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RESEARCH ARTICLE

Host protection against Omicron BA.2.2 sublineages by prior vaccination in spring 2022 COVID-19 outbreak in Shanghai

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Abstract **The Omicron family of SARS-CoV-2 variants are currently driving the COVID-19 pandemic. Here we analyzed the clinical laboratory test results of 9911 Omicron BA.2.2 sublineages-infected symptomatic patients without earlier infection histories during a SARS-CoV-2 outbreak in Shanghai in spring 2022. Compared to an earlier patient cohort infected by SARS-CoV-2 prototype strains in 2020, BA.2.2 infection led to distinct fluctuations of pathophysiological markers in the peripheral blood. In particular, severe/critical cases of COVID-19 post BA.2.2 infection were associated with less pro-inflammatory macrophage activation and stronger interferon alpha response in the bronchoalveolar microenvironment. Importantly, the abnormal biomarkers were significantly subdued in individuals who had been immunized by 2 or 3 doses of SARS-CoV-2 prototype-inactivated vaccines, supporting the estimation of an overall 96.02% of protection rate against severe/critical disease in the 4854 cases in our BA.2.2 patient cohort with traceable vaccination records. Furthermore, even though age was a critical risk factor of the severity of COVID-19 post BA.2.2 infection, vaccination-elicited protection against severe/critical COVID-19 reached 90.15% in patients aged ⩾ 60 years old. Together, our study delineates the pathophysiological features of Omicron BA.2.2 sublineages and demonstrates significant protection conferred by prior prototype-based inactivated vaccines.**

Keywords SARS-CoV-2; COVID-19; host response; bronchoalveolar lavage fluid (BALF)

Introduction

The Omicron family of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) subvariants are currently driving the spread of the coronavirus disease 2019 (COVID-19) around the globe. The first Omicron member (Pango lineage# BA.1.1.529) emerged in South Africa in November 2021 [1] with a constellation of mutations dramatically increasing the virus' infectivity and immunity-evasion capability [2–4]. Since then, continuous evolution of the viral genome has generated a series of Omicron sublineages, with members of the BA.2, BA.5, and BQ.1 sublineages being the most widely distributed strains up to date. The high infectivity and transmissibility of these variants have caused large community outbreaks of COVID-19 even in places where non-pharmaceutical public health interventions (NPI; e.g., contact tracing and social distancing) were effective in containing local spreading of ancestral SARS-CoV-2 strains. For example, from March 1 to June 27, 2022, a large-scale community outbreak of BA.2.2 variant in the city of Shanghai led to 649 655 infected cases (including 591 518 asymptomatic and 58 137 symptomatic cases),

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among whom 588 individuals died from or with the COVID-19 [5,6]. Though the majority of Omicron variants-infected individuals either appeared asymptomatic or exhibited relatively mild symptoms (e.g., cough, fever, and headache, etc.), the changing landscape of the Omicron genome arouses considerable uncertainty as to whether more transmissible and virulent strains would emerge in the coming waves of the pandemic [7], making it necessary to closely monitor and investigate the clinical features of SARS-CoV-2 subvariants over time, evaluate the benefit of current therapeutic and prophylactic options, and adjust the public health strategies correspondingly.

With respect to the pathophysiology of SARS-CoV-2, studies over the past three years have provided a comprehensive view of its infection route and reciprocal interactions with the host [8,9]. Generally speaking, SARS-CoV-2 enters the human body via the respiratory system, where it encounters the first line of host defense responses in the mucus and epithelia of the respiratory tract and then in the lung. These responses are often sufficient to clear off the virus in one or two weeks and limit COVID-19 to relatively mild or moderate respiratory disease-like symptoms. However, in a small percentage of patients (< 5%) with defective immunity (e.g., seniors, patients with autoimmunity diseases or cancer, etc.), SARS-CoV-2 infection may trigger aberrant cytokine releases from the bronchoalveolar microenvironment, leading to heightened systemic immune responses that, if not properly controlled, lead to severe lymphopenia, thrombosis, multi-organ failures (e.g., lung, heart, liver, and kidney), and even death [10,11]. Compared to ancestral strains, the Omicron variants were reportedly less prone to spread beyond the upper respiratory tract, a feature that might explain the observed high percentage of asymptomatic or mild/moderate COVID-19 cases after Omicron variants infection [12]. However, it remains unclear how Omicron variants cause severe COVID-19, and whether the pathology of severe COVID-19 is the same as or different between Omicron- and ancestral strain(s)-infected patients. Moreover, before Omicron's emergence, a large fraction of the human population has been immunized with vaccines based on ancestral SARS-CoV-2 strain(s). Even though antibodies elicited by these vaccines do not effectively neutralize Omicron variants (especially newer ones such as BA.2, BA.5, and their derivatives) *in vitro* and *in vivo* [4,13–16], prior-immunized individuals appear much less likely to develop severe COVID-19 during recent infection waves, suggesting the existence of vaccine-induced cellular immunity against aberrant host immune responses to Omicron variants [17]. However, the duration and efficacy of such protection are incompletely understood.

In this study, we sought to characterize the pathophysiological features of the Omicron BA.2.2 sublineages, based on analysis of the clinical laboratory tests and immunization records of a cohort of 9911 patients who were diagnosed as symptomatic COVID-19 cases during the outbreak of the spring of 2022 in Shanghai. Our analysis indicated that a number of clinically relevant factors—such as age, vaccination, lymphocytopenia, coagulation disorder, and liver dysfunction—were associated with severe COVID-19. Longitudinal analysis of the dynamical changes of these factors, as well as mono- and multi-variant analyses of them between COVID-19 severity groups, i.e., mild/moderate versus (vs.) severe/critical cases, revealed that age and immunization status as two critical independent risk factors of severe COVID-19. Last, comparison of these Omicron BA.2.2 sublineages-infected patients with an earlier cohort of 963 patients infected by the SARS-CoV-2 ancestral strain(s) and treated in Shanghai from February to July 2020 [10,18], suggested a number of disease mechanism features of the Omicron BA.2.2 sublineages that diverged from those of SARS-CoV-2 prototype strains.

Methods

Enrollment

A total of 9911 COVID-19 cases from February 27, 2022 to July 1, 2022 were enrolled. The COVID-19 were classified according to the Diagnosis and Treatment Protocol for COVID-19 Patients in China (Trial Version 9). General demographic analysis is shown in Table S1. This study was approved by the Ethics Committee of Shanghai Public Health Clinical Center (No. 2022-S069-01) in accordance with the *Declaration of Helsinki*. Informed consents were obtained from all enrolled patients. Previously enrolled 963 COVID-19 cases from January 21, 2020 to October 17, 2020 were reported before [18].

Clinical laboratory tests

Routine blood tests were conducted as previously reported [18]. The levels of 12 cytokines (including IFN-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, and TNF) were quantified by the BD™ Cytometric Bead Array (human Th1/Th2/Th17 cytokine kit and human inflammatory cytokine kit) according to the manufacture's instruction. The distribution and features of lymphocyte subsets were analyzed with the BD Multitest™ 6-color TBNK by BD FACSCanto™.

RNA-seq

A total of 19 bronchoalveolar lavage fluid (BALF)

samples were collected while the patients were in hospital as previously reported [18]. The nuclear acid in BALF samples was collected using the MGIEasy Nucleic Acid Extraction Kit (MGI tech). RNA was further cleaned with the RNeasy MinElute Cleanup Kit (Qiagen). rRNAs were removed with the KAPA RiboErase Kit (Human/Mouse/Rat). Total-RNA-seq libraries were constructed using the KAPA RNA HyperPrep Kit and sequenced with the BGI-sequencing platform.

Bioinformatics analysis

For SARS-CoV-2 viral sequence analysis, raw sequenced reads were mapped to the SARS-CoV-2 genome using the bowtie2 [19]. For analysis of host transcriptomic features, raw sequenced reads were mapped to the human reference genome (hg38) using the STAR algorithm [20]. Gene read counts were obtained with the Htseq suite [21]. For differential gene expression analysis, the DEseq2 package was used [22]. Cell type composition deconvolution from bulk RNA-seq data was performed using CIBERSORTx [23].

Results

Patient enrollment and study cohorts

We recruited 9638 mild/moderate and 273 severe/critical COVID-9 cases (including three who died during the observation time of one month) infected by BA.2.2 sublineages from the Shanghai Public Health Center (SPHC), the main hospitalization center for symptomatic COVID-19 in Shanghai, which admitted 17.05% (9911/58 137) symptomatic cases during a community outbreak of SARS-CoV-2 between February and July 2022 (Fig. 1A and Table S1; also see Methods for the criteria for disease severity classification). The viral genomes uncovered by our group from 263 randomly sampled patient swabs revealed two closely related strains, both of which were identified as BA.2.2 derivatives according to a phylogenetic analysis using viral genomes of the GAISAID database [24]. One strain was estimated to have infected more than 90% of the patients of our cohort; this dominant strain, later designated as Pango lineage# BA.2.2.1, was characterized by two linked mutations: C26789T (M:G89G, synonymous) and A28119G (ORF8:I76V) in addition to other BA.2.2-characteristic mutations (S:I1221T, ORF1a:T1543I, ORF1a:T4087I) [24]. The other strain was a minor subvariant of BA.2.2 that did not show additional characteristic mutations. Because the BA.2.2.1- and non-BA.2.2.1-infected individuals of our cohort did not exhibit notable differences in terms of symptoms, they were grouped together for subsequent analysis and were collectively referred to the BA.2.2 cohort.

Previously, we studied another cohort of 963 symptomatic COVID-19 patients hospitalized at SPHC between February and July 2020 when the circulating strains were the original SARS-CoV-2 and the D614 variant [10,25] (Table S1, heretofore referred to as prototype cohort). A gross analysis of the age and gender distributions showed that, as compared to the prototype cohort, the BA.2.2 cohort included relatively larger fractions of children (< 10 years: 5.97%/BA.2.2 vs. 0.83%/prototype), seniors (⩾ 60 years: 27.99%/BA.2.2 vs. 12.77%/prototype), and females (45.83%/BA.2.2 vs. 40.19%/prototype) (P < 0.001) (Fig. 1A and Table S1). It is noteworthy that none of the prototype cohort was vaccinated (anti-SARS-CoV-2 vaccination programs were not available in Shanghai until early 2021), while the majority of patients in the BA.2.2 cohort received 1–3 doses of vaccine (ancestral-strain-based inactivated vaccines, produced by Sinopharm or Sinovac; Table S1). Moreover, the patients in the prototype cohort of 2020 were mainly composed of migrant workers who contracted the virus from other cities and then traveled to Shanghai, whereas the patients of the BA.2.2 cohort of 2022 were mainly local Shanghai residents. Thus, other than viral strains, other factors such as vaccination and exposure patterns (large-scale community outbreak in BA.2.2 wave vs. a small mobility population of 963 cases infected by prototype) might have contributed to the demographic shift of BA.2.2-infected populations.

Consistent with recent reports, the overall rate of severe/critical COVID-19 cases in the symptomatic BA.2.2 cohort was relatively low (2.76%), and such cases were predominantly found in people ⩾ 60 years old (Fig. 1B, and Table S1). In addition, we also analyzed distribution of vaccination rates in different age groups among 4854 cases with traceable vaccination records. Interestingly, while vaccination coverage was low in both children less than 10 years old and elder adults (⩾ 60 years old), severe/critical cases were only found in the latter age group (Fig. 1B–1D). Moreover, the percentages of severe/critical cases gradually increased with age, but the slope of such increase was dramatically lower among vaccinated populations (Fig. 1D). For instance, within age groups of 60–69, 70–79, and ⩾ 80 years old, the severe/critical rates were 8.64%, 17.12%, and 20.28% in unvaccinated cases; 6.90%, 12.12%, and 14.29% in 1-dose vaccinated cases, but only 0.76%, 4.76%, and 4.35% in 2-dose vaccinated and 0.58%, 2.60%, and 0.00% in 3-dose vaccinated ones, respectively (Fig. 1D). Based on these results, it was estimated that, while severe/critical COVID-19 were more likely to occur in the elderly population, 2–3 doses of vaccination led to an overall 96.02% protection rate against severe/critical COVID-19 in the present patient series of all age groups, including 90.15% protection rate among elderly population (⩾ 60

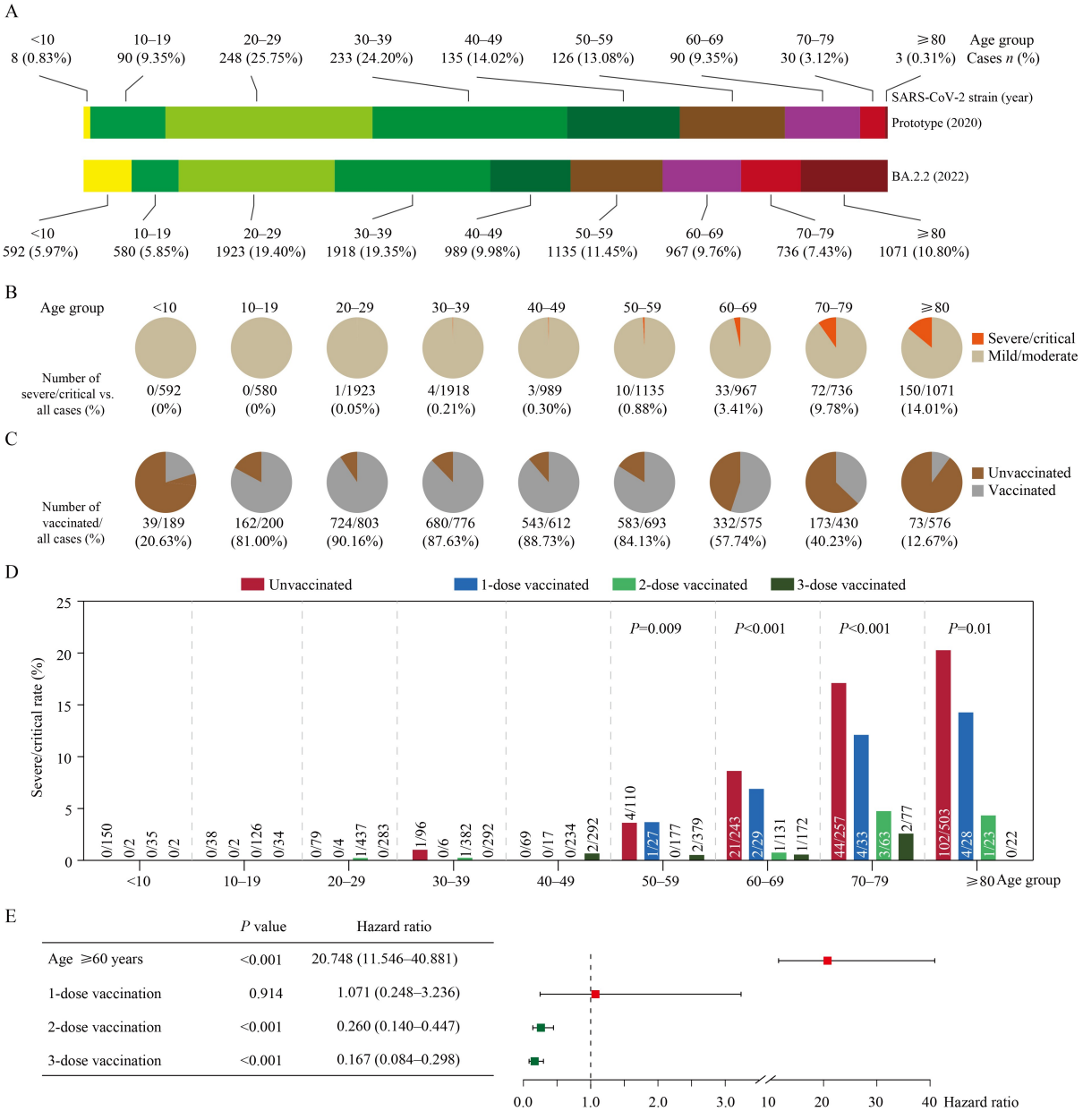

Fig. 1 BA.2.2 cohort and risk factors of severe COVID-19. (A) Numbers and fraction of COVID-19 cases in different age groups. (B) Number and fraction of severe/critical cases in different age groups. (C) Number and fraction of vaccination states in different age groups. (D) Distribution of severe cases in different age groups between vaccinated and non-vaccinated individuals. (E) Risk factor analysis. Multivariate analysis identifies that both age and vaccination were determinate for the severity of COVID-19. Hazard ratio was plotted in the right panel.

years old) (Table S3). Indeed, multi-variant test indicated that both age ($P < 0.001$) and 2-dose ($P < 0.001$) or 3-dose ($P < 0.001$) vaccinations were independent risk factors for severe COVID-19 post BA.2.2 infection (Fig. 1E).

Clinical laboratory test features associated with COVID-19 severity post BA.2.2 infection

For the BA.2.2 cohort, we monitored the pathophysiological presentations of each patient based on routine clinical laboratory tests of the peripheral blood collected at various intervals for up to 30 days. These tests included measurements of a variety of bio-analytes related to host immune response, multi-organ function and damage, coagulation, and cytokine release event during hospitalization. To identify factors that might be prognostic of severe COVID-19, we divided the patients into mild/moderate and severe/critical groups, and compared the results of the samples collected since the first day post-hospitalization (dph1 sample, which were also used for diagnosis). In univariant analyses, many bio-analytes

already showed significant differences between the severe/critical and mild/moderate cases even from the outset of the disease progression (Table S2). In particular, severe/critical cases were characterized by increased levels of factors related to liver function/damage (e.g., albumin, pre-albumin, aspartate aminotransferase) and kidney injury (e.g., creatinine and blood urea nitrogen), as well as decreased levels of those related to host immune response (e.g., significantly reduced lymphocyte counts, including $CD3^+$, $CD4^+$, and $CD8^+$ cell counts, in severe/critical cases) (Table S2). A multi-variant test further indicated that albumin (P=0.024) and high-sensitivity C-reactive protein (HS-CRP) ($P < 0.001$)—which were respectively indicators of liver function/damage and general inflammatory response levels—were independent risk factors for the severity of COVID-19 (Fig. 2A).

We then performed a longitudinal analysis by plotting the laboratory test results over the dphs. Overall, the levels of many parameters significantly deviated from normal ranges in the severe/critical group (composing mostly of cases ⩾ 60 years, 93.40%) further than in the mild/moderate group (Fig. 2B–2F). Moreover, some severe/critical case-related parameters showed distinct temporal profiles between the younger and elderly groups in mild/moderate cases (< 60 years and ⩾ 60 years, respectively) (Fig. 2B–2F). For example, HS-CRP was consistently higher, while the lymphocyte counts were consistently lower, in severe/critical group throughout dph1–30 (Fig. 2B); though these two markers were within or near the normal range in all mild/moderate cases on dph1, their curves in mild/moderate ⩾ 60 years group changed afterwards and converged to those of severe/critical one on dph30, leaving only the mild/moderate < 60 years group within/near the normal ranges. Similar patterns were noticed for dynamic changes of markers of liver dysfunction (albumin and pre-albumin; Fig. 2C), kidney injury (blood urea nitrogen; Fig. 2D), and anemia (hemoglobin and hematocrit; Fig. 2E). On the other hand, the temporal profile of coagulation-related D-dimer was unique: at starting points, its levels in the severe/critical and the mild/moderate ⩾ 60 years groups were both well above the normal range, but near the upper normal limit in the mild/moderate < 60 years group; while its curve in severe/critical remained stable and slightly decreased over time, that of mild/moderate ⩾ 60 years rose significantly and even surpassed severe/critical on dph20–30, and that of mild/moderate < 60 years gradually increased to the same level as severe/critical around dph30 (Fig. 2F). Taken together, these results suggested a potential longitudinal impact of the BA.2.2 sublineages on liver/kidney dysfunction/damage, anemia, and coagulation abnormality in severe/critical COVID-19 cases. In the elderly population, even though many individuals were symptomatically diagnosed as mild/moderate cases, the profiles of some clinical laboratory tests were somewhat similar to those of severe/critical ones, further highlighting age as a critical risk factor for the severity of COVID-19.

Prior immunization provides significant protection to patients from severe COVID-19 after BA.2.2 infection

Since vaccination status was also a major risk factor for severe COVID-19, we analyzed the relationship between the laboratory test results of severe/critical cases and vaccination history. As shown in Fig. 3, the abnormality of bio-analytes in the blood characterizing severe/critical cases was significantly protected to various degrees in vaccinated individuals. Such effect was also observed in mild/moderate cases, especially in elderly people. For example, in routine blood tests, while lymphocyte counts in unvaccinated severe/critical cases stayed below the normal ranges throughout dph1–30, those in the vaccinated group were not only significantly higher but also returned close to normal levels on dph 16–30 (Fig. 3A, left panel). In mild/moderate cases, lymphocyte counts were largely within normal ranges throughout the course of this study, yet their counts in vaccinated cases showed significantly more increases, altered relatively small, than in unvaccinated cases at various time intervals (Fig. 3A, middle and right panels). In comparison, while HS-CRP levels did not seem to be affected by vaccination status in severe/critical cases, their within-normal-range levels in mild/moderate cases were significantly higher than in unvaccinated cases in both < 60 years and elder (⩾ 60 years) individuals (Fig. 3B).

Vaccination-related protection effects were also observed, though varied to some degrees, for other pathophysiological biomarkers. For anemia-related markers, hematocrit and hemoglobin were consistently higher in vaccinated cases compared to unvaccinated cases in all three settings (severe/critical, mild/moderate ⩾ 60 years, and mild/moderate < 60 years) throughout dph1–15 (Fig. 3C), suggesting a general protective effect of vaccination on COVID-19 related-anemia. For liver function-related markers, the levels of albumin and pre-albumin in the severe/critical group showed a somewhat complicated relationship with vaccination status, that is, as compared to unvaccinated cases, vaccination seemed to have enabled albumin and pre-albumin to stay closer to the lower limit of normal ranges on dph1–5, an effect that was absent on dph6–15 and then reversed on dph16–30, so that the below-normal-levels of these two markers were even lower in vaccinated cases (Fig. 3D and 3E, left panel). In comparison, in mild/moderate cases, albumin and pre-albumin levels were consistently higher in vaccinated individuals throughout dph1–30, which were

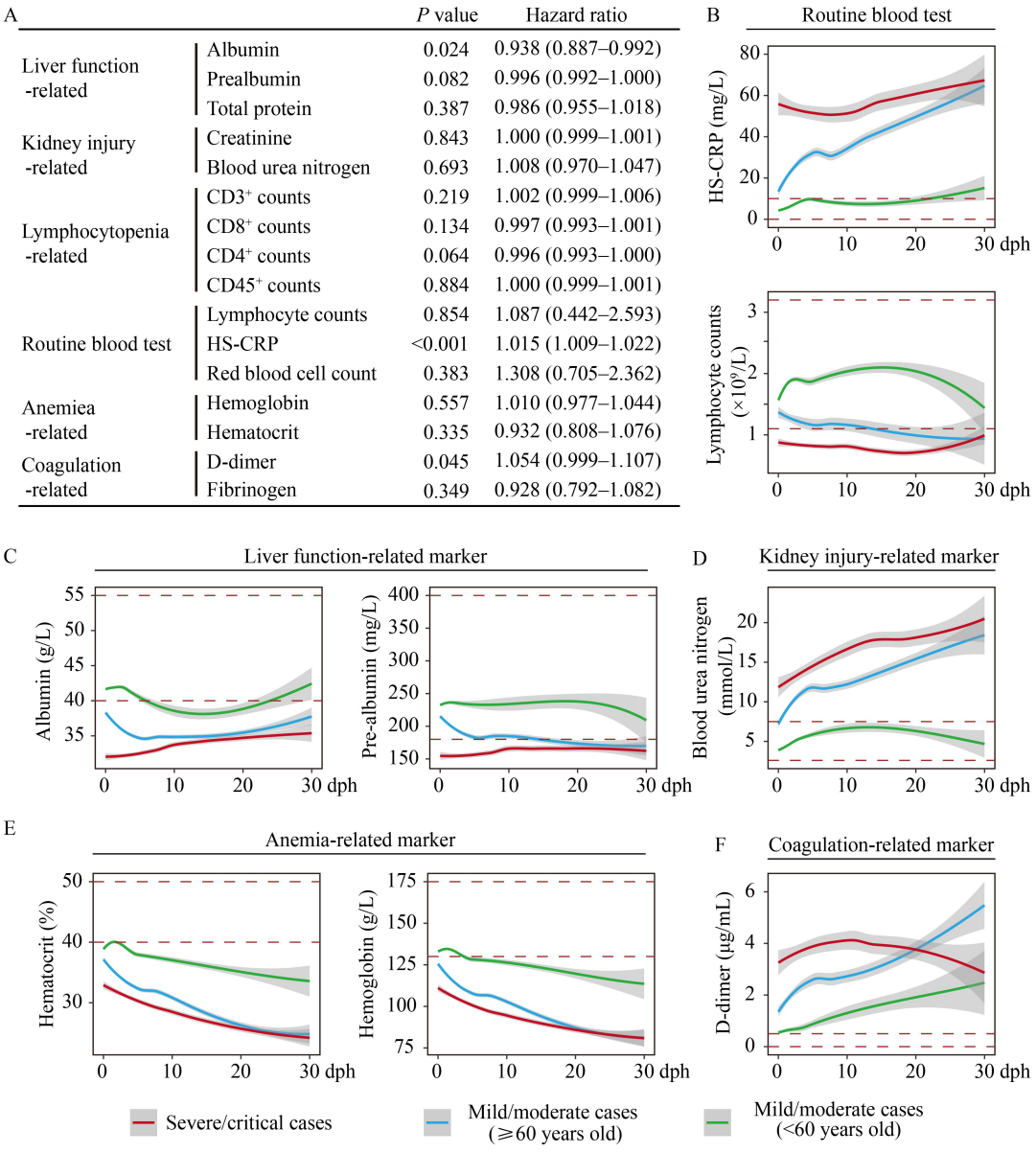

Fig. 2 Clinical laboratory features associated with COVID-19 severity in the BA.2.2 cohort. (A) Multi-variant tests of various clinical parameters in association with severe COVID. (B–F) Longitudinal analysis of a subset of routine blood test, anemia, coagulation, kidney damage, and liver function related features from 1 to 30 days post hospitalization (dph) in severe/critical, younger mild/moderate (< 60 years old), and elder mid/moderate (≥ 60 years old) COVID-19 infected by BA.2.2. Results are plotted by time. (B) Analysis of lymphocyte counts and high-sensitivity C-reactive protein (HS-CRP) on 1–30 dph. (C) Analysis of markers related to liver function/damage. Levels of albumin and pre-albumin are shown. (D) Analysis of markers related to kidney injury. Levels of blood urea nitrogen and creatinine are shown. (E) Analysis of anemia related features. Levels of hematocrit and hemoglobin are shown. (F) Analysis of coagulation related features. Results of D-dimers are shown.

responsible for these markers to return to normal ranges in most of the patients in the mild/moderate ≥ 60 years group (Fig. 3D and 3E, middle and right panels). For kidney-injury related marker, vaccination was associated with significant reduction of blood urea nitrogen levels on dph11–15 in severe/critical group and on dph1–5 in both mild/moderate ≥ 60 years and mild/moderate < 60 years groups (Fig. 3F). Last, for coagulation-related marker, the levels of D-dimer were significantly lower in vaccinated cases than in unvaccinated ones throughout dph1–30 in all patient groups. Besides, while D-dimer levels were above normal-range in the majority of cases in the severe/critical and mild/moderate ≥ 60 years groups, they were closer to (or even within) normal ranges in vaccinated cases in these two groups (Fig. 3G).

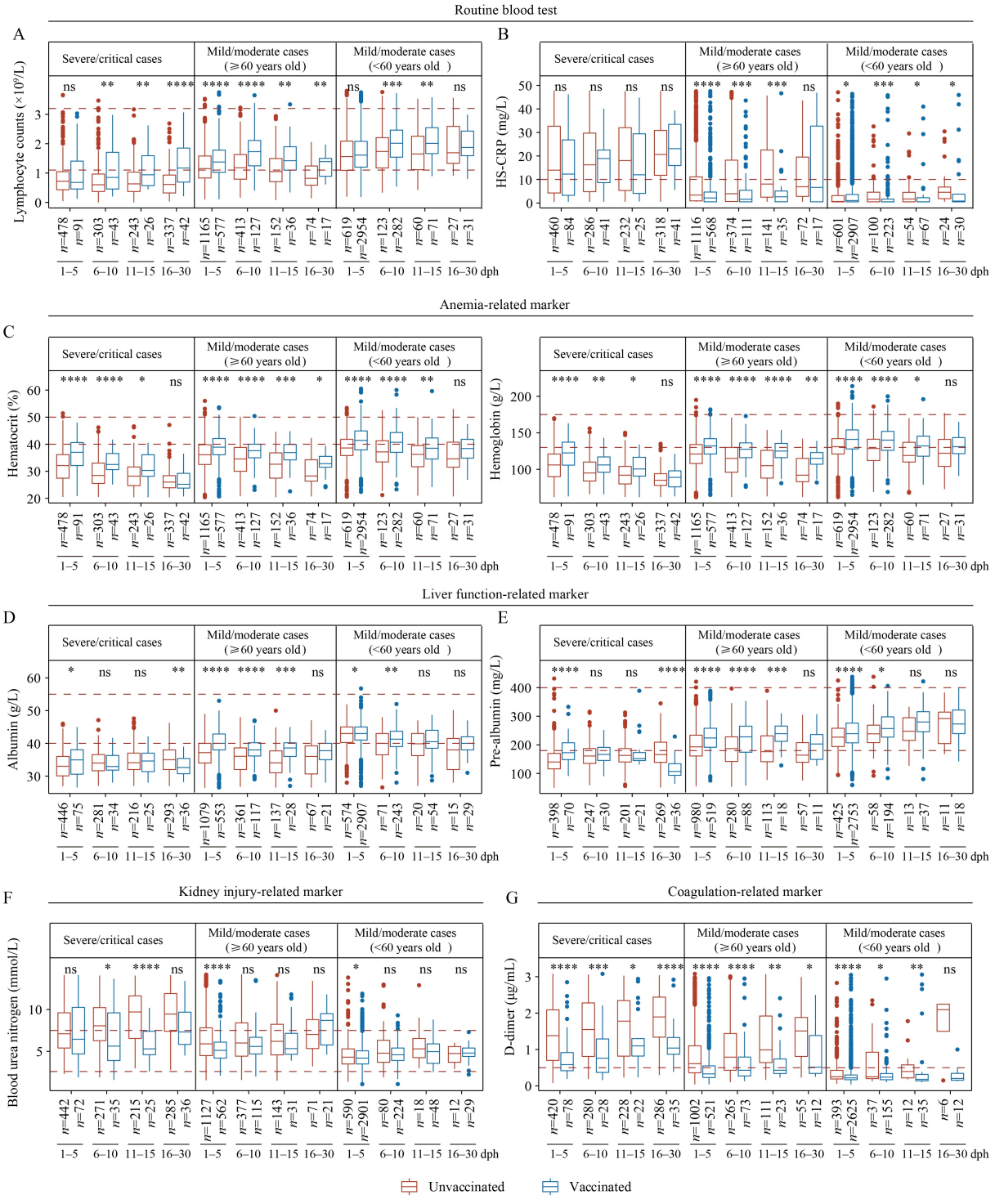

Fig. 3 Vaccination suppressed lymphocytopenia, anemia, coagulation disorder, and kidney injury in severe/critical COVID-19. (A–G) Longitudinal analysis of a subset of routine blood test (lymphocyte counts and HS-CRP), anemia (hemoglobin and hematocrit), liver function (albumin and pre-albumin), kidney injury (blood urea nitrogen), and coagulation (D-dimer) related features from 1 to 30 days post hospitalization (dph) in vaccinated and unvaccinated COVID-19 infected by BA.2.2. Severe/critical, mild/moderate of ⩾ 60 years old, and mild/moderate of < 60 years old COVID-19 cases were enrolled for analysis. Samples were divided into two groups, including the vaccinated and the unvaccinated. Results are plotted by time; results from 1–5, 6–10, 11–15, and 16–30 dph are shown. *n*, the number of samples used for analysis. A patient might have several tests on bio-analytes at multiple time points due to clinical requirement. Wilcox-test: ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.05$, *$P < 0.01$. ns, not significant.

Comparative analysis of clinical laboratory tests between patient cohorts with BA.2.2 and the prototype strain infection

To assess the impact of distinct viral lineages on the clinical presentation of COVID-19, we wondered what were vaccination-independent differences between the BA.2.2 and prototype cohorts. To address this question, we focused on comparing the clinical laboratory tests of 173 and 33 severe/critical naïve cases in the BA.2.2 and prototype cohorts, respectively, none of whom were vaccinated or infected before the time of sample collection. Overall, the patients in naïve BA.2.2 severe/critical cohort (median age 81 years, IQR 72–89 years) were much older than those in the prototype cohort (median age 64 years, IQR 55–71 years) ($P < 0.0001$, Fig. 4A). When the normal ranges of each clinical laboratory test were used as reference, even though the trend of deterioration of the levels of most COVID-19-related bioanalyte levels was similar between these naïve BA.2.2- and prototype-infected severe/critical cases (Fig. 4B–4F and Fig. S2), it was found that those related to white blood cells (elevated neutrophil count and decreased lymphocyte count; Fig. 4B), anemia (hematocrit and hemoglobin levels; Fig. 4C), coagulation (D-dimer, INR levels; Fig. 4D), liver function (albumin level; Fig. 4E), and kidney injury (blood urea nitrogen and creatinine levels; Fig. 4F) deviated in naïve BA.2.2-infected cases further away from normal levels than in prototype-infected cases. It is noteworthy that such differences were already present at early stage of the disease (i.e., dph1–5) and persisted during the remainder of hospitalization (i.e., dph6–30) for anemia-, coagulation-, and kidney-related markers (Fig. 4C, 4D, and 4F). Thus, the observed worse pathophysiological profiles of naïve BA.2.2 severe/critical cases, compared to the severe/critical cases affected by prototype infection, might be due to their occurring in a more elderly population with much weaker basal health conditions.

Additionally, we checked the profiles of 12 cytokines in 1387 cases (including 1222 mild/moderate and 165 severe/critical cases) with naïve BA.2.2 and 840 cases (including 807 mild/moderate and 33 severe/critical cases) with prototype infection (Fig. 4G). As reported earlier, prototype-infected severe/critical cases showed aberrant bursts of IL-12P70, IL-5, and IL-6 between dph5–20, which largely returned to normal levels between dph21–30 (Fig. S3A–S3C). Interestingly, the temporal profiles of these three factors were notably different in BA.2.2-infected severe/critical cases, that is, IL-12P70 levels stayed normal (Fig. S3A), IL-6 levels showed a small increase above normal levels between dph5–20 and then a gradual and notable rise on dph30 (Fig. S3B), whereas IL-5 levels did not increase until after dph20 (Fig. S3C). On the other hand, the levels of IL-8, which appeared normal in prototype-infected severe/critical cases, were instead aberrantly high in BA.2.2-infected cases (Fig. S3D). Last, previous studies suggested that defective type-I, but not type-II, interferon response was responsible for severe COVID-19 cases post prototype-infection. In agreement with this, IFN-γ, but not IFN-α, showed a distinct temporary rise between dph1–20, peaking at about dph10, in prototype severe/critical cases (Fig. S3E and S3F). However, these patterns were not observed in BA.2.2-infected cases; instead, IFN-γ levels stayed largely unchanged, while IFN-α showed a distinct increase between dph1–20 in BA.2.2-infected mild/moderate cases. Together, these results suggested that different cytokine release events were induced by SARS-CoV-2 prototype strain(s) and BA.2.2.

Comparative analysis of the bronchoalveolar microenvironment between patient cohorts with BA.2.2 and the prototype strain infection

To further assess the impact of distinct viral lineages on the host pulmonary immune responses, we conducted RNA-seq analyses using the bronchoalveolar lavage fluid (BALF) samples of 5 prototype- and 19 naïve BA.2.2 cases. Principal component analysis (PCA) suggested a notable difference in the transcriptome profiles between prototype and BA.2.2 groups (Fig. 5A). Gene expression analysis between these two groups identified 536 differentially expressed genes (DEGs). Among them, those related to type I interferon signaling pathway (e.g., *IFIT1*, *IFIT2*, *IFIT3*, *IFITM1*, and *IFITM3*), negative regulation of viral genome replication (e.g., *ISG15*, *ISG20*, *MX1*, *OAS1*, and *OAS3*), and positive regulation of interleukin-8 production (e.g., *DDX58*, *HSPA1A*, *HSPA1B*, and *PRKD2*) appeared to be significantly upregulated mainly in BA.2.2-infected cases (Fig. 5B and 5C), a conclusion supported by the relative enrichment of IFN-α signaling pathway activity in BA.2.2-infected cases according to GSEA analysis (Fig. S4). Moreover, as mentioned earlier, when compared to prototype-infected cases, BA.2.2-infected ones were characterized by elevated levels of IFN-α and IL-8 (Fig. S3D and S3F), which thus might be responsible for the differences in gene expression profiles in the BALF samples.

Last, our previous study of the BALF samples of the prototype cohort indicated alveolar macrophage as a potential main source of elevated cytokines in COVID-19 [18]. We thus compared the cell-type composition of prototype- and BA.2.2-infected BALF samples using a CIBERSORTx-based deconvolution analysis [23]. Interestingly, the overall ratio of M1/M2 macrophages showed no significant differences, but the relative fractions of macrophage subtypes differed in samples

A

Age group	BA.2.2 (severe/critical)	Prototype (severe/critical)	*P* value
Median (range)	81 (72–89)	64 (55–71)	<0.0001[a]
<10, *n* (%)	0 (0.00%)	0 (0.00%)	<0.0001[b]
10–19, *n* (%)	0 (0.00%)	0 (0.00%)	
20–29, *n* (%)	1 (0.37%)	2 (6.06%)	
30–39, *n* (%)	4 (1.47%)	2 (6.06%)	
40–49, *n* (%)	3 (1.10%)	4 (12.12%)	
50–59, *n* (%)	10 (3.67%)	4 (12.12%)	
60–69, *n* (%)	33 (12.09%)	10 (30.30%)	
70–79, *n* (%)	72 (26.37%)	8 (24.24%)	
80–89, *n* (%)	83 (30.40%)	3 (9.09%)	
≥90, *n* (%)	67 (24.54%)	0 (0.00%)	

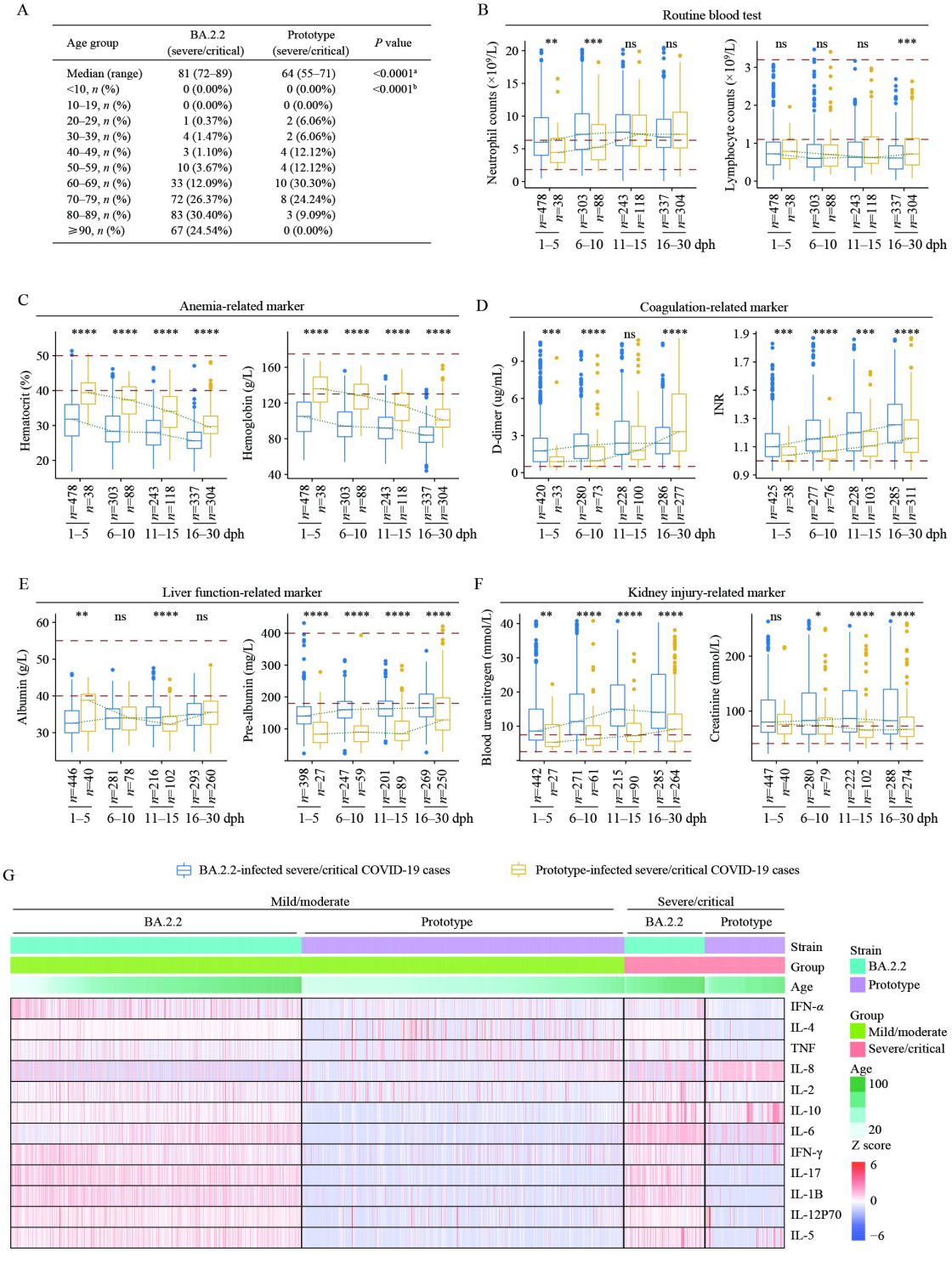

Fig. 4 Comparison of clinical laboratory features of patient cohorts with BA.2.2 and the prototype strain infection. (A) Age distribution in BA.2.2 and prototype infected severe/critical COVID-19. [a]*t*-test, [b]Fisher exact test. (B–F) Longitudinal analysis of a subset of routine blood test, cytokines, coagulation, liver injury, and kidney injury related features from 1 to 30 days post hospitalization (dph) in prototype and BA.2.2 infected severe/critical COVID-19. Prototype and BA.2.2-infected unvaccinated severe/critical COVID-19 cases were enrolled for analysis. Samples were divided into two groups, including the prototype and the unvaccinated BA.2.2 ones. Results are plotted by time, i.e., on 1–5, 6–10, 11–15, and 16–30 dph. Data of neutrophil counts and lymphocyte counts (B), hematocrit and hemoglobin (C), D-dimers and prothrombin time/international normalized ratio (PT/INR) (D), albumin and pre-albumin (E), blood urea nitrogen and creatinine (F) are shown. (G) Heatmap showing relative cytokine levels in prototype and BA.2.2-infected unvaccinated severe/critical COVID-19 cases. *n*, the number of samples used for analysis. A patient might have several tests on bio-analytes at multiple time points due to clinical requirement. Wilcox-test: ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. ns, not significant.

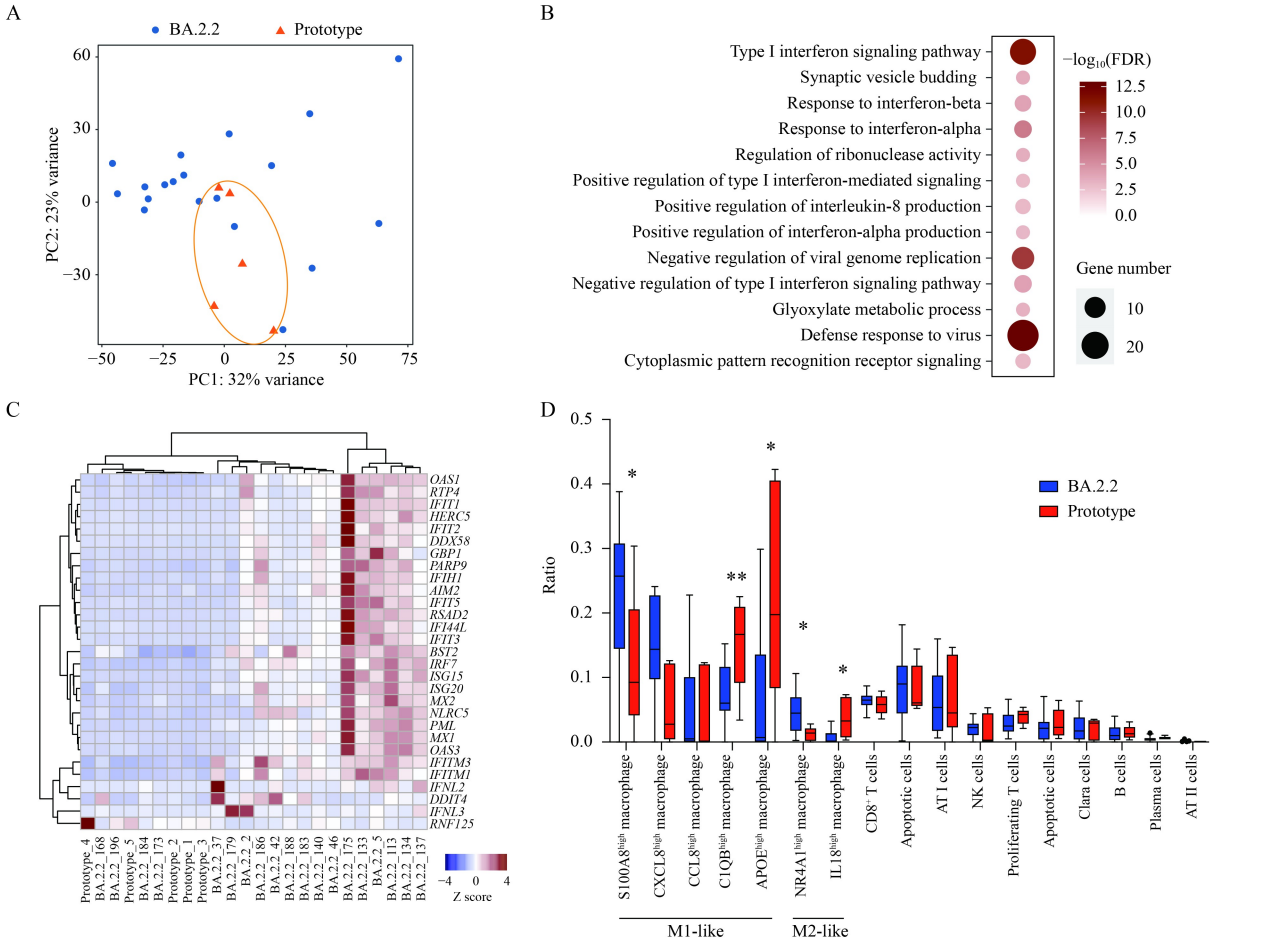

Fig. 5 Elevated virus burden and altered host responses in BALF of BA.2.2 infected patients. (A) Principal component analysis (PCA) of prototype and unvaccinated SARS-CoV-2-infected BALF samples in severe/critical COVID-19 samples. RNA-seq was conducted in the BALF samples of 5 prototype and 19 BA.2.2 infected severe/critical COVID-19 cases. (B) Gene ontology analysis of genes differentially expressed in BALF samples between BA.2.2 and prototype infected COVID-19. FDR values were shown by color. Enriched gene numbers were shown by circle size. (C) Heatmap showing representative differential expressed genes. (D) Cell distribution in the BALF of prototype and BA.2.2-infected severe/critical COVID-19. Relative ratio of 16 cell types were calculated by the CIBERSORT tools using bulk RNA-seq of BALF samples.

between sample groups. Of M1-like macrophages, the S100A8^{high} macrophages were significantly increased, while C1QB^{high} and APOE^{high} macrophages were significantly decreased, in BA.2.2 cases. Meanwhile, in M2-like macrophages, the *NR4A1*^{high} macrophages were significantly increased, while *IL18*^{high} was significantly decreased, in BA.2.2 cases (Fig. 5D). In agreement with these results, GSEA analysis using the DEGs between BA.2.2- and prototype-infected BALF samples showed a relative reduction of pro-inflammatory gene signatures in BA.2.2-infected cases (Fig. S4).

Discussion

Since the emergence of SARS-CoV-2 in human society in late 2019, great strides have been made to ameliorate the impact of this new pathogen on public health worldwide. In particular, NPI measures have shown perhaps the greatest benefit by far in saving human lives during the pandemic. For example, the average life expectancy in China increased from 77.3 years in 2019 to 78.2 years in 2021, which could be attributed to the strong NPI measures implemented in China to quickly detect and limit local spreading of SARS-CoV-2. However, it has been shown that recently evolved Omicron variants of SARS-CoV-2 have become extraordinarily infective, with an R_0 value approaching 18, while the mortality rate is also significantly reduced compared to other strains [26]. In this context, complementing NPI measures with new developments of vaccines and pharmaceutical means should offer a significantly stronger solution to protect life and further reduce the socio-economic impact of the ongoing pandemic. In particular, two pharmaceutical/vaccination strategies have gained great traction in countering against the COVID-19 symptoms: first, stratified treatment/options based on disease severity (i.e.,

palliative care for mild/moderate patients; Paxlovid plus immunotherapy for severe/critical cases) are highly effective in curing most symptomatic patients, including those infected by Omicron sublineages such as BA.2.2; second, vaccines based on ancestral and newer SARS-CoV-2 variants, using a number of different technologies (e.g., inactivated vaccine, recombinant vaccine, nucleic acid vaccines etc.), can offer strong protection against severe COVID-19. Nevertheless, while newly developed anti-viral drugs (e.g., Paxlovid) have shown high efficacy in treating COVID-19 patients with mild to moderate symptoms, their benefit for severe/critical cases remain unclear. In comparison, significant reduction of the rate of severe COVID-19 cases was observed among previously vaccinated individuals. Therefore, anti-viral therapy and immunization could serve as complementary approaches to treat mild/moderate cases and prevent severe/critical cases of COVID-19, respectively.

Previous studies reported that vaccination based on the ancestral SARS-CoV-2 might not be able to prevent infection of SARS-CoV-2, but protects against the severity of the COVID-19 [27–29]. Here, we showed an overall 96.02% of protection rate against severe/critical disease in symptomatic COVID-19 in our BA.2.2 patient cohort, while this could be even higher when asymptomatic cases were included for analysis. However, due to the continuing change of the SARS-CoV-2 genome and the uneven coverage of vaccination across regions and ages, it has been difficult to assess the benefit of vaccination in newer viral strains (especially those of the Omicron sublineages), or the target populations who might benefit the most from population-wide vaccination programs. Toward that end, here we have analyzed the pathophysiological features of Omicron BA.2.2 sublineages and the impact of prior immunization on them, based on the study of clinical laboratory test results of 9911 COVID-19 patients infected by a wave of subvariants of the Omicron BA.2.2 sublineages in Shanghai in spring 2022. Because the patients in our study reported no prior SARS-CoV-2 infection history, they comprise a unique cohort for the study of the pathophysiology of the Omicron BA.2.2 sublineages and the efficacy of prior vaccinations. Overall, our analysis showed that COVID-19 post-BA.2.2 infection was characterized by distinct dynamic patterns of bio-analytes in the peripheral blood that were indicative of anemia, lymphocytopenia, and internal organ functions (e.g., in the liver and kidney). Despite some statistically significant differences, these BA.2.2-characteristic pathophysiological features were similar to those observed in another group of patients in 2020 who were infected by prototype strain(s) of SARS-CoV-2 (prototype cohort) [18]. On the other hand, we identified age and vaccination status as two critical, independent risk factors of the severity of COVID-19 post BA.2.2 infection. As a result, severe/critical cases were primarily found in unvaccinated, elderly population (people $\geqslant$ 60 years old), while the percentages of severe/critical cases in each age group (60–69, 70–79, $\geqslant$ 80 years) decreased with the doses of prior immunization (1–3 doses). It is noteworthy that earlier studies also implicated several comorbidities —such as diabetes and high blood pressure—as independent risk factors. Because these medical conditions are common among the elderly population, they may also contribute to the development of severe COVID-19 post BA.2.2 infection, though their individual contributions could not be clearly separated from age due to the not large enough sample size of our patient cohort.

Recent studies also demonstrated milder symptoms in Omicron-infected COVID-19 compared to ancestral strains of SARS-CoV-2, such as Delta and Alpha [30]; for example, the rates of lung injury and acute respiratory distress syndrome (ARDS) were found to be significantly reduced in Omicron-infected cases comparing to ancestral strains, such as prototype and Delta [31,32], and the risk of long COVID-19 seems to be lower after infection by the Omicron variant than infection by other strains such as the Delta variant [33]. In consistence with these studies, we found that BALF samples in BA.2.2-infected patients showed less pro-inflammatory macrophage responses (i.e., $IL18^{high}$, $C1QB^{high}$, and $APOE^{high}$ macrophages [34,35]) and more anti-inflammatory macrophage response (i.e., $NR4A1^{high}$ macrophage) [36] were observed in unvaccinated patients post BA.2.2 infection (as compared to prototype strain infection), suggesting that SARS-CoV-2 BA.2.2 sublineages might trigger a more balanced innate immune response that, in turn, led to less severe COVID-19. Nevertheless, despite the suggestion that Omicron-infection might be limited to upper respiratory tract, we have found very high SARS-CoV-2 loads in several BALF samples from BA.2.2-infected severe/critical cases (Fig. S5), suggesting that the virus was able to penetrate into the lung in severe conditions. Further studies are warranted to investigate mechanism of the viral spreading in these cases.

In China, inactivated vaccines based on SARS-CoV-2 prototype strains were widely applied since early 2021 [37]. Reassuringly, despite notable divergence of the genome sequences of the Omicron BA.2.2 sublineages from ancestral strains, our study demonstrated significant protection against severe COVID-19-related symptoms in the BA.2.2 patient cohort. Such protection was especially pronounced in those receiving a three-dose vaccination schedule (two priming doses and one booster dose), suggesting that increasing the coverage of vaccination in the elderly population might be especially beneficial to reduce the rate of severe COVID-19. Indeed, a recent survey in Singapore showed that the overall low death

rate of COVID-19 (approximately 0.1%) could be primarily attributed to the higher vaccination rate, especially in elder people [38]. However, in the city of Shanghai, only 62% of people ⩾ 60 years received 2 shots of vaccination and only 38% received 3 shots of vaccination till April 28, 2022 [39]. Even after Shanghai's great effort to use strict NPI measures—including large-scale viral nucleic acid and antigen screening, quarantine of infected cases and close contacts in shelter hospitals and hotels—to contain the recent BA.2.2 outbreak, the vaccination coverage among the elderly Shanghai residents only marginally increased. For example, as of November 14, 2022, only approximately 46.19% of people ⩾ 60 years received 3 shots [40]. Thus, it is conceivable that broadening the coverage of full schedule and/or booster doses would be especially useful in dampening the impact of coming waves of Omicron pandemic in places without full vaccination and/or booster programs. Furthermore, given the observed protection against BA.2.2 by ancestral-strain-based vaccines, we suggest that currently available vaccines could be applied to help promote immunity against currently circulating SARS-CoV-2 strains, including Omicron variants, at least in the short-term. Meanwhile, public policies may be implemented to enable and accelerate the development and testing of new vaccination programs to prepare against the pandemic in the long-term. For example, emergency usage could be granted for multi-valent vaccines against multiple Omicron strains or heterologous immunization with vaccines based on different technologies. Additionally, community-level vaccination programs should be set up to provide/restore timely immunity in case the efficacy of vaccine-elicited immunity wanes over time. These measures, combined with efforts to ensure sufficient supplies of newly developed anti-viral drugs such as Paxlovid, may provide cost-effective venues to supplement and maximize the benefit of NPI measures.

Still, there remains some limitation of the current work. Samples of BALF collected from the vaccinated cases might be applied in future studies to address further the impact of vaccination on the immune response in the respiratory system. The dynamic features of clinical laboratory tests between different viral strains and vaccination conditions post-illness might be monitored in future studies.

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Compliance with ethics guidelines

Ziyu Fu, Dongguo Liang, Wei Zhang, Dongling Shi, Yuhua Ma, Dong Wei, Junxiang Xi, Sizhe Yang, Xiaoguang Xu, Di Tian, Zhaoqing Zhu, Mingquan Guo, Lu Jiang, Shuting Yu, Shuai Wang, Fangyin Jiang, Yun Ling, Shengyue Wang, Saijuan Chen, Feng Liu, Yun Tan, and Xiaohong Fan declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki Declaration* of 1975, as revised in 2000(5). Informed consent was obtained from all patients for being included in the study.

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