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

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ABSTRACT

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 ageing 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 (≥ 1 x 10^9/L and ≥10% of leukocytes) in the population-based Lifelines cohort (n=144676 adults). The prevalence and spectrum of CH were evaluated for individuals ≥60 years with monocytosis (n=167, 0.8%) and controls 1:3 matched for age and sex (n=501). Diagnoses of hematological malignancies were retrieved by linkage to the Netherlands Cancer Registry. Monocyte counts and the prevalence of monocytosis increased with advancing age. Older individuals with monocytosis more frequently carried CH (50.9% versus 35.5%, P<0.001). Monocytosis associated with enrichment of multiple gene mutations (P=0.006) and spliceosome mutations (P=0.007) but not isolated mutated DNMT3A, TET2 or ASXL1. Persistent monocytosis over four years was observed in 30/102 evaluable individuals and associated with higher prevalence of CH (63%). Myeloid malignancies, including one case of CMML, developed in four individuals with monocytosis that all carried CH. In conclusion, monocytosis and CH both occur at 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. Normal monocyte values range between 0.3 and 0.9x $10^9$/L, constituting 2-8% of the total white blood cell (WBC) population in peripheral blood. Abnormal production 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 auto-immune disorders.\(^3\) Besides secondary causes, peripheral blood 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 by a persistent (≥3 months) increase in peripheral blood monocyte counts ≥ 1x$10^9$/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 peripheral blood of otherwise healthy individuals, and clonal hematopoiesis (CH) is increasingly recognized as an important hallmark of the ageing hematopoietic system.\(^9\)\(^-\)\(^12\) CH is associated with a 10-fold increased risk of developing hematological malignancies and has been proposed to represent a pre-phase of myeloid malignancies, especially in the presence of peripheral cytopenias.\(^10\)\(^-\)\(^15\) In a recent population-based study, we found a significantly higher prevalence of CH in older individuals with anemia compared to non-anemic matched controls, 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 non-clonal CMML “pre-phases” has been suggested to be of potential clinical relevance, including a subtype of mild monocyte proliferation with monocyte counts between 0.5 and 1x$10^9$/L.\(^16\)\(^,\)\(^17\) We aimed to test these hypotheses in the “real-world” population-based Lifelines cohort (n=144676 adults). To investigate whether clonal hematopoiesis, in the presence of monocytosis, is indicative of CMML or CMML pre-stages, we determined the presence of clonal gene mutations in all older individuals with monocytosis and 1:3 matched controls.
METHODS

Study population
This study was undertaken within the prospective Lifelines Cohort Study. 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, socio-demographic, 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 the Lifelines cohort is representative of the background population of the northern part of the Netherlands. The Lifelines study 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. Peripheral blood specimens were drawn at study inclusion visit and at the first follow-up visit, which was scheduled after approximately five years. Details regarding laboratory procedures are provided in the Supplementary Appendix.

Cohort selection
For this study, we included all Lifelines participants aged ≥18 years for whom peripheral blood monocyte counts were available at study inclusion visit (n=144,676), including n=21,729 individuals aged ≥60 years. Following current WHO criteria for CMML, monocytosis was defined as a peripheral blood monocyte count ≥1 x 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) (Supplementary 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 smMIPs. 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 Supplementary Methods.

Outcomes
Data on incident hematological malignancies were obtained by linkage of the Lifelines cohort to the nationwide Netherlands Cancer Registry (NCR), censored at 12-2019. The completeness of the NCR is estimated at ≥95%. 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 that were confirmed by histology and/or cytology. Hematological malignancies were identified based on ICD-O codes 9590-9999 (Supplementary 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 06-2020). 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. T-tests and Mann-Whitney tests were used to compare continuous data with parametric and non-parametric distribution respectively. Differences in 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 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 emergence of monocytosis in older individuals
Peripheral blood monocyte counts were available for 144676 Lifelines participants. This population included 60088 men and 84588 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, as compared to females. Upon ageing, an increase in absolute monocyte count was observed in the cohort of individuals ≥60 years (Figure 1A). The prevalence of monocytosis, defined as a peripheral blood monocyte count ≥1 x 10^9/L and ≥10% of total WBC count, increased with advancing age. In total, 167 out of 21729 (0.8%) evaluable Lifelines participants ≥60 years had monocytosis at study inclusion visit. Consistent with the observed sex differences in monocyte count, there was male predominance in monocytosis prevalence (Figure 1B). Peripheral blood counts for community-dwelling individuals ≥60 years with and without monocytosis are presented in Table 1. Platelet count (P=0.004) and hemoglobin levels (P<0.001) were higher for individuals with monocytosis. Individuals with monocytosis had higher levels of mean corpuscular volume (MCV) (P=0.001). Monocytosis further associated with a concomitant increase of total WBC count (P<0.001), neutrophil count (P<0.001) and also lymphocyte count (P<0.001). Finally, monocytosis associated with higher high-sensitivity CRP (hsCRP) levels (P<0.001). Recently, alternative criteria were proposed to define a persistent monocytosis that is suggestive for pre-CMML conditions, including a lower absolute monocyte count or the presence of an absolute but not relative increase in monocytes. When these criteria were applied to our cross-sectional community-based cohort, almost half of all individuals were meeting the proposed cut-off criteria for absolute monocytosis (monocyte count ≥0.5 x 10^9/L, 41%), with a substantial percentage (12%) also having a relative increase in monocyte count ≥10% of the total WBC count (Figure 1C).
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Figure 1. Emergence of monocytosis with ageing 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 cut-off criteria, within the entire evaluable younger (<60 years, n=122 947) and older (≥60 years, n=21 729) Lifelines cohort.

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 study inclusion visit. The flowchart for the nested case-control study is shown in Figure 2. Cases and controls 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 controls. The mean sequencing depth was 9126 consensus reads, and the consensus read depth was >500x for 97.7% of all regions (Supplementary Figure 1).
Figure 2. Nested case-control study design
Flowchart depicting the nested case-control study design with selection of cases with monocytosis and controls from the entire evaluable Lifelines cohort (n=144,676). Monocytosis was defined in accordance with WHO criteria for monocytosis associated with CMML: peripheral blood monocyte counts $\geq 1 \times 10^9$/L and $\geq 10\%$ of WBC. NGS, next-generation sequencing; WBC, white blood cell count.

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 $\leq 10\%$ (Supplementary 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 to controls (35.5\%) (OR 1.88, 95\%CI 1.30-2.72, P<0.001) (Figure 3A, Supplementary Table 2). We additionally corrected the association between monocytosis and CH for baseline differences in hemoglobin, platelet, 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<0.001). In addition, we performed sensitivity analyses restricting to variants $\geq 2\%$ VAF compatible with definition of clonal hematopoiesis of indeterminate potential (“CHIP”)$^{12}$, which confirmed a higher prevalence of CH in monocytosis cases (OR 1.83, 95\%CI 1.23-2.72, P=0.002) (Supplementary Figures 4-6). The prevalence of CH increased with age for both cases with monocytosis and controls (Figure 3B). For individuals with CH, no significant differences were observed in the number of mutated genes (P=0.12) (Figure 3C). Absolute monocyte counts were higher in the presence of CH for individuals with monocytosis (P=0.023) but not for controls (P=0.398) (Figure 3E-F and Supplementary Table 3).
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A. Clonal hematopoiesis (%)

B. Prevalence of CH (%)

C. Number of mutated genes

D. DNMT3A, TET2, ASXL1, SETBP1, SPDEF, SPOP, SMARCD2, CBL, U2AF1, EZH2, RUNX1, CALR, ETNK1, NRAS, IDH2, KIT, KITLG, RUNX1, EZH2, U2AF1, CBL, SETBP1, TET2

E. Absolute monocyte count (x10^9/L)

F. Absolute monocyte count (x10^9/L)

G. Proportion of individuals (%)

H. Clonal hematopoiesis (%)

I. Absolute monocyte count (x10^9/L)
Mutational spectrum associated with monocytosis in older individuals

We next compared the mutational spectrum between cases with monocytosis and controls. Overall, a significant overlap was observed in the mutational spectrum of individuals with monocytosis and controls that represent the 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=0.03). No significant differences were observed with regard to TET2 (P=0.18) and ASXL1 (P=0.10). Monocytosis was associated with a higher proportion of spliceosome mutations (SF3B1, SRSF2 and U2AF1) (7.2%) when compared to controls (2.4%) (P=0.007) (Figure 4A). The clonal trajectory from age-related CH to myeloid neoplasms may occur in a step-wise manner. Isolated mutated DNMT3A, TET2 and ASXL1 are most frequently detected upon ageing. In fact, the prevalence of CH confined to isolated mutated DNMT3A (P=0.42), TET2 (P=0.86) or ASXL1 (P=1.00) (DTA) was comparable between cases with monocytosis and controls (Figure 4B). In contrast, individuals with monocytosis more frequently carried multiple gene mutations (21 out of 167 versus 29 out of 501, P=0.006) or isolated gene mutations other than DTA (P=0.010). 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, Supplementary Figure 3). The combination of TET2 and SRSF2 mutations, which may be specific for a myeloid neoplasm with myelodysplasia and monocytosis6,22, was observed twice in this community-based cohort with monocytosis (n=167) and in three of the controls (n=501). Other mutations observed at moderate frequencies in CMML, including RUNX1, CBL, NRAS/KRAS and SETBP15, were detected in a few (n=4) population-based individuals with monocytosis.

Individuals with persistence of monocytosis have 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=102668. Studying the persistence of monocytosis and its relation to CH was thus limited by loss to follow-up of potential high-risk cases during this
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latency period (Supplementary Table 2). Among 167 individuals with monocytosis at 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 30/102 (29%) evaluable individuals ≥60 years had persistence of monocytosis over this period (Figure 3G). We grouped individuals and their respective 1:3 controls 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 clonal hematopoiesis, as compared to 28% of matched controls (P=0.001) (Figure 3H and Supplementary 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.

Highest absolute monocyte counts at baseline visit were found among individuals with persistent monocytosis, although this was not statistically significant (Figure 3I).

Figure 4. Mutational spectra for individuals with monocytosis and 1:3 matched controls (II)

(A) Pyramid plot indicating the proportion of individuals with detected gene mutations within the monocytosis (green) 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 (green, 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. (C) Highest detected VAF according to mutational spectrum for monocytosis cases (green) and control (blue). 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 median, first and third quartiles. CH, clonal hematopoiesis; DTA, DNMT3A, TET2 or ASXL1; VAF, variant allele frequency.

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 one 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 1717dup 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 white blood cells, 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 (Supplementary Methods). In the T cells, the VAFs were always lower compared to the other fractions, confirming the myeloid bias of all clonal expansions. In the three cases with lower VAFs, the mutational load in the bulk cells, sorted granulocytes and monocytes was comparably low. The VAFs in the sorted CD45+/CD14 monocytic cells were generally less than 3%, indicating that the observed monocytosis could not be explained by a mutation-driven preferential outgrowth of monocytic cells. In the 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, linkage of the Lifelines cohort to the Netherlands Cancer Registry was performed. Incident malignancies could be evaluated for n=21601 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=0.002, Gray's test, sHR 2.91, 95%CI 1.29-6.55, P=0.01, Figure 5A). A total of 6 out of 166 developed a hematological malignancy during follow-up, all were carrying CH. 4 out of 6 developed a myeloid malignancy, only one individual developed CMML. Out of 500 evaluable controls, n=7 developed a hematological malignancy, of whom 5 were carrying CH.

Monocytosis and spliceosome mutations associate with inferior overall survival
Finally, we investigated whether CH affects prognosis for older individuals with monocytosis. The presence of monocytosis was significantly associated with poor survival compared to those without monocytosis (age- and sex corrected HR 2.30, 95% CI 1.65-3.21, P<0.001). 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=0.92) nor for controls (age- and sex corrected HR 1.35, 95% CI 0.80-2.26, P=0.26) (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 overall survival (Figure 5C). However, the presence of spliceosome mutations associated with a higher risk of death in older individuals with monocytosis (P<0.001) (Figure 5D).

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) versus without (n=21435), 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 controls (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), one 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. CH, clonal hematopoiesis; OS, overall survival.
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 (pre-phases 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 ageing.\(^5\) 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 cut-offs used in WHO 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 x 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 pre-stages of CMML in the absence of other co-criteria.

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 controls. 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 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 (MPN) 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 expansion of the mutated clone that may eventually transform to 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 hsCRP and other white blood cell 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 pre-stages from a reactive monocytosis, especially when more liberal criteria (e.g. levels 0.5-1.0 x 10^9/L) to define a peripheral blood 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. 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 et al., that evaluated patients presenting in hematology practice for 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% as compared to 39% in the study by Cargo et al. Although various differences in sequencing technique and sensitivity may be noted between these studies, this is unlikely to explain the substantial differences in 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 as compared to diagnosed CMML patients. The clinical study by Cargo et al. probably included individuals with a more advanced stage, 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.

In conclusion, we identify monocytosis in a substantial proportion of the general ageing population. A higher prevalence of CH was found in those with monocytosis as compared to matched population-based controls, 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.
Table 1. Characteristics and peripheral blood counts of community-dwelling individuals ≥ 60 years with and without monocytosis as well as 1:3 matched controls

<table>
<thead>
<tr>
<th></th>
<th>Absence of monocytosis</th>
<th>Monocytosis N=167</th>
<th>P-value¹</th>
<th>1:3 matched controls N=501</th>
<th>P-value²</th>
<th>N</th>
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<tr>
<td>Age (years)</td>
<td>65.0</td>
<td>68.0</td>
<td>&lt;0.001</td>
<td>68.0</td>
<td>1.00</td>
<td>21729</td>
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<td></td>
<td>[62.0;69.0]</td>
<td>[63.0;72.0]</td>
<td></td>
<td>[63.0;72.0]</td>
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<tr>
<td>Male sex</td>
<td>9756 (45.2%)</td>
<td>123 (73.7%)</td>
<td>&lt;0.001</td>
<td>369 (73.7%)</td>
<td>1.00</td>
<td>21729</td>
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<td></td>
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<tr>
<td>Monocyte count (10⁹/L)</td>
<td>0.50 (0.15)</td>
<td>1.12 (0.12)</td>
<td>&lt;0.001</td>
<td>0.53 (0.15)</td>
<td>&lt;0.001</td>
<td>21729</td>
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<tr>
<td>WBC count (10⁹/L)</td>
<td>5.83 (1.46)</td>
<td>8.60 (1.72)</td>
<td>&lt;0.001</td>
<td>6.08 (1.56)</td>
<td>&lt;0.001</td>
<td>21727</td>
</tr>
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<td>Neutrophil count (10⁹/L)</td>
<td>3.14 (1.08)</td>
<td>4.88 (1.51)</td>
<td>&lt;0.001</td>
<td>3.37 (1.22)</td>
<td>&lt;0.001</td>
<td>21729</td>
</tr>
<tr>
<td>Basophil count (10⁹/L)</td>
<td>0.03 (0.02)</td>
<td>0.04 (0.03)</td>
<td>&lt;0.001</td>
<td>0.03 (0.02)</td>
<td>&lt;0.001</td>
<td>21729</td>
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<tr>
<td>Eosinophil count (10⁹/L)</td>
<td>0.19 (0.13)</td>
<td>0.25 (0.15)</td>
<td>&lt;0.001</td>
<td>0.21 (0.14)</td>
<td>0.001</td>
<td>21729</td>
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<td>Lymphocyte count (10⁹/L)</td>
<td>1.96 (0.59)</td>
<td>2.30 (0.77)</td>
<td>&lt;0.001</td>
<td>1.94 (0.59)</td>
<td>&lt;0.001</td>
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<td>Hemoglobin (g/dL)</td>
<td>14.2 (1.15)</td>
<td>14.6 (1.34)</td>
<td>&lt;0.001</td>
<td>14.5 (1.25)</td>
<td>0.348</td>
<td>21727</td>
</tr>
<tr>
<td>Erythrocyte count (10⁹/L)</td>
<td>4.70 (0.37)</td>
<td>4.77 (0.43)</td>
<td>0.038</td>
<td>4.79 (0.39)</td>
<td>0.736</td>
<td>21727</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.43 (0.03)</td>
<td>0.44 (0.04)</td>
<td>&lt;0.001</td>
<td>0.43 (0.03)</td>
<td>0.153</td>
<td>21727</td>
</tr>
<tr>
<td>Platelet count (10⁹/L)</td>
<td>240 (56.3)</td>
<td>254 (64.9)</td>
<td>0.004</td>
<td>229 (51.7)</td>
<td>&lt;0.001</td>
<td>21707</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>90.9 (3.96)</td>
<td>92.0 (4.16)</td>
<td>0.001</td>
<td>90.8 (4.21)</td>
<td>0.002</td>
<td>21727</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.40</td>
<td>3.20</td>
<td>&lt;0.001</td>
<td>1.40</td>
<td>&lt;0.001</td>
<td>7316</td>
</tr>
<tr>
<td></td>
<td>[0.80;2.90]</td>
<td>[1.65;7.75]</td>
<td></td>
<td>[0.80;2.70]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of medications used¹</td>
<td>2.00</td>
<td>3.00</td>
<td>&lt;0.001</td>
<td>2.00</td>
<td>&lt;0.001</td>
<td>21729</td>
</tr>
<tr>
<td></td>
<td>[0.00;4.00]</td>
<td>[1.00;5.00]</td>
<td></td>
<td>[0.00;4.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concurrent cytopenia*</td>
<td>2434 (11.3%)</td>
<td>15 (8.98%)</td>
<td>0.412</td>
<td>66 (13.2%)</td>
<td>0.194</td>
<td>21708</td>
</tr>
<tr>
<td>Concurrent cytosis²</td>
<td>480 (2.2%)</td>
<td>38 (22.8%)</td>
<td>&lt;0.001</td>
<td>13 (2.6%)</td>
<td>&lt;0.001</td>
<td>21707</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) or median [IQR] for continuous variables and number (%) for categorical variables. WBC, white blood cell; MCV, mean corpuscular volume; N, total number of evaluable individuals ≥60 years; hsCRP, high sensitive CRP. *A concurrent cytopenia was defined as follows: anemia, hemoglobin concentration <12.0 g/dL in women or <13.0 g/dL in men; thrombocytopenia, platelet count <150 x 10⁹/L; neutropenia, absolute neutrophil count <1.8 x 10⁹/L. $ A concurrent cytosis was defined as follows: erythrocytosis, hemoglobin concentration >16.5 g/dL or hematocrit ≥48% in women or hemoglobin concentration >18.5 g/dL or hematocrit ≥52% in men; thrombocytosis, platelet count >400 x 10⁹/L; leukocytosis, white blood cell count >10 x 10⁹/L. # As a proxy for comorbidity. ¹ P-value for the comparison of individuals with and without monocytosis. ² P-value for the comparison between individuals with monocytosis and 1:3 matched controls.
<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNMT3A 2245C&gt;T</td>
<td>SF3B1 1866G&gt;T</td>
</tr>
<tr>
<td></td>
<td>ASXL1 1772dup</td>
<td>DNMT3A 1711_1720del</td>
</tr>
<tr>
<td></td>
<td>VAF reads mutant total</td>
<td>VAF reads mutant total</td>
</tr>
<tr>
<td>bulk</td>
<td>1.6%   56 3500</td>
<td>7.4%   285 3851</td>
</tr>
<tr>
<td>granulocytes</td>
<td>0.9%   6 632</td>
<td>4.6%   139 3019</td>
</tr>
<tr>
<td>T-cells</td>
<td>0.4%   2 538</td>
<td>0.0%   0 3736</td>
</tr>
<tr>
<td>monocytes</td>
<td>0.9%   14 1572</td>
<td>5.3%   246 4656</td>
</tr>
<tr>
<td>bulk</td>
<td>1.7%   22 1294</td>
<td>1.5%   60 4000</td>
</tr>
<tr>
<td>granulocytes</td>
<td>0.0%   0 541</td>
<td>0%     0 218</td>
</tr>
<tr>
<td>T-cells</td>
<td>0.0%   0 1206</td>
<td>0%     0 902</td>
</tr>
<tr>
<td>monocytes</td>
<td>2.9%   48 1670</td>
<td>0%     0 959</td>
</tr>
<tr>
<td>Case 2</td>
<td>Case 3</td>
<td>Case 4</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>DNMT3A 1811G&gt;T</td>
<td>DNMT3A 2371del</td>
<td>SRSF2 284C&gt;A</td>
</tr>
<tr>
<td>VAF reads mutant</td>
<td>VAF reads total</td>
<td>VAF reads mutant</td>
</tr>
<tr>
<td>1,0% 62 6200</td>
<td>0,0% 0 280</td>
<td>0,1% 2 1436</td>
</tr>
<tr>
<td>1,8% 30 1666</td>
<td>0,0% 0 40</td>
<td>1,1% 2 182</td>
</tr>
<tr>
<td>39% 1742 4466 34% 2349 6908</td>
<td>60% 266 444 37% 1870 5056</td>
<td>0,2% 2 832 0,0% 10 12254</td>
</tr>
</tbody>
</table>
Monocytosis and clonal hematopoiesis in community-based individuals

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

Declaration of interests
The authors declare to have no competing interests.

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Data sharing statement
The manuscript is based on data from the Lifelines Cohort Study. Lifelines adheres to standards for data availability. The data catalogue 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.
SUPPLEMENTARY MATERIAL

The supplementary material for this chapter can be accessed online at: https://doi.org/10.1182/bloodadvances.2021006755
REFERENCES

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