Human Immunodeficiency Virus (HIV)-Negative Men Who Have Sex With Men Have Higher CD8⁺ T-Cell Counts and Lower CD4⁺/CD8⁺ T-Cell Ratios Compared With HIV-Negative Heterosexual Men

Sebastiaan O. Verboeket,1,2 Ferdinand W. Wit,1,2 Eveline Verheij,1,2 Rosan A. van Zoest,1,2 Neeltje A. Kootstra,4 Marc van der Valk,1 Jan M. Prins,1 Maarten F. Schim van der Loeff,1,5 and Peter Reiss,1,2,3; on behalf of the AGEhIV Study Group

1Amsterdam UMC, University of Amsterdam, Departments of Global Health and Internal Medicine, Amsterdam Infection and Immunity Institute and Amsterdam Public Health Research Institute, Amsterdam, the Netherlands, 2Amsterdam Institute for Global Health and Development, Amsterdam, the Netherlands, 3HIV Monitoring Foundation, Amsterdam, the Netherlands, 4Amsterdam UMC, University of Amsterdam, Department of Experimental Immunology, Amsterdam Infection & Immunity Institute, Amsterdam, the Netherlands, 5Public Health Service of Amsterdam, Department of Infectious Diseases, Amsterdam, the Netherlands

Background. We previously reported T-cell senescence to be similar in people with human immunodeficiency virus (PWH) with suppressed viremia (predominantly men who have sex with men [MSM]) and human immunodeficiency virus (HIV)-negative otherwise comparable controls but greater than in healthy blood donors. This led us to compare CD4⁺ and CD8⁺ T-cell counts and CD4⁺/CD8⁺ ratios between HIV-negative MSM and men who only have sex with women (MSW) and relate observed differences in behavioral factors and infectious exposures, including cytomegalovirus (CMV) infection.

Methods. In 368 HIV-negative MSM and 72 HIV-negative MSW, T lymphocyte phenotyping was performed 3 times biennially. Baseline CMV serology and sexually transmitted infection (STI) incidence and/or STI seroprevalence, sexual, and substance-use behavior data were collected during study visits.

Results. Men who have sex with men, compared with MSW, had higher CD8⁺ counts (551 vs 437 cells/mm³, P < .001), similar CD4⁺ counts (864 vs 880 cells/mm³, P = .5), and lower CD4⁺/CD8⁺ ratios (1.84 vs 2.47, P < .001). Differences were most pronounced for MSM with >10 recent sex partners and partly explained by higher CMV seroprevalence in MSM.

Conclusions. These findings suggest that factors other than HIV may, in both PWH and certain HIV-negative MSM, contribute to a low CD4⁺/CD8⁺ ratio. Whether this, like in PWH, contributes to comorbidity risk in HIV-negative MSM requires further study.

Keywords. CD4-CD8 ratio; cytomegalovirus; HIV; men who have sex with men; sexual and gender minorities.

Since the beginning of the human immunodeficiency virus (HIV) epidemic, CD4⁺ (CD4) and CD8⁺ T lymphocyte (CD8) counts and the CD4/CD8 ratio have been studied as potential predictive markers of disease progression in people with HIV (PWH). We now know that CD4 count restoration after combination antiretroviral therapy (cART) initiation is strongly correlated not only with a lower likelihood of developing acquired immune deficiency syndrome (AIDS), but also non-AIDS-associated comorbidities [1, 2]. Furthermore, although evidence remains inconclusive [3], studies have suggested a low CD4/CD8 ratio during long-term cART to be predictive for the development of non-AIDS-associated comorbidities [4–9]. People with HIV remain at increased risk of such comorbidities despite sustained viral suppression on cART [10, 11]. In HIV-negative populations, a low CD4/CD8 ratio with an expansion of CD8⁺ T cells that express CD57 or lack expression of CD28 negative populations, a low CD4/CD8 ratio with an expansion of CD8⁺ T cells that express CD57 or lack expression of CD28 has been described as reflecting part of an “immune risk phenotype” [12, 13]. Thus, low CD4/CD8 ratios, in both HIV-positive and HIV-negative populations, have been associated with morbidity [14] and mortality [15–17]. An in-depth analysis of T-cell senescence and activation was performed on a limited sample of AGE, IV participants [18] and in those enrolled in the COBRA (CoMorBidity in Relation to AIDS) study linked to it [19]. Both cohorts include an HIV-negative control group, with a majority of men who have sex with men (MSM), to ensure good comparability with the HIV-positive index group. A second control group of blood bank donors was only included within the COBRA cohort, who had low risk of bloodborne infectious
diseases. CD4+ and CD8+ T-cell senescence, as measured by the expression of CD57 or loss of CD27 and CD28 expression, was comparable between HIV-positive and HIV-negative participants but higher for both groups when compared with blood bank donors. This result suggests increased T-cell senescence also in HIV-negative COBRA/AGEhIV participants. Cytomegalovirus (CMV) seropositivity was an important driver of this increased T-cell senescence in both HIV-positive and HIV-negative COBRA participants. Likewise, CMV seropositivity has also been associated with high CD8 counts and low CD4/CD8 ratios in PWH [20].

In many countries, including the Netherlands, the majority of PWH are MSM [21]. Men who have sex with men are not only at increased risk of acquiring HIV but also of acquiring a wide range of other sexually transmitted infections (STIs). These not only include treatable bacterial STIs such as syphilis, gonorrhea, and chlamydia but also chronic viral infections such as viral hepatitis and herpesvirus infections, including CMV [22]. Cytomegalovirus seropositivity in particular [23], but also transient bacterial STIs [24, 25], have each been associated with a decreased CD4/CD8 ratio and an increased CD8 count.

In view of our earlier observation, we evaluated CD4 and CD8 counts and CD4/CD8 ratios in a larger group of HIV-negative individuals, comparing MSM and men who only have sex with women (MSW). We evaluated to what extent any observed differences were related to sexual and behavioral factors, exposure to STIs, and CMV infection.

**METHODS**

**Study Population**

The prospective AGEhIV Cohort Study investigates age-related comorbidities and their risk factors in HIV-positive and HIV-negative individuals aged ≥45 years [10]. Human immunodeficiency virus-positive participants were recruited from the Academic Medical Center's ambulatory HIV clinic, and HIV-negative participants were recruited from the sexual health clinic and the Amsterdam Cohort Studies on HIV/AIDS at the Public Health Service of Amsterdam [26]. Participants undergo standardized biennial screening to evaluate the presence of, or risk factors for, age-associated comorbidities. Most participants in the HIV-negative cohort are MSM, with a limited number of MSW and women. For the current analysis, only HIV-negative men with data available on sexual behavior were included (Figure 1). Human immunodeficiency virus-positive participants and women were not included to allow evaluation of the effects of being an MSM independent from the effects of gender and HIV. Participants with HIV seroconversion during follow-up were excluded from the time of seroconversion onward. All participants provided written informed consent; the study protocol was approved by the local ethics review committee and is registered at www.clinicaltrials.gov (identifier NCT01466582).

**Measurements**

Data from baseline and 2 follow-up study visits were available for analysis, comprising 4 years of follow-up. During each study visit, information about sexual behavior, smoking, alcohol use, and recreational drug use were obtained through standardized questionnaires inquiring about lifetime exposure and exposure during the 6 months before the study visits. In addition, during each study visit, percentages and absolute counts of T, B, and natural killer (NK) cells and CD4 and CD8 subpopulations of T cells in peripheral blood were determined using BD Multitest 6-color TBNK reagent with BD Trucount Tubes (BD Biosciences) by flow cytometry (BD FACSCanto II; BD Biosciences). Serology for HIV (4th-generation antibody/
antigen test) and syphilis (Treponema pallidum particle agglutination assay and rapid plasma reagin) were also performed at each visit. Chronic hepatitis B (hepatitis B surface antigen positive) and hepatitis C (hepatitis C virus ribonucleic acid [RNA] positive) statuses were determined only at baseline. In stored baseline plasma samples, CMV-immunoglobulin (IgG titer (ELISA-VIDITEST; VIDIA, Praha, Czech Republic) and levels of intestinal fatty acid binding protein ([I-FABP] a biomarker for intestinal integrity), soluble (s)CD14, sCD163 (markers of monocyte activation), and interleukin (IL)-6 (marker of inflammation) were determined using enzyme-linked immunosorbent assays (ELISAs) (DuoSet ELISA; R&D Systems). Data on syphilis, chlamydia, and gonorrhea diagnosed at the municipal sexual health clinic during each of the 3 study visits were extracted from clinical records. For a subset of participants (n = 92) (Supplementary Table 1.1), data regarding percentages of activated (CD38+/HLA-DR+) and senescent (CD27−/CD28−) T-cells within total CD4 and CD8 T-cell populations were available for analyses we previously published [18, 19].

Definitions
Men who have sex with men was defined as men who reported having had ≥2 male lifetime sex partners, and MSW was defined as men with <2 lifetime male sex partners, given that repeated exposures were the primary focus of interest. After finding large differences in CD4/CD8 ratios between MSW and MSM, but also between MSM with high versus low numbers of recent sex partners in a preliminary analysis, we decided, for this analysis, to further stratify MSM into high- and low-risk categories for the exploration of potential factors driving differences between study groups. High-risk as opposed to low-risk MSM were defined as MSM who reported a mean of >10 vs ≤10 sex partners in the 6 months before each of the 3 study visits, respectively, using the number of sex partners as proxy for the risk of both STIs and HIV.

Statistical Analysis
Characteristics of MSW, low-risk MSM, and high-risk MSM were compared using Wilcoxon rank-sum and χ² tests as appropriate. Differences in loge-transformed CD4/CD8 ratios and loge-transformed CD4 and CD8 counts between study groups were determined using linear mixed-effects models to account for repeated measurements. Models included a random intercept and random slope for time since baseline for each participant. We subsequently included potential confounding and/or mediating factors in the models following a stepwise forward variable selection process to evaluate which factors were driving any observed differences in lymphocyte subsets between subgroups. The variables body mass index (BMI), recent gonorrhea diagnosis, recent chlamydia diagnosis, and those concerning sexual and substance use behaviors were time updated. To allow differentiating between effects caused by different behavioral patterns between participants (between-participant variation) versus changes in an individual’s behavior over time (within-participant variation), we used a technique called “within subject centering” [27]. This means continuous predictor variables were split into 2. One dummy variable was fixed in time and consisted of the participant’s calculated mean of that variable over the 3 study visits, for the analysis of between-participant variation. A second dummy variable consisted of the deviation from the individual’s mean at a particular study visit, for the analysis of within-participant variation. Because the covariates CMV seropositivity, HCV RNA status, and I-FABP levels were only measured at the baseline visit, we also performed sensitivity analyses using baseline-data only. Additional mixed-effects models were built to evaluate associations of activation and senescence markers with absolute T-cell counts and the mean differences of these markers among MSW, low-risk MSM, and high-risk MSM, for the subgroup of participants in whom these markers were available [18, 19].

RESULTS
Differences in Participant Characteristics
Seventy-two MSW, 254 low-risk MSM, and 114 high-risk MSM were included in this analysis, 74%, 78%, and 82% of whom, respectively, completed 4 years of follow-up (Figure 1). High-risk MSM were slightly younger than low-risk MSM (Table 1). Men who have sex with men generally were more highly educated, used recreational drugs more often, and smoked less frequently compared with MSW. Participants’ estimated lifetime number of sex partners differed substantially: MSW had a median of 25 (interquartile range [IQR], 15–50), compared with 200 (IQR, 50–700) and 1000 (IQR, 500–2000) estimated lifetime sex partners in low-risk MSM and high-risk MSM, respectively. All STI rates were higher in MSM, especially in high-risk MSM; however, chronic hepatitis B and C were rare (prevalence <2% for both in all groups). Baseline CMV seroprevalence was higher in low-risk MSM compared with MSW (76% vs 54%, P < .001) and highest in high-risk MSM (90%, P high-risk MSM vs low-risk MSM = .003). During follow-up, none of the MSW but 2 low-risk MSM and 4 high-risk MSM seroconverted for HIV (incidence rate 0, 2.1, and 9.3 per 1000 person-years of follow-up, respectively).

Higher CD8 Counts and Lower CD4/CD8 Ratios in Men Who Have Sex With Men (MSM), Especially in High-Risk MSM
Including all measurements from all 3 study visits, MSM, compared with MSW, had higher mean CD8 counts (551 vs 437 cells/mm³, P < .001), similar mean CD4 counts (864 vs 880 cells/mm³, P = .5), and lower mean CD4/CD8 ratios (1.84 vs 2.47, P < .001), respectively. Furthermore, high-risk compared with low-risk MSM had higher mean CD8 counts (599 vs 529 cells/mm³, P = .013), similar mean CD4 counts (824 vs 881, P = .097), and mean lower CD4/CD8 ratios (1.59 vs 1.96, P < .001), respectively. We calculated the within-subject mean CD4 count, mean CD8 count, and mean CD4/CD8 ratio, for each participant separately;

Lower CD4/CD8 Ratios in HIV-Negative MSM • JID 2021:224 (1 October) • 1189
### Table 1. Baseline Characteristics of MSM and Low-Risk and High-Risk MSM

| Characteristics                                      | MSW   | Low-Risk MSM (≤10 Partners) | High-Risk MSM (>10 Partners) | P Value MSW vs Low-Risk MSM\(^a\) | P Value Low-Risk MSM vs High-Risk MSM\(^a\) |
|------------------------------------------------------|-------|----------------------------|-----------------------------|-----------------------------------|-----------------------------------------------|
| N                                                    | 72    | 254                        | 114                         |                                   |                                               |
| Age in years                                         | 52 (48–60) | 53 (48–59)                  | 51 (47–56)                  | 1                                 | .02                                           |
| BMI (kg/m\(^2\))                                     | 25 (24–29) | 25 (23–27)                  | 24 (23–26)                  | .009                              | .06                                           |
| Born in the Netherlands                              | 55 (76.4%) | 218 (86.5%)                 | 93 (82.3%)                 | .04                               | .3                                            |
| High educational attainment\(^b\)                   | 32 (45.1%) | 157 (62.5%)                 | 70 (64.2%)                 | .008                              | .8                                            |
| Estimated number of lifetime male sex partners       | 0 (0–0) | 200 (50–700)                | 1000 (500–2000)             | <.001                             | <.001                                         |
| Estimated number of lifetime female sex partners     | 25 (15–50) | 2 (0–5)                     | 2 (0–5)                    | <.001                             | <.001                                         |
| Number of male sex partners prior 6 months           | 0 (0–0) | 2 (1–5)                     | 20 (10–30)                 | <.001                             | <.001                                         |
| Number of recent female sex partners prior 6 months  | 2 (1–3) | 0 (0–0)                     | 0 (0–0)                    | <.001                             | <.001                                         |
| Tobacco smoking during last month                    | 25 (34.7%) | 56 (22.2%)                  | 22 (19.5%)                 | .03                               | .6                                            |
| Heavy daily drinking\(^d\)*                          | 7 (9.7%) | 12 (4.8%)                   | 6 (5.4%)                   | .1                                | .8                                            |
| Recreational drug use\(^e\)                          | 11 (15.5%) | 68 (27.1%)                  | 47 (41.6%)                 | .05                               | .006                                          |
| Daily marijuana use\(^f\)                           | 4 (5.6%) | 11 (4.4%)                   | 2 (1.8%)                   | .7                                | .2                                            |
| Use of MDMA, monthly or more frequent\(^g\)          | 3 (4.2%) | 18 (72%)                    | 14 (12.4%)                 | .4                                | .1                                            |
| Neisseria gonorrhoeae diagnosis at recruitment visit | 1 (1.4%) | 9 (3.6%)                    | 11 (9.6%)                  | .4                                | .02                                           |
| Chlamydia trachomatis diagnosis at recruitment visit | 2 (2.8%) | 17 (6.7%)                   | 10 (8.8%)                  | .2                                | .5                                            |
| Ever contracted syphilis\(^h\)                       | 1 (1.4%) | 53 (20.9%)                  | 32 (28.1%)                 | <.001                             | .1                                            |
| Syphilis diagnosis at recruitment visit\(^i\)        | 1 (1.4%) | 2 (0.8%)                    | 5 (4.4%)                   | .6                                | .02                                           |
| HCV RNA positive                                     | 0 (0.0%) | 2 (0.8%)                    | 2 (1.8%)                   | .5                                | .4                                            |
| HBsAg positive                                       | 0 (0.0%) | 1 (0.4%)                    | 2 (1.8%)                   | .6                                | .2                                            |
| CMV IgG-seropositive                                 | 39 (54.2%) | 193 (76.3%)                 | 102 (89.5%)                | <.001                             | .03                                           |

**Abbreviations:** BMI, body mass index; CMV, cytomegalovirus; HbsAg, hepatitis B surface antigen; HCV, hepatitis C virus; IgG, immunoglobulin G; MDMA, 3,4-methylenedioxyamphetamine; MSM, men who have sex with men; MSW, men who only have sex with women; RNA, ribonucleic acid.

**NOTE:** Given values are median (interquartile range) or n (%).

\(^a\) \(\chi^2\) tests were used for comparison, except for age, BMI, and numbers of sex partners where Wilcoxon rank-sum tests were used.

\(^b\) Finished vocational level education or higher.

\(^c\) Estimated by participant himself.

\(^d\) Drinking > 4 international units of alcohol daily or almost daily.

\(^e\) During prior 6 months.

\(^f\) Treponema pallidum particle agglutination assay positive.

\(^g\) Rapid plasma reagin >1:4.
based on all his available measurements (from at most 3 study visits). Figure 2 shows the distribution of these mean CD4/CD8 ratios and mean CD4 and CD8 counts, stratified by MSW, low-risk MSM, and high-risk MSM. Mean CD8 counts were higher and mean CD4/CD8 ratios lower with higher numbers of recent sex partners, with 5.6% of MSW having a mean CD4/CD8 ratio below 1, as opposed to 9.1% of low-risk MSM and 21.9% of high-risk MSM. A CD4/CD8 ratio <.5, observed at just a single study visit, was found in 4 (2%) low-risk MSM, 1 (1%) high-risk MSM, and no (0%) MSW. The cutoff for having on average >10 recent sex partners (ie, “high-risk”) was derived from the analysis shown in Table 2. Three separate multivariable linear mixed-effects models were used to explore associations between being MSM and the reported number of sex partners in the 6 months before study visits and the absolute CD4 and CD8 counts and CD4/CD8 ratio.

Table 2. Associations of Being MSM and the Reported Number of Sex Partners in the 6 Months Before Study Visits With the Absolute CD4 and CD8 Counts and CD4/CD8 Ratio

| Variable | N | Loge(CD4) Coeff 95% CI | Loge(CD8) Coeff 95% CI | Loge(CD4/CD8) Coeff 95% CI |
|----------|---|------------------------|-------------------------|-----------------------------|
| <2 lifetime male-male sexual contact (MSW) | 181 | ref | ref | ref |
| ≥2 lifetime male-male sexual contact (MSM) | 968 | -0.01 | -0.10 to 0.07 | 0.19** | 0.06 to 0.31 | -0.20** | -0.32 to -0.08 |

Mean number of sex partners during 6 months before study visits, per participant (between participant variation)

| ≤2 sex partners | 410 | ref | ref | ref | ref | ref | ref |
| >2 to ≤10 sex partners | 432 | -0.01 | -0.09 to 0.06 | 0.02 | -0.08 to 0.12 | -0.03 | -0.13 to 0.07 |
| >10 to ≤30 sex partners | 225 | -0.05 | -0.14 to 0.04 | 0.14* | 0.02 to 0.27 | -0.19** | -0.31 to -0.07 |
| >30 sex partners | 82 | -0.08 | -0.21 to 0.05 | 0.14 | -0.06 to 0.32 | -0.21* | -0.39 to -0.04 |

Deviation from individual’s mean number of sex partners reported at current visit (within participant variation)

| ≤5 sex partners higher or lower | 931 | ref | ref | ref | ref | ref | ref |
| >5 sex partners higher | 105 | -0.02 | -0.07 to 0.03 | -0.01 | -0.08 to 0.05 | 0.00 | -0.05 to 0.04 |
| >5 sex partners lower | 113 | -0.02 | -0.07 to 0.03 | -0.02 | -0.09 to 0.05 | 0.00 | -0.04 to 0.05 |

Abbreviations: CI, confidence interval; Coeff, coefficient; MSM, men who have sex with men; MSW, men who only have sex with women; ref, reference category.

Results of multivariable linear mixed-effects models.

Number of measurements per variable category. Estimates are derived from 3 multivariable linear mixed-effects models with the loge transformed absolute CD4 and CD8 counts and loge transformed CD4/CD8 ratio as outcome variables. The models include a per participant random intercept and random slope for time since baseline, using an unstructured covariance matrix. Models also included adjustment for time since baseline and time in the day of blood draw.

*P < .05.
**P < .01.
models were constructed to analyze predictors of CD4 count, CD8 count, and CD4/CD8 ratio. In addition to 2 potential confounding variables (time since baseline and time in the day of blood draw), the models included the following 3 predictor variables of interest: (1) MSW vs MSM; (2) mean number of recent sex partners over all study visits of the participant; and (3) variation in number of recent sex partners between the mean and the actual visit. Being MSM and having either >10 or >30 recent sex partners “on average during study follow-up”, each were independently associated with higher CD8 counts \( (P = 0.003, P > 10 \text{ vs } \leq 2 \text{ partners} = 0.028, P > 30 \text{ vs } \leq 2 \text{ partners} = 0.14) \) and lower CD4/CD8 ratios \( (P = 0.001, P > 10 \text{ vs } \leq 2 \text{ partners} = 0.003, P > 30 \text{ vs } \leq 2 \text{ partners} = 0.017) \). Differences in an individual’s number of recent sex partners during study follow-up were not associated with differences in CD4 or CD8 counts.

**Lower CD4/CD8 Ratio for a Large Part Explained by Higher Cytomegalovirus-Seroprevalence in Men Who Have Sex With Men**

Figure 3 shows results of multivariable models adjusted both for potential confounders and mediators of the differences among MSW, low-risk MSM, and high-risk MSM. The differences in CD4/CD8 ratios and absolute CD8 counts between groups were reduced after addition of baseline CMV serostatus to the models, with the difference in CD4/CD8 ratio between MSW and low-risk MSM remaining borderline statistically significant \( (P = 0.051) \). The differences in CD4/CD8 ratios between high-risk MSM and both low-risk MSM and MSW were attenuated by 26% and 32%, respectively, but remained statistically significant \( (P = 0.003 \text{ and } P < 0.001, \text{ respectively). Supplementary Figure 1 illustrates differences in CD4/CD8 ratios in both CMV-positive and CMV-negative subgroups. Cytomegalovirus IgG titers were not associated with a lower CD4/CD8 ratio, independently of CMV serostatus, and therefore were not included in the models. Although other factors were independently associated with the CD4/CD8 ratio, they had little to no effect on the low-risk MSM and high-risk MSM coefficients. Table 3 shows the associations of the included covariates on the lymphocyte counts in the final multivariable models from Figure 3. Other than baseline CMV serostatus, recent 3,4-methylenedioxymethamphetamine ([MDMA] \( P = 0.02 \)) use, a recent diagnosis of syphilis \( (P = 0.01) \) or gonorrhea \( (P = 0.005) \), and having chronic hepatitis C at baseline \( (P = 0.01) \) were also significantly associated with lower CD4/CD8 ratios. A history of tobacco smoking (expressed as pack-years smoking, \( P = 0.01 \)), a blood draw later in the day (ie, diurnal effect, \( P = 0.009 \)), and time since baseline \( (P < 0.001) \) were associated with a higher CD4/CD8 ratio. Higher I-FABP levels were univariately associated with a lower CD4/CD8 ratio \( (P = 0.009) \) but no longer in the multivariable model \( (P = 0.09) \). Soluble CD14, sCD163, IL-6, high-sensitivity C-reactive protein (hs-CRP), and D-dimer were not associated with the CD4/CD8 ratio (Supplementary Table 2). Sensitivity analyses limited to cross-sectional data from just the baseline visit showed similar results: after adjustment for CMV-serostatus and other covariates, high-risk MSM still had a significantly lower CD4/CD8 ratio \( (P = 0.006) \) and higher CD8 count \( (P = 0.06) \) compared with MSW. Effect sizes were comparable to those found in the full models \[ \log(\text{CD4}/\text{CD8}) \] high-risk MSM vs MSW \(-0.19 \) (baseline only) and \(-0.20 \) (full model); \[ \log(\text{CD8}) \] high-risk MSM vs MSW 0.13 (baseline only) and 0.16 (full model)).

**High CD8 Count and Low CD4/CD8 Ratio Associated With T-Cell Activation and Senescence**

In the subgroup of participants with available data, both high CD8 counts and low CD4/CD8 ratios were strongly associated with
higher percentages of senescent and activated T-cells, in both the CD4 and CD8 compartments (Supplementary Table 1.2). Moreover, the percentages of activated CD4 and CD8 T cells were significantly higher among high-risk MSM compared with MSW and low-risk MSM (Supplementary Table 1.3). Albeit proportions of senescent CD4 and CD8 T-cells were also numerically higher in high-risk MSM compared with MSW and low-risk MSM, these differences were not statistically significant.

**DISCUSSION**

In our analysis, middle-aged HIV-negative MSM, especially MSM with a higher number of sex partners, had lower CD4/CD8 ratios, mostly driven by higher CD8 counts, compared with HIV-negative MSW. These differences for a large part were explained by a higher CMV seroprevalence among MSM and high-risk MSM. The remainder of the differences in relative CD4 and CD8 counts could not fully be explained by other factors measured in our study. Several behavioral characteristics and recent STIs were more prevalent among MSM and also independently associated with a lower CD4/CD8 ratio, but they did not influence the low-risk MSM and high-risk MSM coefficients. The independence of these factors (eg, recent syphilis or gonorrhea or MDMA use) should be interpreted with caution. These factors are also strongly correlated with, and therefore a proxy of, high-risk sexual behavior, which might not be fully adjusted for through the other variables in the models. Vieira et al [25] also reported lower CD4 and
higher CD8 counts in pre-exposure prophylaxis-using MSM diagnosed with asymptomatic chlamydia and/or gonorrhea, which was related to increased CD8 T-cell activation, similar to our findings. Furthermore, repeated infection with different CMV strains could be responsible for increased CD8 counts in MSM. Cytomegalovirus-superinfection with a different strain has been demonstrated to occur in humans, most definitively in the context of women already known to be CMV seropositive before becoming pregnant, delivering a child with symptomatic congenital CMV disease [28, 29]. Likewise, in animal models, CMV superinfection was demonstrated and was related to CD8 T-cell expansion [30, 31]. Other viruses, such as herpes simplex virus 1/2, Epstein-Barr virus, and human herpesvirus 8, are also more prevalent among MSM and could contribute to differences in immunological phenotypes [22, 32, 33]. Finally, Noguera-Julian et al [34] showed MSM to have a different gut microbiome compared with MSW irrespective of their HIV status. We found a trend of higher I-FABP (ie, intestinal integrity marker) in those with lower CD4/CD8 ratios. Differences in the composition of the gut microbiome, for example, caused by specific sexual practices or frequent antibiotic use could hypothetically contribute to the lower CD4/CD8 ratios we observed in MSM. Contrary to our findings, previous studies, but conducted only in HIV-positive persons, have reported higher sCD14, sCD163, hs-CRP, and IL-6 levels associated with a lower CD4/CD8 ratio [4, 35]. Specifically, Serrano-Villar et al [4] reported such associations related only to lower CD4 counts, but not higher CD8 counts, and to be absent in HIV-positive participants with >500 CD4 cells/mm³. In PWH, the association between lower CD4/CD8 ratios and increased innate immune activation markers therefore seems predominantly related to HIV-specific factors, in conjunction with poor CD4 count recovery after cART initiation. The association between a lower CD4/CD8 ratio and higher CD8 T-cell activation in the subgroup of our HIV-negative participants, with higher degrees of T-cell activation in high-risk MSM, resembles findings concerning T-cell activation in HIV-positive individuals [4]. However, our subgroup is insufficiently large and lacks the required follow-up time to demonstrate whether these changes in HIV-negative MSM are associated with increased risk of non-AIDS comorbidity and mortality as was shown in PWH [4].

Strengths and Limitations
The strengths of this study are the relatively large sample of MSM with a sexual risk behavior profile comparable to that of many HIV-positive MSM and the measurement of CD4 and CD8 counts at multiple time points, allowing us to account for the high variability of these indices over time. The extensive data collection including STI-positivity, immune activation markers, and data on sexual behavior and recreational drug use allowed us to explore a multitude of potential factors driving the observed differences in T lymphocyte counts. Important limitations include that data on chlamydia and gonorrhea infections were not systematically collected, implying that reported diagnosed STIs had been treated at the time of lymphocyte phenotyping, and those asymptomatically present during study visits may have remained undiagnosed. Furthermore, CMV and hepatitis B and C status were only determined at baseline. Thus, incident super- or (re)infections with these viruses during follow-up could not be accounted for. Any such undiagnosed infections could have influenced our results, potentially explaining the remainder of the observed effect of being MSM. However, the cross-sectional baseline-only sensitivity analysis suggested other causative mechanisms, because STI data at baseline were complete. Furthermore, the use of self-reported sexual behavior data may potentially under- or overestimate true exposures, because participants may be inclined to provide socially acceptable answers and be subject to recall bias. The strong correlation of self-reported data with biomarkers of sexual risk such as STI incidence suggests that such bias may be limited in our study. Generalizability of these findings to the general population of MSW and MSM is limited given recruitment of participants at a sexual health clinic, who are more likely to have exhibited more sexual risk behavior. Finally, the classification of low-risk and high-risk behavior based on ≤/>10 sex partners was data-driven and therefore might not be reproducible in other cohorts.

CONCLUSIONS
We observed lower CD4/CD8 ratios, mainly resulting from higher CD8 counts, in HIV-negative MSM compared with MSW. We show that these differences could at least partially be explained by higher CMV seroprevalence in MSM. Men who have sex with men have high rates of STIs, but they also have psychosocial stress due to a social minority position [36], often in conjunction with higher rates of recreational drug use. Future studies should attempt to evaluate the possible influence of such factors on immune system changes. In turn, these changes may be related to increased cardiovascular disease risk observed in both HIV-positive and HIV-negative MSM [37, 38]. For example, CMV seroprevalence was much higher among MSM in our study and has been associated with cardiovascular disease development, frailty, and mortality [39–46]. Our findings contribute to the understanding of why in many HIV-positive MSM who are treated with cART and have suppressed viremia, the CD4/CD8 ratio and CD8 counts do not return to general population-based normal values. Many PWH differ in their lifestyle compared with people from the general population. We show that such factors or exposures can impact their immune system. These immunological differences should not mistakenly be attributed only to HIV infection or its treatment in studies using general population control subjects. Our results also warrant further investigation into the effects of (CMV-induced) immunological differences on the occurrence
of age-related comorbidities not only in HIV-positive but also HIV-negative MSM.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Disclaimer. None of the funding bodies had a role in the design or conduct of the study, the analysis and interpretation of the results, the writing of the report, or the decision to publish.

Financial support. This work was funded by The Netherlands Organization for Health Research and Development (ZonMW) (Grant Number 300020007) and AIDS Fonds (Grant Number 2009063). Additional unrestricted scientific grants were received from Gilead Sciences, ViViV Healthcare, Janssen Pharmaceuticals N.V., and Merck & Co.

Potential conflicts of interest. P. R., through his institution, has received independent scientific grant support from Gilead Sciences, Janssen Pharmaceuticals Inc., Merck & Co., and ViViV Healthcare and has served on scientific advisory boards for Gilead Sciences, ViViV Healthcare, Merck & Co., and Teva Pharmaceutical Industries, for which honoraria were all paid to his institution. F. W. W. has served on scientific advisory boards for ViViV and Gilead Sciences. R. A. v. Z. has received travel grants from Gilead Sciences and was a speaker at an event sponsored by Gilead Sciences for which her institution received remuneration. M. F. S. v. d. L. has received independent scientific grant support from Sanofi Pasteur, MSD Janssen Infectious Diseases and Vaccines, and Merck, served on the advisory board of GSK, and received nonfinancial support from Stichting Pathologie Onderzoek en Ontwikkeling. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

AGEhiV Cohort Study Group
Scientific Oversight and Coordination. P. Reiss (principal investigator), F. W. N. M. Wit, M. van der Valk, J. Schouten, K. W. Kooij, R. A. van Zoest, E. Verheij, S. O. Verboeket, B. C. Elsenga (Amsterdam University Medical Centers [Amsterdam UMC], University of Amsterdam, Department of Global Health and Amsterdam Institute for Global Health and Development [AIGHD]), M. Prins (coprincipal investigator), M.F. Schim van der Loeff, L. del Grande, V. Olthof, I. Agard (Public Health Service of Amsterdam, Department of Infectious Diseases).

Data Management. S. Zaheri, M. M. J. Hillebregt, Y. M. C. Ruijs, D. P. Benschop, A. el Berkaoui (HIV Monitoring Foundation).

Central Laboratory Support. N. A. Kootstra, A. M. Harskamp-Holwerda, I. Maurer, M. M. Mangas Ruiz, A. F. Girigorie, B. Boeser-Nunnink (Amsterdam UMC, Laboratory for Viral Immune Pathogenesis and Department of Experimental Immunology).

Project Management and Administrative Support. W. Zikkenheiner, F. R. Janssen (AIGHD).

Participating HIV Physicians and Nurses. S. E. Geerlings, A. Goorhuis, J. W. R. Hovius, F. J. B. Nellen, T. van der Poll, J. M. Prins, P. Reiss, M. van der Valk, W. J. Wiersinga, M. van Vugt, G. de Bree, F. W. N. M. Wit; J. van Eden, A. M. H. van Hes, F. J. J. Pijnappel, A. Weijzenfeld, S. Smallhout, M. van Duinen, A. Hazenberg (Amsterdam UMC, Division of Infectious Diseases).

Other Collaborators. P. G. Postema (Amsterdam UMC, Department of Cardiology); P. H. L. T. Bisschop, M. J. M. Serlie (Amsterdam UMC, Division of Endocrinology and Metabolism); P. Lips (Amsterdam UMC); E. Dekker (Amsterdam UMC, Department of Gastroenterology); N. van der Velde (Amsterdam UMC, Division of Gastroenterology); J. M. R. Willemse, L. Vogt (Amsterdam UMC, Division of Nephrology); J. Schouten, P. Portegies, B. A. Schmand, G. J. Geurtsen (Amsterdam UMC, Department of Neurology); F. D. Verbraak, N. Demirkaya (Amsterdam UMC, Department of Ophthalmology); I. Visser (Amsterdam UMC, Department of Psychiatry); A. Schadé (Amsterdam UMC, Department of Psychiatry); P. T. Nieuwkerk, N. Langebeek (Amsterdam UMC, Department of Medical Psychology); R. P. van Steenwijk, E. Dijkers (Amsterdam UMC, Department of Pulmonary Medicine); C. B. L. M. Majoie, M. W. A. Caan (Amsterdam UMC, Department of Radiology); H. W. van Lunsen, M. A. F. Nievaard (Amsterdam UMC, Department of Gynaecology); B. J. H. van den Born, E. S. G. Stroes (Amsterdam UMC, Division of Vascular Medicine); W. M. C. Mulder, S. van Oorsprong (HIV Vereniging Nederland).

References
1. Lundgren JD, Babiker AG, Gordin F, et al.; INSIGHT START Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med 2015; 373:795–807.
2. El-Sadr WM, Lundgren J, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006; 355:2283–96.
3. Trickey A, May MT, Schommers P, et al.; Antiretroviral Therapy Cohort Collaboration (ART-CC). CD4:CD8 ratio and CD8 count as prognostic markers for mortality in human immunodeficiency virus-infected patients on antiretroviral therapy: the antiretroviral therapy cohort collaboration (ART-CC). Clin Infect Dis 2017; 65:959–66.
4. Serrano-Villator S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral
therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 2014; 10:e1004078.

5. Serrano-Villar S, Pérez-Elias MJ, Dronda F, et al. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. PLoS One 2014; 9:e85798.

6. Serrano-Villar S, Moreno S, Fuentes-Ferrer M, et al. The CD4:CD8 ratio is associated with markers of age-associated disease in virally suppressed HIV-infected patients with immunological recovery. HIV Med 2014; 15:40–9.

7. Lo J, Abbara S, Shrutman L, et al. Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. AIDS 2010; 24:243–53.

8. Sigel K, Wisnivesky J, Crothers K, et al. Immunological and infectious risk factors for lung cancer in US veterans with HIV: a longitudinal cohort study. Lancet HIV 2017; 4:e67–73.

9. Mussini C, Lorenzini P, Cozzi-LePreti A, et al.; Icona Foundation Study Group. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. Lancet HIV 2015; 2:e98–106.

10. Schouten J, Wit FW, Stolte IG, et al.; AGEhIV Cohort Study Group. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. Clin Infect Dis 2014; 59:1787–97.

11. Guaraldi G, Orlando G, Zona S, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. Clin Infect Dis 2011; 53:1120–6.

12. Vasto S, Colonna-Romano G, Larbi A, Wikby A, Caruso C, Pawelec G. Role of persistent CMV infection in configuring T cell immunity in the elderly. Immun Ageing 2007; 4:2.

13. Olsson J, Wikby A, Johansson B, Löfgren S, Nilsson BO, Ferguson FG. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev 2001; 121:187–201.

14. Dutta A, Uno H, Lorenz DR, Wolinsky SM, Gabuzda D. Low T-cell subsets prior to development of virus-associated cancer in HIV-seronegative men who have sex with men. Cancer Causes Control 2018; 29:1131–42.

15. Huppert FA, Pinto EM, Morgan K, Brayne C. Survival in a population sample is predicted by proportions of lymphocyte subsets. Mech Ageing Dev 2003; 124:449–51.

16. Ferguson FG, Wikby A, Maxson P, Olsson J, Johansson B. Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. J Gerontol A Biol Sci Med Sci 1995; 50:B378–82.
30. Hansen SG, Powers CJ, Richards R, et al. Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. Science 2010; 328:102–6.

31. Trgovcich J, Kincaid M, Thomas A, et al. Cytomegalovirus reinfections stimulate CD8 T-memory inflation. PLoS One 2016; 11:e0167097.

32. Chuerduangphui J, Proyrungroj K, Pientong C, et al. Prevalence and anatomical sites of human papillomavirus, Epstein-Barr virus and herpes simplex virus infections in men who have sex with men, Khon Kaen, Thailand. BMC Infect Dis 2018; 18:509.

33. van Baarle D, Hovenkamp E, Dukers NH, et al. High prevalence of Epstein-Barr virus type 2 among homosexual men is caused by sexual transmission. J Infect Dis 2000; 181:2045–9.

34. Noguera-Julian M, Rocafort M, Guillén Y, et al. Gut microbiota linked to sexual preference and HIV infection. EBioMedicine 2016; 5:135–46.

35. Vita S, Lichtner M, Marchetti G, et al.; for ICONA Foundation Study Group. Brief report: soluble CD163 in CMV-infected and CMV-uninfected subjects on virologically suppressive antiretroviral therapy in the ICONA cohort. J Acquir Immune Defic Syndr 2017; 74:347–52.

36. Meyer IH. Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: conceptual issues and research evidence. Psychol Bull 2003; 129:674–97.

37. Swartz JA. The relative odds of lifetime health conditions and infectious diseases among men who have sex with men compared with a matched general population sample. Am J Mens Health 2015; 9:150–62.

38. Hatzenbuehler ML, McLaughlin KA, Slopen N. Sexual orientation disparities in cardiovascular biomarkers among young adults. Am J Prev Med 2013; 44:612–21.

39. Gkrania-Klotsas E, Langenberg C, Sharp SJ, Luben R, Khaw KT, Wareham NJ. Seropositivity and higher immunoglobulin G antibody levels against cytomegalovirus are associated with mortality in the population-based European prospective investigation of Cancer-Norfolk cohort. Clin Infect Dis 2013; 56:1421–7.

40. Gkrania-Klotsas E, Langenberg C, Sharp SJ, Luben R, Khaw KT, Wareham NJ. Higher immunoglobulin G antibody levels against cytomegalovirus are associated with incident ischemic heart disease in the population-based EPIC-Norfolk cohort. J Infect Dis 2012; 206:897–903.

41. Haarala A, Kähönen M, Lehtimäki T, et al. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: the Cardiovascular Risk in Young Finns Study. Clin Exp Immunol 2012; 167:309–16.

42. Hui J, Qu YY, Tang N, et al. Association of cytomegalovirus infection with hypertension risk: a meta-analysis. Wien Klin Wochenschr 2016; 128:586–91.

43. Margolick JB, Bream JH, Nilles TL, et al. Relationship between T-cell responses to CMV, markers of inflammation, and frailty in HIV-uninfected and HIV-infected men in the multicenter AIDS cohort study. J Infect Dis 2018; 218:249–58.

44. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. Am J Epidemiol 2010; 172:363–71.

45. Tang N, Li JW, Liu YM, et al. Human cytomegalovirus infection is associated with essential hypertension in Kazakh and Han Chinese populations. Med Sci Monit 2014; 20:2508–19.

46. Wang GC, Kao WH, Murakami P, et al. Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. Am J Epidemiol 2010; 171:1144–52.