Received: 28 November 2023 | Accepted: 13 February 2024

DOI: 10.1002/hem3.58

HemaSphere EHA EUROPEAN HEMATOLOGY ASSOCIATION

LETTER

Clonal hematopoiesis and COVID-19 hospitalization in Danish adults

Celia Burgos Sequeros[1] | Morten Tulstrup[2,3] | Sofie Bliddal[4] | Karina Meden Sørensen[5] | Ioanna Nissen[6] | Omid Rezahosseini[7] | Patrick Terrence Brooks[6] | Bjarke Feenstra[6,8] | Anne Ortved Gang[2,9] | Frank Geller[6,8] | Annemette Hald[10] | Zitta Barrella Harboe[7,9] | Marie Helleberg[10,11] | Jakob S. Jespersen[3,12] | Anne-Mette Lebech[9,10] | Birgitte Lindegaard[7,9] | Trine H. Mogensen[13,14] | Maria Elizabeth Engel Møller[6] | Claus Henrik Nielsen[15] | Carsten Utoft Niemann[2,9] | Daria Podlekareva[16] | Adin Sejdic[7] | Erik Sørensen[6] | Rebecca Svanberg Teglgaard[2,6] | Niels Tommerup[17] | Nina Weis[9,18] | Søren Brunak[1] | Ole Birger Vestager Pedersen[9,19] | Karina Banasik[1,20] | Ulla Feldt-Rasmussen[4,9] | Susanne Dam Nielsen[9,10] | Sisse Rye Ostrowski[6,9] | Kirsten Grønbæk[2,3,9]

Correspondence: Kirsten Grønbæk (Kirsten.Groenbaek@regionh.dk)

Clonal hematopoiesis of indeterminate potential (CHIP) refers to the nonmalignant clonal expansion of blood stem cells that carry somatic mutations in myeloid cancer-associated genes.[1] The main driver of CHIP progression is age, but other factors like smoking or chemotherapy exposure can also have significant effects.[2] CHIP has been associated with an increased risk of hematological malignancies and a range of other age-related conditions, including severe infections and death.[1,3] The basis for this link is suggested to be an altered inflammatory response, which could occur as a result of mutations that impair the regulation of proinflammatory factor secretion from myeloid cells.[4–6]

Over 3 years have passed since the World Health Organization declared the coronavirus disease 2019 (COVID-19) a global

[1]Translational Disease Systems Biology, Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[2]Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[3]Biotech Research and Innovation Center (BRIC), University of Copenhagen, Copenhagen, Denmark
[4]Department of Endocrinology and Metabolism, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[5]Danish National Biobank, Statens Serum Institut, Copenhagen, Denmark
[6]Department of Clinical Immunology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
[7]Department of Respiratory Medicine and Infectious Diseases, Copenhagen University Hospital, North Zealand, Hillerød, Denmark
[8]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark
[9]Department of Clinical Medicine, Faculty of Health and Clinical Sciences, University of Copenhagen, Copenhagen, Denmark
[10]Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[11]Center of Excellence for Health, Immunity and Infections, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[12]The Finsen Laboratory, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[13]Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark
[14]Department of Biomedicine, Aarhus University, Aarhus, Denmark
[15]Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[16]Department of Respiratory Medicine and Infectious Diseases, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark
[17]Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark
[18]Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Copenhagen, Denmark
[19]Department of Clinical Immunology, Zealand University Hospital, Køge, Denmark
[20]Department of Gynecology and Obstetrics, Copenhagen University Hospital, Hvidovre, Copenhagen, Denmark

pandemic. Caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), 773 million infections and 6.9 million deaths have been reported worldwide as of January 2024.[7] The clinical presentation of SARS-CoV-2 infection varies widely, ranging from asymptomatic to more severe responses that result in acute respiratory distress syndrome, intensive care unit (ICU) admission, or death.[8] The hyperinflammatory response that characterizes the severe form of COVID-19,[9] as well as the significantly higher risk of older, more comorbid males to suffer from it,[10] closely resembles the CHIP phenotype and thus has raised the question of whether CHIP might influence COVID-19 severity. Several studies have addressed this,[11–17] but their widely variable study designs, phenotype definitions, and patient cohorts have generated mostly conflicting results.

The current matched case–control study included 470 Danish individuals with a positive SARS-CoV-2 polymerase chain reaction (PCR) test between March 1, 2020 and December 1, 2021. They were selected to be over 60 years of age at the time of the PCR test and to have no record of a hematological malignancy previously associated with CHIP (ICD10:C81-90/92-96) in the Danish National Patient Registry (DNPR). Five individuals with chronic lymphoid leukemia (ICD10:C91.1) were identified, but still included in the selection, as this type of cancer has not been associated with a higher CHIP prevalence.[18] As part of the Danish COVID-19 Genetic Consortium,[19] all participants had an available biobank sample for genetic analysis. Scientific ethics permission for this study was obtained from the Danish National and Capital Region Ethics Committees (NVK-2003947 and H-20026501). Written consent was required from all living individuals. Individuals who died due to COVID-19 before January 6, 2021 were included without consent, as approved by the regional Ethics Committee. More information about the Consortium and the ethical approvals can be found in Supporting Information S2: Methods.

We assessed the association between CHIP and COVID-19 severity in our population using univariable and multivariable logistic regressions. We adjusted the models by including a selection of lifestyle and clinical covariates known to be associated with both CHIP progression and severe COVID-19[10]: body mass index (BMI), smoking status (active smoking vs. smoking in the past or never), diabetes, cardiovascular disease, lung disease, cancer, and prior chemo- and/or radiotherapy treatment. These data were extracted from hospital-administered diagnoses and treatments in the DNPR, self-reported questionnaires,[19] patient records from hospital admissions, the national intensive care unit database, and the COVIMUN study database. More details on participant information retrieval and missing data handling are given in Supporting Information S2: Methods and in Supporting Information S1: Figures 1–3. Statistical analyses were performed using R.[20] Covariate effects can be found in Supporting Information S1: Figures 4 and 5.

To identify CHIP mutations, we performed deep targeted sequencing of peripheral whole blood for 31 genes recurrently mutated in myeloid cancer, using a custom capture-based panel from Twist Biosciences. We limited our analysis to variants with a variant allele fraction (VAF) ≥ 2%. More details regarding CHIP typing are provided in the Supporting Information S2: Methods.

In the primary analysis, we compared 235 individuals who had been hospitalized within 14 days of the positive SARS-CoV-2 PCR test, to 235 with no hospitalization. Hospitalized cases and nonhospitalized controls were matched one-to-one on sex, age at the time of the positive SARS-CoV-2 PCR test (±12 months), and sample age (±14 months), defined as the number of months between the blood sample collection and the positive SARS-CoV-2 PCR test (Figure 1A and Supporting Information S2: Methods). The matching by age and sex was performed due to the known strong associations with both CHIP prevalence and COVID-19 severity. The matching by blood sample age aimed at eliminating any bias that could have arisen from the expansion of CHIP over time, as blood samples were collected over several years at the biobanks. We chose to further adjust the regressions for blood sample age to account for any residual effects of the variable; sex and age were considered sufficiently matched for additional adjustments to be necessary (Supporting Information S1: Figures 6 and 7).

We identified a total of 187 mutations in 143 out of 470 study participants (30.4%). The most frequently mutated genes were *DNMT3A*, *TET2*, *ASXL1*, *PPM1D*, and *TP53* (Figure 1B). CHIP was observed in 81 (34.5%) of the 235 hospitalized cases, and 62 (26.4%) of the 235 nonhospitalized controls. Multiple mutations were found in 22 (9.4%) cases and eight (3.4%) controls. Clones with a VAF ≥ 10% were detected in 32 (13.6%) cases and 18 (7.7%) controls. CHIP mutations within DNA repair genes (*PPM1D* and/or *TP53*) were seen in 13 (5.5%) cases and eight (3.4%) controls. Mutations within *PPM1D*, previously found to be associated with severe COVID-19 in the large study by Kessler et al.,[11] were present in 10 (4.3%) cases and three (1.3%) controls. In the covariate-adjusted model (Figure 1C), presenting multiple CHIP clones—compared to one or none—or large (VAF ≥ 10%) mutation(s)—compared to small (VAF < 10%) or none—was statistically significantly associated with COVID-19 hospitalization. The effects of carrying any CHIP clone, having mutations in DNA repair genes (*PPM1D* and/or *TP53*), or in *PPM1D* specifically were not statistically significant. However, all effect size estimates were positive, suggestive of an overrepresentation of the different CHIP phenotypes among hospitalized COVID-19 patients.

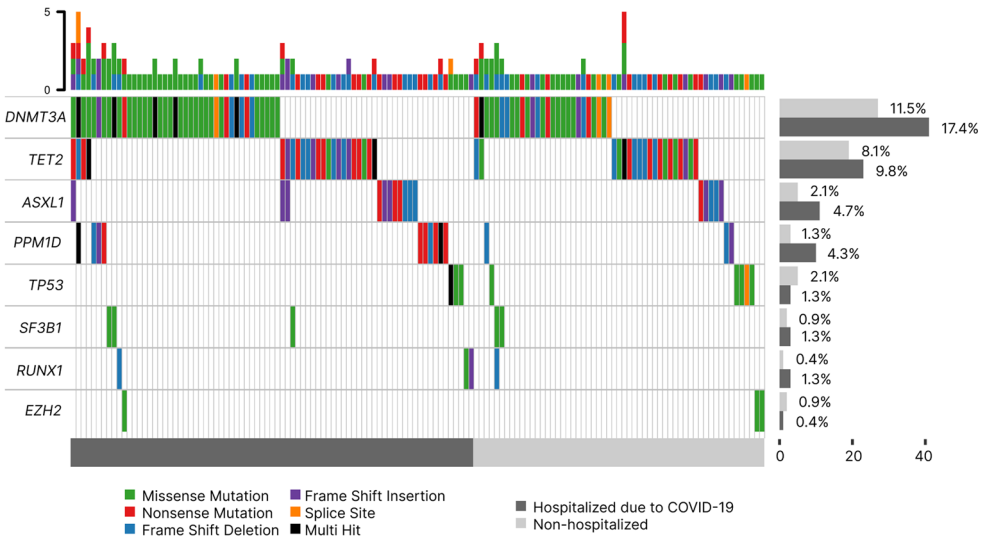
We also performed a secondary analysis on the subset of hospitalized participants, where we compared 123 patients who had received a general admission to 112 who had been further admitted into the ICU within 14 days of the positive test (28 patients) or had died at the hospital within 30 days of the test (84 patients; Figure 2A,B). Importantly, participants' sex, age at the time of the positive SARS-CoV-2 PCR test, and sample age were no longer matched between the two outcome groups in this modified setup, so we included them as covariates in the adjusted regressions. We did not find any statistically significant associations between carrying CHIP, multiple clones, large (VAF ≥ 10%) mutation(s), or mutation(s) in a DNA repair gene, and an ICU admission or in-hospital death, compared to a general admission (Figure 2C). There was only a borderline significant overrepresentation of mutations in *PPM1D* among participants with an ICU admission or an in-hospital death.

In summary, we observed that the risk of COVID-19 hospitalization increased with the presence of multiple or large (VAF ≥ 10%) CHIP clone(s) in this study of 470 Danish individuals, PCR-confirmed positive for SARS-CoV-2 while 60–89 years old. These results are consistent with those of the large study by Kessler et al.,[11] but not of the smaller one by Zhou et al.[13] In the subset of 235 COVID-19 hospitalized cases, we did not find CHIP to be a risk factor for ICU admission or in-hospital death, in contrast to general admission, which is also consistent with findings by Duployez et al.,[14] Hameister et al.,[15] Petzer et al.,[16] Miller et al.,[12] and Del Pozo-Valero et al.[17] However, these results should be interpreted with caution as our study was designed as a matched case–control study between hospitalized and nonhospitalized COVID-19. The previously observed association between *PPM1D* CHIP and severe COVID-19 by Kessler et al.[11] was only suggestively replicated in our data and may grant further investigation. The main limitations of our study include the collection of blood samples across several years, which could bias our measurement of CHIP but is mitigated through

(A)

		Total (n=470)	Hospitalized cases (n=235)	Non-hospitalized controls (n=235)	Missing & imputed (%)
Female participants, n (%)		254 (54)	127 (54)	127 (54)	-
Age at time of positive SARS-CoV-2 PCR test, median (quartiles), in years		70.6 (65.5 – 74.9)	70.7 (65.5 – 75.0)	70.6 (65.4 – 74.8)	-
Blood sample age , median (quartiles), in months		-5.7 (-30.3 – 0.3)	-0.3 (-27.5 – 0.3)	-7.1 (-34.3 – 0.2)	-
BMI, median (quartiles)		26.3 (23.2 – 29.8)	27.3 (23.8 – 32.3)	25.3 (22.8 – 28.4)	25 (5.3)
Active smoking, n (%)		26 (5.5)	13 (5.5)	13 (5.5)	50 (10.6)
Comorbidity, n (%)	Diabetes	90 (19.1)	24 (10.2)	66 (28.1)	-
	Cardiovascular disease or hypertension	303 (64.5)	143 (60.9)	160 (68.1)	-
	Lung disease	125 (26)	49 (20.9)	76 (32.3)	-
	Cancer	97 (20.6)	35 (14.9)	62 (26.4)	-
Prior chemo- or radiotherapy, n (%)		54 (11.5)	38 (16.2)	16 (6.8)	-

(B)



(C)

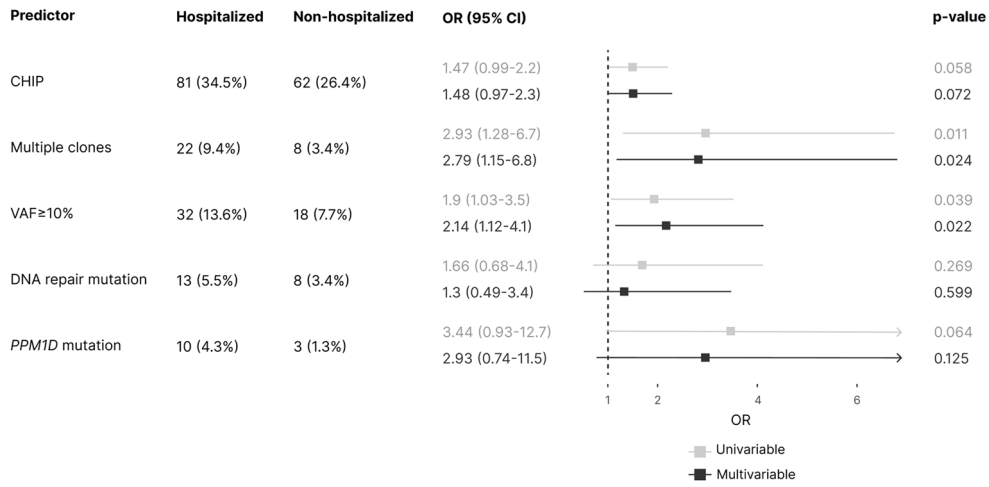
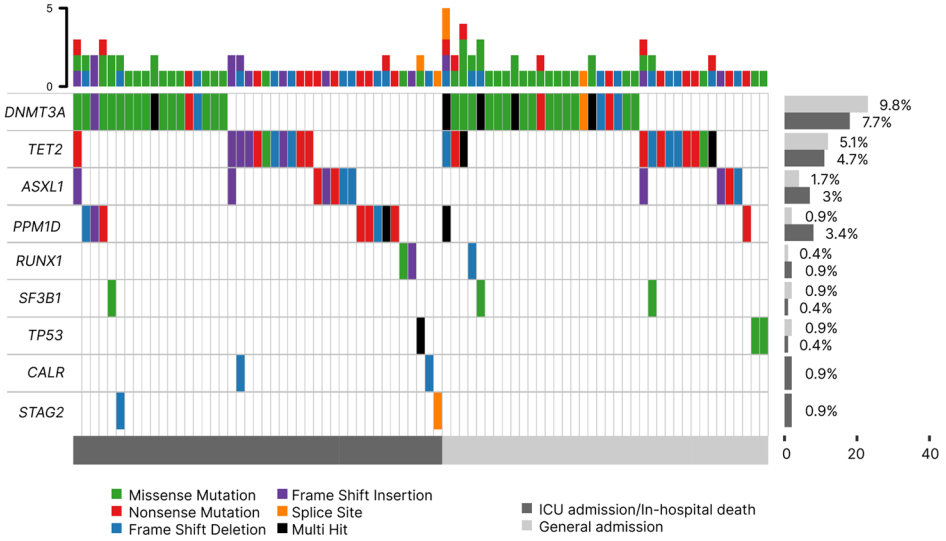


FIGURE 1 Clonal hematopoiesis of indeterminate potential (CHIP) and coronavirus disease 2019 (COVID-19) hospitalization. (A) Characteristics of the matched case–control severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive study participants. Cases and controls were matched by sex, age at the time of the SARS-CoV-2 polymerase chain reaction (PCR) test, and blood sample age. Blood sample age was calculated as the number of months between the blood sample collection and the PCR test. Negative values indicate that the blood sample was taken before the PCR test, and vice versa. (B) Oncoplot of co-mutation patterns and bar plot of mutation prevalence in each CHIP gene. Only genes with a mutation prevalence ≥0.5% are shown. Mutations in the oncoplot are colored by variant type, as displayed in the legend. COVID-19 hospitalizations and nonhospitalizations are represented in dark and light gray, respectively. (C) Associations between CHIP and COVID-19 hospitalizations. CHIP status was evaluated based on the presence of: (1) any CHIP versus no CHIP, (2) multiple mutations versus no CHIP or a single mutation, (3) large (variant allele frequency [VAF] ≥ 10%) clone(s) versus no CHIP or small (VAF < 10%) clones, (4) mutation(s) in a DNA repair gene (*PPM1D* and/or *TP53*) versus no CHIP or mutation(s) elsewhere, and (5) mutation(s) in *PPM1D* versus no CHIP or mutation(s) elsewhere. In gray, the results of the univariable analysis are shown; in black, those of the multivariable analysis adjusted for blood sample age, body mass index, active smoking, diabetes, cardiovascular disease, lung disease, cancer, and prior chemo- and/or radiotherapy treatment. BMI, body mass index; CI, confidence interval; ICU, intensive care unit; OR, odds ratio.

(A)

		Total (n=235)	General admission (n=123)	ICU admission/In-hospital death (n=112)	Missing & imputed (%)
Female participants, n (%)		127 (54)	69 (56.1)	58 (51.8)	-
Age at time of positive SARS-CoV-2 PCR test, median (quartiles), in years		70.7 (65.5-75.0)	69.6 (64.5 – 73.6)	71.9 (67.0 – 75.5)	-
Sample age , median (quartiles), in months		-0.3 (-27.5 – 0.3)	0.1 (-27.9 – 0.4)	-3.1 (-27.3 – 0.2)	-
BMI, median (quartiles)		27.3 (23.8 – 32.3)	26.8 (23.8 – 30.9)	28.4 (23.9 – 33.2)	18 (7.7)
Active smoking, n (%)		13 (5.5)	6 (4.9)	7 (6.3)	44 (18.7)
Comorbidity, n (%)	Diabetes	66 (28.1)	24 (18.5)	42 (40)	-
	Cardiovascular disease or hypertension	160 (68.1)	99 (76.2)	61 (58.1)	-
	Lung disease	76 (32.3)	43 (33.1)	33 (31.4)	-
	Cancer	62 (26.4)	34 (26.2)	28 (26.7)	-
Prior chemo- or radiotherapy, n (%)		38 (16.2)	22 (17.9)	16 (14.3)	-

(B)



(C)

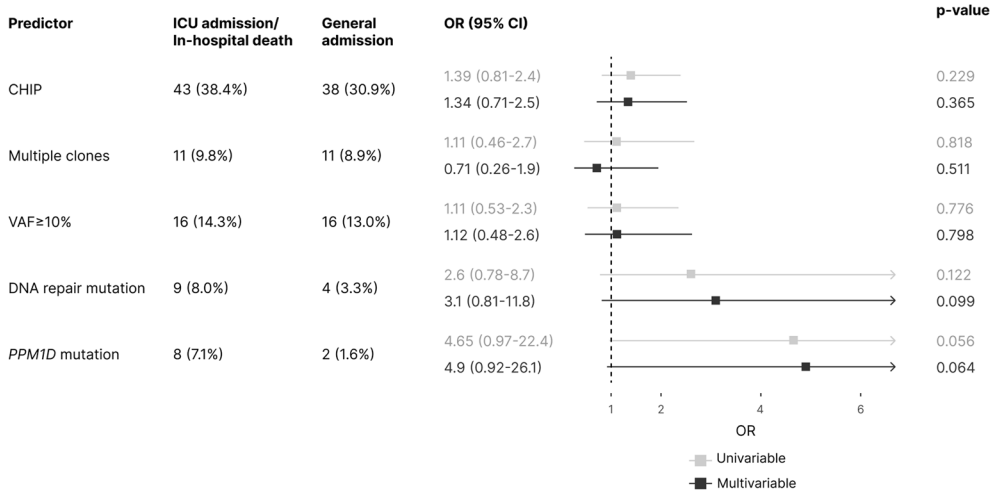


FIGURE 2 Clonal hematopoiesis of indeterminate potential (CHIP) and intensive care unit (ICU) admission or in-hospital death, in coronavirus disease 2019 (COVID-19) hospitalized study participants. (A) Characteristics of the COVID-19 hospitalized study participants, by type of admission. Blood sample age was calculated as the number of months between the blood sample collection and the polymerase chain reaction (PCR) test. Negative values indicate that the blood sample was taken before the PCR test, and vice versa. (B) Oncoplot of co-mutation patterns and bar plot of mutation prevalence in each CHIP gene. Only genes with a mutation prevalence ≥0.5% are shown. Mutations in the oncoplot are colored by variant type, as displayed in the legend. ICU admissions/in-hospital deaths and general admissions are represented in dark and light gray, respectively. (C) Associations between CHIP and ICU admissions/in-hospital deaths. CHIP status was evaluated based on the presence of: (1) any CHIP versus no CHIP, (2) multiple mutations versus no CHIP or a single mutation, (3) large (variant allele frequency [VAF] ≥ 10%) clone(s) versus no CHIP or small (VAF < 10%) clones, (4) mutation(s) in a DNA repair gene (*PPM1D* and/or *TP53*) versus no CHIP or mutation(s) elsewhere, and (5) mutation(s) in *PPM1D* versus no CHIP or mutation(s) elsewhere. In gray, the results of the univariable analysis are shown; in black, those of the multivariable analysis adjusted for sex, age at the time of the positive severe acute respiratory syndrome coronavirus 2 PCR test, blood sample age, body mass index, active smoking, diabetes, cardiovascular disease, lung disease, cancer, and prior chemo- and/or radiotherapy treatment. BMI, body mass index; CI, confidence interval; OR, odds ratio.

matching; the use of self-reported and hospital-recorded lifestyle and clinical information, which can have different accuracies; and our inability to differentiate individuals with past smoking habits from those who have never smoked. Overall, the findings presented add to our understanding of the effect of CHIP on inflammation and infectious diseases, and emphasize the importance of disease prophylaxis such as vaccinations in CHIP carriers.

ACKNOWLEDGMENTS

We are deeply grateful to all of the patients for their participation.

AUTHOR CONTRIBUTIONS

Morten Tulstrup and Kirsten Grønbæk conceived and designed the study. Sofie Bliddal, Ioanna Nissen, Sisse Rye Ostrowski, and Karina Meden Sørensen secured participant consent and samples from biobanks. Jakob Schmidt Jespersen handled library preparation and DNA sequencing. Morten Tulstrup handled variant calling and filtering. Celia Burgos Sequeros, Karina Banasik, Sofie Bliddal, Omid Rezahosseini, and Morten Tulstrup planned the statistical analyses. Ioanna Nissen and Ole Birger Vestager Pedersen were in charge of ethical and data permissions and secured data from the Danish registers. Sofie Bliddal collected data from patients' records. Celia Burgos Sequeros analyzed the data and wrote the first draft of the manuscript. Celia Burgos Sequeros, Morten Tulstrup, and Kirsten Grønbæk interpreted the data. All co-authors contributed to the sample or data collection and critically revised the manuscript.

CONFLICT OF INTEREST STATEMENT

Kirsten Grønbæk has received research funding and/or consultancy fees from Janssen Pharma and GSK. Carsten Utoft Niemann has received research funding and/or consultancy fees from Abbvie, AstraZeneca, Janssen, Octapharma, Beigene, Genmab, CSL Behring, Takeda, Lilly, and MSD. Anne-Mette Lebech has received unrestricted research grant from Gilead and consultancy fees from GSK, MSD, and Pfizer.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

FUNDING

This study was supported by a research grant from the European Hematology Association (EHA COVID-19 in Hematology Research Grant 2020). In addition, the study is part of the Danish Research Center for Precision Medicine in Blood Cancers (Danish Cancer Society Grant R223-A13071). A grant from Sygesikring Danmark (2020-0178) was given to the Danish COVID-19 Genetic Consortium. Celia Burgos Sequeros, Karina Banasik, and Søren Brunak acknowledge the Novo Nordisk Foundation (Grant numbers: NNF17OC0027594 and NNF14CC0001). This work was also supported by a grant from the Ministry of Higher Education and Science, Copenhagen, Denmark (0238-00006B). This research has been conducted using the Danish National Biobank resource. UFR's research salary was sponsored by Kirsten and Freddy Johansen's Fund.

ORCID

Jakob S. Jespersen http://orcid.org/0000-0002-4930-5900
Kirsten Grønbæk http://orcid.org/0000-0002-1535-9601

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498. doi:10.1056/NEJMoa1408617
2. Joo L, Bradley CC, Lin SH, Scheet PA, Nead KT. Causes of clonal hematopoiesis: a review. *Curr Oncol Rep*. 2023;25(3):211-220. doi:10.1007/s11912-023-01362-z
3. Zekavat SM, Lin SH, Bick AG, et al. Hematopoietic mosaic chromosomal alterations increase the risk for diverse types of infection. *Nat Med*. 2021;27(6):1012-1024. doi:10.1038/s41591-021-01371-0
4. Li X, Zhang Q, Ding Y, et al. Methyltransferase Dnmt3a upregulates HDAC9 to deacetylate the kinase TBK1 for activation of antiviral innate immunity. *Nature Immunol*. 2016;17(7):806-815. doi:10.1038/ni.3464
5. Zhang Q, Zhao K, Shen Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature*. 2015;525(7569):389-393. doi:10.1038/nature15252
6. Sano S, Oshima K, Wang Y, et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the il-1β/nlrp3 inflammasome. *J Am Coll Cardiol*. 2018;71(8):875-886. doi:10.1016/j.jacc.2017.12.037
7. World Health Organization. *Coronaavirus (COVID-19) Dashboard*. WHO; 2024. https://covid19.who.int
8. Xie N, Zhang W, Chen J, Tian F, Song J. Clinical characteristics, diagnosis, and therapeutics of COVID-19: a review. *Curr Med Sci*. 2023;43(6):1066-1074. doi:10.1007/s11596-023-2797-3
9. Gustine JN, Jones D. Immunopathology of hyperinflammation in COVID-19. *Am J Pathol*. 2021;191(1):4-17. doi:10.1016/j.ajpath.2020.08.009
10. Center for Disease Control and Prevention. *Underlying Medical Conditions Associated with Higher Risk for Severe COVID-19: Information for Healthcare Professionals*. CDC; 2023. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html
11. Kessler MD, Damask A, O'Keeffe S, et al. Common and rare variant associations with clonal haematopoiesis phenotypes. *Nature*. 2022;612(7939):301-309. doi:10.1038/s41586-022-05448-9
12. Miller PG, Fell GG, Foy BH, et al. Clonal hematopoiesis of indeterminate potential and risk of death from COVID-19. *Blood*. 2022;140(18):1993-1997. doi:10.1182/blood.2022018052
13. Zhou Y, Shalhoub R, Rogers SN, et al. Clonal hematopoiesis is not significantly associated with COVID-19 disease severity. *Blood*. 2022;140(14):1650-1655. doi:10.1182/blood.2022015721
14. Duployez N, Demonchy J, Berthon C, et al. Clinico-biological features and clonal hematopoiesis in patients with severe COVID-19. *Cancers*. 2020;12(7):1992. doi:10.3390/cancers12071992
15. Hameister E, Stolz SM, Fuhrer Y, et al. Clonal hematopoiesis in hospitalized elderly patients with COVID-19. *Hemasphere*. 2020;4(4):e453. doi:10.1097/HS9.0000000000000453
16. Petzer V, Schwendinger S, Haschka D, et al. Clonal hematopoiesis in patients with Covid-19 is stable and not linked to an aggravated clinical course. *Am J Hematol*. 2021;96(9):E331-E333. doi:10.1002/ajh.26251
17. Del Pozo-Valero M, Corton M, López-Rodríguez R, et al. Age-dependent association of clonal hematopoiesis with COVID-19 mortality in patients over 60 years. *GeroScience*. 2023;45(1):543-553. doi:10.1007/s11357-022-00666-5
18. Niroula A, Sekar A, Murakami MA, et al. Distinction of lymphoid and myeloid clonal hematopoiesis. *Nat Med*. 2021;27(11):1921-1927. doi:10.1038/s41591-021-01521-4
19. Bliddal S, Banasik K, Pedersen OB, et al. Acute and persistent symptoms in non-hospitalized PCR-confirmed COVID-19 patients. *Sci Rep*. 2021;11(1):13153. doi:10.1038/s41598-021-92045-x
20. R Core Team. *R: A Language and Environment for Statistical Computing*. R Core Team; 2021. https://www.R-project.org/