



Fatal cases after Omicron BA.1 and BA.2 infection: Results of an autopsy study

Bruno Märkl^{1,*}, Sebastian Dintner¹, Tina Schaller¹, Eva Sipos¹, Elisabeth Kling², Silvia Miller¹, Francisco Farfán López¹, Przemyslaw Grochowski¹, Nic Reitsam¹, Johanna Waidhauser³, Klaus Hirschbühl³, Oliver Spring⁴, Andre Fuchs⁵, Thomas Wibmer⁶, Peter Boor⁷, Martin Beer⁸, Claudia Wylezich⁸

¹ Pathology, Medical Faculty, University of Augsburg, Augsburg, Germany

² Microbiology, Medical Faculty, University of Augsburg, Augsburg, Germany

³ Hematology and Oncology, Medical Faculty, University of Augsburg, Augsburg, Germany

⁴ Anesthesiology and Operative Intensive Care Medicine, Medical Faculty, University of Augsburg, Augsburg, Germany

⁵ Internal Medicine III – Gastroenterology and Infectious Diseases, Medical Faculty, University of Augsburg, Augsburg, Germany

⁶ Public Health Department, City of Augsburg, Augsburg, Germany

⁷ Institute of Pathology, RWTH Aachen University Hospital, Aachen, Germany

⁸ Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald, Germany

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ABSTRACT

Objectives: Omicron lineages BA.1/2 are considered to cause mild clinical courses. Nevertheless, fatal cases after those infections are recognized but little is known about risk factors.

Methods: A total of 23 full and three partial autopsies in deceased with known Omicron BA.1/2 infections have been consecutively performed. The investigations included histology, blood analyses, and molecular virus detection.

Results: COVID-19-associated diffuse alveolar damage was found in only eight cases (31%). This rate is significantly lower compared with previous studies, including non-Omicron variants, where rates between 69% and 92% were observed. Neither vaccination nor known risk factors were significantly associated with a direct cause of death by COVID-19. Only those patients who were admitted to the clinic because of COVID-19 but not for other reasons had a significant association with a direct COVID-19 –caused death ($P > 0.001$).

Conclusion: Diffuse alveolar damage still occurred in the Omicron BA.1/BA.2 era but at a considerably lower frequency than seen with previous variants of concern. None of the known risk factors discriminated the cases with COVID-19-caused death from those that died because of a different disease. Therefore, the host's genomics might play a key role in this regard. Further studies should elucidate the existence of such a genomic risk factor.

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Introduction

The Omicron variant of concern (VOC) of SARS-CoV-2 is characterized by both high infectivity and transmissibility. Despite this, it causes a rather mild clinical course of COVID-19 compared with

the other VOCs [1–3]. In concordance, animal experiments showed reduced pathogenicity of the Omicron variants BA.1 and BA.2 compared with other VOCs. This includes less prominent loss of weight and lower viral burden in the upper and lower respiratory tracts in hamsters, angiotensin-converting enzyme-2 (ACE2)-wildtype mice, and K18-hACE2 transgenic mice [4]. In hamsters, the Delta variant was dominant over the BA.1 lineage of Omicron. In ferrets, COVID-19 infection was even abortive [5]. Meanwhile, five lineages (BA.1, 2, 3, 4, and 5) have been identified and characterized [6].

* Corresponding author: Bruno Märkl, Institute of Pathology and Molecular Diagnostics, Stenglinstrasse 2, 86156 Augsburg

E-mail address: bruno.maerkl@uka-science.de (B. Märkl).

As the Omicron variant is associated with a significant immune escape, both the effectiveness of current vaccines and the immunity of convalescents are hampered [7–10]. Despite this immune escape, booster vaccination has also been reported to reduce the mortality of COVID-19 caused by Omicron [11]. Although a considerable number of studies regarding the Wuhan strain and non-Omicron variants report effects on nearly all human organ systems, the direct cause of death is COVID-19 pneumonia with different stages of diffuse alveolar damage (DAD) [12,13]. The involvement of the vasculature seems to be responsible, in part, for these severe lung injuries [14]. A dysfunctional immune reaction after SARS-CoV-2 infection that causes immediate release of cytokines and a self-amplifying mechanism leading to a cytokine storm is likely causative of the COVID-19 involvement of many organs without direct viral interaction [15]. Given the data on reduced pathogenicity of the Omicron variant compared with previous VOCs, the question arises of whether and to what extent COVID-19 pneumonia is prevalent and of the cause of death in the deceased with Omicron SARS-CoV-2-infection. To address this, we analyzed the cases of the Augsburg autopsy study with confirmed Omicron variant BA1/2 infections.

Material and methods

Case collection

The Omicron study cohort comprises 26 deceased with proven infection with one of the known Omicron lineages of SARS-CoV-2 between January 2022 and May 2022. All patients were treated at the University Medical Center of Augsburg. In total, 170 autopsy cases of infections of other SARS-CoV-2 variants, partially included in previously published studies, served as controls [12,16,17]. Full autopsies were performed in 23 cases, while in three cases, the relatives restricted the autopsy to a minimally invasive approach (Figure 2).

Informed consent was obtained from the next of kin. The study was approved by the ethics committee of the Ludwig Maximilian University Munich (Project numbers 20–426; 22-0469)

Autopsy, sample collection, histology

The procedures used for the autopsy, sample collection, and histology have been described previously [16]. In brief, autopsies were performed within a body bag with respect to adequate safety rules [18,19]. Full autopsies included the opening of all body cavities and careful inspection and tissue sampling of all organs. In partial autopsies, larger tissue samples from the thoracic and abdominal organs were obtained from epigastric access. Regarding the causes of death, we classified the diagnosis that led directly to death. Importantly, this is different from the World Health Organization definition, which includes COVID-19 as a cause of death in cases when an existing disease is exacerbated due to COVID-19 [20]. Histological analyses are based on hematoxylin & eosin and periodic acid-Schiff stains. No immunohistochemical staining was performed.

RNA-in situ hybridization

These techniques have also been described previously [17]. In brief, RNA-in situ hybridization (ISH) was performed on representative lung samples from all Omicron cases using SARS-CoV-2 RNA-specific antisense probes designed and synthesized by Advanced Cell Diagnostics (ACD, Palo Alto, CA, USA; Cat. No: 848568). The RNAscope ISH assays were conducted on the Leica BOND-RX System (Leica, Germany) using the RNAscope 2.5 LS Reagent kit-BROWN (ACD, Cat. No: 322100). Chromogen detection and hema-

toxylin counterstaining were performed using a bond polymer refine detection kit (Leica, Cat. No.: DS9800).

Quantitative reverse transcription–polymerase chain reaction (RT-qPCR)

The RT-qPCR assay was described earlier. Briefly, RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) sections using the Maxwell CSC RNA FFPE Kit (AS1360, Promega) and swabs using the Maxwell 16 LEV Blood DNA Kit (AS1290, Promega) on a Maxwell system (Promega Corporation, Madison, WI, USA). An MS2 Phage control was added to the samples before the extraction of the RNA. A negative control containing only MS2 Phage was prepared and used for RT-qPCR after extraction. RT-qPCR was performed on a QuantStudio 5 Dx real-time PCR instrument (Thermo Fisher, Carlsbad, CA, USA) using the Taq-Path COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher, Pleasanton, TX, USA). The cycle threshold (Ct) values were classified into six categories (<10; 11–17; 18–24; 25–29; 30–40; negative). In cases where viral whole-genome sequencing failed, the Omicron BA.1 lineage was determined by S-Gene target failure. S-Gene negative cases were assigned to the BA.1 group. Viral dissemination with widespread viral RNA detection was defined previously [17].

Viral whole-genome sequencing/variant determination

SARS-CoV-2 whole-genome sequences were generated using a generic metagenomics workflow [21] which was combined with a capture enrichment procedure using SARS-CoV-2 specific myBaits (Daicel Arbor Biosciences, Ann Arbor, USA), if necessary [22]. In some cases, the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific, Germany) was applied using the Ion Chef instrument. After quality checks and quantification of generated sequencing libraries, they were pooled together and sequenced on an Ion Torrent S5XL instrument (Thermo Fisher Scientific, Germany) with Ion 530 sequencing chips and chemistry for 400 base pair reads. Raw sequencing data were analyzed using the Genome Sequencer Software Suite (version 2.6; Roche, Mannheim, Germany <https://roche.com>), with default software settings for quality filtering and mapping, using the SARS-CoV-2 reference sequence Wuhan-Hu-1 (MN908947). SARS-CoV-2 lineages were determined with the Pangolin COVID-19 Lineage Assigner [23]. The obtained SARS-CoV-2 genome sequences were aligned together with sequences retrieved from GenBank and GISAID using MAFFT version 7.38837, as implemented in Geneious version 10.2.3 (Biomatters, Auckland, New Zealand). Phylogenetic trees were constructed with PhyML version 3.038, using the general time reversible (GTR)+GAMMA+ I model with 100 bootstrap replications, and MrBayes version 3.2.639, using the GTR model with eight rate categories and a proportion of invariable sites in the Geneious software package. The Bayesian analysis was performed for 1,000,000 generations and sampled every 1000 generations for four simultaneous chains. The SARS-CoV-2 genome sequences generated in this study are available under accession numbers OP430881–OP430898.

Statistics

Categorical data were compared using the chi-square or Fisher's exact test depending on the group sizes. Depending on the distribution status and group numbers, the Student's *t*-test, the Mann-Whitney Rank sum test, or analysis of variance were used to compare continuous data. Correlations between nonconstant variables were calculated using Spearman rank order correlation. A *P*-value <0.05 was considered significant. All analyses were calculated using the Sigma Plot 13.0 software package (Systat, San Jose, CA, USA).

Results

Study cohort, vaccination status, causes of death

During the study period, 138 patients died from a proven SARS-CoV-2 infection at the University Medical Center in Augsburg. The study cohort comprises 26 consecutively collected cases, resulting in an autopsy rate of 19%. The demographic and clinicopathological data are summarized in Table 1. Overall, six (23%) of the deceased were nonvaccinated, three (12%) were partially vaccinated, and 17 patients were fully vaccinated (65%), including 10 (38%) cases with one and two (8%) with two booster vaccinations. The rate of fully vaccinated persons among the inhabitants of the city of Augsburg was 77% (July 2022) [24], which means that the group of fully vaccinated persons is slightly underrepresented in this study but without statistical significance ($P = 0.253$). Based on the autopsy results, in the group of un- or partially vaccinated patients ($n = 9$), only one person died directly due to COVID-19 pneumonia (11%), compared to seven (41%) ($P = 0.190$) in the group of fully vaccinated patients ($n = 17$), resulting in a total rate of 31% COVID-19-caused deaths. The corresponding rates in previous studies that served as controls were 92% and 69% for nonvaccinated and fully vaccinated patients, respectively [17] (Supplementary Table S1). The non-COVID-19 causes of death are summarized in Table 1. Seven out of those 18 patients died because of a non-

COVID, very likely bacterial, infection. The remaining 11 individuals succumbed to an acute exacerbation of cardiovascular comorbidity. In 13 patients who administratively belonged to the Public Health Department of the city of Augsburg, the cause of death provided to the official disease surveillance system was determined. Compared to the autopsy results, one case was consistently classified as caused by COVID-19. In eight cases, COVID-19 was classified divergently. In four cases, death was stated not to be because of COVID-19 per the autopsy results.

Organ involvement: Histology, RNA-ISH, PCR

The histological evaluation of all lung tissue samples revealed typical COVID-19-associated severe DAD as previously described [12] with coexisting acute and proliferative stages in several areas/lobes in eight (31%) cases (Figure 1a/b). In only one (C56) of these cases, a fibrotic stage was reached. In those eight cases, consecutive respiratory failure was the direct cause of death. Those eight were the only ones where a direct COVID-19-caused death was verifiable. In two additional cases, a mild nonfatal DAD was identified but did not directly cause the deaths. Severe acute pneumonia with dense infiltration of one or even two entire lobes was found in eight cases (31%) (Figure 1d). Overlap with severe, partly organizing DAD occurred in only one of those acute pneumonia cases (Figure 1c). Aspergillosis was also identified in one of these

Table 1
Clinicopathological data.

	Entire Omicron-study-group n = 26	Non-COVID-19 death n = 18	COVID-19 death n = 8	P-value
Median age (years)	82 (52 - 92)	83 (52 - 92)	80 (58 - 90)	0.656
Sex (female: male)	1: 3	1: 2	0: 8	0.132
Median number of comorbidities	4 (0 - 7)	4 (0 - 7)	4 (0 - 5)	0.683
Cancer	8 (31%)	5 (28%)	3 (38%)	0.667
COVID-19 hospital admission ^a	8 (31%)	1 (6%)	7 (88%)	< 0.001
Median body mass index (kg/m ²)	28 (16 - 48)	28 (16 - 48)	29 (19 - 33)	0.662
Hint for immuno suppression	11 (42%)	7 (39%)	4 (50%)	0.683
Invasive ventilation	6 (23%)	3 (17%)	3 (38%)	0.33
Omicron variant BA.1	21 (81%)	15 (83%)	6 (75%)	0.628
Omicron variant BA.2	5 (19%)	3 (17%)	2 (25%)	
Nonvaccinated	6 (23%)	5 (28%)	1 (13%)	0.190 ^c
Partial vaccinated	3 (12%)	3 (17%)	0	
Twice vaccinated	5 (19%)	3 (17%)	2 (25%)	
One booster vaccination	10 (38%)	6 (33%)	4 (50%)	
Two booster vaccinations	2 (8%)	1 (6%)	1 (13%)	
Median Ct-value nasophar (initial)	27 (24 - 38)	27 (24 - 38)	27 (24 - 29)	0.903
Median Ct-value nasophar (Autopsy)	20 (10 - 34)	22 (10 - 34)	19 (13 - 30)	0.594
IgA-levels (normal: 70-400) [mg/dl]	241 (14- 453)	402 (166 - 453)	212 (14 - 426)	0.077
IgG-levels (normal: 700-1600) [mg/dl]	759 (286 - 1360)	1185 (286 - 1340)	692 (491 - 1360)	0.338
Highest C-reactive protein (normal: <0.5) [mg/dl]	11 (0.8 - 43)	10 (0.8 - 43.0)	12.0 (1.7 - 27.4)	0.598
Highest procalcitonin (normal: <0.5) [ng/ml]	0.9 (0.1 - 55)	0.4 (0.1 - 55)	1.2 (0.1 - 25.0)	0.535
Highest interleukin-6 (normal: <15) [pg/ml]	500 (17 - 3560)	392 (17 - 3560)	728 (31 - 2570)	0.620
Time from first symptom to death (days)	9 (1 - 44)	9 (1 - 23)	17 (5 - 44)	0.096
Time from first positive polymerase chain reaction to death (days)	7 (1 - 51)	5 (1 - 51)	17 (1 - 44)	0.094
Remdesivir therapy		1 (5%)	4 (50%)	0.020
Anakinra therapy		1 (5%)	0 (0%)	1.000
Antibody ^b treatment		4 (22%)	4 (50%)	0.197
Dexamethasone treatment		7 (39%)	6 (75%)	0.202
Hospitalization length	5 (1 - 66)	5 (1 - 66)	7 (1 - 29)	1.000
Non-COVID-19 direct causes of death:				
Acute pneumonia		5	n.a.	
Cardiac failure		7	n.a.	
Probably arrhythmia		1	n.a.	
Mesenterial infarction		1	n.a.	
Pulmonary embolism		1	n.a.	
Myocardial infarction		1	n.a.	
Sepsis		2	n.a.	

Ct, cycle threshold; Ig, immunoglobulin.

^a Number of patients with hospital admission due primarily to COVID-19;

^b Sotrovimab, tocilizumab or fluvoxamine have been applied singular or in combination;

^c P-value was calculated between un-/incomplete vaccinated vs at least two vaccinations or booster received.

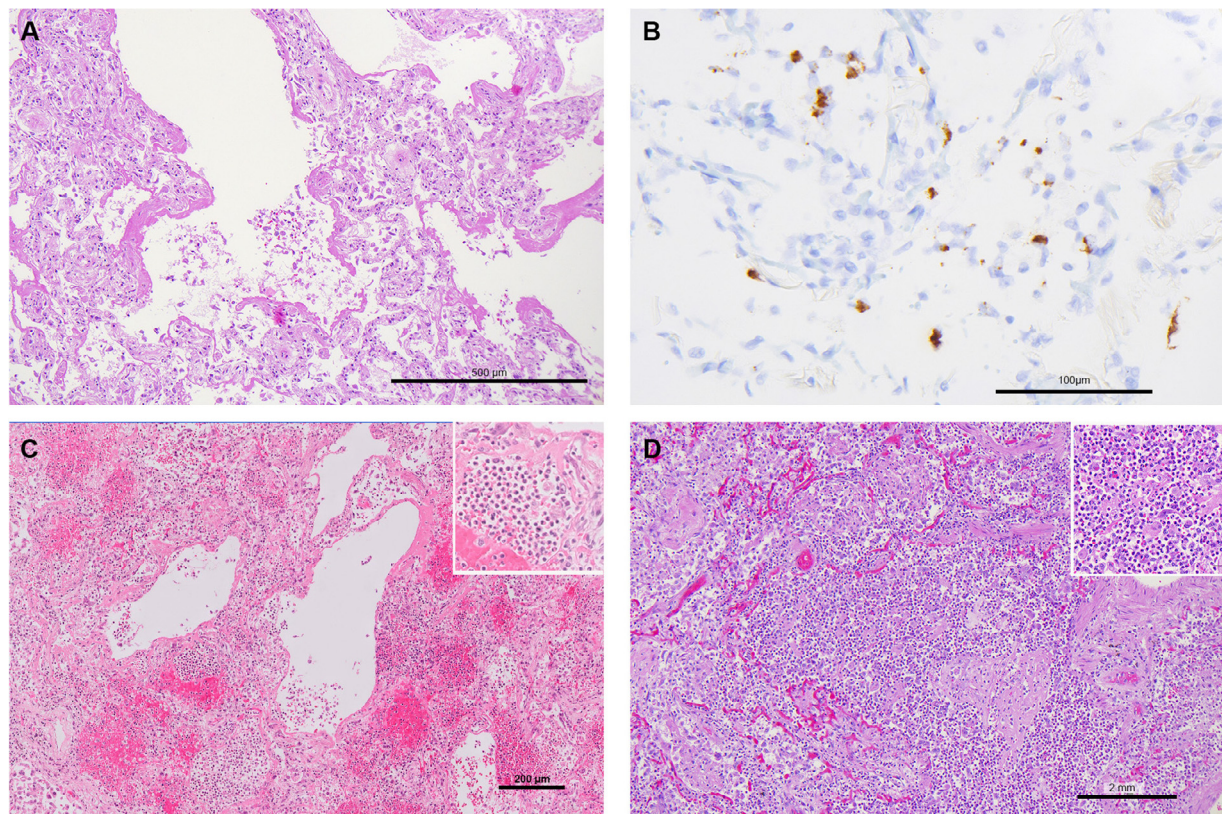


Figure 1. (a) H&E stain; acute DAD with alveolar spaces aligned with hyaline membrane. (b) SARS-CoV-2 RNA-in-situ hybridization; detection of viral RNA within the lung parenchymal. (c) H&E stain; simultaneous development of DAD and acute pneumonia with dense neutrophilic infiltration. Insert: higher magnification, same case. (d) H&E stain; severe acute pneumonia with parenchymal destruction and dense neutrophilic infiltration. Insert: higher magnification, same case. DAD, diffuse alveolar damage; H&E, hematoxylin & eosin.

cases. In concordance with previous investigations [16], no other organs showed alterations that could be classified as SARS-CoV-2-specific on the level of conventional light microscopy. SARS-CoV-2 identification by RNA-ISH was performed in samples from all lungs and revealed positive results in ten cases, with a highly significant correlation with the results of the RT-PCR ($P < 0.001$) from the lung samples (Figure 2b). The median Ct-value of the nasopharyngeal RT-PCR was 20 (range: 10–43). In five cases (19%), viral dissemination within the organ system, as previously defined and reported [17], is in the same range as in our series of non-VOC [16] but significantly lower than in our previous analyses of vaccinated non-Omicron cases ($P = 0.014$). Only one of these five cases belongs to the group of COVID deaths.

Serum analyses and sequencing

In 14 cases, the serum anti-SARS-spike and the anti-SARS-nucleocapsid antibodies were evaluated (Figure 2b). In all except one case, anti-SARS-spike antibodies were identified. This was especially true in all investigated fully vaccinated cases. In only five cases, anti-nucleocapsid antibodies were found, and only two of these belonged to the fully vaccinated group. Other serum values, including IL6, are summarized in Table 1.

All infections could be assigned either to the SARS-CoV-2 Omicron BA.1 ($n = 21$) or BA.2 ($n = 5$) lineages. Detailed assignment to sublineages was possible for cases that provided a full or nearly full genome sequence (Figure 2a). In one case (C54), no RNA was available, but this case occurred when BA.1 sublineages were circulating. None of the clinic-pathological or outcome parameters correlated with the attributed Omicron lineage.

Comparison between cases with and without direct COVID-19-related deaths

The comparison between cases with and without direct COVID-19-related deaths revealed no discriminating factor except the clinical presentation. Seven out of eight (88%) fatal cases presented as COVID-19 on admission, while the admission in only 6% of nonfatal cases was directly COVID-19-related ($P < 0.001$). Patients who died directly because of COVID-19 received more often a specific therapy even if this difference was significant only for Remdesivir (Table 1). The vaccination status did not significantly differ between the groups. In the 94% of remaining cases, the hospital admission was primarily because of other diseases. There was a clear trend toward a more prolonged period between first symptoms/positive PCR testing and death in COVID-19-caused deaths compared to other causes of death ($P = 0.096$ and 0.094). Immune globulin levels tended to be decreased in COVID-19 deaths, with a statistical trend for immunoglobulin A ($P = 0.077$). All other investigated clinicopathological data, including age, sex, body mass index, comorbidities, cancer history or hint at immune suppression did not differ between the two groups. (Table 1).

Discussion

In here we present the results of an autopsy study of the deceased with proven SARS-CoV-2 Omicron BA.1 or BA.2 infection. This study delivers three main findings. First, the frequency of direct COVID-19-caused deaths decreased considerably from initially 92% (non-Omicron, nonvaccinated COVID-19 deceased [16]) to 72% (non-Omicron, partially or fully vaccinated COVID-19 deceased, [17]) to 31% in the current study (Omicron) (Supplementary

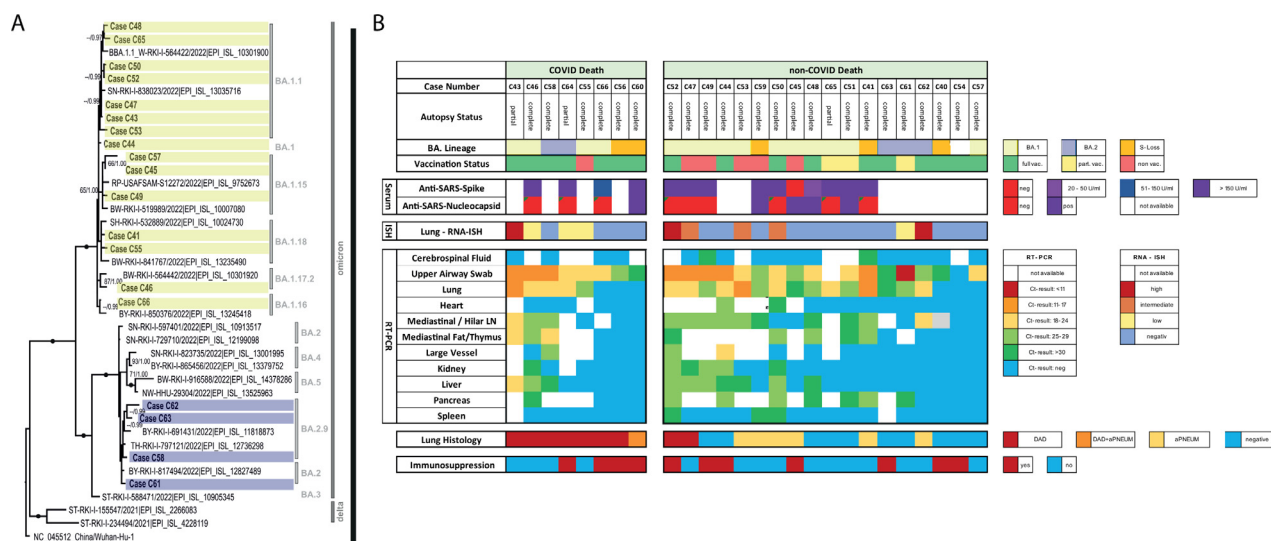


Figure 2. (a) Phylogenetic tree representing cases in which the whole viral genome could be sequenced. Dots indicate bootstrap values of 100/1.00 (Maximum Likelihood/MrBayes). Support values above 50% are given. (b) Autopsy-status, viral variant lineages, anti-SARS-antibody titer, and viral infection in different organs by RT-qPCR and RNA-ISH (for lungs only). aPNEUM, severe acute pneumonia; immunosuppression includes low immune globulin level, cancer, and drug-related; Ct, cycle threshold; COVID-19 Death, cause of death is directly COVID-19 related; DAD, diffuse alveolar damage; ISH, in situ hybridization; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; S-Loss, S-Gene loss determined by RT-qPCR. Note: this is not identical with the World Health Organization definition. Lung histology indicates the occurrence of DAD and acute pneumonia. In cases C52 and C47, DAD was mild without respiratory impairment. Note: Cases are not sorted in a consecutive manner but by grade of viral dissemination.

Table 1). In this context, it is worth emphasizing that in this study, as in the previously performed studies, the inclusion criterion was death with confirmed SARS-CoV-2 infection regardless of the extent of COVID-19-related symptoms. Second, although it is a rare scenario, severe fatal COVID-19 caused by Omicron BA.1/BA.2 does not differ regarding organ involvement and morphology compared to previous strains. Third, no convincing factor could be identified to discriminate between patients who were prone to develop severe fatal COVID-19 and the majority of individuals who died with SARS-CoV-2 infection but due to a different disease.

SARS-CoV-2 Omicron lineages differ in several terms from those of previous prevalent strains and VOCs [1,3] with increased infectivity and a relevant immune escape. Infections are related to a lower risk for hospitalization and severe clinical courses, including death [1,3,25–28]. This is in concordance with our result of a significantly reduced rate of direct COVID-19-caused deaths and a high rate of breakthrough infections in 65%. In addition, seven out of eight fatal COVID-19 courses were fully vaccinated, and two had even received the second booster. This underlines the ability of the Omicron lineages to escape efficiently from ancestral vaccine-derived immunity. We do not believe that our finding justifies the conclusion that vaccination promoted fatal courses. We suppose that it is rather caused by chance ($P = 0.190$), but it questions whether at the time available vaccinations had the potential to effectively prevent severe disease. However, at this point, it must be emphasized that our study is limited by a small number of cases. This is also because of an autopsy rate of only 19%, which is considerably decreased compared to previous studies, with 86% [16] and 55% [17], respectively. A further limitation of all autopsy studies is that all tissue-based analyses reflect a snapshot with limited potential to reconstruct the previous course.

Large population-based investigations report reduced effectiveness of vaccines in preventing fatal course. However, a relevant protective effect is still reported [1,28,29]. The results of this study allow for no direct transfer to the general population. However, they suggest that in the era of Omicron BA.1/BA.2, some patients with the Omicron variant still die due to classical COVID-19 pneu-

monia that shows the same morphology as with previous SARS-CoV-2 variants. This is an important finding because, as discussed above, the Omicron variant is known to be attenuated regarding its potential to cause severe illness. Animal experiments using BA.1 and BA.2 strains show exclusively mild courses with almost no symptoms [4,5]. Therefore, it could be questioned whether people infected with an Omicron variant die directly from the consequences of COVID-19 at all.

The low rate of direct COVID-19-caused death, however, may influence the official statistics of disease control institutions. Based on notifications according to the German infection control law, we identified eight out of 13 cases that were officially recorded as COVID-19-caused deaths, divergently from the results of autopsies, which may indicate a systematic problem. The establishment of regions with officially mandated postmortal investigations in a high frequency, like in Hamburg during the first wave of the pandemic, could be an approach to solving this [30]. Another potential approach is to gather these data on a national level, for example, in registries such as the German Registry of COVID-19 Autopsies (www.DeRegCOVID.ukaachen.de).

It would be interesting to identify factors associated with fatal SARS-CoV-2 infections. However, only the reason for hospital admission and the hospital stay time difference between the groups of cases with and without death because of COVID-19. All other relevant parameters, including factors related to immune suppression, showed similar characteristics. Even low Ct-values in airway samples occurred in cases without the development of DAD. It seems that fatal courses of BA.1/2 infections are triggered by a factor that is different from those of previous SARS-CoV-2 variants, which were connected with age, comorbidities, obesity, immune suppression, and vaccination status [17,31]. Increased susceptibility could be caused by the genetics of the host. For influenza A, several genetic variations can be identified that cause inborn errors of immunity. These affect viral replication and the inflammatory response in different parts of the immune system [32,33]. Genetic variations can influence the manifestation of many infections at different levels, such as viral load, organ involvement, chronicity,

malignant transformation, and severity of the acute disease [34]. Very recently, a meta-analysis addressed the aspect of individual genetic susceptibility to COVID-19. Variants of four genes could be observed that are associated with an increased rate of infection, as well as variants in five genes that heighten the risk of a severe clinical course. These genes code for the angiotensin-converting enzyme, the angiotensin-II receptor-1, and tumor necrosis factor- α . The question of whether the different viral variants are affected similarly remains unanswered [35].

A high rate (31%) of unusually severe acute pneumonia affecting one or two entire lobes was an unexpected observation in this study. Fungal coinfections in autopsy cases of those deceased after long-term treatment of COVID-19 were previously described [36]. In our previously published autopsy series of deceased vaccinated patients, we identified four out of 29 (14%) cases with pulmonary aspergillosis, while in our current series, fungal infection was observed in only one (4%) case. The reported prevalence numbers of bacterial co-/superinfections in SARS-CoV-2 infections differ considerably between studies and range from <1% to >50%, depending on the clinical setting and the definition [37]. There are several hypotheses about how SARS-CoV-2 infections could heighten the risk of bacterial or fungal superinfections. Epithelial disruption could enhance viral adherence, but an even more significant effect might be caused by immunogenic reactions, such as reduction of type I and III interferons, which are believed to be counterproductive regarding the defense against bacterial infections [38,39]. The question of whether the high rate of severe acute pneumonia is just an incidental accumulation, or indeed an enhanced susceptibility to superinfections, cannot be answered by this study. However, these data, together with divergent results reported in the literature, underline the need to address this issue in a separate study.

In conclusion, this study confirms that classic COVID-19 pneumonia can be caused by Omicron BA.1/2 infections but at a considerably lower rate compared with previously prevalent variants. The underlying mechanism that leads to such a fatal clinical course remains unclear and needs to be elucidated in further studies, potentially including analysis of the host's genome. Low rates of fatal courses might also influence the correctness of national statistics. The establishment of model regions with monitoring of the ongoing pandemic with autopsies at high frequency to be embedded in a registry such as the German COVID-19 autopsy registry [40], could be an appropriate approach to gain insight rapidly into an ongoing pandemic.

Declaration of competing interest

The authors have no competing interests to declare.

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Author contributions

Bruno Märkl: Study design, Data analysis, Data collection, Writing
Sebastian Dintner: Data analysis, Data collection, Writing
Tina Schaller: Data collection, Critical review
Eva Sipo: Data collection, Critical review
Elisabeth Kling: Data collection, Critical review
Silvia Miller: Data collection, Critical review
Francisco Farfán López: Data collection, Critical review
Przemyslaw Grochowski: Data collection, Critical review
Nic Reitsam: Data interpretation, Critical review
Johanna Waidhauser: Data collection, Critical review
Klaus Hirschbühl: Data analysis, Data collection, Data collection, Critical review
Oliver Spring: Data collection, Critical review
Andre Fuchs: Data interpretation, Critical review
Thomas Wibmer: Data analysis, Data collection
Peter Boor: Data collection, Data interpretation, Critical review
Martin Beer: Study design, Data analysis, Data collection, Critical review
Claudia Wylezich: Study design, Data analysis, Writing

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ijid.2022.12.029](https://doi.org/10.1016/j.ijid.2022.12.029).

References

- Mayr FB, Talisa VB, Castro AD, Shaikh OS, Omer SB, Butt AA. COVID-19 disease severity in US Veterans infected during Omicron and Delta variant predominant periods. *Nat Commun* 2022;13:3647. doi:[10.1038/s41467-022-31402-4](https://doi.org/10.1038/s41467-022-31402-4).
- Maslo C, Friedland R, Toubkin M, Laubscher A, Akaloo T, Kama B. Characteristics and outcomes of hospitalized patients in South Africa during the COVID-19 omicron wave compared with previous waves. *JAMA* 2022;327:583–4. doi:[10.1001/jama.2021.24868](https://doi.org/10.1001/jama.2021.24868).
- Wolter N, Jassat W, Walaza S, Welch R, Moultrie H, Groome M, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. *Lancet* 2022;399:437–46. doi:[10.1016/S0140-6736\(22\)00017-4](https://doi.org/10.1016/S0140-6736(22)00017-4).
- Halfmann P, Iida S, Iwatsuki-Horimoto K, et al. SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. *Nature* 2022;603:687–92. doi:[10.1038/s41586-022-04441-6](https://doi.org/10.1038/s41586-022-04441-6).
- Barut T, Halwe NJ, Taddeo A, Kelly JN, Schön J, Ebert N, et al. The spike gene is a major determinant for the SARS-CoV-2 Omicron-BA.1 phenotype. *Nat Commun* 2022;13:5929. doi:[10.1038/s41467-022-33632-y](https://doi.org/10.1038/s41467-022-33632-y).
- Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, et al. Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. *Nat Med* 2022;28:1785–90. doi:[10.1038/s41591-022-01911-2](https://doi.org/10.1038/s41591-022-01911-2).
- Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* 2022;602:654–6. doi:[10.1038/s41586-021-04387-1](https://doi.org/10.1038/s41586-021-04387-1).
- Hoffmann M, Krüger N, Schulz S, Cossmann A, Rocha C, Kempf A, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. *Cell* 2022;185:447–456.e11. doi:[10.1016/j.cell.2021.12.032](https://doi.org/10.1016/j.cell.2021.12.032).
- Zhang L, Li Q, Liang Z, Li T, Liu S, Cui Q, et al. The significant immune escape of pseudotyped SARS-CoV-2 variant Omicron. *Emerg Microbes Infect* 2022;11:1–5. doi:[10.1080/22221751.2021.2017757](https://doi.org/10.1080/22221751.2021.2017757).
- Lu L, Mok BWY, Chen LL, Chan JMC, Tsang OTY, Lam BHS, et al. Neutralization of severe acute respiratory syndrome coronavirus 2 omicron variant by sera from BNT162b2 or CoronaVac vaccine recipients. *Clin Infect Dis* 2022;75:e822–6. doi:[10.1093/cid/ciab1041](https://doi.org/10.1093/cid/ciab1041).
- Kiss Z, Wittmann I, Polivka L, Surján G, Surján O, Barcza Z, et al. Nationwide effectiveness of first and second SARS-CoV-2 booster vaccines during the delta and omicron pandemic waves in Hungary (HUN-VE 2 study). *Front Immunol* 2022;13:905585. doi:[10.3389/fimmu.2022.905585](https://doi.org/10.3389/fimmu.2022.905585).
- Schaller T, Hirschbühl K, Burkhardt K, Braun G, Trepel M, Märkl B, et al. Postmortem examination of patients with COVID-19. *JAMA* 2020;323:2518–20. doi:[10.1001/jama.2020.8907](https://doi.org/10.1001/jama.2020.8907).
- Satturwar S, Fowkes M, Farver C, Wilson AM, Eccher A, Girolami I, et al. Postmortem findings associated with SARS-CoV-2: systematic review and meta-analysis. *Am J Surg Pathol* 2021;45:587–603. doi:[10.1097/PAS.0000000000001650](https://doi.org/10.1097/PAS.0000000000001650).
- Jonigk D, Werlein C, Acker T, Aepfelbacher M, Amann KU, Baretton G, et al. Organ manifestations of COVID-19: what have we learned so far (not only) from autopsies? *Virchows Arch* 2022;481:139–59. doi:[10.1007/s00428-022-03319-2](https://doi.org/10.1007/s00428-022-03319-2).

- [15] Mangalmurti N, Hunter CA. Cytokine storms: understanding COVID-19. *Immunity* 2020;**53**:19–25. doi:10.1016/j.immuni.2020.06.017.
- [16] Hirschtbühl K, Dintner S, Beer M, Wylezich C, Schlegel J, Delbridge C, et al. Viral mapping in COVID-19 deceased in the Augsburg autopsy series of the first wave: a multiorgan and multimethodological approach. *PLoS One* 2021;**16**:e0254872. doi:10.1371/journal.pone.0254872.
- [17] Hirschtbühl K, Schaller T, Märkl B, Claus R, Sipos E, Rentschler L, et al. High viral loads: what drives fatal cases of COVID-19 in vaccinees? – an autopsy study. *Mod Pathol* 2022;**35**:1013–21. doi:10.1038/s41379-022-01069-9.
- [18] Brandner JM, Boor P, Borchering L, Edler C, Gerber S, Heinemann A, et al. Contamination of personal protective equipment during COVID-19 autopsies. *Virchows Arch* 2022;**480**:519–28. doi:10.1007/s00428-021-03263-7.
- [19] Hirschtbühl K, Schaller T, Kling E, Märkl B, Claus R. Autopsy of patients with COVID-19: a balance of fear and curiosity. *Pathol Res Pract* 2020;**216**:153039. doi:10.1016/j.prp.2020.153039.
- [20] World Health Organization *International Guidelines for certification and classification (Coding) of COVID-19 as cause of death*; 2020 https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19.pdf (accessed 04 May 2022).
- [21] Wylezich C, Papa A, Beer M, Höper D. A versatile sample processing workflow for metagenomic pathogen detection. *Sci Rep* 2018;**8**:13108. doi:10.1038/s41598-018-31496-1.
- [22] Wylezich C, Schaller T, Claus R, Hirschtbühl K, Märkl B, Kling E, et al. Whole-genome analysis of SARS-CoV-2 samples indicate no tissue specific genetic adaptation of the virus in COVID-19 patients' upper and lower respiratory tract. *Diagn Microbiol Infect Dis* 2021;**101**:115520. doi:10.1016/j.diagmicrobio.2021.115520.
- [23] O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evol* 2021;**7**:veab064. doi:10.1093/ve/veab064.
- [24] Augsburg Co Corona information site; 2022 <https://www.augsburg.de/umwelt-soziales/gesundheit/coronavirus/englisch#c1082673> (accessed 21 July 2022).
- [25] Lino A, Cardoso MA, Martins-Lopes P, Gonçalves HMR. Omicron - the new SARS-CoV-2 challenge? *Rev Med Virol* 2022;**32**:e2358. doi:10.1002/rmv.2358.
- [26] Tian D, Sun Y, Xu H, Ye Q. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *J Med Virol* 2022;**94**:2376–83. doi:10.1002/jmv.27643.
- [27] Kumar S, Thambiraja TS, Karuppanan K, Subramaniam G. Omicron and Delta variant of SARS-CoV-2: a comparative computational study of spike protein. *J Med Virol* 2022;**94**:1641–9. doi:10.1002/jmv.27526.
- [28] Bager P, Wohlfahrt J, Bhatt S, Stegger M, Legarth R, Møller CH, et al. Risk of hospitalisation associated with infection with SARS-CoV-2 omicron variant versus delta variant in Denmark: an observational cohort study. *Lancet Infect Dis* 2022;**22**:967–76. doi:10.1016/S1473-3099(22)00154-2.
- [29] Sheikh A, Kerr S, Woolhouse M, McMenamin J, Robertson C. Severity of omicron variant of concern and effectiveness of vaccine boosters against symptomatic disease in Scotland (EAVE II): a national cohort study with nested test-negative design. *Lancet Infect Dis* 2022;**22**:959–66. doi:10.1016/S1473-3099(22)00141-4.
- [30] Edler C, Schröder AS, Aepfelbacher M, Fitzek A, Heinemann A, Heinrich F, et al. Dying with SARS-CoV-2 infection—an autopsy study of the first consecutive 80 cases in Hamburg, Germany. *Int J Legal Med* 2020;**134**:1275–84. doi:10.1007/s00414-020-02317-w.
- [31] Booth A, Reed AB, Ponzio S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: a global systematic review and meta-analysis. *PLoS One* 2021;**16**:e0247461. doi:10.1371/journal.pone.0247461.
- [32] Clohisey S, Baillie JK. Host susceptibility to severe influenza A virus infection. *Crit Care* 2019;**23**:303. doi:10.1186/s13054-019-2566-7.
- [33] Novak T, Hall MW, McDonald DR, Newhams MM, Mistry AJ, Panoskaltis-Mortari A, et al. RIG-I and TLR4 responses and adverse outcomes in pediatric influenza-related critical illness. *J Allergy Clin Immunol* 2020;**145**:1673–1680.e11. doi:10.1016/j.jaci.2020.01.040.
- [34] Kenney AD, Dowdle JA, Bozzacco L, McMichael TM, St Gelais CS, Panfil AR, et al. Human genetic determinants of viral diseases. *Annu Rev Genet* 2017;**51**:241–63. doi:10.1146/annurev-genet-120116-023425.
- [35] Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med* 2020;**26**:1017–32. doi:10.1038/s41591-020-0968-3.
- [36] Evert K, Dienemann T, Brochhausen C, Lunz D, Lubnow M, Ritzka M, et al. Autopsy findings after long-term treatment of COVID-19 patients with microbiological correlation. *Virchows Arch* 2021;**479**:97–108. doi:10.1007/s00428-020-03014-0.
- [37] Omoush SA, Alzyoud JAM. The prevalence and impact of coinfection and superinfection on the severity and outcome of COVID-19 infection: an updated literature review. *Pathogens* 2022;**11**. doi:10.3390/pathogens11040445.
- [38] Bengoechea JA, Bamford CG. SARS-CoV-2, bacterial co-infections, and AMR: the deadly trio in COVID-19? *EMBO Mol Med* 2020;**12**:e12560. doi:10.15252/emmm.202012560.
- [39] Rynda-Apple A, Robinson KM, Alcorn JF. Influenza and bacterial superinfection: illuminating the immunologic mechanisms of disease. *Infect Immun* 2015;**83**:3764–70. doi:10.1128/IAI.00298-15.
- [40] von Stillfried S, Bülow RD, Röhrig R, Boor P, Böcker J, Schmidt J, et al. First report from the German COVID-19 autopsy registry. *Lancet Reg Health Eur* 2022;**15**:100330. doi:10.1016/j.lanepe.2022.100330.