–6 months	**0.57**	**0.50–0.64**	**0.79**	**0.69–0.90**	0.95	0.79–1.14	0.99	0.81–1.21

Measurements of variables:

All adjusted analyses were adjusted for cohort, year of hemoglobin measurement, HIV acquisition risk factor, hepatitis B virus coinfection, BMI, and concurrent medications including zidovudine, ribavirin and interferon use in addition to all covariates listed in this table

Time fixed variables included sex, race and ethnicity (due to small numbers, Asian/PI was collapsed into the other/unknown category), and HIV acquisition risk, all other variables were time varying based on hemoglobin measure and associated MCV

Values with P < 0.05 are bolded

* Missing values for smoking, CD4 count, viral load, and BMI were imputed using multiple imputation by chained equations

increase in macrocytic anemia; however these studies were conducted in developing countries with possibly higher utilization of ZDV [24, 25]. Among observations signaling macrocytic anemia in our population, 39% were among individuals on ZDV. We observed a reduction in the proportion of macrocytic anemia, in part attributable to the reduced usage of ZDV, as demonstrated by our subgroup analysis removing hemoglobin measurements while on ZDV [22, 26].

The median age of females with anemia was younger than males within our study, likely associated with blood loss from menstruation among young females. Lower CD4 counts have been associated with amenorrhea and/or irregular menstrual cycles [27]. It is therefore possible that as women maintain ART, they are less likely to experience the normocytic anemia of chronic disease and instead are more likely to experience microcytosis due to iron loss from menstruation [11]. We found that among

Table 3 Crude (cPR) and adjusted (aPR) prevalence ratios of risk factors associated with Microcytic anemia compared to Normocytic anemia (2007–2017), stratified by sex

Characteristic	Crude prevalence ratio males (N = 21,634 in 9485 males with HIV)		Adjusted prevalence ratio males		Crude prevalence ratio females (N = 10,532 among 3669 females with HIV)		Adjusted prevalence ratio females	
	cPR	95% CI	aPR	95% CI	cPR	95% CI	aPR	95% CI
Anemia severity								
Mild anemia	Ref	Ref	Ref		Ref		Ref	
Moderate anemia	**1.62**	**1.43–1.83**	**1.66**	**1.46–1.89**	**2.12**	**1.90–2.37**	**2.24**	**2.00–2.49**
Severe anemia	**2.24**	**1.85–2.70**	**2.31**	**1.86–2.87**	**2.91**	**2.52–3.36**	**3.25**	**2.80–3.78**
Age (per 10-year increase) at hemoglobin measure	1.00	0.93–1.08	1.03	0.92–1.16	**0.85**	**0.80–0.90**	**0.88**	**0.83–0.94**
Race and ethnicity								
Non-Hispanic White	Ref	Ref	Ref		Ref		Ref	
Non-Hispanic black	**2.00**	**1.73–2.32**	**1.32**	**1.01–1.72**	**1.55**	**1.26–1.90**	**1.23**	**1.00–1.50**
Non-Hispanic asian	**4.72**	**3.66–6.09**	**3.14**	**2.22–4.46**	**1.83**	**1.09–3.05**	1.43	0.83–2.46
Hispanic	**1.30**	**1.03–1.65**	1.04	0.71–1.51	**1.56**	**1.17–2.09**	1.11	0.84–1.47
Other/unknown	**1.75**	**1.23–2.51**	1.44	0.86–2.41	1.46	0.96–2.21	1.12	0.76–1.65
Comorbidities at hemoglobin measure								
Hepatitis C infection	0.91	0.71–1.16	0.93	0.66–1.31	**0.70**	**0.58–0.86**	0.92	0.73–1.17
Chronic kidney disease	0.88	0.72–1.09	**0.73**	**0.58–0.93**	**0.57**	**0.44–0.73**	**0.51**	**0.39–0.67**
Diabetes	**1.18**	**1.04–1.33**	1.08	0.97–1.20	1.00	0.85–1.19	1.03	0.86–1.25
Hypercholesterolemia	**0.80**	**0.70–0.93**	**0.87**	**0.77–0.98**	0.92	0.79–1.08	1.02	0.86–1.20
Hypertension	1.00	0.89–1.12	0.99	0.89–1.10	**0.83**	**0.73–0.96**	0.89	0.76–1.04
Non-AIDS defining Cancer	1.19	0.91–1.56	0.92	0.68–1.25	**0.66**	**0.48–0.92**	**0.62**	**0.45–0.86**
History of clinical AIDS diagnosis	0.94	0.76–1.17	0.94	0.74–1.18	0.86	0.74–1.01	0.87	0.75–1.00
*Ever smoking	0.93	0.79–1.09	0.95	0.77–1.17	**0.86**	**0.76–0.98**	**0.87**	**0.76–0.99**
HIV Co-factors at hemoglobin measure								
*Detectable Viral Load (> 200 copies/mL)	**1.20**	**1.06–1.35**	1.12	0.98–1.28	**1.15**	**1.03–1.28**	1.11	0.99–1.25
*Low CD4 count (≤ 200 cells/mm^3)	1.06	0.92–1.23	1.00	0.86–1.17	0.99	0.87–1.14	**0.83**	**0.71–0.97**
Not on ART at hemoglobin measure	**1.36**	**1.09–1.69**	**1.27**	**1.03–1.57**	1.16	0.96–1.40	1.03	0.88–1.21
Time on ART								
> 1 year	Ref	Ref	Ref		Ref		Ref	
6 months-1 year	0.95	0.75–1.20	0.96	0.77–1.21	1.04	0.88–1.23	1.00	0.85–1.17
0–6 months	1.04	0.87–1.24	0.98	0.82–1.18	**0.74**	**0.62–0.89**	**0.69**	**0.59–0.81**

Measurements of variables:

All adjusted analyses were adjusted for cohort, year of hemoglobin measurement, HIV acquisition risk factor, BMI, hepatitis B virus coinfection, and concurrent medications including zidovudine, ribavirin and interferon use in addition to all covariates listed in this table

Time fixed variables included sex, race and ethnicity (due to small numbers, Asian/PI was collapsed into the other/unknown category), sex, HIV acquisition risk group, and cohort. And HIV acquisition risk, all other variables were time varying based on hemoglobin measure and associated MCV

Values with P < 0.05 are bolded

* Missing values for smoking, CD4 count, viral load, and BMI were imputed using multiple imputation by chained equations

women aged 50 years and older the risk of microcytic anemia is reduced compared with women under age 50, which can be used as a rough estimation for the effect of menopause.

The prevalence of macrocytic anemia increased with age irrespective of sex, a finding also seen among non-PWH populations [7, 8]. One hypothesis for this is that as individuals age their RBC do not survive as long and younger RBC tend to have larger volumes [28]. We confirmed previous observations that smoking was associated with macrocytosis [29]. Liver disease is also known to result in macrocytosis [30], which is consistent with our observation that those with HCV were more likely to have macrocytic anemia. Anemia among individuals with CKD is typically either microcytic or normocytic, with macrocytosis often characterized by

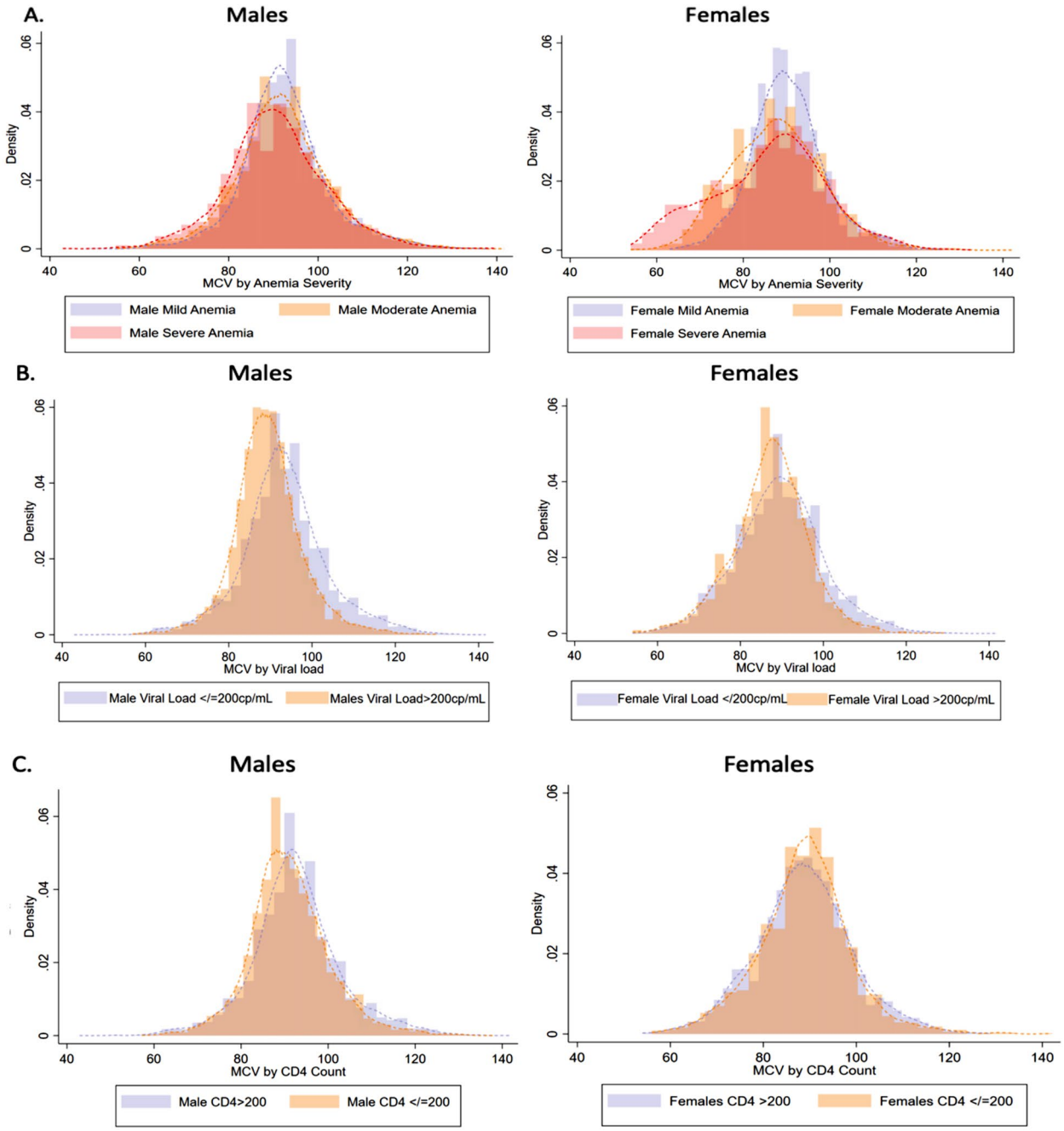

Fig. 2 Density histogram with kernel density estimation of MCV (normocytic (80–100 fL), macrocytic (> 100 fL), and microcytic (< 80 fL)) among anemic PWH stratified by sex for **A** anemia severity **B** HIV viral suppression **C** CD4 count **D** ART use

other underlying conditions such as nutritional deficiencies, medications, or bone marrow disorders [31]. We identified both males and females with CKD were more likely to have macrocytic (vs. normocytic) anemia compared with those without CKD. In our study, PWH with CKD were more likely to be older (median age 55 vs. 47 years) and on ART for longer durations (median 8.3 vs. 5.8 years), which may in-part account for this difference.

We identified that PWH who had higher viral loads (> 200 copies/mL) were more likely to have either microcytic or normocytic anemia, possibly related to both iron deficiency and/or anemia of chronic disease. A previous study of South African women identified a reduction of

MCV by 1.78 fL with acute HIV infection [32]. Iron deficiency anemia more commonly results in microcytic anemia, whereas anemia of chronic disease commonly results in normocytic anemia; however, there is overlap and the two can also occur concurrently [23, 33]. People with anemia of chronic disease and concurrent iron-deficiency anemia are more likely to have microcytosis and more severe anemia than those with anemia of chronic disease alone [23, 33], possibly explaining our finding of increased anemia severity among PWH with microcytosis, particularly in females.

Irrespective of sex, non-Hispanic Black PWH had a higher prevalence of microcytic (vs. normocytic) anemia compared with non-Hispanic White PWH, which supports observations in the general population that iron deficiency is more common among Black individuals [34, 35]. Differences in the menarche and menstruation cycles by race may result in greater iron deficiency among Black women compared with White women [35]. Genetic disorders affecting hemoglobin and resulting in microcytic anemia are more prevalent among people from Africa or of African descent (thalassemia and sickle cell disease) and Asia (thalassemia) [11, 36, 37].

Our findings are only generalizable to PWH who have both successfully linked into HIV care and initiated ART. We cannot discern a mixed contribution of macrocytic and microcytic anemia resulting in a normocytic result compared to a truly normocytic anemia. Factors such as alcohol use, nutritional status, socioeconomic status, hemoglobinopathies and treatment of comorbidities may lead to residual confounding. We did not collect data on menstruation, pregnancy, contraception use, and menopause, which are likely to impact hemoglobin and MCV among women. We are unable to assess temporality or causality of identified associations on MCV and this is an area which would benefit from further research. Further laboratory studies such as iron studies, vitamin B12, folate and reticulocytes are needed to characterize the underlying cause of anemia among PWH and evaluate the impact of HIV. Future studies comparing MCV categories and risk factors of PWH with people without HIV are needed to assess the contribution of HIV infection and its associated complications.

Conclusion

The proportion of PWH with microcytic, normocytic, and macrocytic anemia fluctuated over time, associated with changes in HIV care and therapeutics. The risk factors for these different anemia types varied significantly by sex, therefore investigation into underlying causes of anemia should be considered by sex. HIV care programs should also consider age, race/ethnicity, comorbidities, and HIV factors such as CD4 count, viral load, and duration of ART as risk factors for microcytic, normocytic, and macrocytic anemia among PWH who have initiated ART. As anemia remains highly prevalent and associated with poor outcomes among PWH, we have highlighted the utility of using MCV to provide insight into the trends and risk factors for type-specific anemia over time. As MCV categories are indicative of underlying factors driving anemia, awareness of the prevalence and risk factors associated with MCV categories may lead to more prompt screening, diagnoses, and treatment of anemia among PWH.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BMI	Body mass index
CKD	Chronic kidney disease
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration rate
FL	Femtolitre
GEE	General estimating equations
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HTN	Hypertension
IeDEA	International epidemiology databases to evaluate AIDS
IDU	Injection drug use
IQR	Interquartile range
MSM	Men who have sex with men
MCV	Mean corpuscular volume
NA-ACCORD	North American AIDS cohort collaboration on research and design
NADC	Non-AIDS defining cancer
PR	Prevalence ratio
PWH	People with HIV
RBC	Red blood cells
WHO	World Health Organization
ZDV	Zidovudine

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12981-024-00641-4.

Additional file 1.

Author contributions

All authors contributed to the concept and design of this work as well as the acquisition and interpretation of the data. The statistical analysis and initial drafting of the manuscript was performed by Dr. Lang with supervision from Dr. Althoff. Critical revision of the manuscript was conducted by all listed authors. All listed authors were integral to the completion of this manuscript, participating in the planning and execution of this analysis.

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Availability of data and materials

Complete data for this study cannot be publicly shared because of legal and ethical restrictions. The NA-ACCORD Principles of Collaboration requires submission and approval of a concept sheet that describes the intended research project for which data are being requested. The NA-ACCORD Executive Committee and the Steering Committee (composed of principal investigators of contributing cohorts) must approve the concept sheet and elect to have their data included for the research project. A signed Data User Agreement is required before data can be released. Guidance for how to obtain NA-ACCORD data are outlined on the NA-ACCORD website (www.naaccord.org/collaboration-policies).

Declarations

Ethics approval and consent to participate

Each cohort has approval by their respective local institutional review boards (IRB), and by the Johns Hopkins School of Medicine. Approval for this work was obtained from the University of Calgary IRB REB20-2094.

Consent for publication

All authors of this manuscript have consented to publish this work.

Competing interests

Dr. Althoff is a consultant to the *All of Us* Research Program and serves on the scientific advisory board for Trio Health. Dr. Gill serves as an ad hoc advisor on Canadian National HIV advisory boards of Merck, Gilead and ViiV. Dr. Gebo is a paid consultant for the Aspen Institute, UptoDate and Teach for America. All other authors have no competing disclose.

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