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blood advances

Monocytosis and its association with clonal hematopoiesis in community-dwelling individuals

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Key Points

- Monocytosis associates with a higher frequency of CH with spliceosome and multiple gene mutations, but not isolated DNMT3A/TET2/ASXL1.
- Few community-based individuals with monocytosis and CH develop myeloid malignancy (among 166 individuals: n = 4 cases including n = 1 CMML).

Monocytosis may occur in numerous inflammatory conditions but is also the defining feature of chronic myelomonocytic leukemia (CMML). Clonal somatic mutations detectable in CMML may occur with aging in otherwise healthy individuals, so-called "clonal hematopoiesis" (CH). We investigated whether the combination of CH and monocytosis would represent an early developmental stage of CMML. We studied community-dwelling individuals with monocytosis ($\geq 1 \times 10^9$/L and ≥10% of leukocytes) in the population-based Lifelines cohort (n = 144 676 adults). The prevalence and spectrum of CH were evaluated for individuals ≥60 years with monocytosis (n = 167 [0.8%]), and control subjects 1:3 matched for age and sex (n = 501). Diagnoses of hematological malignancies were retrieved by linkage to the Netherlands Cancer Registry (NCR). Monocyte counts and the prevalence of monocytosis increased with advancing age. Older individuals with monocytosis more frequently carried CH (50.9% vs 35.5%; $P < .001$). Monocytosis is associated with enrichment of multiple gene mutations ($P = .006$) and spliceosome mutations ($P = .007$) but not isolated mutated *DNMT3A*, *TET2*, or *ASXL1*. Persistent monocytosis over 4 years was observed in 30/102 evaluable individuals and associated with a higher prevalence of CH (63%). Myeloid malignancies, including 1 case of CMML, developed in 4 individuals with monocytosis who all carried CH. In conclusion, monocytosis and CH both occur at an older age and do not necessarily reflect clonal monocytic proliferation. In a fraction of older subjects with monocytosis, CH might constitute early clonal dominance in developing malignant myelomonocytic disease. Mutational spectra deviating from age-related CH require attention.

Introduction

Monocytes are part of the myeloid lineage that expands with age and represent key players in inflammatory and immune reactions.[1,2] Normal monocyte values range between 0.3 and 0.9×10^9/L, constituting 2% to 8% of the total white blood cell (WBC) population in peripheral blood (PB). Abnormal production

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The manuscript is based on data from the Lifelines cohort study. Lifelines adheres to standards for data availability. The data catalog of Lifelines is publicly accessible at www.lifelines.nl. All international researchers can obtain data at the Lifelines research office (research@lifelines.nl), for which a fee is required.

The full-text version of this article contains a data supplement.

and accumulation of monocytes may occur in numerous conditions. Monocytosis may be explained by inflammatory conditions ("reactive monocytosis"), as present in acute stress, myocardial infarction, chronic infection, systemic inflammation, and autoimmune disorders.[3] Besides secondary causes, PB monocytosis is the defining phenotypic hallmark of chronic myelomonocytic leukemia (CMML), a chronic myeloid neoplasm with both myelodysplastic and myeloproliferative features that occurs almost exclusively in older individuals.[3-5] In addition, monocytosis may accompany other myeloid malignancies. Current World Health Organization (WHO) criteria for CMML define monocytosis as a persistent (≥3 months) increase in PB monocyte counts $\geq 1 \times 10^9$/L in combination with a relative excess of monocytes comprising ≥10% of the WBC count that cannot be explained by secondary causes.[4] Driver gene mutations are detected in the majority of patients with CMML and most frequently involve *SRSF2*, *TET2*, and *ASXL1* genes, although none of these are CMML-specific.[5-8] In current WHO criteria, clonal genetic lesions in the presence of unexplained, persistent monocytosis can support a diagnosis of CMML, even in the absence of typical myelodysplastic and/or myeloproliferative features.[4]

Recently, clonally expanded genetic lesions have been identified in PB of otherwise healthy individuals, and clonal hematopoiesis (CH) is increasingly recognized as an important hallmark of the aging hematopoietic system.[9-12] CH is associated with a 10-fold increased risk of developing hematological malignancies and has been proposed to represent a prephase of myeloid malignancies, especially in the presence of peripheral cytopenias.[10,12-15] In a recent population-based study, we found a significantly higher prevalence of CH in older individuals with anemia compared with nonanemic matched control subjects, with enrichment of *TP53* and *SF3B1* gene mutations.[11]

CH in the context of monocytosis might represent the earliest clonal expansion preceding CMML. The existence of such early clonal and nonclonal CMML "prephases" has been suggested to be of potential clinical relevance, including a subtype of mild monocyte proliferation with monocyte counts between 0.5 and 1×10^9/L.[16,17] We aimed to test these hypotheses in the "real-world" population-based Lifelines cohort (n = 144 676 adults). To investigate whether clonal hematopoiesis in the presence of monocytosis is indicative of CMML or CMML prestages, we determined the presence of clonal gene mutations in all older individuals with monocytosis and 1:3 matched control subjects.

Methods

Study population

This study was undertaken within the prospective Lifelines cohort. Lifelines is a multidisciplinary prospective population-based cohort study of 167 729 persons living in the north of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. It has been shown that Lifelines is representative of the background population of the northern part of The Netherlands.[18-20] Lifelines is conducted according to the principles of the Declaration of Helsinki, and informed consent was obtained from all study participants. The local medical ethical committee approved the study protocol. PB specimens were drawn at the study inclusion visit and at the first follow-up visit, which was scheduled after approximately 5 years. Details regarding laboratory procedures are provided in the supplemental Appendix.

Cohort selection

For this study, we included all Lifelines participants aged ≥18 years for whom PB monocyte counts were available at the study inclusion visit (n = 144 676), including n = 21 729 individuals aged ≥60 years. Following current WHO criteria for CMML,[4] monocytosis was defined as a PB monocyte count $\geq 1 \times 10^9$/L and ≥10% of the total WBC count. For evaluation of CH, all individuals with monocytosis aged ≥60 years (n = 167) were included. A control cohort was selected by 1:3 matching for age and sex. All available DNA samples for cases and controls were subjected to error-corrected next-generation sequencing.

Targeted error-corrected next-generation sequencing

Target regions in 27 driver genes were covered with a panel of single-molecule tagged molecular inversion probes (MIPs) (supplemental Table 1). Paired-end sequencing of MIP libraries was performed on the NovaSeq 6000 platform (Illumina, San Diego, CA). We called somatic variants with the following criteria: ≥1% variant allele frequency (VAF) and ≥10 mutant unique single-molecule MIPs. Subsequently, recurrent artifacts and polymorphisms were excluded by inspection and curation of variants. Details regarding panel design, library preparation, and data analysis are outlined in the supplemental Methods.

Outcomes

Data on incident hematological malignancies were obtained by linkage of the Lifelines cohort to the nationwide Netherlands Cancer Registry (NCR), censored December 2019. The completeness of the NCR is estimated at ≥95%.[21] Linkage of records was performed using pseudonyms of the last name (8 letters), date of birth, sex, and postal code at the time of malignancy development (6 letters). The NCR only includes malignant diagnoses confirmed by histology and/or cytology. Hematological malignancies were identified based on the International Classification of Disease for Oncology codes 9590 to 9999 (supplemental Methods). Individuals with a recorded history of hematological malignancy were excluded from analyses for malignancy development. Survival status of participants was ascertained by consulting the Municipal Persons Records Database (last consultation June 2020). Overall survival (OS) was defined as the time from study inclusion until death or last follow-up.

Statistical analyses

Data are presented as mean (SD) or median (range) for continuous variables and number (%) for categorical variables. Student *t* tests and Mann-Whitney tests were used to compare continuous data with parametric and nonparametric distribution, respectively. Differences in the mutational spectrum were assessed using Fisher's exact test. The Kaplan-Meier estimator was used for visual comparison of OS, with statistical differences reported from log-rank tests. Cumulative incidences of hematological malignancies were visualized using the Aalen-Johansson estimator and compared using Gray's test. We additionally performed multivariable Fine-Gray regression to calculate subdistribution hazard ratios (sHR) for malignancy development, taking into account the competing event of

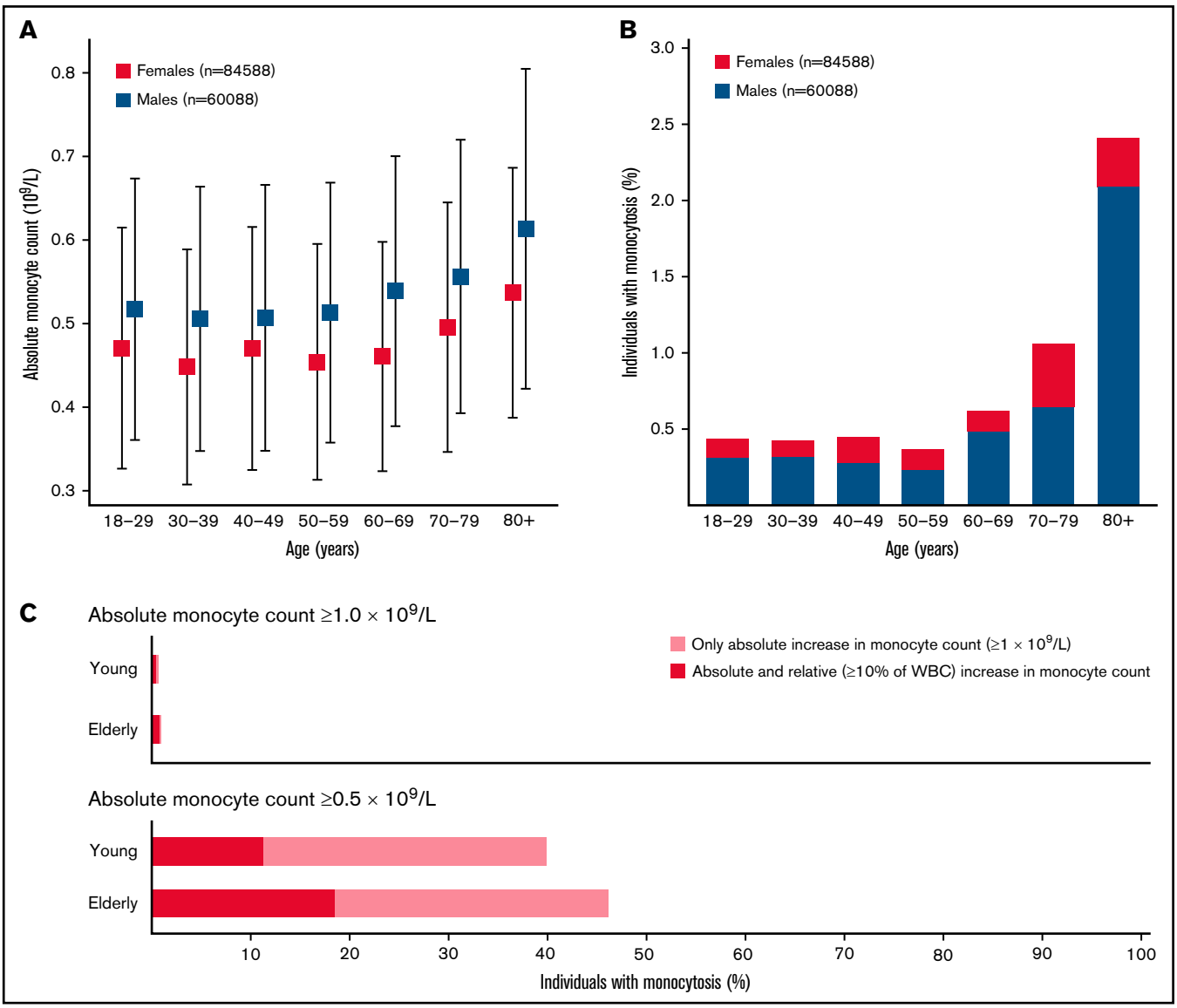

Figure 1. The emergence of monocytosis with aging in community-dwelling individuals. (A) Change in absolute monocyte counts with increasing age for evaluable male (n = 60 088) and female (n = 84 588) participants in the Lifelines cohort. Squares denote means, with error bars representing standard deviations of monocyte counts in the respective age category. (B) Prevalence of monocytosis according to age for the entire evaluable Lifelines cohort (n = 144 676). Bars are colored according to the sex of individuals with monocytosis: male (blue) or female (red). (C) Prevalence of monocytosis according to different proposed cutoff criteria within the entire evaluable younger (<60 years, n = 122 947) and older (≥60 years, n = 21 729) Lifelines cohort.

death and correction for age and sex. Cox proportional hazard regression was used to obtain risk estimates for OS when evaluated in multivariable models with correction for age and sex. Hazard ratios (HR) are presented along with the corresponding 95% confidence interval (CI). Statistical analyses were performed using R statistical computing software (supplemental Methods).

Results

Age-related changes in monocyte count and the emergence of monocytosis in older individuals

PB monocyte counts were available for 144 676 Lifelines participants. This population included 60 088 men and 84 588 women, with a median follow-up of 8.6 years (range, 0-13.8 years). Higher monocyte counts were observed for male participants across all ages compared with females. Upon aging, an increase in absolute monocyte count was observed in the cohort of individuals ≥60 years (Figure 1A). The prevalence of monocytosis, defined as a PB monocyte count ≥1 × 10^9/L and ≥10% of total WBC count, increased with advancing age. In total, 167 out of 21 729 (0.8%) evaluable Lifelines participants ≥60 years had monocytosis at the study inclusion visit. Consistent with the observed sex differences in monocyte count, there was male predominance in monocytosis prevalence (Figure 1B). PB counts for community-dwelling individuals ≥60 years with and without monocytosis are presented in Table 1. Platelet count (P = .004) and hemoglobin levels (P < .001)

Table 1. Characteristics and PB counts of community-dwelling individuals at least 60 years old with and without monocytosis as well as 1:3 matched control subjects

	Absence of monocytosis (n = 21 562)	Monocytosis (n = 167)	*P* value*	1:3 matched control subjects n = 501	*P* value†	N‡
Age (y)	65.0 (62.0-69.0)	68.0 (63.0-72.0)	<.001	68.0 (63.0-72.0)	1.00	21 729
Male sex, n (%)	9 756 (45.2)	123 (73.7)	<.001	369 (73.7)	1.00	21 729
Monocyte count (10^9/L)	0.50 (0.15)	1.12 (0.12)	<.001	0.53 (0.15)	<.001	21 729
WBC count (10^9/L)	5.83 (1.46)	8.60 (1.72)	<.001	6.08 (1.56)	<.001	21 727
Neutrophil count (10^9/L)	3.14 (1.08)	4.88 (1.51)	<.001	3.37 (1.22)	<.001	21 729
Basophil count (10^9/L)	0.03 (0.02)	0.04 (0.03)	<.001	0.03 (0.02)	<.001	21 729
Eosinophil count (10^9/L)	0.19 (0.13)	0.25 (0.15)	<.001	0.21 (0.14)	.001	21 729
Lymphocyte count (10^9/L)	1.96 (0.59)	2.30 (0.77)	<.001	1.94 (0.59)	<.001	21 729
Hemoglobin concentration (g/dL)	14.2 (1.15)	14.6 (1.34)	<.001	14.5 (1.25)	.348	21 727
Erythrocyte count (10^9/L)	4.70 (0.37)	4.77 (0.43)	.038	4.79 (0.39)	.736	21 727
Hematocrit (L/L)	0.43 (0.03)	0.44 (0.04)	<.001	0.43 (0.03)	.153	21 727
Platelet count (10^9/L)	240 (56.3)	254 (64.9)	.004	229 (51.7)	<.001	21 707
MCV (fL)	90.9 (3.96)	92.0 (4.16)	.001	90.8 (4.21)	.002	21 727
hsCRP (mg/L)	1.40 (0.80-2.90)	3.20 (1.65-7.75)	<.001	1.40 (0.80-2.70)	<.001	7 316
Number of medications used§	2.00 (0.00-4.00)	3.00 (1.00-5.00)	<.001	2.00 (0.00-4.00)	<.001	21 729
Concurrent cytopenia‖, n (%)	2 434 (11.3)	15 (8.98)	.412	66 (13.2)	.194	21 708
Concurrent cytosis¶, n (%)	480 (2.2)	38 (22.8)	<.001	13 (2.6)	<.001	21 707

hsCRP, high-sensitivity C-reactive protein; MCV, mean corpuscular volume.
Data are presented as mean (SD) or median (IQR) for continuous variables and number (%) for categorical variables.
**P* value for the comparison of individuals with and without monocytosis.
†*P* value for the comparison between individuals with monocytosis and 1:3 matched control subjects.
‡Total number of evaluable individuals ≥60 years.
§As a proxy for comorbidity.
‖A concurrent cytopenia was defined as follows: anemia, Hb concentration <12.0 g/dL in women or <13.0 g/dL in men; thrombocytopenia, platelet count <150 × 10^9/L; neutropenia, absolute neutrophil count <1.8 × 10^9/L.
¶A concurrent cytosis was defined as follows: erythrocytosis, Hb concentration >16.5 g/dL or hematocrit ≥48% in women or Hb concentration >18.5 g/dL or hematocrit ≥52% in men; thrombocytosis, platelet count >400 × 10^9/L; leukocytosis, WBC count >10 × 10^9/L.

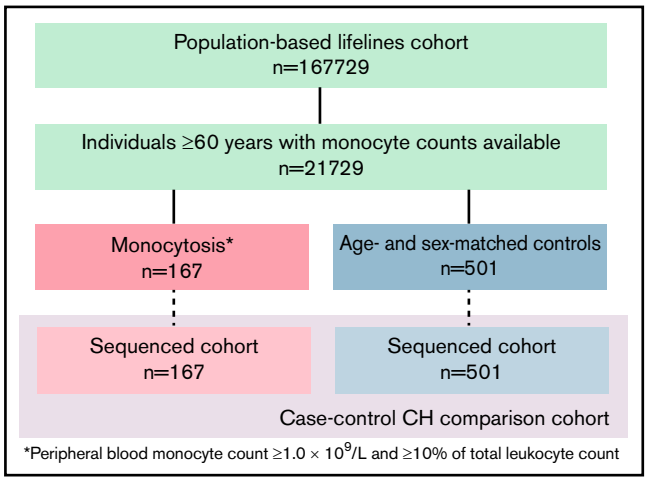

Figure 2. Nested case-control study design. Flowchart depicting the nested case-control study design with a selection of cases with monocytosis and control subjects from the entire evaluable Lifelines cohort (n = 144 676). *Monocytosis was defined in accordance with WHO criteria for monocytosis associated with CMML: PB monocyte counts ≥1 × 10^9/L and ≥10% of WBC. NGS, next-generation sequencing.

were higher for individuals with monocytosis. Individuals with monocytosis had higher levels of mean corpuscular volume (P = .001). Monocytosis further associated with a concomitant increase of total WBC count (P < .001), neutrophil count (P < .001), and also lymphocyte count (P < .001). Finally, monocytosis associated with higher high-sensitivity C-reactive protein (concentrations (P < .001). Recently, alternative criteria were proposed to define a persistent monocytosis that is suggestive of pre-CMML conditions, including a lower absolute monocyte count or the presence of an absolute but not relative increase in monocytes.[16] When these criteria were applied to our crosssectional community-based cohort, almost half of all individuals (41%) were meeting the proposed cutoff criteria for absolute monocytosis (monocyte count ≥0.5 × 10^9/L), with a substantial percentage (12%) also having a relative increase in monocyte count ≥10% of the total WBC count (Figure 1C).

Monocytosis in older individuals from the general population associates with a higher prevalence of CH

Guided by the fact that both CMML and CH predominantly occur in older individuals, we studied the prevalence and spectrum of CH in all individuals ≥60 years with monocytosis at the study inclusion

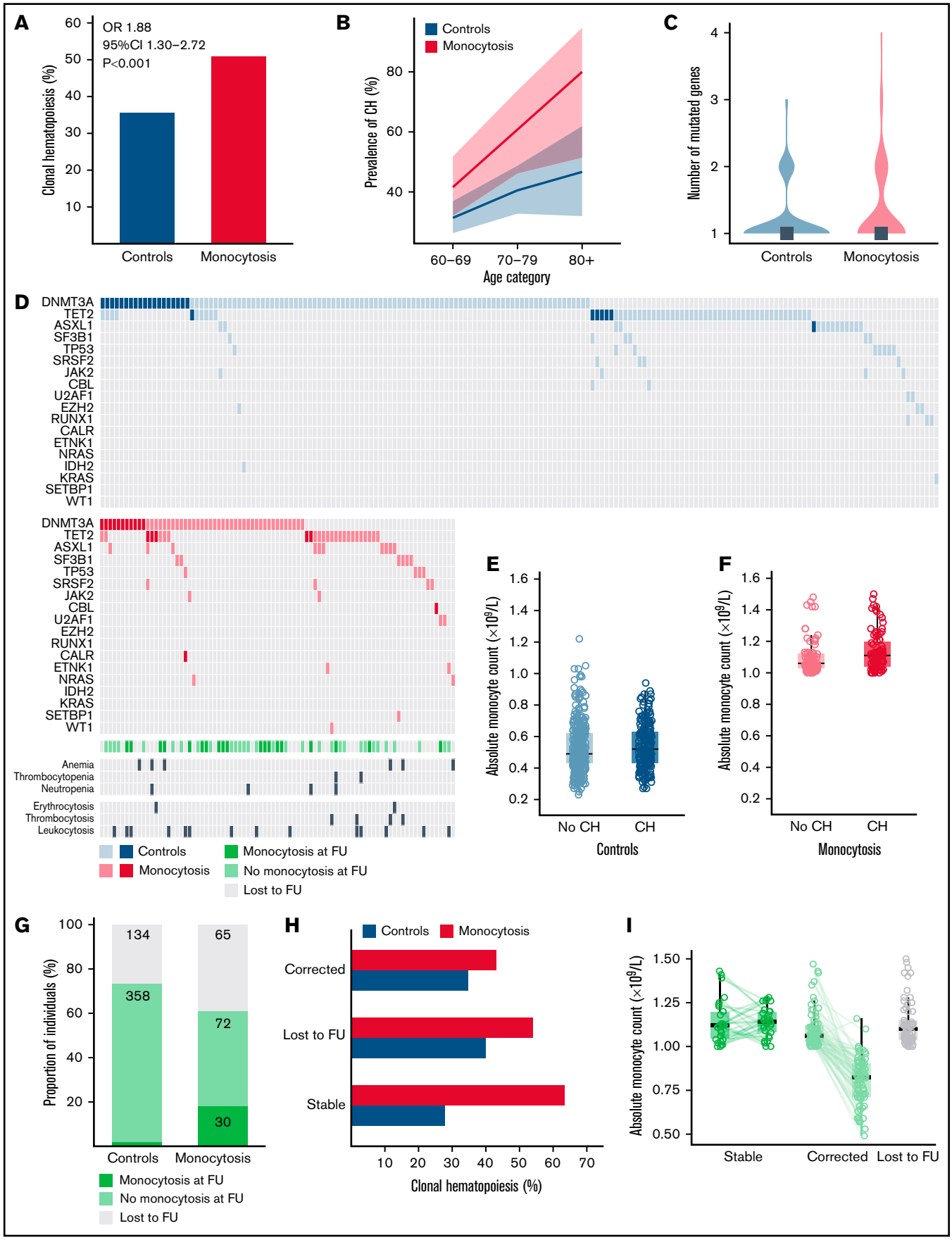

Figure 3.

visit. The flowchart for the nested case-control study is shown in Figure 2. Cases and control subjects were fully matched for age and sex (Table 1). We obtained data on CH by error-corrected next-generation sequencing of 27 genes for all 167 monocytosis cases and 501 matched control subjects. The mean sequencing depth was 9126 consensus reads, and the consensus read depth was >500× for 97.7% of all regions (supplemental Figure 1). This revealed 379 mutations in leukemia-associated genes. In agreement with previous population-based cohorts, mutations were most frequently detected in *DNMT3A*, *TET2*, and *ASXL1* genes. The majority of detected gene mutations were present at low VAF ≤10% (supplemental Figure 2). As a primary outcome for the nested case-control analyses, a higher prevalence of CH was found in the cohort with monocytosis (50.9%) as compared with control subjects (35.5%) (odds ratio [OR], 1.88; 95% confidence interval [CI], 1.30-2.72; $P < .001$) (Figure 3A and supplemental Table 2). We additionally corrected the association between monocytosis and CH for baseline differences in hemoglobin, platelet, and neutrophil and lymphocyte counts using a multivariable logistic regression model, resulting in a comparable risk estimate (OR, 2.00; 95% CI, 1.33-3.03; $P < .001$). In addition, we performed sensitivity analyses restricting to variants ≥2% VAF compatible with the definition of clonal hematopoiesis of indeterminate potential,[12] which confirmed a higher prevalence of CH in monocytosis cases (OR, 1.83; 95% CI, 1.23-2.72; $P = .002$) (supplemental Figures 4-6). The prevalence of CH increased with age for both cases with monocytosis and control subjects (Figure 3B). For individuals with CH, no significant differences were observed in the number of mutated genes ($P = .12$) (Figure 3C). Absolute monocyte counts were higher in the presence of CH for individuals with monocytosis ($P = .023$) but not for control subjects ($P = .398$) (Figure 3E-F and supplemental Table 3).

Mutational spectrum associated with monocytosis in older individuals

We next compared the mutational spectrum between cases with monocytosis and control subjects. Overall, a significant overlap was observed in the mutational spectrum of individuals with monocytosis and control subjects representing background age-related CH (Figure 3D). We subsequently evaluated the association of monocytosis with individual gene mutations. A higher proportion of individuals with monocytosis carried *DNMT3A* variants ($P = .03$). No significant differences were observed with regard to *TET2* ($P = .18$) and *ASXL1* ($P = .10$). Monocytosis was associated with a higher proportion of spliceosome mutations (*SF3B1*, *SRSF2*, and *U2AF1*) (7.2%) when compared with control subjects (2.4%) ($P = .007$) (Figure 4A). The clonal trajectory from age-related CH to myeloid neoplasms may occur in a stepwise manner. Isolated mutated *DNMT3A*, *TET2*, and *ASXL1* are most frequently detected upon aging. In fact, the prevalence of CH confined to isolated mutated *DNMT3A* ($P = .42$), *TET2* ($P = .86$), or *ASXL1* ($P = 1.00$) (DTA) was comparable between cases with monocytosis and control subjects (Figure 4B). In contrast, individuals with monocytosis more frequently carried multiple gene mutations (21 out of 167 vs 29 out of 501; $P = .006$) or isolated gene mutations other than DTA ($P = .010$). Higher VAFs, consistent with increased clonal outgrowth, were found for individuals with mutational spectra involving multiple mutated genes, but this was observed both in the monocytosis and control cohort (Figure 4C-D and supplemental Figure 3). The combination of *TET2* and *SRSF2* mutations, which may be specific for a myeloid neoplasm with myelodysplasia and monocytosis,[6,22] was observed twice in this community-based cohort with monocytosis (n = 167) and in 3 of the control subjects (n = 501). Other mutations observed at moderate frequencies in CMML, including *RUNX1*, *CBL*, *NRAS*/*KRAS*, and *SETBP1*,[5] were detected in a few (n = 4) population-based individuals with monocytosis.

Individuals with persistence of monocytosis have a higher prevalence of CH and biased mutational profile

WHO criteria for CMML require the persistence of peripheral monocytosis over a 3-month period. A follow-up for Lifelines participants was available after a median period of 3.8 years and included monocyte counts for n = 102 668. Studying the persistence of monocytosis and its relation to CH was thus limited by the loss to follow-up of potential high-risk cases during this latency period (supplemental Table 2). Among 167 individuals with monocytosis at the study inclusion visit, 102 could be evaluated for long-term persistence of monocytosis (eg, detection of monocytosis at baseline and follow-up visit). In total, 30 out of 102 (29%) evaluable individuals ≥60 years had persistence of monocytosis over this period (Figure 3G). We grouped individuals and their respective 1:3 control subjects based on the persistence of monocytosis over time. Prevalence of CH was highest in those with persistent monocytosis over time: 19 (63%) of individuals with persistent monocytosis carried CH, as compared with 28% of matched control subjects ($P = .001$) (Figure 3H and supplemental Figure 4). When following current WHO diagnostic criteria, this would translate into an estimated prevalence of 1.85 in 10.000 for (undiagnosed) CMML in community-dwelling individuals. The highest absolute monocyte counts at baseline visits were found among individuals with persistent monocytosis, although this was not statistically significant (Figure 3I).

Figure 3 (continued) Mutational spectra for individuals with monocytosis and 1:3 matched control subjects (I). (A) Prevalence of CH among all individuals with monocytosis (n = 167) compared with 1:3 matched control subjects (n = 501). (B) Prevalence of CH according to age for individuals with monocytosis and control subjects. Shaded areas represent 95% CIs. (C) Violin plot showing the distribution in the number of mutated genes for individuals with CH in the monocytosis (red) and control (blue) cohort. Gray rectangles indicate the median number. (D) The mutational landscape for the control (blue, top) and monocytosis (red, bottom) cohort. A darker shade indicates multiple mutations in the same gene. Grouping of samples according to the presence of monocytosis after ∼4 years of follow-up and the presence of peripheral cytopenia or cytosis (supplemental Methods) is indicated. (E-F) Absolute monocyte counts according to the presence of CH in the control (left) and monocytosis (right) cohort. (G) Detection of monocytosis after a median of ∼4 years among individuals with monocytosis and 1:3 matched control subjects. The proportion of individuals lost to follow-up is shown in gray. (H) Prevalence of CH among individuals with monocytosis stratified according to the stability of monocytosis over time and compared with respective 1:3 matched control subjects. (I) Absolute monocyte counts stratified according to the stability of monocytosis over time.

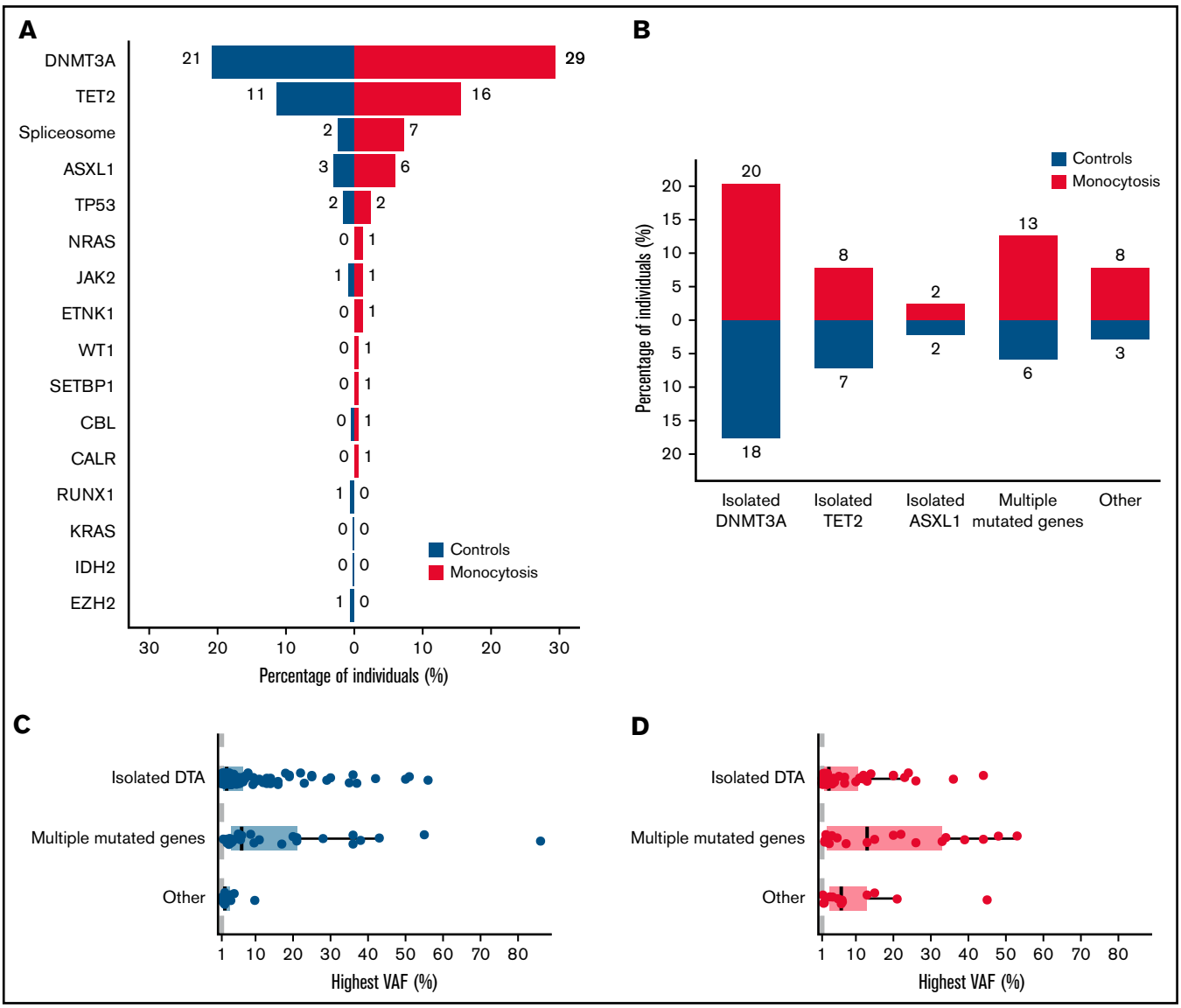

Figure 4. Mutational spectra for individuals with monocytosis and 1:3 matched control subjects (II). (A) Pyramid plot indicating the proportion of individuals with detected gene mutations within the monocytosis (red) and control (blue) cohort. The category of spliceosome mutations includes SF3B1, SRSF2, and U2AF1. The proportion of individuals carrying the gene mutation is given. (B) Bar plot showing the proportion of monocytosis cases (red, top) and controls (blue, bottom) with mutational spectra confined to mutated DNMT3A, TET2, or ASXL1, or multiple mutated genes. The category "other" denotes isolated gene mutations other than DNMT3A, TET2, or ASXL1. The proportion of individuals for each category is given. Highest detected VAF according to mutational spectrum for monocytosis cases (red, C) and controls (blue, D). Individuals were classified as carrying CH confined to mutated DNMT3A, TET2, or ASXL1 (isolated DTA), CH involving multiple mutated genes, and other isolated gene mutations (other). Boxes represent the median and first and third quartiles. DTA, DNMT3A, TET2, or ASXL1.

Is the monocytosis explained by a proliferation of monocytes carrying gene mutations?

To determine whether the monocytosis was explained by a biased monocytic outgrowth of progenitor cells carrying clonal mutations, we performed subfraction sequencing for a selection of cases with CH and monocytosis (Table 2). Three cases with lower VAFs were selected (cases 1-3) and 1 case with higher VAFs (case 4). The mutational profiles were as follows: case 1: *DNMT3A* 2245C>T, 1.6%, and *SF3B1* 1866G>T, 7.4%; case 2: *ASXL1* 1772dup, 1.7%, *DNMT3A* 1711_1720del, 1.5%, and *DNMT3A* 1811G>T, 1.0%; case 3: *DNMT3A* 2371del, 1.8%; and case 4: *SRSF2* 284C>A, 39%, and *TET2* 3732_3733del, 34%. DNA was isolated from bulk WBCs, as well as sorted granulocytes ($CD45^+/CD15^+$), monocytes ($CD45^+/CD14^+$), and T cells ($CD45^+/CD3^+$). Sequencing was performed on the bulk and subfractions with ultra-high depth for the identified variants (supplemental Methods). In the T cells, the VAFs were always lower compared with the other fractions, confirming the myeloid bias of all clonal expansions. In the 3 cases with lower VAFs, the mutational load in the bulk cells, sorted granulocytes, and monocytes were comparably low. The VAFs in the sorted CD45/CD14 monocytic cells were generally <3%, indicating that the observed monocytosis could not be explained by a mutation-driven preferential outgrowth of monocytic cells. In the

Table 2. Mutational load in WBC subfractions for a selection of community-dwelling subjects with monocytosis and clonal hematopoiesis

	Mutation	1						2						3			4					
		DNMT3A 2245C>T			SF3B1 1866G>T			ASXL1 1772dup			DNMT3A 1711_1720del			DNMT3A 1811G>T			DNMT3A 2371del			SRSF2 284C>A			TET2 3732_3733del		
Case	Fraction	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads	VAF (%)	Mutant reads	Total reads
1	Bulk	1.6	56	3500	7.4	285	3851	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Granulocytes	0.9	6	632	4.6	139	3019	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	T cells	0.4	2	538	0.0	0	3736	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Monocytes	0.9	14	1572	5.3	246	4656	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	Bulk	–	–	–	–	–	–	1.7	22	1294	1.50	60	4000	1.0	62	6200	–	–	–	–	–	–	–	–	–
	Granulocytes	–	–	–	–	–	–	0.0	0	541	0	0	218	0.0	0	280	–	–	–	–	–	–	–	–	–
	T cells	–	–	–	–	–	–	0.0	0	1206	0	0	902	0.1	2	1436	–	–	–	–	–	–	–	–	–
	Monocytes	–	–	–	–	–	–	2.9	48	1670	0	0	959	2.7	38	1426	–	–	–	–	–	–	–	–	–
3	Bulk	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.8	30	1666	–	–	–	–	–	–
	Granulocytes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0	0	40	–	–	–	–	–	–
	T cells	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.1	2	182	–	–	–	–	–	–
	Monocytes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.1	8	380	–	–	–	–	–	–
4	Bulk	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	39.0	1742	4466	34.0	2349	6 908
	Granulocytes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	60.0	266	444	37.0	1870	5 056
	T cells	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	2	832	0.0	10	12 254
	Monocytes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	53.0	584	1104	48.0	5397	11 345

case with higher mutational load and a combination of *TET2* and *SRSF2* mutations (case 4), the VAFs in both the monocyte and granulocyte cell fractions were high, indicating that most, if not all, granulocytes and monocytes carried mutations. For this individual, the monocytosis might be explained by a clonal expansion of cells carrying mutations.

Hematological malignancies developing in community-based individuals with monocytosis

To investigate the incidence of hematological malignancies for individuals with monocytosis, a linkage of the Lifelines cohort to the NCR was performed. Incident malignancies could be evaluated for n = 21 601 older Lifelines participants, of whom 166 with monocytosis after a median follow-up of 7.7 (range, 6.1-11.4) years. Monocytosis associated with a higher risk of developing a hematological malignancy ($P = .002$, Gray's test; sHR, 2.91; 95% CI, 1.29-6.55; $P = .01$) (Figure 5A). A total of 6 out of 166 developed a hematological malignancy during follow-up, and all were carrying CH. Four out of 6 developed a myeloid malignancy, and only 1 individual developed CMML. Out of 500 evaluable control subjects, n = 7 developed a hematological malignancy, of whom 5 were carrying CH.

Monocytosis and spliceosome mutations associate with inferior OS

Finally, we investigated whether CH affects the prognosis of older individuals with monocytosis. The presence of monocytosis was significantly associated with poor survival compared with those without monocytosis (age- and sex-corrected HR, 2.30; 95% CI, 1.65-3.21; $P < .001$). The presence of CH was not associated with a higher risk of death for these individuals with monocytosis (age- and sex-corrected HR, 1.04; 95% CI, 0.512-2.12; $P = .92$) nor for control subjects (age- and sex-corrected HR, 1.35; 95% CI, 0.80-2.26; $P = .26$) (Figure 5B). Although these results should be interpreted cautiously due to low numbers, we evaluated the prognostic relevance of mutational spectra in community-based subjects with monocytosis. The number of mutated genes did not affect OS (Figure 5C). However, the presence of spliceosome mutations is associated with a higher risk of death in older individuals with monocytosis ($P < .001$) (Figure 5D).

Discussion

This is the first study assessing the occurrence of monocytosis in an unbiased and prospective cohort of community-dwelling individuals. To investigate the potential presence of pre-CMML conditions among older individuals with clonal monocytosis, we studied the relation between monocytosis and clonal hematopoiesis in a nested case-control design. Our results help to distinguish mutational spectra of importance in the context of monocytosis and show that the presence of CH in individuals with monocytosis is not sufficient to diagnose (prephases of) CMML in asymptomatic or community-dwelling individuals.

Monocytosis was detected in a substantial proportion of older individuals, and its prevalence increased with age. CMML is characterized by a strong male predominance, especially with aging.[5] In this population-based cohort, we also observed higher monocyte counts and a higher prevalence of monocytosis in males. The definition of monocytosis was in accordance with current cutoffs used in WHO

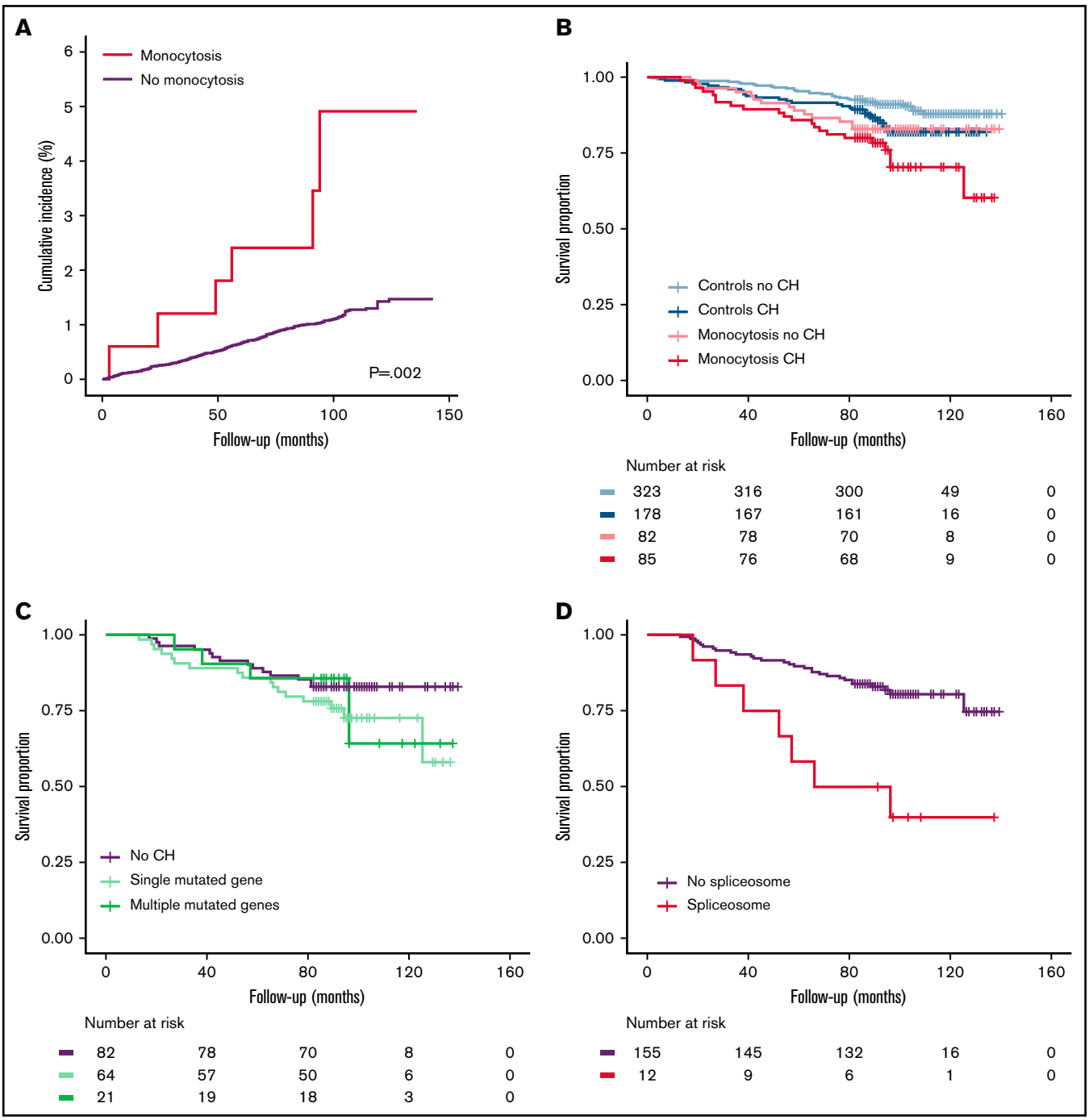

Figure 5. Development of hematological malignancies and risk of all-cause mortality for individuals with monocytosis. (A) Cumulative incidence of hematological malignancies for older individuals with monocytosis (n = 166) vs without (n = 21 435), as derived from linkage to the Netherlands Cancer Registry. Individuals with a recorded history of hematological malignancy were excluded from this analysis. (B) Kaplan-Meier plot for OS of older individuals with monocytosis (n = 167) and 1:3 matched control subjects (n = 501), stratified according to the presence of CH. (C) Kaplan-Meier plot for OS of individuals with monocytosis, stratified according to the number of mutated genes: no CH (n = 82), 1 mutated gene (n = 64), or multiple mutated genes (n = 21). (D) Kaplan-Meier plot for OS of individuals with monocytosis (n = 167), stratified according to the presence of spliceosome mutations. The category of spliceosome mutations includes SF3B1, SRSF2, and U2AF1.

criteria to define monocytosis in CMML,[4] except for the evaluation of 3-month persistence of monocytosis, the evaluation of which was hampered by the large time interval for follow-up blood counts. When applying less stringent proposed criteria that have been proposed in the literature (eg, monocytosis ≥10% of WBC count and ≥0.5 × 10^9/L) to define mild but relevant monocytosis,[16,17] we identified a much higher proportion of individuals with monocytosis (up to 12%). The high prevalence of monocytosis according to

these criteria indicates that these may not be clinically useful to define prestages of CMML in the absence of other cocriteria.

Mutational screening in this case-control cohort revealed a higher prevalence of CH in community-dwelling individuals with monocytosis, especially in cases with long-term persistent monocytosis. Isolated mutations in genes most commonly involved in age-related CH (*DNMT3A*, *TET2*, and *ASXL1*) were detected at comparable frequencies in individuals with monocytosis and control subjects. In addition, the combination of monocytosis and CH did not necessarily involve a clonal expansion of mutated monocytes. Thus, although both monocytosis and DTA mutations develop with advancing age, these phenomena are not necessarily related. In contrast, the presence of monocytosis is associated with a higher prevalence of spliceosome mutations. These mutations occur in >50% of MDS and CMML patients, and aberrant splicing is considered one of the key drivers of MDS and myeloproliferative neoplasm disease pathophysiology.[8,23] In addition, we identified a significantly higher proportion of individuals with monocytosis carrying combinations of gene mutations. Acquisition of additional mutations may contribute to the expansion of the mutated clone that may eventually transform into malignant disease, including CMML. We propose that individuals with dysregulated monocyte counts in combination with a mutational signature that deviates from common age-related CH may be at risk for an early stage in the development of malignant myeloid disease.

Only a small proportion of older individuals with monocytosis developed a myeloid malignancy. It might be that a long latency period is required for a very early stage of clonal monocytosis to develop into malignant disease. In addition, supported by a higher level of inflammatory marker high-sensitivity C-reactive protein and other WBC counts, a major proportion of individuals in this study probably suffered from reactive monocytosis despite the presence of CH. Clearly, CH in the presence of monocytosis does not directly imply the presence of a clonal monocyte proliferation with malignant potential. This stresses the fact that mutational screening should not be used as an isolated screening tool to distinguish CMML, oligomonocytic CMML, and other potential CMML prestages[16] from a reactive monocytosis, especially when more liberal criteria (eg, concentrations of 0.5 to 1.0 $\times 10^9$/L) to define a PB monocytosis are applied and other diagnostic criteria are not met. Indeed, this also questions whether all clonal genetic lesions in the presence of unexplained, persistent monocytosis may support a diagnosis of CMML, as it is currently used in the 2016 WHO criteria. For example, we previously described the clonal evolution of a CMML case which was remarkably stable and whose diagnosis could be questioned in hindsight.[24] The combination of *TET2* and *SRSF2* mutations, in contrast, was associated with clonal expansion of monocytic cells, which confirms the specificity of this mutational spectrum for myelomonocytic disorders.

This population of community-dwelling individuals, as well as the results from this study, are considerably different from the cohort presented by Cargo and colleagues[25] that evaluated patients presenting in hematology practice for the evaluation of monocytosis. In that study, individuals with clinically significant monocytosis but not (yet) meeting WHO criteria were found to have a mutational spectrum and clinical outcome indistinguishable from WHO-defined CMML. The median VAF in our study was 2.7%, compared with 39% in the study by Cargo and colleagues. Although various differences in sequencing technique and sensitivity may be noted between these studies, this is unlikely to explain the substantial differences in the mutational spectrum associated with monocytosis. Our cohort comprised unselected community-dwelling individuals for whom monocytosis was detected incidentally at study inclusion, without known associated health complaints. Indeed, there was a relatively low prevalence of accompanying cytopenias compared with diagnosed CMML patients. The clinical study by Cargo and colleagues probably included individuals with a more advanced stage and more suspect of overt clonal myeloid disease. Thus, the meaning of CH in the context of monocytosis presumably depends on the clinical burden of monocytosis and may very well be a continuum of (stepwise) clonal outgrowth and progression.

Conclusions

We identify monocytosis in a substantial proportion of the general aging population. A higher prevalence of CH was found in those with monocytosis than in matched population-based control subjects, especially when the monocytosis was persistent over time. Although not necessarily reflecting a clonal monocyte population, in a fraction of subjects with monocytosis, CH might constitute very early clonal dominance in the development of malignant myelomonocytic disease. Clinical attention seems warranted for cases with spliceosome gene mutations or with multiple mutated genes. Longitudinal studies are needed to track the evolutionary trajectory of cases with monocytosis and age-related CH and decide on the added value of a clinical follow-up for these cases.

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Authorship

Contribution: G.H. and J.H.J. were principal investigators and involved in all aspects of the study, including design and collection and interpretation of data; I.A.v.Z. and A.O.d.G. contributed to study design, collection, and analysis and interpretation of the data; T.N.K.-S. performed sorting and sequencing of subfractions; I.A.v.Z. wrote the first version of the manuscript; and B.A.v.d.R., M.M.v.d.K., A.G.D., A.D., J.J.S., and L.M. were involved in the interpretation of the data.

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