samples from Portugal sequenced in May 2022 (*6*) and in a publicly available MPXV sequence dataset (A. Nekrutenko et al., unpub. data, https://virological.org/t/mpxv-intrahost-variation-in-the-context-of-apobec-deamination-an-initial-look/856), suggesting that this pattern might be a general pattern of evolution for the 2022 MPXV outbreak. However, in contrast to the previous findings (*6*; A. Nekrutenko et al., unpub. data, https://virological.org/t/mpxv-intrahost-variation-in-the-context-of-apobec-deamination-an-initial-look/856), both the major and minor SNV genotypes from patient 1 could be found fixed in previously reported MPXV sequences.

In conclusion, we demonstrate intrahost MPXV variation within a single lesion from one of the patients with infection introduced to Finland. Most of the sequence reads in that sample contained APOBEC3-related mutations, which may have emerged from the ancestral minor variant present in this sample. However, because the majority and minority nucleotides in that sample are also found fixed in sequences from other countries, we cannot resolve whether this observation relates to contemporary APOBEC3-driven evolution or to co-infection.

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New Postmortem Perspective on Emerging SARS-CoV-2 Variants of Concern, Germany

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We performed autopsies on persons in Germany who died from COVID-19 and observed higher nasopharyngeal SARS-CoV-2 viral loads for variants of concern (VOC) compared with non-VOC lineages. Pulmonary inflammation and damage appeared higher in non-VOC than VOC lineages until adjusted for vaccination status, suggesting COVID-19 vaccination may mitigate pulmonary damage.

[1]These authors contributed equally to this article.

SARS-CoV-2 emerged in 2020, and after rapid global spread, virus variants emerged that had adaptation or immune evasion mutations and were classified as variants of concern (VOCs). Although the first VOCs, Alpha (B.1.1.7) and Delta (B.1.617.2), showed enhanced transmissibility (*1*), Omicron (B.1.1.529) lineages carry mutations that provide strong immune evasion after infection with previous lineages or mRNA vaccination (*2*). Because autopsy data are lacking, differences in SARS-CoV-2–related pulmonary disease and tropisms have not been well studied. In this study, we performed full autopsies of persons

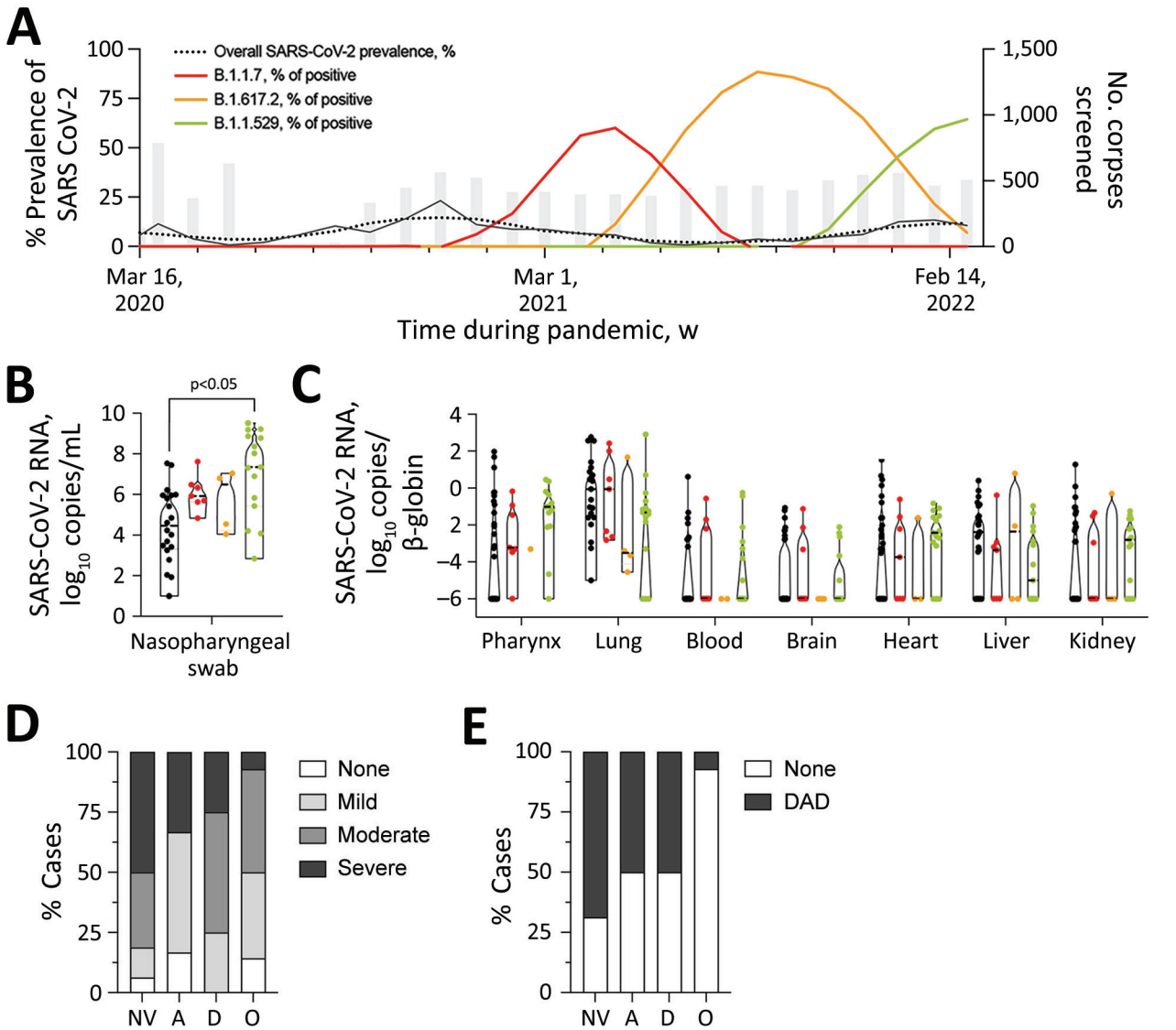

Figure. Prevalence of SARS-CoV-2 variants, pulmonary inflammation, and diffuse alveolar damage in corpses autopsied during 2020 and 2022 at the Institute of Legal Medicine and crematoria in Hamburg, Germany. A) Overall prevalence of corpses positive for SARS-CoV-2 mRNA and prevalence of B.1.1.7 (Alpha), B.1.617.2 (Delta), and B.1.1.529 (Omicron) variants of concern as the percentage of SARS-CoV-2 mRNA-positive corpses are depicted; 3-point centered moving averages are shown. Gray bars indicate monthly number of corpses screened for SARS-CoV-2 mRNA. B, C) Number of SARS-CoV-2 mRNA copies in different autopsy specimens. Median and interquartile ranges of viral mRNA loads were stratified according to virus variants in nasopharyngeal swabs (B) and different organs (C). Nasopharyngeal and organ viral loads for non-VOC and B.1.1.7 were published in part previously (*7*,*8*). Black dots are non-VOC lineages. Pairwise comparisons were performed by using the Kruskal-Wallis H test and Dunn post-hoc analysis. D, E) Percentage of cases that had pulmonary inflammation or alveolar damage caused by different SARS-CoV-2 lineages: NV, Non-VOC lineage; A, Alpha B.1.1.7 lineage; D, Delta B.1.617.2 lineage; O, Omicron B.1.1.529 lineage. D) We scored microscopic pulmonary inflammation as follows: none, 0; mild, 1; moderate, 2; or severe, 3 on the basis of a Likert-like scale and calculated percentages of cases within each group for each SARS-CoV-2 variant. E) Percentage of corpses infected with non-VOC lineages (n = 16) and the VOC lineages B.1.1.7 (Alpha, n = 6), B.1.617.2 (Delta, n = 4), and B.1.1.529 (Omicron, n = 14) that had DAD in lungs. DAD, diffuse alveolar damage; VOC, variant of concern.

Table. Baseline characteristics of persons whose deaths were associated with SARS-CoV-2 infection, grouped according to SARS-CoV-2 virus variant in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Variable	Non-VOC lineages	B.1.1.7†	B.1.617.2†	B.1.1.529†	p value	Cohort total
No. corpses	23	7	4	15	NA	49
Age, y, median (IQR)	76.0 (70.0–85.0)	75.0 (52.0–77.0)	50.5 (42.5–70.0)	75.0 (58.0–87.0)	0.29	75.0 (63.0–85.0)
Sex					0.31	
M	15 (65.2)	4 (57.1)	2 (50.0)	5 (33.3)	NA	26 (53.1)
F	8 (34.8)	3 (42.9)	2 (50.0)	10 (66.7)	NA	23 (46.9)
BMI, kg/m^2, median (IQR)	25.3 (20.7–31.9)	29.5 (26.1–34.8)	38.4 (16.5–42.9)	22.6 (18.8–23.6)	0.02	24.8 (20.7–31.0)
COVID-19 deaths	20 (87.0)	6 (85.7)	3 (75.0)	3 (20.0)	<0.0001	32 (65.3)
No. underlying conditions, median (IQR)	4.0 (3.0–4.0)	2.0 (2.0–3.0)	3.0 (2.0–5.0)	4.0 (2.0–5.0)	0.24	3.5 (2.0–4.0)
Place of death					0.23	
Home	5 (21.7)	3 (42.9)	0 (0.0)	5 (33.3)	NA	13 (26.5)
Normal ward	9 (39.1)	2 (28.6)	2 (50.0)	2 (13.3)	NA	15 (30.6)
ICU	5 (21.7)	2 (28.6)	2 (50.0)	2 (13.3)	NA	11 (22.4)
Other	4 (17.4)	0 (0.0)	0 (0.0)	6 (40.0)	NA	10 (20.4)
PMI, d, median (IQR)	1.0 (0.0–1.0)	3.0 (1.0–6.0)	1.0 (1.0–1.5)	0.0 (0.0–1.0)	0.03	1.0 (0.0–2.0)
Vaccination	0 (0.0)	1 (7.7)	1 (7.7)	11 (84.6)	<0.0001	13 (27.1)

*Corpses were admitted to the Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, for autopsy during 2020–22. Values are no. (%) unless otherwise noted. BMI, body mass index; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; PMI, postmortem interval (time elapsed from death until cooling at 4°C); VOC, variant of concern.
†VOC lineages B.1.1.7 (Alpha), B.1.617.2 (Delta), and B.1.1.529 (Omicron).

who died from COVID-19 and conducted comprehensive analyses to characterize COVID-19–related cases macroscopically and microscopically.

All corpses admitted to the Institute of Legal Medicine (n = 8,578) and crematoria (n = 1,705) in Hamburg, Germany, during March 3, 2020–March 31, 2022, were screened for SARS-CoV-2 mRNA by quantitative reverse transcription PCR (*3*). We found a total of 808 SARS-CoV-2 RNA-positive corpses; median monthly prevalence was 6.54% in 2020, 5.28% in 2021, and 12.50% in 2022 (Figure, panel A). In comparison, the median monthly prevalence of SARS-CoV-2 in Hamburg's general population was 0.10% in 2020, 0.37% in 2021, and ≈5.20% in 2022 (https://de.statista.com/statistik/daten/studie/1106006/umfrage/entwicklung-der-fallzahl-des-coronavirus-in-hamburg). A considerably higher prevalence of virus in deceased persons than in the general population can be explained by an older age in postmortem cohorts, targeted transport of clinically suspected or confirmed COVID-19 deaths to the Institute of Legal Medicine in 2020, and underreporting of overall SARS-CoV-2 prevalence because of limited availability of molecular testing.

We further characterized SARS-CoV-2 RNA-positive samples by using multiplexed typing quantitative reverse transcription PCR (*4*,*5*). The occurrence of VOCs among corpses (B.1.1.7 for December 2020–March 2022, B.1.617.2 beginning in May 2021, and B.1.1.529 beginning in November 2021) reflected their occurrence within the general population but had a 2–4 week delay (https://www.leibniz-liv.de/de/aktuelles/covid-19/daten-der-hamburg-surveillance-plattform).

Of the 808 COVID-19–associated deaths (determined by postmortem SARS-CoV-2 RNA detection), we autopsied 49 corpses and collected multiorgan tissue samples for more detailed analyses. We included 23/49 consecutive deceased persons infected with non-VOC lineages and 26/49 consecutive deceased persons infected with VOCs (B.1.1.7, n = 7; B.1.617.2, n = 4; and B.1.1.529, n = 15) (Table). We processed formalin-fixed paraffin-embedded tissue for histologic and immunohistochemical staining and cryopreserved tissue for molecular analysis as previously described (*3*,*6*).

The median nasopharyngeal SARS-CoV-2 RNA load was 5.82 (interquartile range [IQR] 4.08–7.31) $\log_{10}$ copies/mL (Figure, panel B). Nasopharyngeal and organ viral loads for non-VOC and B.1.1.7 were published in part previously (*7*,*8*). We observed strong evidence for a difference in mean nasopharyngeal viral loads by virus variant (p = 0.01); by using multiple comparisons, we observed a difference in means between B.1.1.529 and non-VOC lineages (p = 0.002; Figure, panel B). This result supports increased infectivity of B.1.1.529 compared with non-VOC lineages (*9*). An association between nasopharyngeal virus load and virus variant at the 5% significance level did not persist in a multivariable

model adjusted for the deceased's vaccination status, which might be because of the small sample size (Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/29/3/22-1297-App1.pdf). Of note, the pulmonary virus load was strongly associated with viremia (odds ratio 2.21, 95% CI 1.34–3.63; p = 0.002) and mRNA detection in peripheral organs (odds ratio 1.54, 95% CI 1.10–2.16; p = 0.01) in univariable logistic regression models. However, normalized SARS-CoV-2 RNA loads in peripheral organs did not differ between virus variants (Figure, panel C).

Experienced pathologists performed single-blind histologic assessments. We detected SARS-CoV-2 nucleocapsid protein in the lungs of 25/41 (61%) cases. Using a Likert-like scale, we found the median abundance of SARS-CoV-2 nucleocapsid protein (0, not detected; 1, low abundance; 2, intermediate abundance; 3, high abundance) was 1 (IQR 0–2) for non-VOC lineage, 1.5 (IQR 1–2) for B.1.1.7, 0.5 (IQR 0–1) for B.1.617.2, and 0 (IQR 0–1) for B.1.1.529 cases (p = 0.03) (Appendix Figure).

We detected mild to strong inflammatory changes in the lungs of 36/40 (90%) cases and microscopic signs of diffuse alveolar damage (DAD), indicating acute respiratory distress syndrome, in 17/40 (43%) cases (Figure, panels D, E). As in recent animal experiments (*10*), pulmonary changes, such as inflammation and DAD, were associated with virus variant at the 5% level (Appendix Tables 3, 4) but not with nasopharyngeal or pulmonary viral load (inflammatory changes, p>0.05; DAD, p>0.05). An association between virus variants and inflammatory changes or DAD at the 5% level did not persist in a multivariable model adjusted for the deceased's vaccination status (Appendix Tables 5–7).

In conclusion, our data confirm higher SARS-CoV-2 mRNA loads in nasopharynges of deceased persons who were infected with the B.1.1.529 VOC lineage, but we observed no differences in pulmonary or tertiary organ viral loads. However, pulmonary inflammation appeared higher and DAD more frequent in non-VOC than VOC lineages until adjustment for vaccination status. Our results suggest that prior vaccination, rather than reduced virulence of virus variants, might convey protection against pulmonary inflammation and acute respiratory distress syndrome during SARS-CoV-2 infections.

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Possible Mpox Protection from Smallpox Vaccine–Generated Antibodies among Older Adults

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Smallpox vaccination may confer cross-protection to mpox. We evaluated vaccinia virus antibodies in 162 persons ≥50 years of age in Spain; 68.5% had detectable antibodies. Highest coverage (78%) was among persons 71–80 years of age. Low antibody levels in 31.5% of this population indicates that addressing their vaccination should be a priority.

As the 2022 mpox outbreak spread worldwide, protection against smallpox has become a focus of interest because smallpox vaccination might provide some protection against monkeypox virus (*1*). Massive vaccination with live vaccinia virus vaccines was conducted in most countries before smallpox was eradicated in 1980 (*2*), meaning a substantial proportion of persons ≥50 years of age as of 2022 might be protected against both diseases. One suggested approach to mpox protection during the current outbreak has been to administer smallpox vaccine to close contacts of infected persons (*3*,*4*). However, before taking this approach if the outbreak spreads to additional persons, concerns need to be addressed about whether smallpox vaccination provides real cross-protection and, if so, whether protection has waned over time.

We conducted a serologic study among 162 persons ≥50 years of age in Spain who had probably received smallpox vaccination to determine the seroprevalence of vaccinia virus antibodies (VVAbs). We included 10 unvaccinated persons <40 years of age as controls, avoiding persons 40–49 years of age to eliminate possible interference in findings from persons of those ages possibly having been immunized against smallpox in the final years of vaccination. Our aim was to ascertain the presence of residual vaccinia virus immunity among adult/elderly persons. The study was approved by the ethics committee of the Eastern Health Area of Valladolid (cod: PI 22–2798) and research performed according to the Declaration of Helsinki. We obtained written informed consent from participants before sampling.

We used the Anti-Vaccinia virus IMV/Envelop protein/H3L/p35 IgG ELISA (Alpha Diagnostic International, https://www.4adi.com) to detect IgG against the vaccinia envelope protein H3L/p35, following manufacturer specifications (Appendix, https://wwwnc.cdc.gov/EID/article/29/3/22-1231-App1.pdf). VVAb levels were expressed in units per milliliter. We stratified results by age group: 50–60, 61–70, 71–80, and >80 years.

Seroprevalence differed by age group. We found no VVAbs among the control group. Seroprevalence increased with age, until it dropped dramatically

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New Postmortem Perspective on Emerging SARS-CoV-2 Variants of Concern, Germany

Appendix

Appendix Table 1. Multivariable analysis of nasopharyngeal viral loads and SARS-CoV-2 variants in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	1.94 (0.47–3.41)	0.01
B.1.617.2	1.47 (–0.46–3.40)	0.13
B.1.1.529	2.93 (1.19–4.66)	0.001

*Linear regression model where nasopharyngeal viral load (log copies/mL) was the dependent variable and SAR-CoV-2 variant was the independent variable. Variants were determined by multiplexed typing quantitative reverse transcription PCR. VOC, variant of concern.
†95% CIs are robust for heteroscedasticity. Global F-test, p = 0.01.

Appendix Table 2. Multivariable analysis of nasopharyngeal viral loads, SARS-CoV-2 variants, and vaccination status in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	1.43 (–0.04–2.91)	0.06
B.1.617.2	1.10 (–0.76–2.95)	0.24
B.1.1.529	1.83 (–0.63–4.28)	0.14
Vaccination (reference: none)	1.50 (–0.43–3.43)	0.13

*Linear regression model where nasopharyngeal viral load (log copies/mL) was the dependent variable and SAR-CoV-2 variant and vaccination status (0/1) were independent variables. Variants were determined by multiplexed typing quantitative reverse transcription PCR. VOC, variant of concern.
†95% CIs are robust for heteroscedasticity.

Appendix Table 3. Multivariable analysis of inflammatory changes and SARS-CoV-2 variants in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	−0.75 (–1.84–0.34)	0.17
B.1.617.2	−0.25 (–1.15–0.65)	0.58
B.1.1.529	−0.82 (–1.49 to –0.15)	0.02

*Linear regression model where inflammatory changes were the dependent variable and SAR-CoV-2 variants were the independent variables. Variants were determined by multiplexed typing quantitative reverse transcription PCR. VOC, variant of concern.
†95% confidence intervals are robust for heteroscedasticity. Global F-test, p = 0.10.

Appendix Table 4. Multivariable logistic regression analysis of diffuse alveolar damage and SARS-CoV-2 variants in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	–0.79 (–2.73–1.15)	0.43
B.1.617.2	–0.79 (–3.04–1.47)	0.49
B.1.1.529	–3.35 (–5.67–1.03)	0.01

*Diffuse alveolar damage was the dependent variable and SAR-CoV-2 variants were the independent variables. Variants were determined by multiplexed typing quantitative reverse transcription PCR. Composite Wald test, p = 0.05. VOC, variant of concern.
†95% CIs were calculated on the basis of robust standard errors.

Appendix Table 5. Multivariable analysis of inflammatory changes, SARS-CoV-2 variants, and vaccination status in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	–0.65 (–1.92–0.62)	0.30
B.1.617.2	–0.02 (–0.75–0.71)	0.95
B.1.1.529	–0.17 (–1.01–0.67)	0.68
Vaccination (reference: none)	–0.91 (–1.67 to –0.15)	0.02

*Linear regression model where inflammatory changes were the dependent variable and SAR-CoV-2 variants and vaccination status were independent variables. Variants were determined by multiplexed typing quantitative reverse transcription PCR. VOC, variant of concern.
†95% CIs are robust for heteroscedasticity.

Appendix Table 6. Multivariable logistic regression analysis of diffuse alveolar damage, SARS-CoV-2 variants, and vaccination status in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

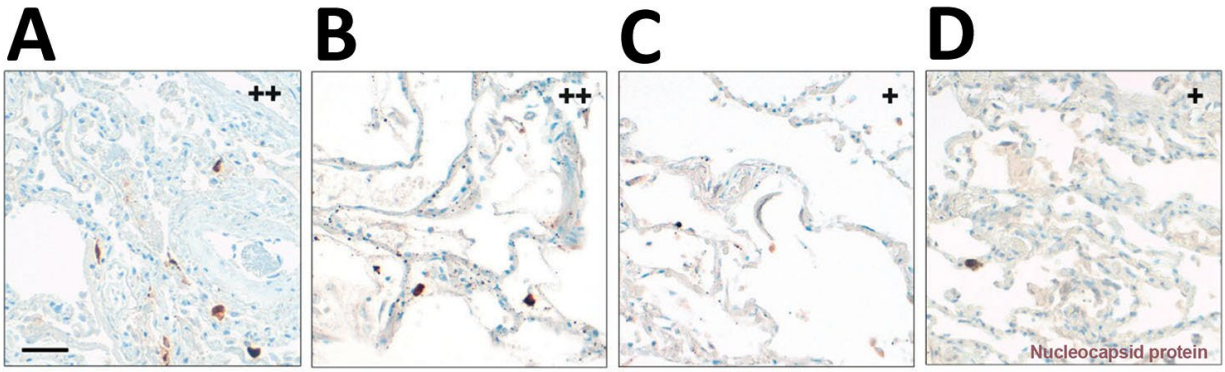
Virus variant (reference: non-VOC lineage)	β coefficient (95% CI)†	p value
B.1.1.7	−1.19 (−3.31–0.92)	0.27
B.1.617.2	−0.10 (−2.77–2.58)	0.94
B.1.1.529	−1.89 (−4.43–0.66)	0.15
Vaccination (reference: none)‡	ND	NA

*Diffuse alveolar damage was the dependent variable and SAR-CoV-2 variants and vaccination status were the independent variables. Variants were determined by multiplexed typing quantitative reverse transcription PCR. NA, not applicable; ND, not done; VOC, variant of concern.
†95% CIs were calculated on the basis of robust standard errors.
‡Vaccination status predicted the outcome perfectly because of data separation and small sample size.

Appendix Table 7. Baseline characteristics of persons whose deaths were associated with SARS-CoV-2 infection, grouped according to vaccination status in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany*

Characteristics	Unvaccinated	Vaccinated	p value	Overall cohort
No. patients†	35	13	NA	48
Age, y, median (IQR)	75.0 (55.0–82.0)	85.0 (65.0–87.0)	0.03	75.0 (60.5–85.0)
Sex			0.18	NA
M	21 (60.0)	5 (38.5)	NA	26 (54.2)
F	14 (40.0)	8 (61.5)	NA	22 (45.8)
BMI, kg/m^2, median (IQR)	25.5 (20.7–34.6)	23.0 (19.3–24.8)	0.08	24.2 (20.5–31.4)
COVID-19 cause of death	27 (77.1)	4 (30.8)	0.01	31 (64.6)
No. pre-existing medical conditions, median (IQR)	3.5 (2.0–4.0)	4.0 (3.0–5.0)	0.24	4.0 (2.0–4.0)
Place of death			0.07	NA
Home	10 (28.6)	2 (15.4)	NA	12 (25.0)
Normal ward	11 (31.4)	4 (30.8)	NA	15 (31.2)
ICU	10 (28.6)	1 (7.7)	NA	11 (22.9)
Other	4 (11.4)	6 (46.2)	NA	10 (20.8)
PMI, d, median (IQR)	1.0 (0.0–2.0)	0.0 (0.0–1.0)	0.13	1.0 (0.0–2.0)
Pulmonary inflammation (scores 0–3), median (IQR)‡	2.0 (1.5–3.0)	1.0 (1.0–2.0)	0.01	2.0 (1.0–3.0)
DAD	16 (57.1%)	0 (0.0%)	0.001	16 (41.0%)

*Corpses were admitted to the Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, for autopsy during 2020–22. Values are no. (%) unless otherwise noted. BMI, body mass index; DAD, diffuse alveolar damage; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; PMI, postmortem interval (time elapsed from death until cooling at 4°C).
†Data for 1 patient is missing because of unknown vaccination status.
‡Microscopic changes in inflammation on the basis of a Likert-like scale: none, 0; mild, 1; moderate, 2; or severe, 3.



Appendix Figure. Representative immunohistochemical staining of SARS-CoV-2 nucleocapsid protein in lungs of corpses in study of new postmortem perspective on emerging SARS-CoV-2 variants of concern, Germany. Single-blind histological assessments were performed by experienced pathologists. We detected SARS-CoV-2 nucleocapsid protein in the lungs of 25/41 (61%) cases. Specimens from corpses infected with (A) non-variant of concern lineages (n=16) and variant of concern lineage (B) B.1.1.7 (Alpha, n = 6), (C) B.1.617.2 (Delta, n =4), and (D) B.1.1.529 (Omicron, n = 15) showed pulmonary virus protein abundance and pulmonary damage. Scale bar is 50 μm.