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Clonal hematopoiesis in patients with Covid-19 is stable and not linked to an aggravated clinical course

To the Editor:

The strength of host immune reaction to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) determines the severity of corona virus disease 2019 (Covid-19). While a basic antiviral immune response involving innate immune mechanisms and an induction of adaptive immunity is needed to resolve the disease, particularly innate myeloid cells can also induce a dysregulated, excessive, aberrant and non-effective host immune response in severe Covid-19.[1] The latter is termed "hyperinflammation," "cytokine storm," "macrophage activation syndrome" or "cytokine release syndrome." Hyperinflammation contributes to the pathogenesis of Covid-19 related acute respiratory distress syndrome (ARDS), which is associated with a fatal outcome in the majority of patients, but may also induce multi-organ damage, which is the second most common cause of death in Covid-19. Thus, a better understanding of underlying host-intrinsic factors driving immune dysfunction is warranted for the immediate identification of patients at risk and eventually also for selecting novel targeted therapies.

Thus, SARS-CoV-2 has been found to infect multiple cells expression ACE-2 receptors including human monocytes, macrophages and dendritic cells, emphasizing their contribution to disease pathology.[2] Of relevance, clonal hematopoiesis (CH; defined as somatic variants in the peripheral blood at a variant allele frequency ≥2%) is considered to imprint inflammation in myeloid cells, which may perpetuate acute (e.g., infectious-triggered) and chronic inflammatory processes.[3,4] Moreover, CH is associated with some of the comorbidities relevant for higher risk of severe Covid-19, especially age and cardiovascular disease. Augmented injury in cardiovascular tissues was attributed to increased inflammatory tension in clonal myeloid and T cells, which drive pro-inflammatory circuits via NLRP3-inflammasome activation and subsequent IL-1β production.[5]

Moreover, while published data regarding the risk of patients with hematological malignancies to acquire Covid-19 are discordant, these papers consistently show that these patients have a higher case fatality rate.[6] In addition, case reports of acute leukemia and concomitant Covid-19 are increasingly reported. Especially newly diagnosed, refractory or relapsed cases pose a challenge, as these patients often need immediate therapy. However, diagnostic work-up prior to therapy initiation is essential for the selection of the optimal patient-tailored therapy. For example, for acute myeloid leukemia, besides a complete blood count, flow cytometric analysis and a bone marrow smear, genetic work-up including molecular genetics is indispensable for proper classification and risk assessment. Indeed, the American Society of Hematology recommends starting with induction chemotherapy in eligible patients regardless of Covid-19 status. Thus, knowledge about the influence of viral infections in general and Covid-19 specifically, on molecular analyses and their dynamics during fulminant infection would help to better manage and interpret findings of such newly diagnosed/relapsed/refractory leukemia patients. However, to the best of our knowledge there are currently no reports on the dynamics of aberrations detected during acute (viral) infections.

Against this background, we herein investigated the prevalence and clinical impact of CH in Covid-19. Moreover, we analyzed clonal size during the disease and upon its resolution.

In total, 169 patients were screened for participation in this study. Nine patients turned out to be SARS-CoV-2 PCR negative, and thus were excluded from further analysis. Of the remaining 160 patients, 43 patients were included during hospitalization due to Covid-19 (median time to blood test for CH analysis: 24 days; range: 0–57 days), 117 patients, who were either treated as outpatients or also being hospitalized during the acute phase, were included when they came for follow-up evaluation performed between 31–119 days (median: 56 days) after initial diagnosis of Covid-19 (Figure S1). Eight patients were analyzed at two or more time points. Both trial protocols were approved by the institutional review board at Innsbruck Medical University (EK-Nb: 1091/2020 and 1103/2020). Informed consent was obtained from each patient. Laboratory analyses and complete blood count were assessed by standard methods as part of patient care at the Medical University of Innsbruck.

Detection of CH variants was performed by next generation sequencing on MiSeq devices using the TrueSight Myeloid sequencing panel from Illumina. For data analysis, the SeqNext software of JSI Medical Systems was utilized. Only coding variants not frequent in the general population with a variant allele frequency (VAF) between 2% and 45% were included in the dataset. Further details on genetic analysis are given in the supplemental file.

Statistical analysis was performed using GraphPad Prism software. In order to assess correlation between disease groups and CH, chi-square test was used. For comparison between disease groups, two-sided Mann–Whitney *U* test was used, because of non-normal variable distributions as assessed by Shapiro–Wilk test. p values <0.05 were considered significant.

The median age of the study cohort is 57 years with 62% male individuals. Further relevant baseline characteristics of our study cohort are shown in Table S1. To characterize the interplay between CH, inflammation and Covid-19 course, we divided our patient cohort into "severe Covid-19" and "non-severe Covide-19" based on the following criteria: Patients, requiring ≥2 L/min of supplemental oxygen support at any given time point were classified as "severe Covid-19." As shown in Figure S2, the severity of Covid-19 is related to hyperinflammation, as "non-severe Covid-19" patients had lower CRP and IL-6 levels than "severe Covid-19" patients.

Note, CH was present in 31 patients (19.4%), with a total of 46 variants affecting 11 genes (Figure 1(A)). Variants affecting

DNMT3A and *TET2* were the most abundant followed by various other mutated genes at much lower frequencies (Figure S3). Nine patients (29%) had more than one variant (Figure S2C). Compared to studies utilizing genome-wide or exome-wide sequencing approaches the prevalence of CH is higher in our study cohort.[4] As previously discussed by Buscarlet et al., this is probably due to the higher sensitivity of targeted resequencing.[7] However, the age and gene distribution of CH in our study cohort resembles the previously published frequencies very well. Higher percentages of patients with CH were found among higher age groups, except for those aged 80 years and higher, consisting only of only six patients, thus making interpretations limited (Figure 1(B)).

Of the study cohort, 99 patients (61.9%) had non-severe Covid-19 while 61 (38.1%) had severe Covid-19 according to our criteria. Of the latter, 37 patients (23.1%) had to be admitted to an intensive care unit due to respiratory insufficiency (Table S1). However, the presence of CH was not associated with severe Covid-19 (Figure 1(C); *p* value: 0.678), which complements previously published data.[8] In fact, median VAF was even significantly higher in the "non-severe COVID-19" group (median: 5.5% vs. 2.7%; Figure 1(D)). Thus, in contrast to previous studies linking higher VAFs (especially ≥10%) to a higher risk of hematological as well as cardiovascular disease risk, which was mainly explained by CH-mediated inflammatory imprinting in circulating leukocytes, CH appears to be irrelevant for the severity of Covid-19.[3] In addition, we could not define a certain pattern of variants associated with Covid-19 in general or with severe Covid-19 (Figure 1(E)). The representative character of our patient cohort is supported by the known association of both, male gender and cardiovascular co-morbidity with "severe Covid-19." However, when we only analyzed CH positivity in these subgroups, no link to disease severity was detected (Table S1).

Of interest, therapy with a human monoclonal antibody targeting IL-1β (canakinumab) in Covid-19 has been reported to cause a reduction in inflammation, and an improved respiratory function with reduced oxygen need, implicating a potential role of IL-1β in severe Covid-19.[9] However, a randomized trial utilizing another IL-1β neutralizing drug (Anakinra) did not show any benefit.[10] Thus, the pathogenic role of IL-1β for severe Covid-19 as well as therapeutic neutralization as potential treatment needs to be further determined. Though the NLRP-3 inflammasome has been linked to CH and vascular risk, we here claim that CH is not a driving force of hyperinflammation, in particular not of NLRP-3/IL-1β induction. Accordingly, a broad inflammatory range with multiple signals has been characterized in patients suffering from severe Covid-19, where IL-1β was not among the biomarkers found to be associated with mortality.[11]

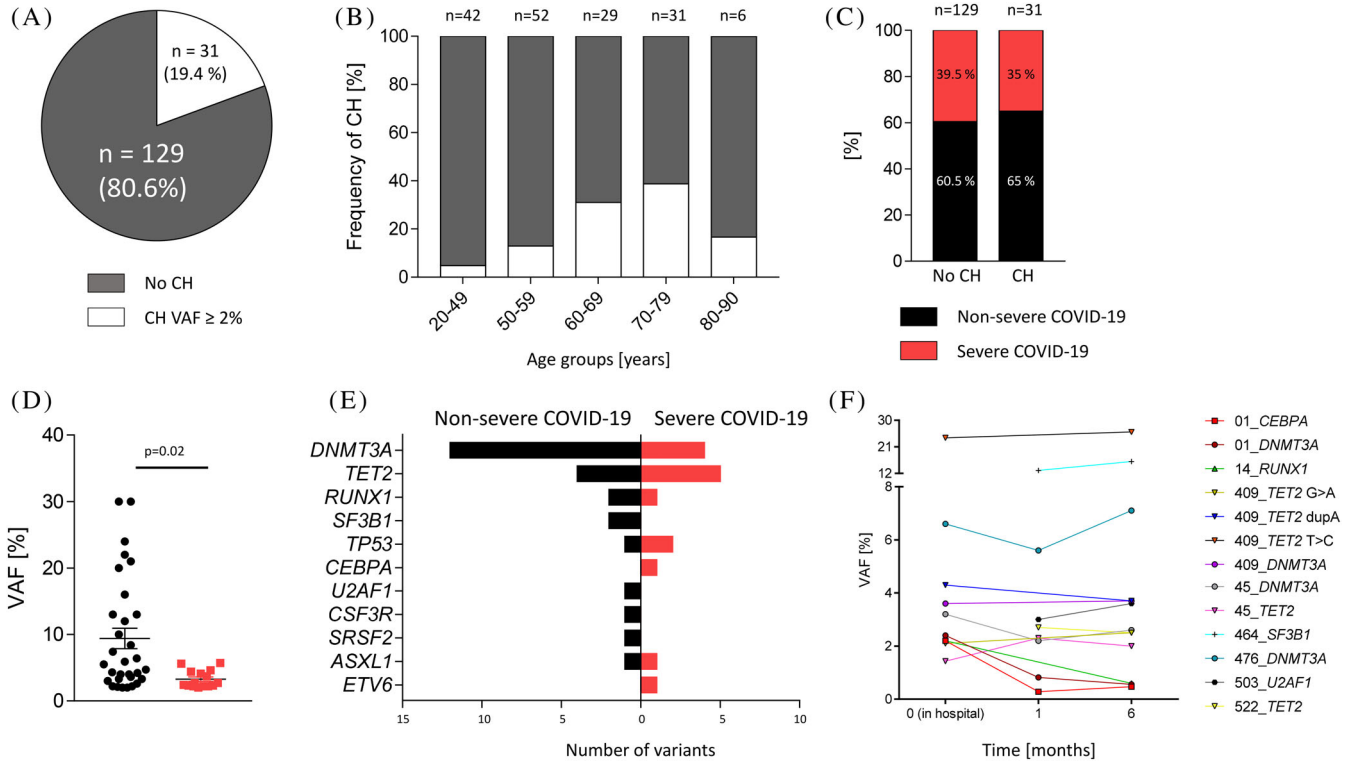

FIGURE 1 Prevalence, dynamics and outcome of patients with clonal hematopoiesis (CH) and Covid-19. (A) Number and proportion of patients affected by CH . (B) Frequency of CH among age groups in Covid-19 patients. (C) Number and proportion of patients suffering from non-severe or severe Covid-19. Statistical significance was calculated using chi-square test; *p* value: 0.610. (D) Variant allele frequencies (VAF) of 46 variants detected in patients with non-severe ($n = 20$) or severe Covid-19 ($n = 11$). Statistical significance was calculated using two-sided Mann–Whitney *U* test because of non-normal variable distributions as assessed by Shapiro–Wilk test. (E) Contribution of individual mutated genes to the spectrum of CH detected in individuals with Covid-19. Black bars indicate a non-severe Covid-19 course, red bars a severe Covid-19 course. Bars indicate the number of variants among all CH-positive patients. (F) Dynamics of variant allele frequency in eight patients during a six-month follow-up period

Next, we analyzed the dynamics of VAF of 15 variants in eight individuals (Figure 1(F)). Nine of these variants (60%) could be reproduced in all analyzed samples. Four additional variants (27%) could be reproduced in all samples after manual inspection. In these cases, the variants were filtered during data analysis pipeline because of low VAF or coverage. Only two variants (13%) could not be reproduced. As shown in Figure S4, blood counts changed over time according to disease status, which had no obvious influence on the VAF of the detected aberrations.

In summary, although the study has a limited sample size, we demonstrate that CH is neither over-represented in Covid-19, nor does it influence disease severity, even though CH has been linked to an inflammatory imprinting phenotype in leukocytes. Moreover, Covid-19 does not modulate the clonal composition in affected individuals, as in patients with CH the VAF is stable during the course of the disease and its resolution.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Verena Petzer, Simon Schwendinger, David Haschka and Verena Vogi performed research. Verena Petzer, Simon Schwendinger, David Haschka, Verena Vogi, Piotr Tymoszuk, Dominik Wolf and Emina Jukic performed data analysis. Verena Petzer, David Haschka, Francesco Burkert, Sabina Sahanic, Thomas Sonnweber, Rosa Bellmann-Weiler, Judith Loeffler-Ragg, Ivan Tancevski recruited patients. Verena Petzer, Simon Schwendinger, David Haschka, Dominik Wolf and Emina Jukic wrote the manuscript. Johannes Zschocke, Guenter Weiss, Dominik Wolf and Emina Jukic supervised the study. All authors read and corrected the manuscript.

PATIENT CONSENT

Informed consent was obtained from each patient.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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