JAIDS Journal of Acquired Immune Deficiency Syndromes Publish Ahead of Print
DOI: 10.1097/QAI.0000000000003502

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THE ASSOCIATION OF ANEMIA WITH SURVIVAL AMONG PEOPLE WITH HIV FOLLOWING ANTIRETROVIRAL INITIATION IN THE NA-ACCORD 2007-2016

Running title: Association of anemia with survival among PWH

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Word Count: 3602/3500

Key Words: Anemia, Survival, People with HIV, NA-ACCORD, Mortality, Mean corpuscular volume.

Presented part of this work at CROI 2023 (Feb 19-22) in Seattle Washington, USA

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Authors 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.

Conflicts of Interest/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. Dr. Thorne is a paid consultant for Gilead and UptoDate. All other authors have no conflicts to disclose.

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

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released. Guidance for how to obtain NA-ACCORD data are outlined on the NA-ACCORD website (www.naaccord.org/collaboration-policies).

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Source of Funding:

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was supported by National Institutes of Health grants U01AI069918, F31AI124794, F31DA037788, G12MD007583, K01AI093197, K01AI131895, K23EY013707, K24AI065298, K24AI118591, K24DA000432, KL2TR000421, N01CP01004, N02CP055504, N02CP91027, P30AI027757, P30AI027763, P30AI027767, P30AI036219, P30AI050409, P30AI050410, P30AI094189, P30AI110527, P30MH62246, R01AA016893, R01DA011602, R01DA012568, R01AG053100, R24AI067039, R34DA045592, U01AA013566, U01AA020790, U01AI038855, U01AI038858, U01AI068634, U01AI068636, U01AI069432, U01AI069434, U01DA036297, U01DA036935, U10EY008057, U10EY008052, U10EY008067, U01HL146192, U01HL146193, U01HL146194, U01HL146201, U01HL146202, U01HL146203, U01HL146204, U01HL146205, U01HL146208, U01HL146240, U01HL146241, U01HL146242, U01HL146245, U01HL146333, U24AA020794, U54GM133807, UL1RR024131, UL1TR000004, UL1TR000083, UL1TR002378, Z01CP010214 and Z01CP010176; contracts CDC-200-2006-18797 and CDC-200-2015-63931 from the Centers for Disease Control and Prevention, USA; contract 90047713 from the Agency for Healthcare Research and Quality, USA; contract 90051652 from the Health Resources and Services Administration, USA; the Grady Health System; grants CBR-86906, CBR-94036, HCP-97105 and TGF-96118 from the Canadian Institutes of Health Research, Canada; Ontario Ministry of Health and Long Term Care, and the Government of Alberta, Canada. Additional support was provided by the National Institute Of Allergy And Infectious Diseases (NIAID), National Cancer Institute (NCI), National Heart, Lung, and Blood Institute (NHLBI), Eunice Kennedy Shriver National Institute Of Child Health

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& Human Development , National Human Genome Research Institute (NHGRI), National Institute for MH (NIMH) and National Institute on Drug Abuse (NIDA), National Institute On Aging (NIA), National Institute Of Dental & Craniofacial Research (NIDCR), National Institute Of Neurological Disorders And Stroke , National Institute Of Nursing Research (NINR), National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institute on Deafness and Other Communication Disorders (NIDCD), and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

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ABSTRACT

Background

Anemia is an independent predictor of mortality, which may be utilized as a signal of deteriorating health. We estimated the association between anemia severity categories and mortality following the initiation of antiretroviral therapy (ART) among people with HIV (PWH) in North America.

Methods

Within the NA-ACCORD, annual median hemoglobin measurements between 01/01/2007-12/31/2016 were categorized using World Health Organization criteria into mild (11.0-12.9g/dL men, 11.0-11.9g/dL women), moderate (8.0–10.9g/dL men/women) and severe (<8.0g/dL men/women) anemia. Discrete time-to-event analyses using complementary log-log link models estimated mortality hazards ratios adjusted for demographics, comorbidities, and HIV clinical markers with 95% confidence intervals for the association between anemia and mortality.

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Results

Among 67,228 PWH contributing a total of 320,261 annual median hemoglobin measurements, 257,293 (80%) demonstrated no anemia, 44,041 (14%) mild, 18,259 (6%) moderate, and 668 (0.2%) severe anemia during follow-up. Mortality risk was 5.6-fold higher among PWH with (vs. without) anemia. The association was greater among males (aHR=5.8 [5.4, 6.2]) versus females (aHR=4.1 [3.2, 5.4]). Mortality risk was 3.8-fold higher among PWH with mild anemia, 13.7-fold higher with moderate anemia, and 34.5-fold higher with severe anemia (vs. no anemia). Median hemoglobin levels significantly declined within 4 years prior to death, with the maximum decrease the year prior to death. Macrocytic anemia was associated with an increased and microcytic anemia a decreased mortality risk (vs. normocytic anemia).

Conclusions

Anemia among PWH who have initiated ART is an important predictive marker for mortality with macrocytic anemia having an increased and microcytic anemia a decreased association with mortality compared with normocytic anemia.

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INTRODUCTION:

Despite the use of effective antiretroviral therapy (ART), virologic suppression and immune recovery, anemia is highly prevalent among people with HIV (PWH)[1,2]. Prior to effective ART, anemia was associated with HIV disease severity leading to poor quality of life and higher risk of death[3-5]. Even following ART initiation in PWH, anemia has remained an independent predictor of mortality, with improved survival following resolution of anemia[6,7].

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Data on the association of anemia with survival among PWH is predominately from either pre-ART or early-ART eras[4,6,8], with many of the recent studies being conducted in low- or middle-income countries, where anemia risk factors, prevalence and outcome may not be generalizable elsewhere[9,10]. Anemia among North American PWH has been assessed as part of the Veterans Aging Cohort Study (VACS) Index, which is a risk assessment tool that provides a score to predict the risk of mortality among PWH who have initiated ART[11-13]. Within the VACS Index 1.0, PWH with hemoglobin levels <10g/dL are assigned the most points out of any other included category resulting in an estimated 17% increased risk in all-cause 5-year mortality when holding other categories at baseline[11-13]. Anemia remains a dominant predictor in VACS Index 2.0 which is based on a more recent sample of patients, and which treats hemoglobin as a continuous variable[14,15]. However, the VACS Index is focused on an estimation of overall risk of mortality and does not characterize anemia as an independent predictor of mortality[15,16].

In both the general population and PWH, women are known to have a higher prevalence of anemia[17]. Anemia in young women is more prevalent due to menstruation leading to iron deficiency anemia and therefore believed to be associated with better outcomes when compared with men with anemia[18,19]. There is little data on the association of anemia on mortality among PWH following initiation of ART and evaluating HIV viral suppression, particularly when stratified by anemia severity categories, sex and by mean corpuscular volume (MCV). The MCV documents the average size of red blood cells (RBC), which is used to classify anemia types and provide context into potential underlying mechanisms[20]. Anemia among PWH may be an early indicator of mortality and therefore understanding this association can help care providers identify and intervene on those at highest risk.

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We aimed to a) estimate the association of mortality by anemia, anemia severity categories, and MCV categories among PWH in North America who had initiated ART, and b) identify differences by sex on the association of anemia on mortality.

METHODS

Study population

The North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) is a consortium of longitudinal HIV cohort studies 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[21]. NA-ACCORD eligibility criteria include successful linkage into clinical care (defined in NA-ACCORD as ≥2 HIV care visits in 12 months). Participating cohorts submit data annually to the Data Management Core (DMC, University of Washington, Seattle WA), where data undergo quality checks, and are transferred to the Epidemiology/Biostatistics Core (EBC, Johns Hopkins University, Baltimore MD). Each participating cohort has approval from their respective local institutional review boards (IRB). This study was approved by the University of Calgary IRB.

The source population for our nested study was PWH (≥18 years) participating in one of 17 NA-ACCORD-contributing clinical cohorts that also contributed hemoglobin measures. Individual-level selection criteria for our study population included those who had initiated ART (defined as the first date of observed ART prescription), were observed in care from Jan 1, 2007– Dec 31, 2016 (study period) and had at least one measured hemoglobin during the study period. Of those

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excluded for not having a hemoglobin measurement (3,163 (3.5%)), less than 1% of these individuals also had a CD4 count, or viral load measured during the study period, likely reflective of their level of engagement in HIV care.

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Anemia Ascertainment

Time varying anemia severity categories were included in time-to-event analysis each year using annual median hemoglobin measurements. Annual median hemoglobin measurements were categorized based on World Health Organization (WHO) definitions as no anemia (≥13g/dL men, ≥12g/dL women) or mild anemia (11.0-12.9g/dL men, 11.0-11.9g/dL women), moderate (8.0–10.9g/dL) or severe (<8.0g/dL)[22]. In a sensitivity analysis annual median hemoglobin was utilized as a continuous variable. The MCV associated with the lowest hemoglobin measurement annually was categorized as normocytic (80-100 femtolitre (fL)), macrocytic (>100 fL), or microcytic (<80 fL) for each year of participant follow-up. Therefore, PWH could contribute data to various anemia severity and MCV categories over the study period.

Outcome: All-cause Mortality

Death was defined as all-cause mortality occurring during the study period as well as within each year of median hemoglobin observation. Deaths in the NA-ACCORD are ascertained through linkages with: (1) provincial, state, or national death registries (includes the US National Death Index); (2) medical record abstraction after notification of death to the attending physician; (3) outreach to family and/or friends; and (4) monitoring of obituaries. Mortality rates were comparable between cohorts with and without registry linkages[23].

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Covariates of interest

Sex was defined as sex at birth. Race and ethnicity were grouped into non-Hispanic white, non-Hispanic Black, Hispanic, and other/unknown categories. HIV acquisition risk group was determined at enrollment into the individual cohorts; 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 prior to each year of observation using NA-ACCORD operationalized definitions for hypertension (HTN), diabetes mellitus (DM), chronic kidney disease (CKD)[24], hypercholesterolemia and non-AIDS defining cancer (NADC)[25] (**Supplemental Table 1**). Hepatitis B (HBV) and hepatitis C (HCV) infection were parameterized as ever/never.

Other time-varying covariates included history of clinical AIDS diagnosis, CD4 count (cells/mm^3), HIV RNA (copies/mL), and current ART use. The median annual CD4 count, and HIV viral load were calculated each year of observation. Individuals with missing CD4 count or HIV viral load measurement within the year were excluded. Individuals were categorized as having an ART interruption if they had a gap in ART prescriptions for 3 months or more and were not censored. All covariates were selected *a priori* based on clinical knowledge and review of relevant literature.

Statistical Analysis

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To minimize the risk of falsely assuming complete event ascertainment from electronic health records, we used an "observation windows" approach[26]. Study entry (baseline) was defined as the date the cohort began observing patients, observation window open date for all covariates, patient enrollment date into the NA-ACCORD, date of ART initiation, or January 1, 2007, whichever came last. Study exit was defined as the earliest date the cohort stopped observing patients, observation window close date for all covariates, the date the patient was lost-to follow up (defined as their last CD4 or HIV RNA measurement prior to a gap of >18 months), date of death, or December 31, 2016, whichever came first.

The difference in median annual hemoglobin measurements were visualized between those who were censored at study exit compared with those who died, looking back each year from either the year of censoring or death. All-cause mortality rates were calculated (per 100 person-years (PY)) by dividing the number of deaths by number of observation years. Discrete (yearly) time-to-event analyses with complementary log-log link regression models estimated crude (cHR) and adjusted hazards ratios (aHR) and 95% confidence intervals (CI) for death by time-varying annual anemia severity categories. Subgroup analyses were conducted stratified by 1) sex and 2) only including PWH with anemia to assess the association of different MCV categories on mortality. P-value <0.05 with two-tailed tests were considered statistically significant. All analyses were performed using STATA version 16.0 (College Station, TX).

RESULTS

Of 320,261 total annual median hemoglobin observations from 67,228 PWH meeting inclusion criteria (**Supplemental Figure 1**), 257,293 observations (80%) were not anemic, 44,041(14%)

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had mild anemia, 18,259 (6%) had moderate anemia, and 668 (0.2%) had severe anemia (**Table 1**). A total of 300,108 PY were observed with similar mean follow-up time in those with any anemia compared to those without anemia (3.63 years vs. 3.74 years, respectively). Among PWH within the youngest assessed age group (18-39 years), 14% had median hemoglobin measures categorized as anemic whereas 39% of PWH within the oldest assessed age group (≥70 years) had median measures of anemia.

Among median hemoglobin measures, 31% of measurements in female PWH were categorized as anemic with 18% in males. The median CD4 count was 517 cells/mm^3 and viral load was 48 copies/mL among all participants during years of observation in the study. Among PWH without anemia, 7% had CD4 counts <200 cells/mm^3 and among PWH with severe anemia, 48% had CD4 counts <200 cells/mm^3. Anemia was significantly more common among PWH with HCV (30% vs. 17%), HBV (23% vs. 19%), DM (33% vs. 18%), HTN (27% vs. 15%), CKD (33% vs. 17%), as well as an AIDS defining illness (27% vs. 17%).

During the study period there were 5,638 deaths in PWH; 1,478 (26%) were among PWH without anemia and 4,160 (74%) were among PWH with anemia based on the median annual hemoglobin measurement the year of death **(Supplemental Table 2)**. Of PWH with median annual measurements of mild anemia 3% died, moderate anemia 14% died, and severe anemia 28% died within the same year. PWH who died had lower median CD4 counts within the year of death (273 cells/mm^3 vs. 521 cells/mm^3), were more likely to have a median viral load of >200 copies/mL (37% vs. 17%) and to have had a clinical AIDS diagnosis (47% vs. 24%).

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Change in Annual Median Hemoglobin

The annual median hemoglobin of PWH who survived during the entire study period remained relatively unchanged with a maximum change of 0.1g/dL (**Figure 1**). The annual median hemoglobin of PWH who died during the study period decreased by a total of 1.1g/dL. The largest change occurred in the year prior to death where the median annual hemoglobin declined by a median of 0.6g/dL. Among PWH who died (n=5,638), 1,153 (20%) did not have hemoglobin measurements the year prior; of those that did, 3,182 (71%) had a lower median hemoglobin than the year prior. A significant decline in annual median hemoglobin among PWH who died compared to those who were censored out of the study was identified within the 4 years prior to death (P<0.001), with a greater decline seen among males compared with females (**Figure 1**). As an HIV viral load >200 copies/mL was associated with both increased prevalence of anemia and an increased risk of mortality we evaluated if the change in annual median hemoglobin was associated with HIV viral replication. The difference in median annual viral load measures were assessed between those who were censored at study exit compared with those who died. The annual median change in viral load remained 0 copies/mL looking back each year from study exit among both PWH who were censored and those who died. Therefore, the identified decrease in median hemoglobin prior to death was not associated with a change in median HIV viral load.

Mortality Rates (MR) in PWH by Anemia status and its Severity

The mortality rate (MR) (per 100 PY) for PWH without anemia was 0.61 [0.58, 0.64] and 7.30 [7.08, 7.52] for those with anemia, (mild: MR=3.56 [3.38, 3.75], moderate: MR=16.03 [15.41, 16.66], and severe: MR=36.44 [31.54, 42.11]) (**Table 2**). The mortality rate was greater among

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males with anemia (mild: MR=4.00 [3.80, 4.22], moderate: MR=20.11 [19.30, 20.96], severe: MR=48.47 [41.47, 56.65]), compared to females with anemia (mild: MR=1.00 [0.78, 1.29], moderate: MR=5.71 [5.05, 6.46], severe: MR=14.53 [9.89, 21.34]).

Risk of Death among PWH with Anemia and by Anemia Severity Category

Anemia was associated with an increased risk of death among PWH who had initiated ART (aHR=5.62 [5.24, 6.04]) (**Table 3**). This increased risk of death by anemia was greater among males (aHR=5.78 [5.37, 6.23]) than females (aHR=4.13 [3.17, 5.40]). With increasing severity of anemia, the risk of death increased (**Figure 2**). Among males, 2% died the same year they had median annual hemoglobin measurements of 12-13g/dl, whereas 35% of those who had median annual hemoglobin measurements of <8g/dl died. Among females, 1% died the same year they had median annual hemoglobin measurements of 11-12g/dl, whereas 13% of those who had median annual hemoglobin measurements of <8g/dl died (**Figure 2**). Per each 1g/dL lower median hemoglobin the adjusted risk of death increased by 72% (aHR=1.72 [1.69-1.74]), 74% (aHR=1.74 [1.72-1.77]) among males and 65% (aHR=1.65 [1.56-1.75]) among females. The risk of death increased by 3.8-fold for PWH with mild anemia, 13.7-fold with moderate anemia and 34.5-fold with severe anemia compared with PWH with no anemia (**Table 3**).

Overall, females with HIV had a 37% (aHR=0.63 [0.57-0.71]) reduced risk of mortality compared with males with HIV (**Supplemental Table 3**). To evaluate the differences by sex on the association of anemia on mortality, a stratified analysis was done by birth sex (**Table 3**). Males with mild anemia had a 4-fold (aHR=3.99 [3.69-4.31]) increased risk of death, with moderate anemia a 15-fold (aHR=14.82 [13.72-16.01]) increased risk and with severe anemia a

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38-fold (aHR=38.13 [32.00-45.44]) increased risk of death compared with males with no anemia. Females with mild anemia had a 2-fold (aHR=2.04 [1.48-2.83]) increased risk of death, moderate anemia a 7-fold (aHR=7.28 [5.64-9.40]) increased risk and with severe anemia a 16-fold (aHR=15.89 [10.03-25.16]) increased risk of death compared with females with no anemia.

Risk of Death among PWH with Anemia by MCV Category

Among PWH with anemia, macrocytic vs. normocytic anemia was associated with a greater risk of death (aHR=1.40 [1.22-1.61]) whereas microcytic vs. normocytic anemia conferred relative protection (aHR=0.57 [0.46-0.70]). In analyses stratified by sex, the increased risk of death among females with macrocytic anemia attenuated (aHR=1.33 [0.95-1.88]) and the protective association with microcytic anemia vs. normocytic was more pronounced (aHR=0.44 (0.29-0.67)].

DISCUSSION

Following the initiation of contemporary ART, anemia has remained a predictor of mortality among PWH in North America. Mortality risk increases significantly as anemia severity increases and with each 1 g/dL decline in median annual hemoglobin measurements. Males with mild, moderate, and severe anemia have lower survival probabilities compared with females of the same anemia severity category. Within the 4 years prior to death the median annual hemoglobin begins to decline with the sharpest decrease within the year prior to death for both males and females. The change in annual median hemoglobin did not have a corresponding change in annual median HIV viral load. Anemia remains a significant predictor for mortality,

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which differs by sex and severity of anemia and can be utilized clinically as an early signal of deteriorating health.

Prior studies in the pre- or early-ART era found anemia was associated with increased mortality among PWH, we found this remains true even in the current treatment era[4,6,7,27]. Like CD4 counts and viral loads, hemoglobin level is an independent prognostic marker[4,6]. In the 1990's, Mocroft and colleagues identified a continual decrease in hemoglobin levels among PWH who died, while hemoglobin levels remained stable among those who survived, suggesting that hemoglobin is predictive of survival[6]. Over 12-months of follow-up, they found the hazards of death increased by 57% for each 1g/dL reduction in hemoglobin[6]. Compared to these earlier studies, we identified a reduced magnitude of association between anemia and mortality, yet anemia remains a significant predictor of mortality. The VACS Index which has been validated within the NA-ACCORD population also identified an increasing association between anemia severity and mortality, however anemia in this index was not studied as an independent predictor of mortality nor evaluated for sex-based differences in mortality[11,12,15,16].

Males with HIV and anemia had a higher risk of mortality across all anemia severity categories when compared with females. Younger females likely have a higher prevalence of anemia because of blood loss through menses, typically leading to iron deficiency which decreases following menopause[18]. In contrast, anemia among men is not related to a physiologic process but instead likely reflects underlying pathology [28]. Therefore, the difference in mortality outcomes may be because of the underlying etiology of anemia being different between men and women, which is also identified through mortality differences in age categories by sex. This

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association may in part also explain the difference in mortality risk by MCV categories of anemia as the most common cause of microcytic anemia is iron deficiency which is more prevalent among menstruating females[39]. However, as anemia was significantly associated with all-cause mortality among both males and females, identification of anemia should prompt clinicians to investigate the underlying cause, specifically if the anemia is moderate/severe or progressive.

We identified a reduced mortality risk among PWH with microcytic vs. normocytic anemia, an association not identified by other studies[36,37]. One study conducted in Sub-Saharan Africa identified that microcytosis at the time of HIV diagnosis was associated with a higher 12-month mortality[36]. However, microcytosis was found to have no association with survival in the Women's interagency HIV study (WIHS)[37]. There remains little data on the association of MCV on mortality among PWH, however this has been studied in different populations. A Swedish study of anemia in the general population identified the highest mortality with macrocytic anemia with no significant differences noted between microcytic and normocytic anemia[17]. In keeping with our findings, macrocytosis has been associated with a higher mortality among other subpopulations including those with malignancies, heart failure, end stage renal disease and critical illness[32-35]. It has been suggested that macrocytosis is associated with higher mortality as it likely results from impaired DNA synthesis leading to abnormalities of RBC nuclear maturation and therefore reflects bone marrow function[34]. Macrocytosis is known to increase with age and is associated with multiple comorbidities, nutritional deficiencies, drugs, and toxins[35,38].

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Despite increasing age being a risk factor for anemia, older age itself is not a cause of anemia[29]. Anemia among older adults is associated with an increased risk of hospitalization and all-cause mortality[30]. As PWH are an aging population in North America[31] and both age and HIV are associated with increasing prevalence of anemia, clinicians should be aware of the association with mortality to prompt timely screening, investigation, and treatment.

We identified a significant association of anemia on mortality among PWH, however whether anemia is an independent risk factor for death or a surrogate marker of underlying pathology increasing the risk of death remains unanswered. In the VACS Index anemia is an important predictor of mortality independent of other measured biomarkers of organ system injury. Low hemoglobin concentrations reduce carrying capacity and delivery of oxygen which may contribute to adverse outcomes[41]. Alternatively, anemia results from many chronic conditions which increase mortality. Anemia appears to be a significant prognostic marker for mortality among PWH, particularly among males, which increases considerably with worsening anemia severity. These findings highlight the importance of regular screening for investigations into the cause, and treatment of anemia among PWH.

There are limitations to this work. We did not have information on causes of death, which would be a valuable subject for future studies as well as provide further information on the context of the association of anemia on survival. Our definition of anemia and anemia severity categories are based on WHO criteria, which does not account for possible racial/ethnic variations in normal hemoglobin ranges. We used the median hemoglobin annually to categorize anemia to limit measurement bias as individuals with anemia are more likely to have repeated hemoglobin

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measurements, however this would not capture hemoglobin fluctuations over the year. As enrollment criteria into the NA-ACCORD includes only individuals successfully linked into care and initiated on ART, our findings are not generalizable to PWH who do not link to HIV care. There may also be selection bias in that individuals who never had a hemoglobin measurement were excluded from this study. There is likely to be residual confounding impacting the identified association of anemia on death, particularly active drug use or alcohol misuse, socioeconomic status, traumatic events, diet and nutritional status, which were not measured. We used the eGFR calculation adjusted for race, new data demonstrates limitations with this approach and therefore we may have underestimated kidney disease among Black PWH. We were unable to identify the underlying etiology of anemia, which likely would result in mortality risk differences. Further studies are needed to characterize mortality risk by different underlying etiologies of anemia among PWH to improve risk stratification.

The strengths of this work include the use of a large, well-characterized, and representative cohort of PWH who are linked to HIV care in the United States and Canada. Due to our longitudinal study design and large participant numbers, we were able to assess differences in mortality by anemia severity, particularly severe anemia, which is a rare exposure (0.2% in our study) as well as stratification by sex and evaluation of MCV categories. The retrospective longitudinal data provided the ability to evaluate median hemoglobin trends prior to death as well as provide longitudinal risk estimates, which is novel and clinically relevant to care providers.

CONCLUSIONS

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Among both male and female PWH who have initiated ART, anemia is an important predictive factor for death. The association of anemia with death increased with worsening anemia severity and was significantly greater among males compared with females who were predominantly pre-menopausal. Macrocytic anemia is associated with a higher mortality risk whereas microcytic anemia a lower mortality risk compared with normocytic anemia. Median hemoglobin levels decreased significantly within the 4 years prior to death. Clinicians should be aware of the association between anemia and mortality following ART initiation, as well as the differences by sex and MCV categories. Regular screening for anemia and among those with anemia, timely investigation and treatment remains a critical aspect of HIV management.

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Figure Legends:

Figure 1: Change in Annual Median Hemoglobin looking back from time of censoring or death comparing PWH in the NA-ACCORD comparing those censored at the time of study exit compared to those who died.

Figure 2: Annual Survival among Males and Females with HIV in the NA-ACCORD by median annual hemoglobin measurement among those with anemia. Anemia was defined as <13g/dL males, <12g/dL females.

Supplemental tables and figures---http://links.lww.com/QAI/C335

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Table 1: Characteristics of study population per year of median hemoglobin measure by anemia status among 67,228 people with HIV in the NA-ACCORD (2007-2016)

Characteristic	Total Annual Median Hemoglobin Measures N=320,261	No Anemia N=257,293 (80%)	Mild Anemia N=44,041 (14%)	Moderate Anemia N=18,259 (6%)	Severe Anemia N=668 (0.2%)
Year of Measurement					
2007-2010	131,182 (41%)	103,477 (40%)	19,391 (44%)	8,101 (44%)	213 (32%)
2011-2013	106,544 (33%)	85,950 (33%)	14,399 (33%)	5,991 (33%)	204 (31%)
2014-2016	82,535 (26%)	67,866 (26%)	10,251 (23%)	4,167 (23%)	251 (38%)
Age at Measurement (years, median (IQR)	51 (43-58)	50 (42-57)	55 (47-62)	54 (45-62)	50 (40-59)
18-39 years	54,992 (17%)	47,142 (18%)	5,027 (11%)	2,660 (15%)	163 (24%)
40-49 years	90,536 (28%)	76,797 (30%)	9,404 (21%)	4,187 (23%)	148 (22%)
50-59 years	106,057 (33%)	84,621 (33%)	15,489 (35%)	5,752 (32%)	195 (29%)
60-69 years	55,852 (17%)	40,971 (16%)	10,623 (24%)	4,146 (23%)	112 (17%)
≥70 years	12,824 (4%)	7,762 (3%)	3,498 (8%)	1,514 (8%)	50 (7%)
Mean Corpuscular Volume					
Normocytic	117,126 (37%)	99,866 (39%)	11,924 (28%)	5,151 (28%)	185 (28%)
Microcytic	5,364 (2%)	1,982 (1%)	1,579 (4%)	1,649 (9%)	163 (24%)
Macrocytic	19,803 (6%)	16,159 (6%)	2,503 (6%)	1,094 (6%)	47 (7%)
Missing	177,968 (56%)	139,286 (54%)	28,035 (64%)	10,374 (57%)	273 (41%)
Sex at Birth					
Male	282,658 (88%)	231,314 (90%)	37,602 (85%)	13,284 (73%)	458 (69%)
Female	37,603 (12%)	25,979 (10%)	6,439 (15%)	4,975 (27%)	210 (31%)
Race/Ethnicity					
Non-Hispanic White	147,384 (46%)	129,138 (50%)	13,390 (30%)	4,729 (26%)	127 (19%)
Non-Hispanic Black	129,729 (41%)	91,386 (36%)	26,184 (59%)	11,676 (64%)	483 (72%)
Hispanic	33,139 (10%)	28,124 (11%)	3,504 (8%)	1,473 (8%)	38 (6%)
Other/Unknown	10,009 (3%)	8,645 (3%)	963 (2%)	381 (2%)	20 (3%)
HIV acquisition risk					
MSM	99,069 (31%)	88,914 (35%)	7,621 (17%)	2,410 (13%)	124 (19%)
IDU	59,572 (19%)	42,626 (17%)	11,955 (27%)	4,841 (27%)	150 (22%)
Heterosexual	46,404 (14%)	34,475 (13%)	7,178 (16%)	4,538 (25%)	213 (32%)
Other/Unknown	115,216 (36%)	91,278 (35%)	17,287 (39%)	6,470 (35%)	181 (27%)
Diabetes Mellitus	43,769 (14%)	29,395 (11%)	9,509 (22%)	4,701 (26%)	164 (25%)
CKD (eGFR<60mL/min/1.73m^2)	33,444 (10%)	22,376 (9%)	7,425 (17%)	3,512 (19%)	131 (20%)
NADC	21,509 (7%)	13,856 (5%)	4,665 (11%)	2,881 (16%)	107 (16%)
Hypertension	127,989 (40%)	93,557 (36%)	23,664 (54%)	10,433 (57%)	335 (50%)
Hepatitis C Virus	61,197 (19%)	43,115 (17%)	12,174 (28%)	5,708 (31%)	200 (30%)
Hepatitis B Virus	19,921 (6%)	15,418 (6%)	3,040 (7%)	1,404 (8%)	59 (9%)
Diagnosis of AIDS	78,888 (25%)	57,511 (22%)	13,320 (30%)	7,758 (42%)	299 (45%)
Median CD4 per year	517 (338-718)	545 (374-743)	421 (241-623)	300 (138-504)	210 (64-440)

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(cells/mm^3) (IQR)					
≤200cells/mm^3	33,894 (11%)	18,587 (7%)	8,591 (20%)	6,394 (35%)	322 (48%)
Median Viral Load per year (copies/mL) (IQR)	48 (40-75)	48 (40-75)	50 (40-193)	66 (40-2957)	77 (40-25219)
≥200copies/mL	55,763 (17%)	37,960 (15%)	10,921 (25%)	6,582 (36%)	300 (45%)
ART Interruption	599 (0%)	451 (0%)	103 (0%)	44 (0%)	1 (0%)

Footnotes:

Abbreviations:

AIDS, Acquired immunodeficiency syndrome
ART, antiretroviral therapy
CKD, chronic kidney disease
eGFR, estimated glomerular filtration rate, measured in mL/min/1.73m^2
HIV, human immunodeficiency virus
IDU, injection drug use
MSM, men who have sex with men
NA-ACCORD, North American AIDS Cohort Collaboration on Research and Design
NADC, Non-AIDS defining Cancer

Intersex PWH were excluded from analysis due to lack of standardized hemoglobin categorization for anemia, however these individuals did not meet criteria for anemia with either male or female WHO criteria.
P-values for all characteristics <0.01

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Table 2: Incident rate per 100 person-years of mortality by anemia status and sex among people with HIV in the NA-ACCORD.

Anemia Status	Sex	Incident Rate /100 person-years	95% Confidence Interval
No Anemia	Total	0.61	0.58, 0.64
	Male	0.63	0.60, 0.66
	Female	0.41	0.34, 0.50
Any Anemia	Total	7.30	7.08, 7.52
	Male	8.24	7.98, 8.51
	Female	3.20	2.88, 3.56
Mild Anemia	Total	3.56	3.38, 3.75
	Male	4.00	3.80, 4.22
	Female	1.00	0.78, 1.29
Moderate Anemia	Total	16.03	15.41,16.66
	Male	20.11	19.30, 20.96
	Female	5.71	5.05, 6.46
Severe Anemia	Total	36.44	31.54, 42.11
	Male	48.47	41.47, 56.65
	Female	14.53	9.89, 21.34

Footnotes:

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Intersex PWH were excluded from analysis due to lack of standardized hemoglobin categorization for anemia, however these individuals did not meet criteria for anemia with either male or female WHO criteria.

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Table 3: Crude (cHR) and adjusted (aHR) hazard ratios of risk factors associated with all-cause mortality among People with HIV (PWH) in the NA-ACCORD and stratified by sex.

	Crude Hazards Ratio (n=320,261)		**Adjusted Hazards Ratio (aHR)** (n=320,261)		**aHR among Males** (n=282,654)		**aHR among Females** (n= 37,603)	
Characteristic	**cHR**	**95% CI**	**aHR**	**95% CI**	**aHR**	**95% CI**	**aHR**	**95% CI**
No Anemia	Ref		Ref		Ref		Ref	
Any Anemia	11.66	10.99, 12.37	5.62	5.24, 6.04	5.78	5.37, 6.23	4.13	3.17, 5.40
No Anemia	Ref		Ref		Ref		Ref	
Mild Anemia	5.71	5.31, 6.14	3.79	3.51, 4.09	3.99	3.69, 4.31	2.04	1.48, 2.83
Moderate Anemia	25.35	23.77, 27.03	13.72	12.88, 14.94	14.82	13.72, 16.01	7.28	5.64, 9.40
Severe Anemia	59.49	50.99, 69.40	34.54	29.34, 41.33	38.13	32.00, 45.44	15.89	10.03, 25.16
[1]MCV among PWH with Anemia (n=62,968)					**[1]Males with Anemia (n=51,344)**		**[1]Females with Anemia (n=11,619)**	
Normocytic	Ref		Ref		Ref		Ref	
Microcytic	0.61	0.49, 0.74	0.57	0.46, 0.70	0.66	0.52, 0.83	0.44	0.29, 0.67
Macrocytic	1.50	1.64, 1.72	1.40	1.22,1.61	1.43	1.23, 1.67	1.33	0.95, 1.88
Missing MCV	1.34	1.23, 1.45	0.94	0.83, 1.05	1.01	0.89, 1.14	0.72	0.53, 0.99

Adjusted models include: Time-fixed covariates sex at birth, race/ethnicity, HIV acquisition risk and cohort. Time varying covariates: year of hemoglobin measurement, anemia severity category, age, comorbidities at or prior to hemoglobin measurement (diabetes, hypertension, non-AIDS defining cancer, chronic kidney disease, AIDS diagnoses, HCV, HBV), median CD4 count ($\leq$200cells/mm^3), Median HIV RNA (>200 copies/mL), ART use.

[1]MCV analysis, AZT use within the same month of the MCV observation was also included in the adjusted models

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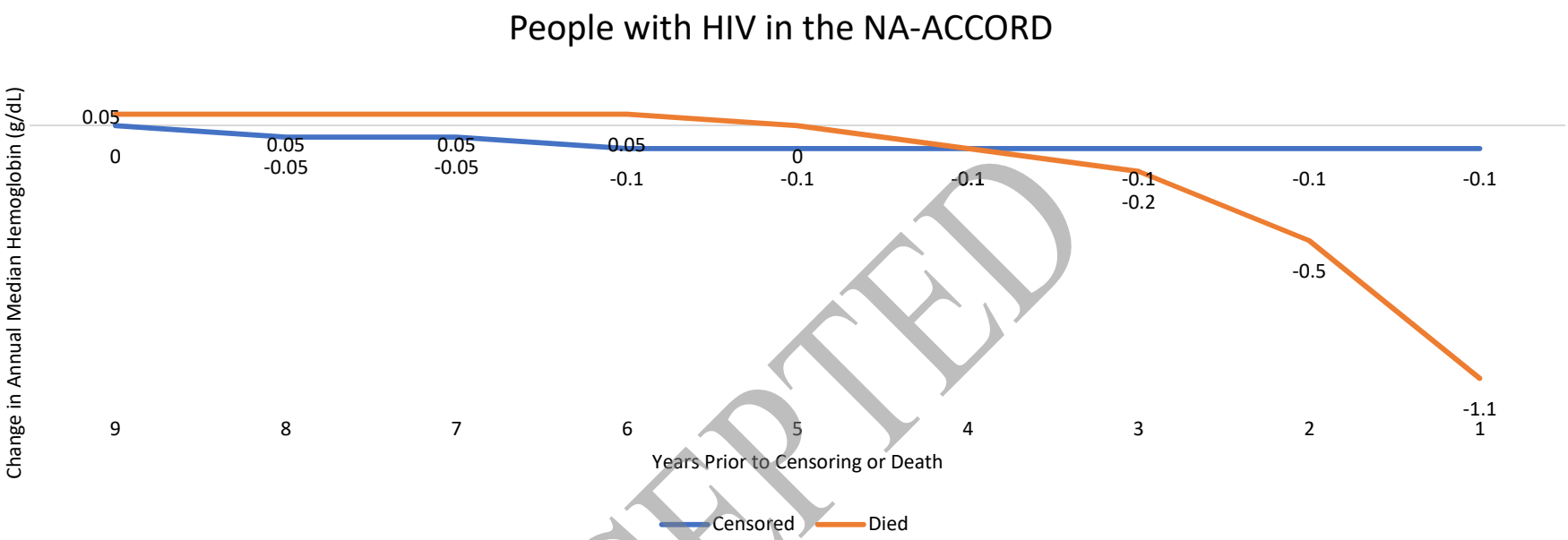

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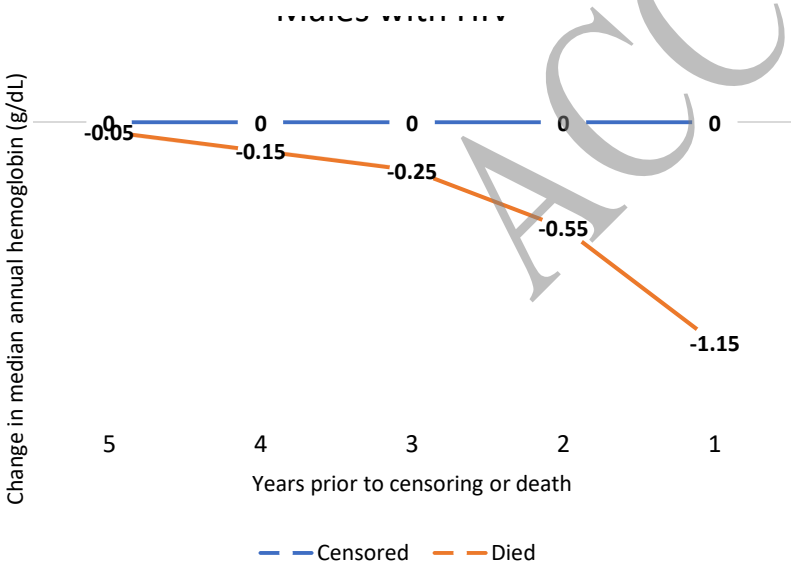
NA-ACCORD comparing those censored at the time of study exit compared to those who died.



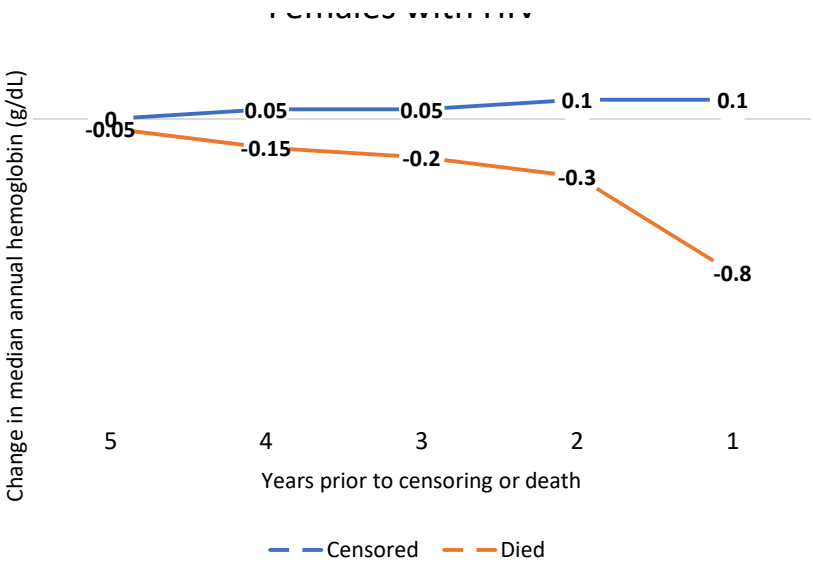


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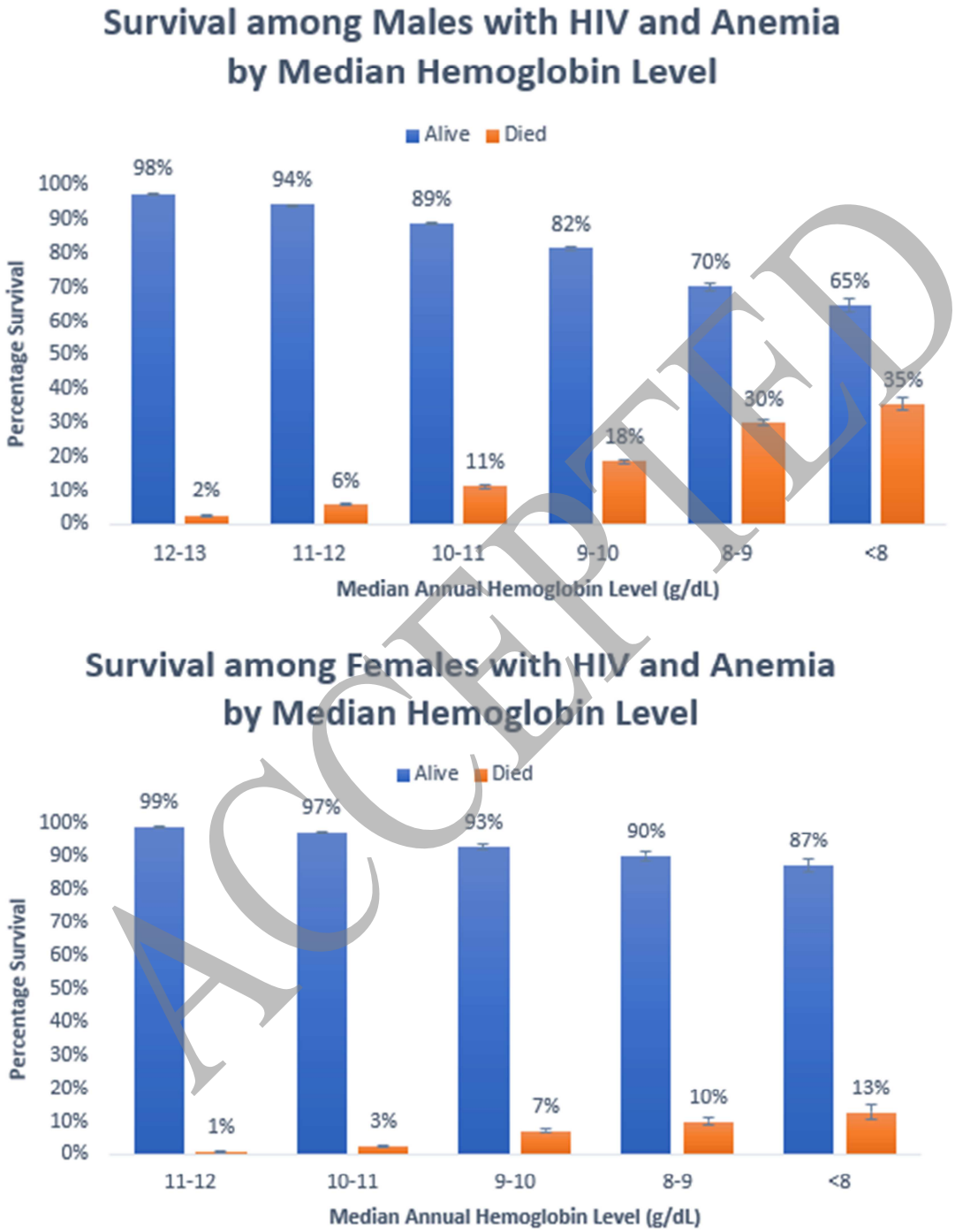
Figure 2: Annual Survival among Males and Females with HIV in the NA-ACCORD by median annual hemoglobin measurement among those with anemia.



Footnotes: There were 3,819 deaths among Males and 341 deaths among Females over the 10 year observation period (2006-2016) among those who were anemic. Anemia was defined as <13g/dL males, <12g/dL females.

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