Clinical impact of clonal hematopoiesis on severe COVID-19 patients without canonical risk factors

Clonal hematopoiesis of indeterminate potential (CHIP) refers to a population of myeloid stem cells with acquired gene mutations, but does not fulfill the diagnostic criteria for hematologic malignancy.[1] There is growing evidence supporting the role of CHIP mutations in altered immune function through effector cells such as monocytes/macrophages and their dysregulated cytokine/chemokine expression.[2,3] Since the immunopathogenesis of such an adverse outcome of CHIP largely shares that of severe COVID-19,[4] such as high levels of circulating pronflammatory cytokines or dysregulated monocytes and macrophages,[5,6] several previous studies have examined whether CHIP might contribute to the progression of COVID-19.[7-9] However, due to the weak significance of association or lack of stratified analysis, it is still unknown whether the presence of CHIP in previously healthy COVID-19 patients has a clinical impact on the disease severity. In this study, we aimed to verify the clinical implications of CHIP in COVID-19 severity, especially in patients without canonical clinical risk factors for severe COVID-19 by analyzing clinical and laboratory characteristics using clustering and statistical examinations including interaction term.

In order to investigate the clinical impact of CHIP on COVID-19 severity, a total of 243 laboratory-confirmed COVID-19 patients between February 2020 and January 2021 in four tertiary hospitals in the Republic of Korea were analyzed according to the severity of COVID-19 and the presence of CHIP (Figure 1A). Informed consent was waived or obtained with the approval of Institutional Review Boards (IRB) of four respective hospitals (IRB Nos. 2003-141-1110, B-2006/616-409, 2008-050, 2020-04-069-001, and IRB-21-269). The severity of COVID-19 was stratified with an ordinal scale 1 to 8 with some modifications.[10,11] Cases with the highest ordinal scale 3 (need for supplementary oxygen therapy via nasal cannula) or more were classified as severe COVID-19, while others were classified with mild ones.[10-12] The presence of CHIP was determined by sequencing 44 target genes defined as CHIP genes based on the previous studies.[13] A variant allele frequency[4,14] of more than 2% was set as the cut-off. Among the patients, 50 patients had CHIP (20.6%) (*Online Supplementary Table S1*). The most commonly mutated gene was *DNMT3A* (24 variants), followed by *TET2* (11 variants) and *ASXL1* (7 variants).

After distinguishing COVID-19 severity and the presence of CHIP, we collected clinical, laboratory, and radiological characteristics of the patients. Those characteristics included body mass index (BMI), presence of comorbidities such as diabetes mellitus (DM), hypertension, the concentration of hematopoietic cells, and chest X-ray score quantified by deep-learning-based method (http://tisepx.com/; TiSepX COVID-19, MEDICALIP, Korea). A part (112/243) of the clinical information of the patients on demographics, underlying diseases, the severity of COVID-19, and the presence or absence of CHIP (7 variables among 19 which used in the below clustering analysis) was previously included in the report by Bolton *et al.*[7]

The baseline characteristics of the patients are shown in the *Online Supplementary Table S1*. The median age was 72 (interquartile range [IQR], 62—81) and 65 (IQR, 52—75) years in patients with or without CHIP, respectively, as consistent with a common aging-related property of CHIP. In patients with or without CHIP, 80.0% (40/50) and 63.7% (123/193) of patients underwent severe COVID-19, respectively. None of the patients in the present study had a history or current hematologic malignancy.

In order to precisely examine the clinical impact of CHIP in patients with COVID-19, we conducted a clustering analysis with t-Stochastic Neighbor Embedding (t-SNE) for baseline characteristics and examined the distribution of CHIP among clusters (Figure 1B). All continuous clinical information was transformed into a range of 0 to 1 using a logistic function, and comorbidity status was dichotomized into 0 and 1 for absence and presence. Dimension reduction steps were subsequently applied to the converted data, including distance metric, correlation, and principal component analysis. Patients were then projected on the t-SNE plane. Based on a Monte Carlo reference-based consensus clustering (M3C) algorithm,[15] patients were grouped into eight different clusters. The clustering analysis well categorized patients according to the COVID-19 severity. Cluster S1 to S5 comprises severe cases since these clusters were marked by representative clinical, laboratory, and radiological properties such as higher ordinal scales, peak C-reactive protein levels in serum, and peak chest X-ray scores than cluster M1 to M3 (unpaired *t*-test, adjusted $P<0.001$) (Figure 1C; *Online Supplementary Figure S1A*).

In order to further characterize the clinical characteristics of individual severe clusters, we investigated the presence or absence of comorbidities for severe clusters S1 to S5 (Figure 1D). Based on the prediction of COVID-19 severity through logistic regression only with putative risk factors (*Online Supplementary Figure S1B*), we focused on DM, CHIP, and hypertension in downstream analysis. For each severe cluster, we examined the enrichment of CHIP compared to the canonical risk factors including DM and hypertension. Cluster S2, S4, and S1 were characterized by the presence of hypertension, DM or both risk factors, respectively (Figure

1D). Interestingly, cluster S3 tended to have a significantly higher rate of CHIP than other risk factors (11/24, 45.8% *vs.* 0/24, 0.0%; chi-squared test, *P*=5.93e-4) (Figure 1D).

The presence of a particular type of severe cases enriched by CHIP without canonical risk factors led us to hypothesize that CHIP may contribute to severe COVID-19 in its own way. In order to test the statistical significance of the impact of CHIP on COVID-19 severity in patients without comorbidities, we stratified all patients by the presence of any of the prevalent canonical risk factors such as DM, hypertension, and BMI ≥30.0. When adjusted for age and sex, the risk of severe COVID-19 tended to be higher in CHIP (+) patients than in CHIP (-) patients in the canonical risk factor-absent subgroup (adjusted odds ratio 7.35; 95% confidence interval [CI]: 1.71-9.38e+07, permutation *P*=5.5e-3) (Table 1). In order to examine the significance of

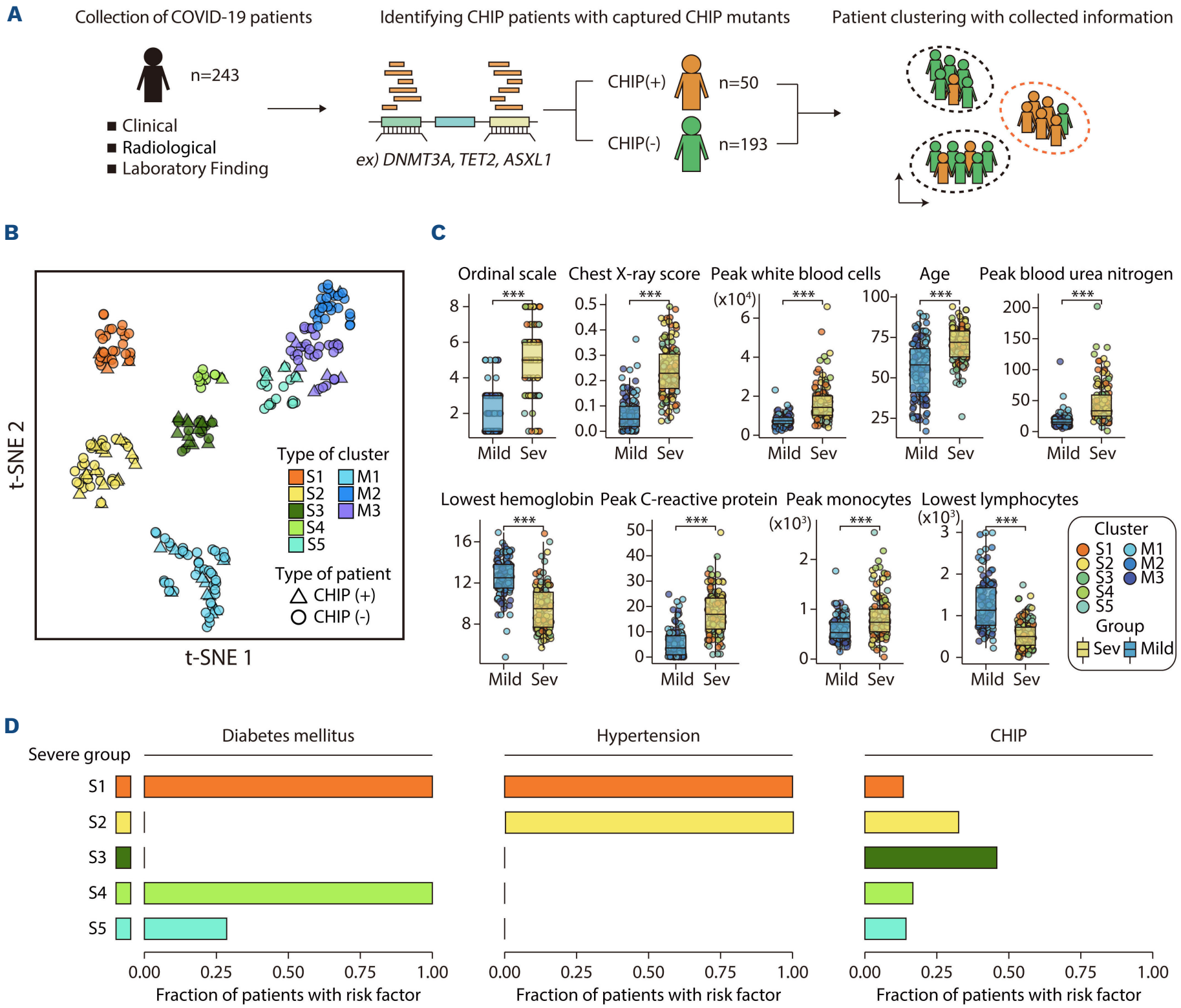

Figure 1. Clustering analysis for clinical characteristics of severe COVID-19. (A) Overview of the study design. (B) Clustered patients on the t-SNE plane based on clinical characteristics. Total 243 patients were projected to the t-SNE plane with the converted clinical data. Eight distinct clusters were identified using a M3C algorithm named S1 (n=30), S2 (n=43), S3 (n=24), S4 (n=12), S5 (n=21), M1 (n=52), M2 (n=27) and M3 (n=34). Each dot indicates individual patients with different shapes. Triangle, with clonal hematopoiesis of indeterminate potential (CHIP). Circle, without CHIP. (C) Boxplots showing 9 representative clinical characteristics between Mild and Severe groups. For the boxplots, the box represents the interquartile range (IQR) and the whiskers correspond to the highest and lowest points within 1.5 × IQR. Statistical significance was examined using an unpaired *t*-test (*adjusted *P*<0.05, **adjusted *P*<0.01 and ***adjusted *P*<0.001). The color indicates the identity of clusters and groups. Sev: Severe group. Mild: Mild group. (D) Bar plots showing fractions of patients with diabetes mellitus, hypertension, or CHIP for clusters S1 to S5. Color indicates the identity of a cluster.

such a difference in CHIP effects along with the canonical risk factors, we formally tested the interaction effects by using a logistic regression. The difference takes a permutation *P* value of 0.01, suggesting statistically significant difference in CHIP effects between two groups. The results of patient clustering and statistical evidence indicate that CHIP is an independent risk factor for severe COVID-19 in patients without canonical risk factors.

Bolton *et al.* recently reported that CHIP is significantly associated with severe COVID-19,[7] especially in patients carrying non-putative driver mutations. However, they could only identify a modest statistically meaningful association between COVID-19 severity and the presence of CHIP. Duployez *et al.* showed a higher prevalence of CHIP in severe COVID-19 patients than in age-matched hematologic malignancy-free cohorts, but they did not show that CHIP affects the rate of orotracheal intubation or death.[8] In another study conducted by Hameister *et al.* involving 102 hospitalized patients with COVID-19, the presence of CHIP was not associated with severe COVID-19.[9] However, the study lacked a stratified analysis or an adjustment for possible interactions. By analyzing thorough clinical, radiological, and laboratory characteristics, as well as the presence of CHIP, we successfully clarified that there is a distinct CHIP-driven severe COVID-19 subgroup defined as patients without canonical risk factors.

Several issues may warrant prudence in the interpretation of the results of the present study. First, we arbitrarily introduced a composite term canonical risk factors with prevalent underlying diseases to examine the interaction between clinical risk factors and CHIP. Second, the proportion of severe COVID-19 was high in the present study since it was conducted in tertiary hospitals in Korea.

In conclusion, by coupling the clustering method to complemented statistical analysis, our study demonstrated that CHIP is an independent risk factor for COVID-19, especially in patients without canonical risk factors. Our findings might provide a better understanding of the previously unexplained exacerbation of clinical conditions for COVID-19 patients without canonical risk factors.

Table 1. A significant interaction between clonal hematopoiesis of indeterminate potential and canonical risk factors for severe COVID-19.

	Incidence of severe COVID-19		aOR (95% CI)†
	CHIP (+)	CHIP (-)	
Total, N (%)	40/50 (80.0)	123/193 (63.7)	1.24 (0.55-3.41)
Canonical risk factor (+), N (%)	25/34 (73.5)	89/122 (73.0)	0.69 (0.26-2.06)
Canonical risk factor (-), N (%)	15/16 (93.8)	34/71 (47.9)	7.35 (1.71-9.38e+07)

†Adjusted for age and sex. CHIP: clonal hematopoiesis of indeterminate potential; aOR: adjusted odds ratio; CI: confidence interval obtained from 10,000 bootstrap results. Canonical risk factors are body mass index ≥ 30.0, diabetes mellitus and hypertension. If the patient has at least 1 canonical risk factor, it is allocated to a present, and if not, regarded as an absent group.

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https://doi.org/10.3324/haematol.2022.280621

Received: January 3, 2022.
Accepted: September 9, 2022.
Prepublished: September 15, 2022.

Disclosures

YK is a CEO of GenomeOpinion. SK, CS and HI are employed by GenomeOpinion. All other authors have no conflicts of interest to disclose.

Contributions

CKK, BC, YK and IJ conceived the study. CKK, BC, SK, CS, SHY and KK performed data analysis. CKK, EC, JJ, PGC, WBP, ESK, HBK, NJK, M-dO, HI, JK, YHL, JL, JYL, JHM and K-HS contributed to the collection of clinical information and samples. CKK, BC, HC, YK

and IJ contributed to data interpretation. CKK and BC prepared the manuscript with assistance from YK and IJ. All authors read and commented on the manuscript and approved it for submission.

Acknowledgments

We thank all our laboratory members for their support and critical suggestions throughout this work. Genome opinion provided the study team with targeted panel (LifeEx© CH panel) sequencing and CHIP variant analysis and technical insights for variant analysis.

Funding

This work was funded by the SUHF Fellowship (to IJ) and the Ministry of Science and ICT through the National Research Foundation in the Republic of Korea (NRF-2020R1A2C4001464 to IJ).

Data-sharing statement

We support data-sharing of individual participant data. The individual participant data that underlie the results reported in this article (text, Tables, Figures and *Online Supplementary Appendix*) will be shared after deidentification. The raw data will be available for 1 year after the publication of this article. Researchers who provide a scientifically sound proposal will be allowed access to the individual participant data. We used publicly available software for all analysis and followed standardized processes. No custom code was used for analysis in the study.

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