

## MGUS and clonal hematopoiesis show unrelated clinical and biological trajectories in an older population cohort

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### Abstract:

Monoclonal gammopathy of undetermined significance (MGUS) and clonal hematopoiesis (CH) are 2 preclinical clonal expansions of hematopoietic cells, whose prevalence rises with age reaching almost 10% in people of 70 years and older. The increased risk of myeloid malignancies in patients with myeloma is well defined, and the study of the association between these CH and MGUS could help explain this phenomenon. Here, we analyzed a fully clinically annotated dataset of 777 older subjects (median age, 91 years-old) previously screened for prevalence of CH. The prevalence of MGUS and CH was 9.6% and 17.3%, respectively. We detected CH in 9.7% of the MGUS patients, and MGUS in 5.5% of the CH patients. We did not find a significant correlation between the presence of MGUS and CH. Furthermore, the 2 conditions showed a differential association with clinical and laboratory covariates, suggesting that MGUS and CH may represent age-associated unrelated clonal drifts of hematopoietic cells. Confirmatory studies are needed to assess the relevance of CH in plasma cell disorders.

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# **MGUS and clonal hematopoiesis show unrelated clinical and biological trajectories in an older population cohort**

## **Running head: MGUS and CH association in an older cohort**

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**Key points:**

- **In a large older cohort, MGUS and CH associate with different clinical and laboratory variables**
- **MGUS and CH do not cooccur frequently and rather follow 2 unrelated clonal trajectories within the bone marrow.**

## Abstract

Monoclonal gammopathy of undetermined significance (MGUS) and clonal hematopoiesis (CH) are 2 preclinical clonal expansions of hematopoietic cells, whose prevalence rises with age reaching almost 10% in people of 70 years and older. The increased risk of myeloid malignancies in patients with myeloma is well defined, and the study of the association between these CH and MGUS could help explain this phenomenon. Here, we analyzed a fully clinically annotated dataset of 777 older subjects (median age, 91 years-old) previously screened for prevalence of CH. The prevalence of MGUS and CH was 9.6% and 17.3%, respectively. We detected CH in 9.7% of the MGUS patients, and MGUS in 5.5% of the CH patients. We did not find a significant correlation between the presence of MGUS and CH. Furthermore, the 2 conditions showed a differential association with clinical and laboratory covariates, suggesting that MGUS and CH may represent age-associated unrelated clonal drifts of hematopoietic cells. Confirmatory studies are needed to assess the relevance of CH in plasma cell disorders.

## Introduction

Cancer is defined by a multi-step acquisition of driver clonal abnormalities resulting in expansion of malignant cell populations. The identification of pre-clinical cancer stages has for long been restricted to cases with phenotypic manifestations, e.g. production of a monoclonal antibody in the case of monoclonal gammopathy of undetermined significance (MGUS).<sup>1</sup> Novel genomic techniques have conversely expanded the knowledge of pre-clinical cancer stage in several solid and hematological tumors.<sup>2,3</sup> Aside from MGUS, sustained by BM clonal plasma cells (PC), a pre-clinical clonal expansion was recently described in hematopoietic stem cells by genomic techniques, a condition called clonal hematopoiesis (CH).<sup>4</sup> This new clonal entity is defined by the presence of somatic gene mutations in white blood cells of people without any sign of hematological malignancy.<sup>4-8</sup> Importantly, a subset of CH cases are referred to as clonal hematopoiesis of indeterminate potential (CHIP) to reflect the presence of driver gene mutations found in leukemia and a higher propensity towards transformation.

Both CH and MGUS are defined as two aging-related entities, as their prevalence increases in the elderly population reaching more than 10% for CH and 6% for MGUS over the age of 70 years.<sup>4-6,9-11</sup> Importantly, patients with CH or MGUS are asymptomatic but at risk of evolution into acute myeloid leukemia (AML) or multiple myeloma (MM), respectively.<sup>4,5,7,10</sup> Recent data highlight shared clinical trajectories of these conditions. The presence of CH has been described as an adverse prognostic factor for MM relapse after treatment.<sup>12</sup> Furthermore, patients with MM are at increased risk of developing myeloid malignancies, both in sequence or synchronously.<sup>13,14</sup> Of note, this risk can be explained by the use of cytotoxic therapy in MM patients undergoing autologous stem cell transplantation, but cannot be explained in asymptomatic patients raising the question as to whether there is a shared clonal phylogeny between these myeloid and PC malignancies.<sup>13</sup> However, data from a large study on myeloid and lymphoid clonal hematopoiesis and their additive risk of development of myeloid and lymphoid malignancies have been published.<sup>15</sup> Interestingly, the presence of CH was not associated with an increased risk of development of MM in this recent paper.<sup>15</sup>

We hypothesized that the study of the association and dynamics of the two precursor conditions CH and MGUS may help shed some light on the possible hierarchical clonal interrelationships between myeloid and PC malignancies. To this end, we analyzed a unique

older population dataset, annotated for both MGUS and CH, and their association with other clinical and laboratory variables.

## Methods

### Study cohort

In this study we analyzed clinical and genomic data derived from 777 subjects enrolled in the “Monzino 80-plus” cohort which was previously used to study dementia in a large population-based study<sup>16</sup> and for subsequent genomic analysis on CH prevalence.<sup>17</sup> The study population and sequencing procedures were already described.<sup>17</sup> For the purpose of our study, we called and filtered variants as described in supplementary methods. We applied a minimum cut-off of 0.02 for variant allele frequency. Written informed consent was obtained prior to blood sampling (ClinicalTrials.gov number: NCT03907553).

### Statistical analysis

Continuous variables were reported by median and range. Prevalence of categorical variables was expressed by absolute numbers and relative frequency. The association between CH and MGUS was evaluated by the Fisher’s exact test. Wilcoxon test was employed to define possible differences in the distribution of continuous variables between patients carrying or not MGUS. Subsequently, using a linear regression model we assessed possible correlations between age as independent continuous variable and other predetermined dependent variables such as hemoglobin, albumin, mean corpuscular volume (MCV) and gamma-globulins concentration. Finally, to better define a possible correlation or anti-correlation of the aforementioned variables with MGUS and CH we implemented uni- and multivariate logistic regression models using MGUS or CH as independent variable. All analyses were performed in R using the “stats” R package version 4.0.4. *P* values were corrected by Bonferroni-Hochberg procedure.

## Results and discussion

Good quality sequence data from unsorted peripheral blood samples were available in 732/777 patients for 43 genes.<sup>17</sup> Serum protein electrophoresis data were available for the entire cohort. The median age of the cohort was 91 years old (range, 81 - 104), significantly higher than other previously published MGUS studies.<sup>11</sup> Other clinical and laboratory features of the cohort are resumed in Table 1. We detected a prevalence of MGUS and CH of 9.6% (75/777) and 17.3% (127/732), respectively. The complete list of identified mutations and the distribution of their allelic frequencies are reported in Supplementary Table 1 and Supplementary Figure 1, respectively. Mutations were filtered for germline and artifacts based on public databases but also using a panel of 32 healthy subjects of max 30 years of age (see Supplementary Methods for details). As expected, people harboring MGUS showed higher levels of gamma-globulins (1.18 g/dl vs 1.05 g/dl, Wilcoxon test, *P* value = .006439) (Supplementary Figure 2A). Surprisingly, no significant association was found between MGUS and age, nor with hemoglobin concentration, albumin levels and MCV (Supplementary Figure 2B-E). We then assessed the impact of age as a continuous variable on specific laboratory features. Of note, albumin and hemoglobin concentration significantly decreased with age (Supplementary Figure 3A-B, *P* values are 8.2e-12 and 8.6e-15, respectively). On the other hand, no significant correlation was found between age and gamma-globulin concentration or MCV (Supplementary Figure 3C-D). A significant anti-correlation between albumin and gamma-globulin levels was also confirmed (*P* value = .0022, Supplementary Figure 3E). Then, we focused on the possible association between MGUS and CH: 7 patients carried both CH and MGUS. The prevalence of CH in patients

with MGUS was 9.7% (7/72), and the prevalence of MGUS in patients with CH was 5.5% (7/127). A Fisher's exact test showed no association between the two clonal events but rather a non-significant trend towards anti-correlation ( $P$  value = .073). To further explore this result, we implemented both uni- and multivariate logistic models using presence/absence of MGUS or CH as independent dichotomic variables. By univariate analysis, high gamma-globulins and low albumin resulted associated with MGUS (Figure 1A and Supplementary Table 2A). In multivariate analysis, the presence of MGUS resulted only significantly associated with higher levels of gamma-globulins (Estimation: 1.48656,  $P$  value =  $7.74 \times 10^{-5}$ ) but not with age, sex, hemoglobin or albumin concentration (Figure 1A and Supplementary Table 2A). This analysis confirmed a trend of anti-correlation between MGUS and CH (Estimation: -0.76713,  $P$  value = .0707) (supplementary table 2A-B). On the other hand, CH as independent variable showed a significant correlation with age (Estimation: 0.076308,  $P$  value = .000158) (Figure 1B and Supplementary Table 2B). As for the type of mutated genes, CH showed an expected pattern. Due to low numbers, we did not find any significant difference in mutational patterns between CH patients with or without MGUS (Supplementary Figure 4).

Because CHIP defines, amongst CH cases, those bearing the known leukemia driver gene mutations and the highest propensity to transformation, we also asked whether the subset of our patients bearing CHIP showed a similar pattern. To this end, we restricted our analysis to cases showing clonality in accordance to the recently published list of gene mutations more frequently related to myeloid neoplasms (M-CHIP) as done in <sup>15</sup>. Again, MGUS and M-CHIP showed association with different clinical and laboratory variables, and no significant association between each other (Supplementary Table 3).

CH is associated with reduced survival, partly -but not predominantly- due to risk of evolution to overt myeloid malignancies.<sup>5,6,12,18</sup> Intriguingly, CH is a risk factor for cerebral-cardiovascular events,<sup>5,6</sup> and is associated with auto-immune diseases in patients undergoing hip replacement for osteoarthritis.<sup>18</sup> Studies have reported an increased risk of MDS/AML development in MGUS<sup>13</sup> and in MM patients undergoing lenalidomide maintenance.<sup>19-21</sup> In this latter case, CH could represent the soil on which the pharmacological intervention induces evolution to a myeloid neoplasm maintaining, at the same time, the plasma cell dyscrasia under control. In asymptomatic cases though, the increased risk may suggest a shared origin of the two malignancies. On the other hand, it has been recently demonstrated that CH represents an independent risk factor for progression in patients with asymptomatic Waldenström macroglobulinemia.<sup>22</sup> However, the association of CH with other pre-clinical BM clonal disorders has not been studied yet in a general patient population, and so the question is still open as to whether there is a hierarchy of clonal conditions and a causal relation between them. Here, interrogating a well annotated dataset of 777 older patients we found no association between CH and MGUS, but on the contrary a tendency towards their mutual exclusivity. Furthermore, the two conditions tended to have different clinical and laboratory co-variables, suggesting they develop along independent biological trajectories. Indeed, our results could be biased by the peculiar features of our cohort, and we may be reporting data on a selected population that may limit their wider application. In fact, our cohort is significantly older than any other study in MGUS and skewed towards the female sex, characterized by a lower prevalence of MGUS and a longer life expectancy. Additionally, we may have selected a somehow favorable subset of patients, while subjects harboring MGUS and CH for a long time could have already died due to disease progression before sampling. However, the lack of association between CHIP and subsequent development of MGUS or MM was recently described by Niroula et al,<sup>15</sup> and our findings here may well be explained by the same biological phenomenon. Moreover, our data in asymptomatic people are also in line with a recent report on the lack of a common progenitor

between myelodysplastic and myeloma clones in rare patients harboring both diseases,<sup>23</sup> and may point at different interactions between these two clonal conditions, i.e. through the microenvironment. In conclusion, our study complements existing evidence in overt cancer conditions and sheds new light on an increasingly over-represented setting of patients, usually understudied and on which biological data are generally scanty.



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## **Authorship contributions**

MDP and NB designed the study; AAG, UL, SM, ER and MT provided samples and clinical data; MCDV, ML, AMar and AM analyzed data; MCDV, ML, AM, ES, LP, AP, LB, AN and NB interpreted data; MCDV, ML and NB wrote the paper. ET performed NGS experiments.

All authors approved the paper for submission.

## **Conflict-of-interest disclosure**

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. NB received honoraria from Celgene, Amgen and Janssen.

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## Tables

**Table 1. Cohort Demographic and Clinico-laboratory Characteristics**

Feature	Value
Number of patients	777
Number of males (%)	198 (25.5%)
Median age (range)	91 (81-104)
Median hemoglobin concentration, g/dl (range)	13.1 (6.7-16.8)
Median MCV, fl (range)	92.2 (58.6-121.8)
Median albumin concentration, g/l (range)	3.58 (1.87-4.78)
Median gamma globulin concentration, g/dl (range)	1.06 (0.35-2.98)
MGUS prevalence	75/777 (9.7%)
CH prevalence	127/732 (17.3%)
M-CHIP prevalence	114/732 (15.5%)
Prevalence of concomitant MGUS and CH	7/732 (1%)
Prevalence of concomitant MGUS and M-CHIP	7/732 (1%)

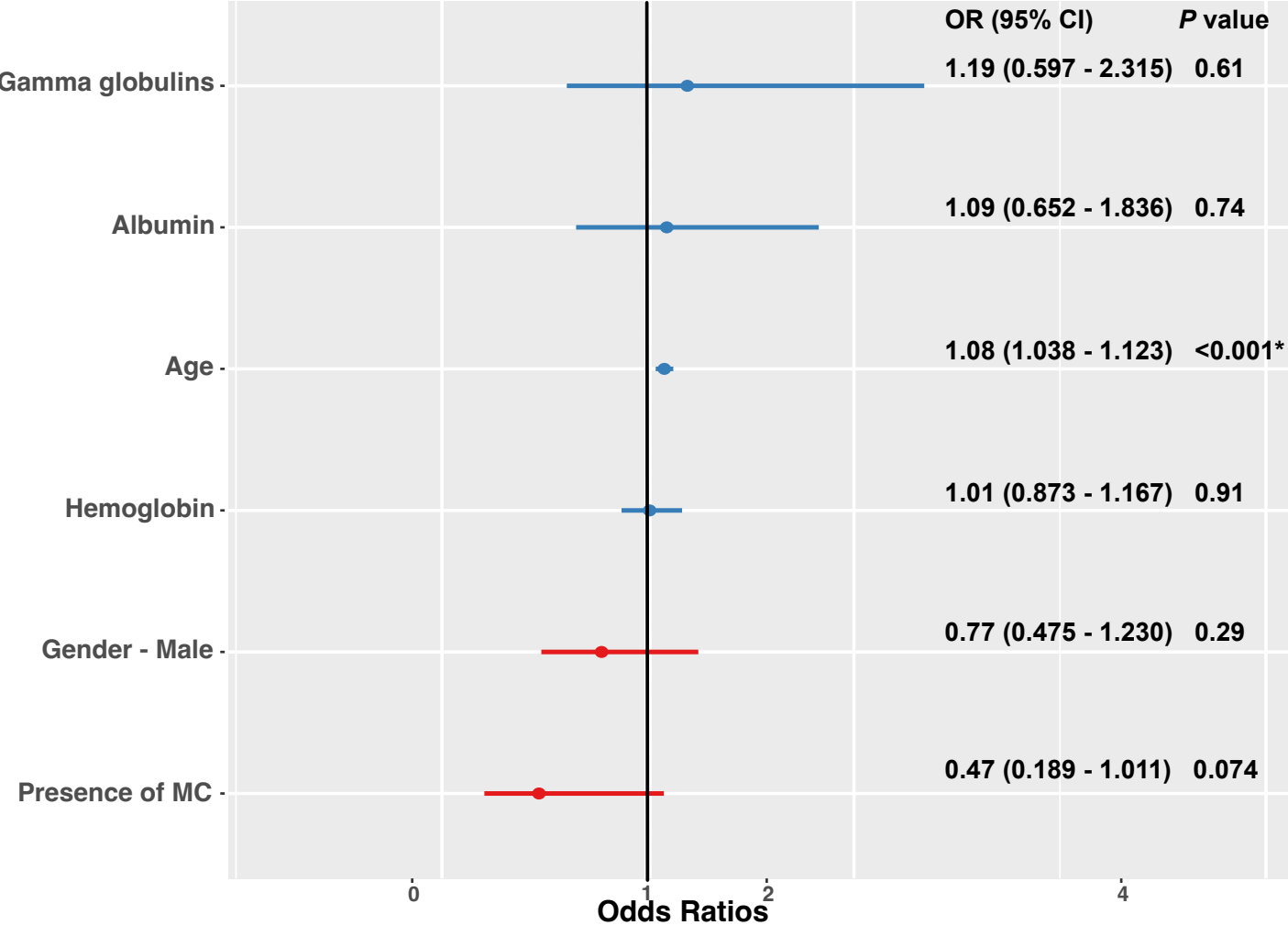
Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; CH, clonal hematopoiesis; M-CHIP, myeloid clonal hematopoiesis of indeterminate potential.

**Figure legend:**

**Figure 1: Forest plots of multivariate analyses to determine the possible association between MGUS and CH.** A) Forest plot using CH as independent variable; B) Forest plot using MGUS as independent variable. Abbreviations: OR: odds ratio; CI: confidence interval.

A

Multivariate analysis: CH as independent variable



B

Multivariate analysis: MGUS as independent variable

