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Impact of clonal hematopoiesis in COVID-19 patients at high risk for adverse clinical outcomes

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Abstract

Purpose Clonal hematopoiesis (CH) describes the aging-associated expansion of mutant hematopoietic cell populations. In various cohorts, CH has been associated with increased morbidity and mortality from non-hematologic diseases such as cardiovascular disease and infections, including COVID-19. Comorbidities placing individuals at risk of complications from these disorders, such as diabetes, also increase in prevalence with age and frequently co-exist with CH. How CH interacts with other aging-associated comorbidities to impact human health remains unknown.

Methods We assessed the impact of CH on the pre-existing end-organ damage and ultimate clinical outcomes among 242 patients hospitalized with COVID-19 at Michigan Medicine from March to June of 2020. In contrast to most previous studies, these patients skewed older with the majority having multiple comorbidities, which placed them at higher risk for end-organ damage and poor clinical outcomes.

Results Overall CH was not significantly associated with increased COVID-19 mortality after controlling for other risk factors, although we did note a borderline-significant association specifically for non-*DNMT3A* CH mutations. In contrast, we observed a significant association between CH and pre-existing chronic kidney disease (CKD), which was strongest for *DNMT3A* mutant CH.

Conclusions These data suggest that the clinical impact of CH is influenced by the specific gene(s) mutated and is further modified by other comorbidities and clinical risk factors frequently present in the elderly.

Keywords Clonal hematopoiesis, COVID-19, Chronic kidney disease

Introduction

Clonal hematopoiesis (CH) is an aging-associated phenomenon which describes the expansion of hematopoietic cell populations harboring somatic mutations in specific genes [1, 2]. Patients with CH are not only at increased risk for the development of hematologic malignancy but also experience increased morbidity and mortality from non-hematologic diseases, including cardiovascular disease [2, 3], COPD [4, 5], and infection [6, 7]. The etiology of these non-hematologic complications from CH remains under investigation but is thought to result from aberrant hyperinflammatory signals arising from the mutant hematopoietic clone

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[8]. As comorbidities such as hypertension and diabetes also increase in prevalence with age, they frequently co-exist with CH. Similar to CH, these comorbidities are associated with aberrant inflammation [9, 10] and place patients at increased risk of end-organ damage [11, 12]. The clinical impact of CH on patients with comorbidities, who are already at high risk for end organ damage and poor clinical outcomes, remains unknown.

COVID-19 is caused by infection with SARS-CoV-2 and has resulted in significant morbidity and mortality with severe cases resulting in pneumonia, acute respiratory distress syndrome, multiple organ failure, and death [13, 14]. Risk factors for increased COVID-19 severity include age, diabetes, hypertension, and obesity. Thus, patients experiencing significant complications from COVID-19 infection would be expected to have high incidences of both CH and co-existing comorbidities such as hypertension and diabetes. We hypothesized that the presence of CH may have a significant impact on the pre-existing end-organ damage present in such high-risk patients and on their ultimate clinical outcomes. We therefore sought to determine the frequency and clinical consequences of CH in patients presenting with complications of COVID-19 severe enough to be hospitalized.

Methods

This study was approved by the University of Michigan institutional review board (HUM00181188). We identified patients hospitalized at Michigan Medicine with COVID-19 from March 2020 to June 2020 with available peripheral blood buffy coat samples. Analyzed patients were > 35 years of age and without the diagnosis of a hematologic malignancy likely to have peripheral blood involvement. Genomic DNA was isolated from peripheral blood buffy coat samples (Qiagen QIAcube; Venlo, Netherlands). Next-generation sequencing was performed using single molecule Molecular Inversion Probes (smMIPS) as previously described [15]. Briefly, the smMIPS capture panel targeted the coding exons (± 5 bp) of 11 genes commonly mutated in age-related clonal hematopoiesis with recurrently mutated hotspots targeted in 4 additional genes (Additional File 1: Table S1). Sequencing reads were aligned to the human reference genome (build 37) with bwa mem [16], and a custom sequencing pipeline (https://github.com/kitzmanlab/mimips) was used for post-alignment processing. Somatic single nucleotide polymorphisms (non-synonymous) and indels within the target space with a minimum variant allele frequency (VAF) of 2.0% were called using LoFreq 2.1.3.1 [17]. Variants were excluded if the sample with the largest VAF had < 5 supporting reads, if they were present at a VAF ≥ 40% (excluding *DNMT3A* R882 mutations), or if, upon blinded manual examination, they were determined to have a VAF similar to samples in which the variant was not called. Somatic mutation calls are listed in Additional File 1: Table S2.

Statistical methods

We fit univariate and multivariate logistic regressions to predict the association of CH with four clinical variables: COVID-19 mortality, pulmonary disease, cardiovascular disease, and chronic kidney disease. We first explored univariate models of raw associations between CH and each variable. We then built multivariate models (Additional File 1: Tables S3-S10) including as covariates demographic information including age (years), sex, and electronic health-recorded ancestry (European, African, or other), and relevant clinical features. For COVID-19 mortality, these covariates included the presence of cardiovascular disease, diabetes, and high BMI (> 30 kg/m^2). Cardiovascular disease was defined as the diagnosis of atherosclerosis or the occurrence of a myocardial infarction, percutaneous intervention, or coronary artery bypass grafting. It also included the diagnosis of congestive heart failure or previous placement of an implantable cardioverter defibrillator. Pulmonary disease was defined as chronic obstructive pulmonary disease (emphysema or chronic bronchitis) or asthma of moderate persistent grade or higher. Patients were characterized as having a history of malignancy if they carried a diagnosis of a malignant cancer not including non-melanomatous skin cancer. For pre-existing pulmonary disease, covariates were the presence of cardiovascular disease and tobacco use (ever smoked). For pre-existing cardiovascular disease, covariates were the presence of pulmonary disease, diabetes, and high BMI. Finally, for pre-existing CKD, covariates were the presence of cardiovascular disease and diabetes. Due to its high degree of overlap with both CKD and cardiovascular disease, hypertension was not included as a covariate in multivariate model creation. For the COVID-19 mortality and CKD associations, we built a second multivariate model in which CH positivity was stratified by the specific gene mutated at the highest VAF (*DNMT3A* or other). We used the same set of selected covariates as in the overall CH models. To protect against bias from the lower number of patients with CH at higher VAF thresholds, all covariate adjusted models with 5.0% as the VAF threshold were Firth corrected [18] using the logistf package in R. The substantive results of models using a 2.0% VAF threshold were not altered by a Firth correction, so for those analyses we present simple logistic regression estimates. All statistical analyses were conducted in R (version 4.2.1).

Results and discussion

Among COVID-19 patients hospitalized at Michigan Medicine and meeting inclusion criteria, we identified 242 with available peripheral blood specimens suitable for genetic analysis, with demographic characteristics shown in Table 1. These patients were largely elderly with a median age of 63.5 (range 36–89) and with a majority having pre-existing chronic medical conditions including diabetes (60.7%) and obesity (50.4%). A significant number exhibited evidence of pre-existing end-organ damage such as cardiovascular disease (41.3%), pulmonary disease (23.1%), and CKD (30.2%). In addition to supportive care, 49.6% of patients received anti-inflammatory therapy, defined as either steroid treatment (above the equivalent of 20 mg prednisone daily for at least 2 days) or anti-IL6 therapy, and 10.3% of patients received remdesivir.

Using ultra-deep targeted sequencing (Methods), we identified CH mutations with a VAF ≥ 2.0% in 57 of the 242 patients (23.6%), with 19 (7.9%) having more than one mutation (Fig. 1A and Additional File 1: Table S2). Consistent with previous population-level studies of aging-related clonal hematopoiesis [1, 2, 19], older patients had higher odds of a CH mutation (per year of age OR = 1.09, 95%CI = [1.06–1.13], $P < 0.001$; Additional File 2: Fig. S1A), and most mutations occurred in *DNMT3A* (Fig. 1B). The percentage of patients with CH trended higher among females ($P = 0.12$) and individuals of European ancestry ($P = 0.25$) when compared to males and individuals of African ancestry (Additional File 2: Fig. S1B). These differences were consistent with the former groups being on average older, reflecting the higher age-adjusted risk of having severe complications of COVID-19 in the latter groups [20, 21]. *DNMT3A* remained the most highly mutated gene regardless of sex (Additional File 2: Fig. S1C) or ancestry (Additional File 2: Fig. S1D).

Table 1 Sample characteristics of hospitalized COVID-19 patients ($N = 242$)

	Median (SD) or % (n)
Age (years)	63.5 (13.1)
Male	58.7 (142)
Ancestry	
African	42.6 (103)
European	41.7 (101)
Other or unreported	15.7 (38)
Tobacco use (ever)	
No	39.7 (96)
Yes	32.6 (79)
Unknown	27.7 (67)
BMI (> 30 kg/m^2)	50.4 (122)
Diabetes	60.7 (147)
History of malignancy	18.2 (44)
COVID-19 mortality (40 days)	18.6 (45)
COVID-19 mortality (100 days)	22.7 (55)
Pulmonary disease	23.1 (56)
Cardiovascular disease	41.3 (100)
Chronic kidney disease (eGFR < 60)	30.2 (73)
CH (VAF ≥ 2.0%)	
Overall	23.6 (57)
DNMT3A dominant	14.5 (35)
CH (VAF ≥ 5.0%)	
Overall	11.6 (28)
DNMT3A dominant	6.6 (16)

We first assessed how CH impacted COVID-19 outcomes in this high-risk population. In observational studies during the early pandemic, most COVID-19 mortality occurred within 40 days of the initial diagnosis [22, 23]. In this cohort, 45 patients (18.6%) died within 40 days of their initial hospitalization at Michigan Medicine with 55 (22.7%) dying within 100 days of this date (Additional File 2: Fig. S2A). Chronological age was the primary risk factor for COVID-19 mortality within 40 days, with the odds of death increasing by 7% per year of age (95%CI = [1.04–1.10], $P < 0.001$). In this cohort, the use of anti-inflammatory or remdesivir therapy was not statistically associated with either the incidence of CH or COVID-19 mortality (data not shown). Although mortality within 40 days was higher in patients with CH (OR = 2.70, 95%CI = [1.35–5.39], $P < 0.01$; Fig. 1C-D; Additional File 1: Table S3), after a multivariate analysis which adjusted for demographics, including age, and comorbidities (cardiovascular disease, diabetes, and high BMI), CH was no longer significantly associated with overall mortality (OR = 1.65, 95%CI = [0.73–3.75], $P = 0.23$; Fig. 1D; Additional File 1: Table S3). We then grouped individuals by which CH gene was mutated at the highest VAF, with *DNMT3A* mutant CH in one group and all other genes combined in the other (due to the lower prevalence of mutations in non-*DNMT3A* genes). While the presence of *DNMT3A* mutant CH was not associated with COVID-19 mortality within 40 days, we did observe a higher rate of COVID-19 mortality among patients with non-*DNMT3A* mutant CH in both univariate (OR = 5.85,95%CI = [2.31–14.8],$P < 0.001$; Fig. 1C-D; Additional File 1: Table S4; Additional File 2: Fig. S2B) and multivariate analysis (OR = 3.03; 95%CI = [1.04–8.84]; $P < 0.05$; Fig. 1D; Additional File 1: Table S4). We observed a similar trend with COVID-19 mortality within 100 days of initial hospitalization at Michigan Medicine (Additional File 1: Table S5).

Prior studies have provided conflicting data regarding the impact of CH on COVID-19 outcomes. Multiple identified no association between CH and COVID-19 severity or mortality [24–28]. In contrast, others have

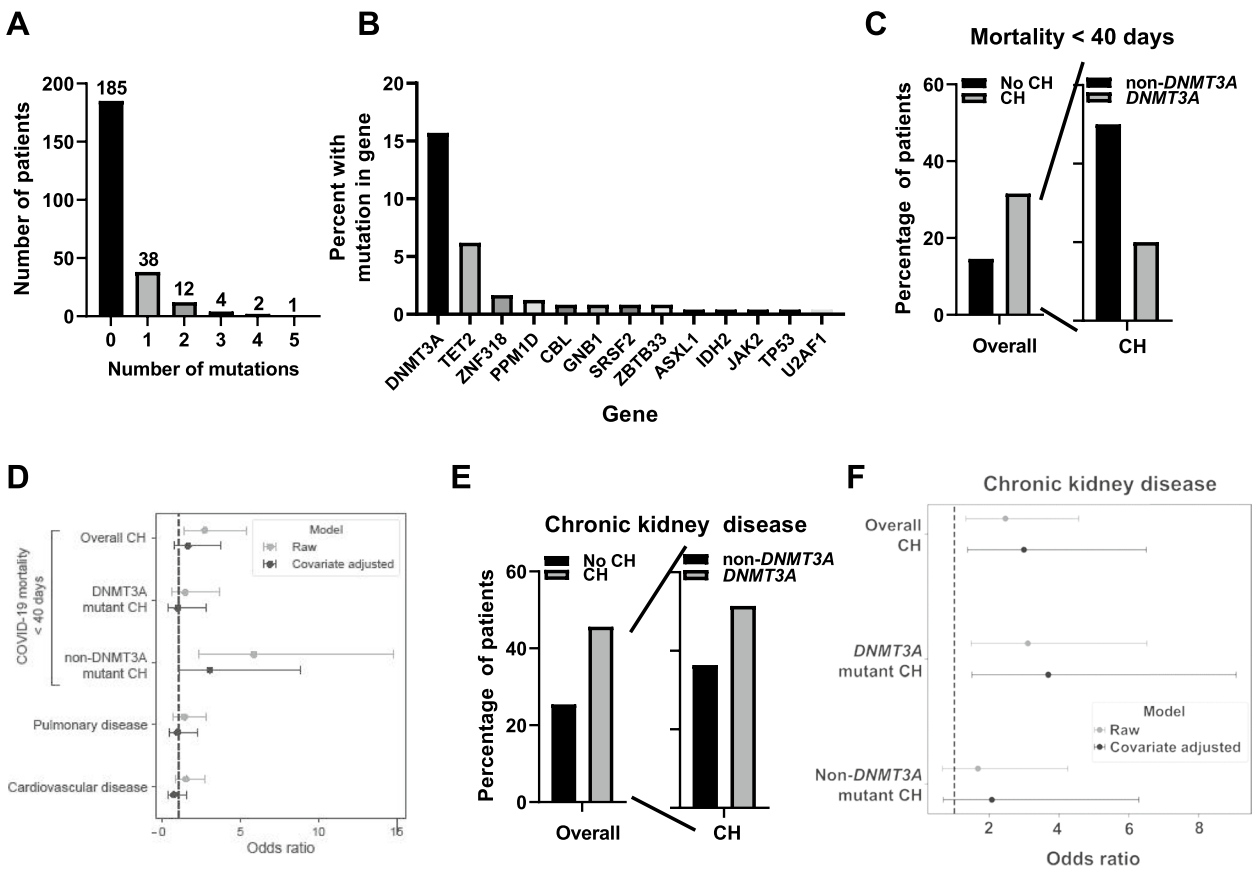

Fig. 1 Clonal hematopoiesis in hospitalized COVID-19 patients. **A** Number of patients with CH at a VAF ≥ 2.0% with the number of mutations per patient identified (x-axis). **B** Percentage of patients with CH in specific genes. **C** Percentage of patients with mortality within 40 days of COVID-19 hospitalization stratified by the presence ($n = 57$) or absence ($n = 185$) of CH, CH with the highest VAF mutation in *DNMT3A* ($n = 35$), or CH with the highest VAF mutation in a gene other than *DNMT3A* ($n = 22$). **D** Forest plot displaying odds ratios (ORs) and 95% confidence intervals (CIs) predicting mortality within 40 days of initial COVID-19 hospitalization from overall CH, *DNMT3A* mutant CH, and non-*DNMT3A* mutant CH measured at VAF ≥ 2.0%. Overall and gene-specific CH results were derived from the same model. Odds ratios predicting pre-existing cardiovascular and pulmonary disease from overall CH at VAF ≥ 2.0% are also shown. Results from both raw (gray) and covariate adjusted (black) logistic regression models are shown. The dashed vertical line indicates the threshold for no effect from CH. **E** Percentage of COVID-19 patients with pre-existing CKD stratified by the presence or absence of CH, CH with the highest VAF mutation in *DNMT3A*, or CH with the highest VAF mutation in a gene other than *DNMT3A*. **F** Forest plot displaying odds ratios (ORs) and 95% confidence intervals (CIs) predicting CKD from overall CH, *DNMT3A* mutant CH, and non-*DNMT3A* mutant CH measured at VAF ≥ 2.0%. Overall and gene specific CH results were derived from the same model. Results from both raw (gray) and covariate adjusted (black) logistic regression models are shown. The dashed vertical line indicates the threshold for no effect from CH

linked CH with enhanced COVID-19 severity. Bolton et al. identified a 1.85-fold increased odds of severe COVID-19 infection among individuals with CH in a population of solid tumor patients and cancer-free controls [6]. Analyzing large population-based studies such as the UK Biobank and FinnGen, Zekavat et al. identified a 1.63-fold increased odds of severe COVID-19 infection in patients with mosaic chromosomal abnormalities (mCAs) [7]. In the study by Bolton et al., patients with malignancy comprised a significant percentage of the cohort. Such patients often have been exposed to significant genotoxic stress with an increased incidence of CH mutations less commonly observed with normal aging [29]. Indeed, in this study most of the increased COVID-19 severity linked to CH was associated with non-putative driver mutations. Interestingly, in our cohort, 44 individuals (18.2%) had a history of malignancy. Of these, 7 had *DNMT3A* mutant CH, and 7 had non-*DNMT3A* mutant CH. Although the numbers are too small to draw definitive conclusions, patients with a history of malignancy mirrored the overall cohort with 6/30 (20%) individuals without CH, no individuals with *DNMT3A* mutant CH, and 3/7 (42.9%) individuals with non-*DNMT3A* mutant CH dying within 40 days of their

initial COVID-19 hospitalization. Likewise, the study by Zekavat et al. defined CH by the presence of mCAs, which frequently occur in individuals lacking the single nucleotide variant (SNV)/indel mutations identified in most CH studies [30].

One possible explanation for these discrepancies is that any true association between CH and COVID-19 severity could be influenced by the type of mutation (e.g., copy number vs point variation) or gene(s) mutated, similar to the CH locus heterogeneity observed with hematologic cancer risk [31, 32] and body mass associations [19]. Indeed, analyzing the UK Biobank, Kessler et al. identified a significant association between COVID-19 severity and *PPM1D* mutant CH, but CH involving other genes did not reach statistical significance [19]. Determining how mutations in specific genes, particularly those mutated less frequently in CH, affect COVID-19 severity in high-risk patients will require larger cohort sizes.

Mouse models of CH have shown mutant myeloid populations to have an aberrant hyperinflammatory signature with abnormal cytokine signaling [3, 33–35]. Baseline elevations in markers of systemic inflammation have also been observed in individuals with CH [36]. As severe COVID-19 infection has been associated with elevated hyperinflammatory signaling [37], we assessed if CH contributed to this process. Consistent with a previous study [24], the presence of CH was not associated with acute elevations in systemic blood inflammatory markers including C-reactive protein, procalcitonin, ferritin, d-dimer, and fibrinogen either in average value or variability (Additional File 2: Fig. S3). CH was also not associated with the leukocytosis frequently observed in severe COVID-19 infection. Thus, mutant hematopoietic clones do not appear to be responsible for the systemic acute hyperinflammation associated with severe COVID-19 infection. It remains to be seen whether CH contributes to more localized tissue-associated inflammatory signaling and its resultant end-organ damage.

We then assessed if CH was associated with an increased risk of pre-existing end-organ damage in patients already at high risk for such complications. In a cohort not enriched with co-existing comorbidities (the MDC study), Jaiswal et al. reported individuals with CH to have an increased risk of cardiovascular disease with an odds ratio of 2.0 [3]. We did not observe a significant association between CH and pre-existing cardiovascular disease in our higher risk patient population after adjusting for demographic and clinical covariates (Fig. 1D; Additional File 1: Table S6). Likewise, we found no significant association between CH and pre-existing pulmonary disease (Fig. 1D; Additional File 1: Table S7). This is likely due to the degree of clinical heterogeneity in cardiovascular and pulmonary conditions carried by patients in this cohort (Methods), with larger and more clinically uniform cohorts necessary to recapitulate these previously identified associations [2–5].

Finally, we investigated the association between CH and pre-existing CKD. We characterized patients as having CKD if they both carried the chart diagnosis of this disease and had a baseline eGFR estimated to be < 60 ml/min prior to COVID-19 diagnosis or the previous receipt of a kidney transplant. The determination of which patients had CKD was made without knowledge of their CH status. Among the 242 patients sequenced, we identified 73 (30.2%) as having pre-existing CKD using these criteria. Unsurprisingly, CKD was significantly associated with the diagnoses of either hypertension or diabetes. CKD was also associated with African (when compared to European) ancestry and male sex (Additional File 1: Tables S8-S10). This is consistent with the former being at increased risk of CKD independent of other known risk factors [38] and the latter being at higher risk of worsening renal function once diagnosed with CKD [39]. In this high-risk population, overall CH nearly tripled the odds of CKD even after correcting for demographics and the risk factors of cardiovascular disease and diabetes (OR = 2.99, 95%CI = [1.38–6.50], $P < 0.01$; Fig. 1E-F; Additional File 1: Table S8). Notably, when splitting by the gene carrying the highest VAF mutation (*DNMT3A* or all other tested genes grouped together), a significant association was limited to *DNMT3A* mutant CH (OR = 3.69, 95%CI = [1.50–9.08], $P < 0.01$; Fig. 1E-F); Additional File 1: Table S9). Similar results were also obtained using the more stringent VAF ≥ 5.0% threshold (Additional File 1: Table S10). Resolving whether this reflects differential biology versus lessened statistical power given that CH mutations in *DNMT3A* are more common than in other genes combined will require future studies. Indeed, some rarer hematopoietic mutations have also been associated with reduced renal function [40].

Previous studies have also provided conflicting data regarding the association between CH and the development of CKD. Vlasschaert et al. provided evidence that, in patients already diagnosed with CKD, the presence of CH is associated with the continued worsening of renal function [41]. Population-based studies, primarily involving individuals at lower risk of CKD, have identified more modest associations between CH and CKD. In the UK Biobank, Dawoud et al. identified a small increase the prevalence of CKD among individuals with CH (OR = 1.02 when using cystatin-C to determine eGFR) [42]. Utilizing data from the TOPMed program, Kestenbaum et al. found individuals with CH to be at modestly increased risk (OR = 1.17) of a 30% eGFR decline [43]. Finally, analyzing the PROVALID cohort, Denicolo et al. did not an find an association between

CH and the development of CKD among diabetic patients [44]. Interestingly, with a CKD prevalence of 30.2%, our patient cohort had more similarities with the CKD cohort assessed by Vlasschaert et al. than with the population-based UK Biobank and TOPMed studies or the PROVALID cohort (which had a CKD prevalence of 4.5%). Thus, in low-risk individuals or individuals with high-risk features that are optimally being treated, CH may have a minimal impact on the development of kidney disease. However, in particularly high-risk patients or individuals with pre-existing renal injury, CH may promote further renal decline. In such cases, it is possible the mutant hematopoietic clone directly contributes towards kidney dysfunction given the recently identified role of aberrant inflammation in the pathogenesis of CKD [45, 46]. Our finding that CH (in particular *DNMT3A* mutant CH) significantly increases the risk of CKD in individuals already at high risk of this disease remains limited by the relatively small size of our patient cohort and its clinical heterogeneity and will need to be replicated on larger, more clinically uniform cohorts. Whether individuals at high risk for future renal dysfunction (e.g. the presence of poorly controlled high-risk comorbidities, albuminuria, or mild eGFR decline) should be routinely screened for the concurrent presence of CH also remains an unanswered question. Finally, the degree to which CH predisposes individuals with primary kidney disorders to the development of CKD remains to be determined.

In this study, we assessed the frequency of CH in a population of hospitalized COVID-19 patients. Due to the nature of this disease, these patients were predominantly elderly with multiple comorbidities. This provided a unique population in which to investigate the clinical consequences of clonal hematopoiesis, with many patients carrying pre-existing conditions that already placed them at high risk for the diseases and complications associated with CH. In sum, we found no significant association between overall CH and COVID-19 mortality after adjusting for age and other risk factors. This is consistent with results from previous studies in which aging-associated CH has often not been associated with worsened COVID-19 outcomes [24–27]. In contrast, the studies linking CH to increased COVID-19 severity may have been enriched for less common CH mutations, leaving open the possibility of gene-specific effects [6, 7, 19]. Consistent with the latter, we observed a borderline-significant association between non-*DNMT3A* mutant CH and COVID-19 mortality among high-risk patients, which will require independent replication in future studies. Additionally, within our higher risk cohort, we observed a significant association between CH and pre-existing CKD, with the effect appearing to be largely driven by mutations in *DNMT3A*. These results suggest that CH may promote further renal decline in patients already at very high risk for chronic renal dysfunction. Overall, these data suggest that the clinical impact of CH is influenced by the specific genes mutated and is further modified by other comorbidities and high-risk features frequently present in the elderly. Understanding how CH interacts with these comorbidities to impact human disease may lead to improved risk stratification and preventative therapeutics.

Abbreviations

CH	Clonal hematopoiesis
CI	Confidence interval
CKD	Chronic kidney disease
OR	Odds ratio
mCA	Mosaic chromosomal abnormality
smMIPS	Single molecule Molecular Inversion Probes
SNV	Single nucleotide variant
VAF	Variant allele frequency

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41231-023-00155-7.

Additional file 1: Table S1. Genes sequenced using custom smMIPS assay. **Table S2.** Identified somatic variants with VAF ≥ 2.0% in COVID-19 patients. **Table S3**. Raw and adjusted associations predicting mortality within 40 days of initial COVID-19 hospitalization at Michigan Medicine in individuals with CH mutations at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting COVID-19 mortality, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. OR = odds ratio. **Table S4**. Raw and adjusted associations predicting mortality within 40 days of initial COVID-19 hospitalization at Michigan Medicine in individuals with CH having their highest VAF mutation in *DNMT3A* versus non-*DNMT3A* genes at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting COVID-19 mortality, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S5**. Raw and adjusted associations predicting mortality within 100 days of initial COVID-19 hospitalization at Michigan Medicine in individuals with CH having their highest VAF mutation in *DNMT3A* versus non-*DNMT3A* genes at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting COVID-19 mortality, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S6.** Raw and adjusted associations predicting pre-existing cardiovascular disease from CH mutations at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting cardiovascular disease, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S7.** Raw and adjusted associations predicting pre-existing pulmonary disease from CH mutations at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting pulmonary disease, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S8.** Raw and adjusted associations predicting pre-existing CKD from CH mutations at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting CKD, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S9.** Raw and adjusted associations predicting pre-existing CKD from CH with their highest VAF mutation in *DNMT3A* versus non-*DNMT3A* genes at VAF ≥ 2.0%. Raw univariate odds ratios reflect individual models predicting CKD, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. **Table S10.** Raw and adjusted associations predicting pre-existing CKD from CH with their highest VAF mutation in

DNMT3A versus non-*DNMT3A* genes at VAF ≥ 5.0%. Raw univariate odds ratios reflect individual models predicting CKD, while covariate adjusted multivariate estimates account for all the listed covariates in one full model. Covariate adjusted estimates are Firth corrected.

Additional file 2: Figure S1. Demographics of hospitalized COVID-19 patients. **Figure S2.** Overall survival of COVID-19 patients from their day of initial hospitalization at Michigan Medicine. **Figure S3.** Elevation of systemic inflammatory markers and leukocytosis in hospitalized COVID-19 patients.

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Authors' contributions
T.N.W., M.A.J., Q.L, C.S., and J.O.K. initiated the project, designed the research, and wrote the paper with input from other authors. T.N.W. and B.B.B. performed the research. T.N.W., C.S., M.A.J., Q.L., and J.O.K. analyzed the data. All authors have read and approved the manuscript.

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Availability of data and materials
Patient data including smMIPS sequencing data have not been publicly released due to privacy concerns. They are available from the authors upon reasonable request dependent upon approval from the University of Michigan institutional review board.

Declarations

Ethics approval and consent to participate
This study was approved by the University of Michigan institutional review board (HUM00181188) with patients enrolled under a waiver of consent.

Consent for publication
This manuscript does not contain identifying images of patients or other personal or clinical details that may compromise patient anonymity.

Competing interests
The authors have no competing or financial conflicts of interest to disclose.

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