TITLE

Risk of reinfection, vaccine protection, and severity of infection with the BA.5 omicron subvariant: a Danish nation-wide population-based study

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SUMMARY

Background: Estimates of immunity and severity for the SARS-CoV-2 omicron subvariant BA.5 are important to assess the public health impact associated with its rapid global spread despite vaccination. We estimated natural and vaccine immunity and severity of BA.5 relative to BA.2 in Denmark, a country with high mRNA vaccination coverage and free-of-charge RT-PCR testing.

Methods: This was an observational cohort study including residents 18 years or older with an RT-PCR test between 10 April and 30 June, 2022, identified in the national COVID-19 surveillance system with information since February, 2020, on RT-PCR tests, whole genome sequencing, vaccinations and hospitalisations with a positive test and COVID-19 as main diagnosis. First, using a case-control design, we calculated the protection of prior PCR-confirmed omicron infection against BA.5 and BA.2 infection and hospitalisation among triple-vaccinated individuals. Second, we compared the vaccination status in BA.5 versus BA.2 cases, and estimated the relative vaccine protection against the two subvariants. Third, the rates of hospitalisation for COVID-19 were compared among those infected with BA.5 versus BA.2. Effects were estimated using logistic regression with adjustment for sex, age, region, PCR test date, comorbidity and, as appropriate, vaccination and prior infection status.

Findings: A total of 2.4% (210/8,678) of the BA.5 cases, 0.7% (192/29,292) of the BA.2 cases and 19.0% (33,972/178,669) of the PCR negative controls had a prior omicron infection which was estimated in the adjusted analyses to offer 92.7% (95% CI: 91.6 to 93.7%) protection against BA.5 infection and 97.1% (96.6 to 97.5%) protection against BA.2 infection. Similarly high levels of protection were found against hospitalisation due to infection with BA.5 (96.4%; 95% CI: 74.2 to 99.5%) and BA.2 (91.2%; 76.3 to 96.7%). Vaccine coverage (3 mRNA doses versus none) was 94.2% (9,307/9,878) and 94.8% (30,581/32,272) among BA.5 and BA.2 cases respectively, although in the adjusted analysis there was weak evidence of slightly higher vaccination coverage among the BA.5 cases (OR: 1.18; 95% CI: 0.99 to 1.42) possibly suggesting marginally poorer vaccine protection against BA.5. The rate of hospitalisation due to COVID-19 was higher among the BA.5 cases (1.9%; 210/11,314) than among the BA.2 cases (1.4%; 514/36,805) with an adjusted OR of 1.69 (95% CI: 1.22 to 2.33) despite low and stable COVID-19 hospitalisation levels during the study period.

Interpretation: The study provides evidence of high levels of protection against BA.5 and BA.2 from a prior omicron infection in triple-vaccinated individuals. However, the protection estimates which were >90% may be too high if the controls were more likely than the cases to have come forward for testing due to reasons other than suspecting COVID-19. Our analysis also showed comparable or slightly weakened vaccine protection against BA.5 infection compared to BA.2 infection. Finally, there was evidence that BA.5 infections were associated with an increased risk of hospitalisation compared with BA.2.

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RESEARCH IN CONTEXT

Evidence before this study

We searched medRxiv, bioRxiv and PubMed for articles between November 1, 2021 and August 1, 2022 using the search term: "(BA.5[Title/Abstract])", and also searched for recent reports from national public health institutes such as NICD in South Africa, INSA in Portugal, UKSHA in the United Kingdom. Several studies have shown markedly increased immune escape of omicron BA.4/5 compared to BA.1 and BA.2 infections indicating changes in the protection afforded by vaccine and prior infection immunity. Thus, two studies from Qatar indicated prior omicron infection affords a high protection against BA.1/2 and BA.4/5 of 85.6-94.9 % and 74.3-83.9%. These estimates are higher than those reported in a meta-analysis of the protection offered by earlier variant infections. With regards to vaccine immunity, a preprint study from Portugal found comparable odds of vaccination among BA.5 and BA.2 cases indicative of a similar vaccine effectiveness for both variants, as also indicated by analysis from the UKSHA. With regards to BA.5 severity, in vitro and in vivo animal studies have highlighted the potential for increased disease severity of BA.4/5 compared to BA.1 and BA.2, but population-based results are sparse. However, a study from Portugal reported higher risk of hospitalisation for BA.5 vs BA.2 among booster-vaccinated individuals (adjusted OR=3.36, 95% CI 1.18-9.63). Comparing different infection waves, a study in preprint from South Africa reported no difference in the risk of severe hospitalisation and death in the BA.4/5 vs the preceding BA.1 wave (HR: 1.12; 0.93-1.34). In Denmark, the share of COVID-19 hospitalisations treated for lower respiratory tract infection have been lower during the BA.5 wave compared to the previous Omicron wave according to data from the health authorities.

Added value of this study

To our knowledge, there is limited available evidence on the vaccine effectiveness against BA.5 compared to BA.2 and, especially, the disease severity of BA.5. Previous studies have largely investigated BA.4 and BA.5 together, some using S-gene "target failure" (SGTF) test result as a proxy for BA.4/5 infection. However, BA.5 have consistently displayed higher growth rates than BA.4 across geographical regions. This study combine national covid-19 surveillance and viral whole-genome sequencing data to estimate the protection afforded by prior infection and vaccination, as well as the severity of BA.5 vs BA.2.

Implications of all the available evidence

The available evidence shows that previous Omicron infection offers significant protection against BA.5 in booster-vaccinated individuals. Evidence also points to comparable or slightly weakened vaccine effectiveness against BA.5 infections relative to BA.2. The impact of the current BA.5 wave may be limited in populations with a high degree of hybrid immunity. The increased risk of hospitalisation after BA.5 infection compared to BA.2 merits further investigation into the disease severity of BA.5 as studies from South Africa and Portugal do not suggest increased risk of severe disease progression and death. This study, and others referenced, also highlight how whole-genome sequencing continue to be a keystone in the surveillance of the SARS-CoV-2 pandemic.

INTRODUCTION

The BA.5 omicron subvariant is rapidly spreading globally including in Denmark despite high vaccination coverage and a large proportion of the population previously infected with the omicron subvariants BA.1 and BA.2. BA.5 was first observed in South Africa[1] in co-circulation with BA.4 where it caused a fifth wave of infections through April and May 2022, and also caused a large infection surge in Portugal [2].

BA.5 have acquired characteristic mutations in the spike protein, including the L452R, F486V mutations and a Q493 reversion, all in the receptor-binding domain. The L452R mutation was most notably present in the delta variant, and has been shown to help evade cellular immunity and increase infectivity[3]. While BA.5 is clearly highly transmissible, there is less clear evidence of its virulence relative to other omicron subvariants. Experiences from South Africa do not suggest an increased COVID-19 disease severity compared with BA.1 and BA.2 as measured by the number of hospital admissions and in-hospital deaths during the BA.4/5 wave[4]. A recent situational report from Portugal also found no evidence of increased risk of hospitalisation with BA.5 compared with earlier omicron subvariants (measured as the crude rate ratio of hospital admissions per case notification)[5]. At the same time both South Africa and Portugal have experienced a rise in all-cause excess mortality during the period of BA.5 predominance [5,6]. Overall, Omicron (B.1.529) replicates most efficiently in the upper parts of the respiratory tract[7] and is associated with less severe disease compared to previous variants of concern [8], however, a study published before the emergence of BA.5 showed that the addition of L452R to omicron enhanced its ability to infect lung tissues of humanised ACE2 mice[9].

Another study found that BA.4/5 replicate more efficiently in human lung cells than BA.2 and is more pathogenic than BA.2 in hamsters[10]. A recent risk assessment from Santé Publique in France evaluated syndromic data on 288 BA.4/5 cases and found that the median disease duration was longer for individuals infected with BA.4/5 compared to BA.1 (median duration 7 days ([interquartile range (IQR); 3-10 days) vs. 4 days (IQR 2-7 days)). They also found a significantly higher proportion of BA.4/5-infected individuals suffering from nasal secretion, nausea, diarrhoea, ageusia and anosmia[11]. However, these results were unadjusted for higher age among the BA.5 cases or differences in vaccination status.

Given the recent surge in SARS-CoV-2 infections caused by BA.5 it is important to establish whether infection with this subvariant is more likely to lead to serious disease than earlier subvariants, and the extent to which vaccination and previous infection protect against infection with BA.5. Using information from wholegenome (WGS) sequencing and national registers in Denmark, we have previously described both vaccine effectiveness, protection of earlier variants against reinfection, and severity of omicron (BA.1 and BA.2), delta, alpha and other previous variants [12-17]. The aims of the present study were to estimate, (1) the protection of a previous infection conveyed against a new infection with BA.5 among triple vaccinated, (2) the relative vaccine protection against infection with BA.5 relative to BA.2, and (3) the severity of infection with BA.5 relative to BA.2.

METHODS

National testing and vaccination programme

During the pandemic Denmark has had one of the highest PCR testing capacities per capita globally with up to a quarter of the population tested every week [18]. Tests are centrally registered and free-of-charge for all citizens. The number of weekly tests performed dropped during the first half of 2022 from around 1.4 million to approximately 60.000 on average during the three-month period from April to June. Close

contacts of infected cases no longer require testing and the rate of screening tests in other population segments have also reduced.

COVID-19 vaccination coverage is high in Denmark. By April 10, 2022 more than 80% of all adults had completed their primary vaccination series and more than 60% had also received a booster dose.[18] Further details of the testing and vaccination strategy are provided in the appendix (p 3).

Genome sequencing strategy and methods

One of the cornerstones of the pandemic surveillance has been the extensive use of WGS (www.covid19genomics.dk) with a community track capacity of ~15,000 per week since 2021 and 4,000 since the end of June 2022 through TCDK in addition to samples from clinics and hospitals (the health care track) sequenced regionally at the Departments of Clinical Microbiology. Since the first BA.5 case identified on April 10, 2022, the proportion of isolates subjected to WGS has been >83% of all positive cases of which 85% have produced genomic data on which variants were called. Further details of the WGS methods are provided in the appendix (p 3).

Data sources

Data were extracted from the national COVID-19 surveillance system maintained at Statens Serum Institut (SSI; Copenhagen, Denmark) described in detail elsewhere [19]. Briefly, individual-level information is linked daily between national registers including the National Patient Register [20] with details of all inpatient and outpatient diagnoses, admission and discharge dates. From here we obtained data on hospital admissions, COVID-19 diagnosis codes as well as comorbidities based on the International Classification of Diseases 10th revision (ICD-10) diagnosis codes (diabetes, adiposity, haematological and other cancers, neurological diseases, kidney diseases cardiovascular diseases, chronic pulmonary diseases, respiratory diseases, and immune deficiency conditions). Further, from the National Vaccination Registry [21] we obtained data with person-level information on all COVID-19 vaccinations administered, while details of sex, age, vital status and address history were obtained from the Civil Registration System [22]. Finally, data were obtained on all SARS-CoV-2 tests conducted by PCR in Denmark since the start of the pandemic from the National Microbiology Database [19].

Study design and statistical methods

The study consisted of three main analyses pertaining to each of the research questions. The first analysis provides an assessment of the protection conveyed against a new omicron infection (studied separately against BA.5 and BA.2) by a previous infection in a fully vaccinated population. The second analysis provides a comparison of the vaccine protection afforded after three mRNA doses against infection with BA.5 versus BA.2. The third analysis investigates the relative risk of hospitalisation after infection with BA.5 compared with BA.2. None of the analyses include cases of the BA.2.12.1 strain.

Study population

The study population in all three analyses was restricted to those over 18 years of age by April 10, 2022 and with uninterrupted residency in Denmark since February 2020 to ensure complete SARS-CoV-2 test and vaccination records. Further restrictions on the study populations are detailed below. Briefly, analysis 1 involves only (triple) *vaccinated* individuals while analysis 2 and 3 involve only SARS-CoV-2 *infected* individuals.

Analysis 1: Protection against reinfection

This was a case-control study involving only those with a complete primary vaccination series and a subsequent booster dose, i.e. three mRNA doses in total with either the BNT162b2 or mRNA-1273 (or a combination of the two). Cases tested positive during the outcome period (April 10, 2022 to June 30, 2022) with the BA.5 subvariant identified through WGS while controls had at least one PCR test during the outcome period, but without testing positive for SARS-CoV-2 [23]. We then compared the proportion among cases and controls that had been exposed to a previous omicron infection, i.e. had a positive SARS-CoV-2 PCR test between January 1, 2022 and February 9, 2022 during which period BA.1 and BA.2 accounted for virtually all infections in Denmark. Those with a positive PCR test outside of this exposure period, and before the outcome period, were excluded from the analysis, as were those without a third mRNA dose by March 27, 2022 (14 days before the start of the outcome period to allow the full effect of vaccination) or with a fourth dose by June 30, 2022.

Protection from a previous infection was estimated with a 95% confidence interval in a logistic regression model and expressed as 1 minus the model-derived odds ratio (OR) analogous to the method of estimating vaccine effectiveness. The model was adjusted for sex, age group (18-24, 25-34, 35-44, 45-54, 55-64, 65-74, 75-84, >85 years), geographical area of residency (five-level categorical variable indicating EU NUTS-2 regions) comorbidity count (four-level categorical variable indicating the number of comorbidities: none, one, two, three or more), and time of PCR sampling (categorical variable indicating week number). Among controls with multiple negative PCR tests during the outcome period, one was randomly selected for inclusion in the analysis. Among the few cases with more than one positive test during the outcome period, only the first positive test was included in the analysis. In a sensitivity analysis to assess the robustness of the findings under an alternative analysis approach, the analysis was repeated using a matched case-control design in which cases and controls were pair-matched on test date, sex and age (further methods detailed in the appendix p 8).

Supplementary analyses:

In two extensions of the main analysis, estimating instead protection from a previous delta or alpha infection, the exposure definition was changed from infection during a period when omicron predominated to infection during July 15, 2021 to November 15, 2021 and March 15, 2021 to June 30, 2021, respectively, when the delta and alpha variant predominated. Finally, all analyses were repeated with cases being those who tested positive during the outcome period with BA.2 rather than BA.5.

Although not a requirement for valid inference that previously infected and uninfected individuals have the same propensity to come forward for testing (i.e. that test rates are independent of exposure status), the OR will generally be biased if the effect of exposure status on test rates is modified by infection status during April-June. The analysis was therefore repeated with hospitalisation as the outcome (defined under Analysis 3) to avoid possible biases due to differences in test rates and reasons for testing.

Analysis 2: Vaccine protection

This analysis involved only infected participants: those infected during the outcome period (April 10, 2022 to June 30, 2022) with either BA.5 or BA.2. The analysis then compared vaccination status across the two groups with differences interpreted as evidence of reduced vaccine protection against one subvariant compared with the other. (Given infection has occurred, the analysis estimates the association between vaccination status and subvariant: If the vaccines protect equally well against BA.2 and BA.5, the ratio of vaccinated to unvaccinated would be identical among the BA.2 and BA.5 cases on expectation.) Only those

with a complete primary vaccination series and a subsequent booster dose by March 27, 2022, i.e. three mRNA doses in total, or those completely unvaccinated against COVID-19 by June 20, 2022, were included in the analysis. The analysis excluded those with a fourth dose by June 30, 2022. The effect of vaccination on the likelihood of an infection being due to BA.5 rather than BA.2 was analysed in a logistic regression and expressed as an OR with a 95% confidence interval. The model was adjusted for prior infection before April 10, 2022 (yes/no) in addition to the other adjustment variables as described for the analysis above.

Supplementary analysis:

As only few people remain unvaccinated in Denmark (~9% of those aged >18 years), a sensitivity analysis was carried out which did not rely on comparison with this group. In the sensitivity analysis, the reference exposure group was changed to those who had completed their primary vaccination series (2 mRNA doses) more than 4.5 months prior to the start of the outcome period but with no booster dose by June 30, 2022.

Analysis 3: Severity of a BA.5 infection

This analysis also involved only infected participants and compared the proportion of cases hospitalised among those infected with BA.5 and BA.2 during the outcome period from April 10, 2022 to June 30, 2022. The effect of subvariant (BA.5 versus BA.2) on the risk of hospitalisation was estimated in a logistic regression with adjustment as described above but with additional adjustment for vaccination status (categorical variable indicating the number of doses received at the time of infection) and prior infection. The analysis included all BA.2 or BA.5 cases in the outcome period, irrespective of COVID-19 vaccination history. Hospitalisations included in the analysis were restricted to those that lasted over 12 hours, had associated ICD-10 primary diagnosis codes B342 or B972 (indicating that COVID-19 was the primary reason for their hospital admission) and occurred no earlier than two days before and no later than 14 days after a positive PCR test.

Supplementary analyses:

A large majority of the adult population has received three COVID-19 mRNA doses. We therefore conducted a subgroup analysis in those who had received three mRNA doses prior to March 27, 2022 excluding anyone with a fourth dose before the end of the outcome period. In another supplementary analysis, the outcome period was extended by advancing the start date to January 1, 2022. Since the delta variant was still in circulation to a limited degree in early 2022 this analysis enabled estimation simultaneously of the effects of BA.5 and the delta variant on hospitalisation using the BA.2 hospitalisation rate as reference. (Those with a previous infection prior to January 1, 2022 were excluded from this analysis to avoid including frequently tested long-term or recurrent hospital patients with a delta infection and repeated positive tests over the winter months.)

Ethical considerations

This study was performed under the authority task of the Danish national infectious disease control institute which allows SSI to perform analyses on data from existing national COVID-19 surveillance systems. According to Danish law, ethical approval or individual consent is not required for anonymised aggregated register-based studies.

Role of funding source

There was no funding source for this study.

RESULTS

Since the start of 2022 the omicron variant has accounted for virtually all SARS-CoV-2 infections in Denmark (Figure 1). Similar to many other countries, Denmark experienced a massive omicron wave between December 2021 and February 2022 with around 35% of the adult population testing positive via PCR during this three-month period (data not shown). Omicron infections were mainly due to the BA.1 subvariant during December 2021 and early January 2022 after which point BA.2 became predominant lasting until the rise of BA.5.

Of the 4,622,106 people over the age of 18 years with residency in Denmark since February 2020, a total of 414,436 were tested by PCR during the outcome period from April 10 to June 30, 2022. Those tested during the outcome period were older, more likely to have comorbidities and to be without a previous PCR-confirmed SARS-CoV-2 infection than those not tested during the outcome period (table 1). Of those tested during the outcome period, 187,347 were included in Analysis 1, 42,150 in Analysis 2 and 48,119 in Analysis 3 (Figure 2). In Analysis 1, cases were somewhat more likely than controls to be without comorbidity. In Analysis 2, the unvaccinated were younger, with less comorbidity, and more likely to have a previous PCR confirmed infection. Finally in Analysis 3, BA.5 cases were more likely than BA.2 cases to have a previous PCR confirmed infection (appendix pp 4-6).

Of the 8,678 triple-vaccinated cases who tested positive for SARS-CoV-2 with a BA.5 infection during the outcome period (between April 10, 2022 and June 30, 2022), only 210 (2.4%) had also tested positive for SARS-CoV-2 between January 1st and February 9th, 2022, when the BA.1 and BA.2 omicron subvariants accounted for almost all infections (table 2). By contrast, among the 178,669 triple-vaccinated controls who tested negative for SARS-CoV-2 during the outcome period, 33,972 (19.0%) had tested positive for SARS-CoV-2 between January 1 and February 9, 2022. The estimated protection was 92.7% (95% CI: 91.6 to 93.7%) suggesting that a previous omicron infection is highly protective against a new infection with BA.5. in a vaccinated population. By comparison, a previous delta or alpha infection provided much weaker protection of 73.4% (65.7 to 79.3%) and 61.2% (49.1 to 70.4%) respectively, against a new infection with BA.5.

In the supplementary analyses estimating protection against BA.2 during the outcome period, a previous omicron infection was even more highly protective against BA.2 than was observed in the above analysis for BA.5, with an estimated protection against BA.2 of 97.1% (96.6 to 97.5%). As in the above analysis of protection against BA.5, a previous infection with the delta or alpha variant protected less well than a previous omicron infection with estimated protection against BA.2 infection of 84.2% (80.7 to 87.1%) and 73.8% (67.8 to 78.6%) respectively. When restricting the outcome to those hospitalised for an infection with BA.5 or BA2, the level of protection from a previous omicron infection was 96.4% (74.2 to 99.5%) and 91.2% (76.3 to 96.7%) respectively (appendix p 7).

In the sensitivity analysis using a matched case-control design the estimates were nearly identical to those in the main analysis. Changing the definition of a reinfection to require a different minimum number of days between repeat positive tests also had little effect on the results. Adjustment for time since vaccination (third dose) similarly had minimal impact on the results (appendix pp 8-11).

In the vaccine analysis, 94.2% of those with a BA.5 infection and 94.8% of those with a BA.2 infection were vaccinated against COVID-19 with three mRNA doses (table 3). When comparing triple-vaccinated with unvaccinated individuals, the adjusted OR for the effect of the vaccine on the likelihood of an infection being due to BA.5 rather than BA.2, was 1.18 (0.99 to 1.42, p=0.064). (The change from the unadjusted estimate of 0.90 (0.82 to 0.99) was largely driven by negative confounding from previous infections since

unvaccinated individuals were more likely to have had a previous infection, and BA.5 cases were more likely than BA.2 cases to have had a previous infection.) When comparing triple-vaccinated individuals with those who had only received two mRNA doses over 4.5 months earlier, the unadjusted and adjusted OR for an infection being due to BA.5 rather than BA.2 were 0.90 (0.81 to 1.00) and 1.12 (0.92 to 1.35, p=0.26) respectively. Overall, there was limited evidence, therefore, that the mRNA vaccines protect less well against BA.5 than BA.2.

Among participants infected with BA.5 during the outcome period from April 10, 2022 to June 30, 2022, 1.9% (210/11,314) were admitted to hospital for COVID-19 whereas 1.4% (514/36,291) among those infected with BA.2 during the same period were hospitalised (table 4). After adjustment, the OR for hospitalisation was 1.69 (1.22; 2.33) among those infected with BA.5 relative to BA.2. The increase in effect size from 1.34 (1.14 to 1.57) in the unadjusted analysis was largely driven by adjustment for age as fewer elderly people were infected with BA.5 than BA.2. The estimate did not change substantially when restricting the analysis to include only triple-vaccinated individuals (OR: 1.66; 95% CI: 1.16 to 2.36). When extending the outcome period, moving the start date back to January 1, 2022 and excluding those with a previous infection, the OR for hospitalisation was 1.83 (1.31 to 2.55) among BA.5 cases relative to BA.2 cases while delta cases were substantially more likely to require hospitalisation compared with BA.2 cases with an OR of 2.86 (1.67 to 4.91).

DISCUSSION

In this study we investigated the risk of BA.5 infection in a population with hybrid immunity, i.e. a previous infection and vaccine immunity, evidence of reduced vaccine protection, and finally, severity of a BA.5 infection relative to earlier strains.

The analyses indicated that a previous omicron infection protected very well against a subsequent infection with BA.5. Similarly, a previous alpha or delta infection offered good protection although to a lesser extent. The level of protection of a previous infection was higher against BA.2 in the same period. In the sensitivity analysis using a matched case-control design, the results were almost identical.

The analysis of vaccine protection against BA.5 infection compared with BA.2 infection did not provide strong evidence of poorer protection against BA.5 than BA.2. The OR estimates of 1.18 (0.99 to 1.42) from the comparison against unvaccinated, and 1.12 (0.92 to 1.35) from the comparison against those who had only received two mRNA doses over 4.5 months earlier, possibly suggest a slightly heightened ability of BA.5 to escape the vaccine protection; however, more data are needed to increase precision around the estimates as both rely on relatively small comparator populations.

The analysis of severity showed evidence of higher hospitalisation rates among BA.5 cases relative to BA.2 cases. As expected, and consistent with our earlier studies, the analysis also showed increased severity from a delta infection with nearly three times the odds of hospitalisation relative to a BA.2 infection [13,15].

Real-world evidence on the disease severity of BA.5 is sparse. In a recent South African study (preprint) the risk of severe hospitalisation (i.e. admission to intensive care or mechanical ventilation or oral/intravenous steroid prescription) and death were similar in the BA.4/5 wave as in the preceding BA.1 wave [24]. In both Portugal and South Africa, the BA.5 wave passed without the overall COVID-19 hospital admissions and deaths exceeding that of the previous omicron wave although Portugal reported excess mortality for a few

weeks.[25] Another recent Portuguese study found a higher hospitalisation rate for BA.5 versus BA.2 among those with a booster vaccination (OR: 3.35; 95% CI 1.18-9.63; 34 BA.5 hospitalisations) [2].

As in our analysis, a recent study from Qatar estimated very high levels of protection of a previous BA.1 infection against infection with BA.2 and vice versa [26]. Studies on the protection of a previous SARS-CoV-2 infection have generally found good protection around or above 80% against reinfection, including our own studies, although a lower level of protection has generally been reported of earlier variant infections against a subsequent omicron infection [16,17,27-29]. In the present study, protection of an alpha or delta infection against omicron was considerably higher than that in our recent cohort analysis of an earlier variant infection against omicron in an *unvaccinated* population (estimates ranging between 19-51%) and also higher than estimates from elsewhere [17,27]. While the comparatively high estimates in this study may reflect a genuine hybrid immunity effect in the vaccinated population, it is possible that those with a previous infection were much more likely –compared to the previously uninfected- to have been tested for reasons other than suspecting COVID-19, which in turn would inflate the estimated levels of protection of a previous infection in our study. Nonetheless, assuming that the number of BA.5 infections observed in our study among those with a previous infection is only half that which would have been observed in the absence of such a bias, the resulting OR estimate would be around 2 x 0.073 = 0.146, and the level of protection still high at around 85%. Importantly, the analysis restricted to cases who were hospitalised for COVID-19, and which is not subject to biases due to testing, still showed very high levels of protection (>90%) of a prior omicron infection against hospitalisation due to infection with either BA.5 or BA.2.

For the vaccine protection analysis (analysis 2), it is important to note that because the remaining unvaccinated group makes up such a small proportion of the population we were unable to assess vaccine effectiveness directly as the ratio of infection rates in vaccinated and unvaccinated individuals. Instead, basing the analysis only upon infected individuals, the analysis compared the vaccination status in the BA.5 and BA.2 infected groups, providing a relative measure of vaccine protection against BA.5 relative to BA.2.

In a recent preliminary analysis from the UK Health Security Agency, a similar analysis strategy was followed comparing those recently vaccinated with a second, third or fourth dose to a baseline group of those vaccinated with a second or third dose more than 25 weeks prior to infection [30]. This analysis did not find differences in the vaccination status among those infected with BA.5 versus BA.2 with an OR of 0.83 (0.64 to 1.08). Importantly in this type of analysis, the OR will be one (on expectation) in the absence of vaccine effectiveness against both sub-variants, and the analysis therefore relies on there being some level of vaccine protection against infection with BA.2, or the relative measure will be non-informative.

Our study was made possible due to the intensive WGS efforts at SSI. However it is possible that some bias exists in the selection of samples for sequencing as not all sequenced samples are selected at random. Second, not all positive cases during the outcome period would have been identified as many were no longer tested. The study also did not include results from the national free-of-charge Rapid Antigen Test (RAT) programme, however these only accounted for approximately 8% of all registered test results (RAT+PCR) during the study period.[18] Third as described above, estimates of protection from a prior infection may be too high if the controls were more likely than the cases to have come forward for testing due to reasons other than suspecting COVID-19. We believe bias from test procedures were largely mitigated in analysis 2 and 3 as they included only PCR-confirmed cases (either BA.2 or BA.5).

Fourth, the analysis did not attempt to take account of the order in which vaccine and natural immunity were acquired; some participants will therefore have been unvaccinated at the time of their prior infection

while others will have received one, two or three vaccine doses. Those with a prior omicron infection were much more likely to have experienced a breakthrough infection than those previously infected with delta or alpha during periods when the vaccination coverage, and number of doses given per person, were much lower. The analysis also did not attempt to assess the effect of waning immunity as a function of time since vaccination or past infection. It was therefore not possible to attribute the weaker protection that was observed among those with a prior alpha or delta infection to reduced cross-reactive immunity with different variant strains rather than a waning effect.

Finally, infection rates varied considerably throughout the first half of 2022 impacting test rates and the age profile of cases, and in turn the proportion of PCR confirmed cases that were hospitalised. By evaluating the adjustment variables, we confirmed that age and time contributed to confounding of the relationship between subvariant and risk of hospitalisation, explaining why a stronger effect was apparent from the adjusted estimate. Importantly, the observation that BA.5 is more severe relative to BA.2 occurred in the context of stable and low absolute numbers of SARS-CoV-2 test positive hospitalisations in Denmark during the study period.

Conclusion/implications

Our study found that a previous omicron infection in triple mRNA-vaccinated individuals offers significant protection against BA.5 infection, including infection leading to hospitalisation. Our analysis also indicated comparable or slightly weakened vaccine protection against BA.5 infection compared with BA.2 infection. Overall, the impact of the current BA.5 wave may be limited in populations with a high degree of hybrid immunity and may be comparable to that of the previous BA.1/BA.2 wave. The increased risk of hospitalisation after a BA.5 infection found in our study merits further investigation into the disease severity of BA.5. This study also highlights how WGS continue to be a cornerstone in the surveillance of the SARS-CoV-2 pandemic.

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CONTRIBUTERS

All authors contributed to either the conception and design of the study, acquisition of data, or data analysis and interpretation. All authors had access to the underlying data and CHH and PB verified all data. CHH, NUF, and PB drafted the manuscript and all authors provided critical revisions and final approval for the decision to submit for publication. All authors agree to be accountable for all aspects of the work in

ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA SHARING

De-identified participant-level data are available for access to members of the scientific and medical community for non-commercial use only. Applications should be submitted to Forskerservice at The Danish Health Data Authority, where they will be reviewed on the basis of relevance and scientific merit. Data are available now, with no defined end date. For the *Forskerservice* website see https://sundhedsdatastyrelsen.dk/da/forskerservice. Consensus sequences from the Danish WGS surveillance is routinely made available at both GISAID (www.gisaid.org) and ENA (www.ebi.ac.uk/ena/).

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545 **TABLES AND FIGURES**

546

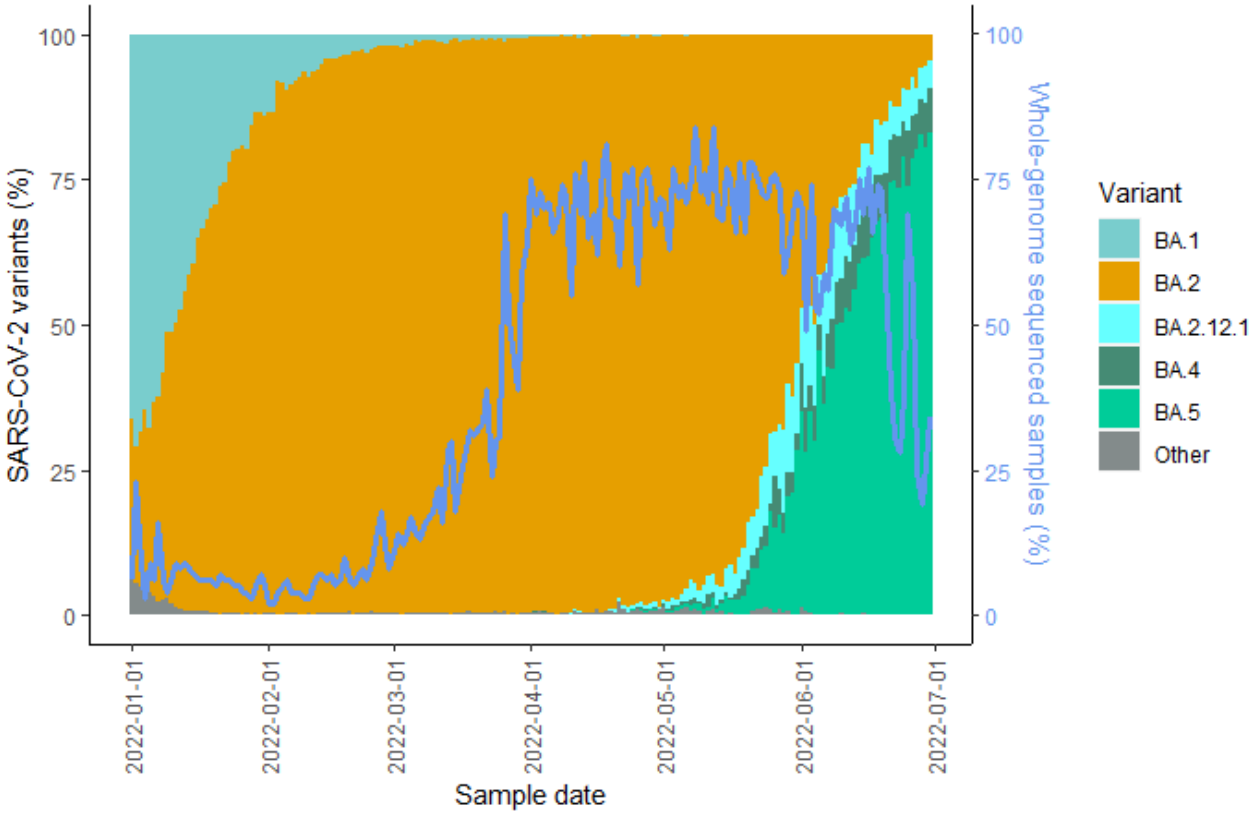

Figure 1 Proportion of cases with wholegenome sequencing and SARS-CoV-2 variants in 2022.

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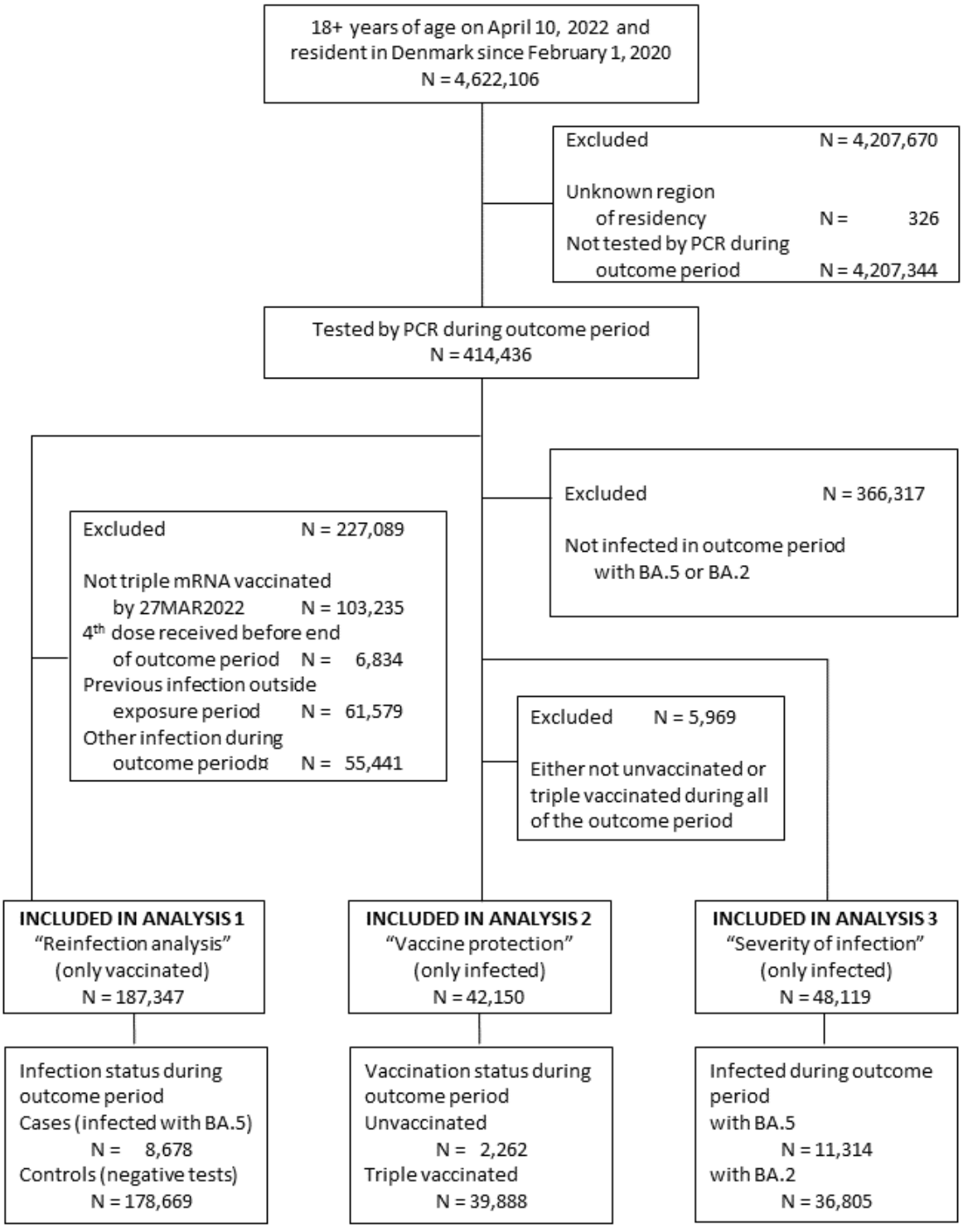

Figure 2 Population included in each of the three main analyses. Outcome period: April 10, 2022 to June 30, 2022. ¤ not shown to be BA.5

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Table 1 Characteristics of the study population, 2022, Denmark.

Population: 18+ years of age on April 10, 2022 and resident in Denmark since February 1, 2020	**ALL**		**Tested by PCR during outcome period**	
	Number	*%*	*Number*	*%*
Total	4,622,106	100	414,436	100
	#			
Female	2,342,597	50.7	226,222	54.6
Male	2,279,462	49.3	188,214	45.4
Age (years)				
18-24	482,695	10.4	30,543	7.4
25-34	746,790	16.2	59,735	14.4
35-44	663,542	14.4	51,806	12.5
45-54	768,454	16.6	68,988	16.7
55-64	763,964	16.5	78,874	19.0
65-74	624,590	13.5	61,873	14.9
75-84	440,840	9.5	45,187	10.9
85+	131,231	2.8	17,430	4.2
Region of residency	#			
Capital	1,451,806	31.4	150,372	36.3
Central Denmark	1,052,396	22.8	80,372	19.4
Northern Denmark	472,794	10.2	36,155	8.7
Zealand	673,221	14.6	63,555	15.3
Southern Denmark	971,563	21.0	83,957	20.3
Migration heritage†				
Denmark	3,982,616	86.2	345,707	83.4
Other European country	295,988	6.4	25,730	6.2
Middle East and North Africa	166,071	3.6	21,304	5.1
Indian subcontinent and Southeast Asia	111,427	2.4	13,594	3.3
Sub-Saharan Africa	37,342	0.8	4,654	1.1
Other	28,559	0.6	3,447	0.8
Number of comorbidities*	#			
None	3,659,772	79.2	297,644	71.8
One	715,355	15.5	79,270	19.1
Two	186,128	4.0	26,465	6.4
Three or more	60,802	1.3	11,057	2.7
COVID-19 vaccinations$				
Unvaccinated	382,479	8.3	31,880	7.7
Only primary vaccination completed – 2 mRNA doses	38,558	0.8	4,011	1.0
Only primary vaccination completed – non-mRNA	5,463	0.1	596	0.1
Primary (mRNA) vaccination + 1 (mRNA) booster dose	3,396,426	73.5	305,731	73.8
Primary (non-mRNA) vaccination + 1 (mRNA) booster dose	147,970	3.2	18,055	4.4
Primary vaccination + 2 booster doses (any type)	36,465	0.8	6,365	1.5
Other¤	614,745	13.3	47,798	11.5
PCR-confirmed SARS-CoV-2 infections‡				
No previous infection	2,164,809	46.8	256,449	61.9
At least 1 previous infection	2,457,297	53.2	157,987	38.1
Infection likely with omicron	1,685,557	(77.9)	111,047	(70.3)
Infection likely with earlier variant	479,252	(22.1)	46,940	(29.7)

$ Vaccinations received by April 10, 2022: mRNA vaccines were either BNT162b2 or mRNA-1273, non-mRNA vaccines included JCOVDEN and ChAdOx1-S. ¤Incomplete primary vaccination or non-mRNA booster doses.*Comorbidities registered in the past 5 years out of the following: diabetes, adiposity, haematological and other cancers, neurological diseases, kidney diseases cardiovascular diseases, chronic pulmonary diseases, respiratory diseases, and immune

deficiency conditions. ‡ Infection status by April 10, 2022. Likely omicron infections were those testing positive after December 20, 2021. # sex and comorbidity data missing for 47 and residency data missing for 326 individuals. †migration heritage defined by country of birth or, if known, mother's country of birth.

Table 2 Protection against BA.5 and BA.2 infection after a prior positive SARS-CoV-2 PCR test, April to June, 2022, Denmark.

	Cases	Controls	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)	Estimated protection, % (95% CI)
	BA.5 cases				**protection against BA.5**
Exposure: prior omicron infection					
Exposed	210 (2.4)	33,972 (19.0)	0.106 (0.092; 0.121)	0.073 (0.063; 0.084)	92.7 (91.6; 93.7)
Unexposed	8,468 (97.6)	144,697 (81.0)	1	1	
Exposure: prior delta infection					
Exposed	65 (0.8)	3,336 (2.3)	0.334 (0.261; 0.427)	0.266 (0.207; 0.343)	73.4 (65.7; 79.3)
Unexposed	8,468 (99.2)	144,697 (97.7)	1	1	
Exposure: prior alpha infection					
Exposed	58 (0.7)	1,878 (1.3)	0.528 (0.406; 0.686)	0.388 (0.296; 0.509)	61.2 (49.1; 70.4)
Unexposed	8,468 (99.3)	144,697 (98.7)	1	1	
	BA.2 cases				**protection against BA.2**
Exposure: prior omicron infection					
Exposed	192 (0.7)	33,972 (19.0)	0.028 (0.024; 0.032)	0.029 (0.025; 0.034)	97.1 (96.6; 97.5)
Unexposed	29,100 (99.3)	144,697 (81.0)	1	1	
Exposure: prior delta infection					
Exposed	100 (0.3)	3,336 (2.3)	0.149 (0.122; 0.182)	0.158 (0.129; 0.193)	84.2 (80.7; 87.1)
Unexposed	29,100 (99.7)	144,697 (97.7)	1		
Exposure: prior alpha infection					
Exposed	98 (0.3)	1,878 (1.3)	0.259 (0.212; 0.318)	0.262 (0.214; 0.322)	73.8 (67.8; 78.6)
Unexposed	29,100 (99.7)	144,697 (98.7)	1	1	

All participants had received 3 mRNA COVID-19 vaccine doses. Cases were infected with either BA5 or BA.2 during the outcome period from April 10, 2022 to June 30, 2022; controls tested negative during the same period. OR denotes odds ratio; CI denotes confidence interval. Unexposed individuals had no positive PCR tests before the start of follow-up on April 10, 2022. *adjusted for age group, time (week number), sex, region and comorbidity.

Table 3 Vaccine protection against infection with BA.5 relative to BA.2, April to June, 2022, Denmark.

Exposure (vaccination status) [$]	Type of infection contracted during outcome period: Infected with BA.5	Type of infection contracted during outcome period: Infected with BA.2	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Three doses versus unvaccinated				
Three doses	9,307 (94.2)	30,581 (94.8)	0.90 (0.82; 0.99)	1.18 (0.99; 1.42)
Unvaccinated	571 (5.8)	1,691 (5.2)	1	1
Three versus two doses				
Three doses	9,307 (94.8)	30,581 (95.3)	0.90 (0.81; 1.00)	1.12 (0.92; 1.35)
Two doses	513 (5.2)	1,515 (4.7)	1	1

All participants were infected with either BA.5 or BA.2. The outcome period was between April 10, 2022 and June 30, 2022. The analysis includes both those with and without a previous infection before April 10, 2022. OR denotes odds ratio; CI denotes confidence interval. *Adjusted for age group, time (week number), sex, region, comorbidity and prior infection (yes/no). $ Three doses: 3 doses of mRNA-1273 or BNT162b2 before March 27, 2022; two doses: completed primary vaccination series >140 days before the outcome period.

Table 4 Severity of BA.5: risk of hospitalisation after infection, April to June, 2022, Denmark.

Exposure (type of infection)	Hospitalised for COVID-19	Cases not hospitalised¤	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Main analysis				
BA.2	514 (1.4)	36,291 (98.6)	1	1
BA.5	210 (1.9)	11,104 (98.1)	1.34 (1.14; 1.57)	1.69 (1.22; 2.33)
Supplementary analysis 1:				
Subgroup analysis in vaccinated (3x mRNA) $				
BA.2	409 (1.3)	30,172 (98.7)	1	1
BA.5	178 (1.9)	9,129 (98.1)	1.44 (1.20; 1.72)	1.66 (1.16; 2.36)
Supplementary analysis 2:				
Extended outcome period ‡				
BA.2	2,362 (1.5)	157,581 (98.5)	1	1
BA.5	203 (2.1)	9,636 (97.9)	1.41 (1.22; 1.62)	1.83 (1.31; 2.55)
Delta	27 (5.1)	499 (94.9)	3.61 (2.45; 5.33)	2.86 (1.67; 4.91)

All participants were infected with SARS-CoV-2. The main and supplementary analysis 1 included BA.2 and BA.5 infections that occurred during the outcome period between April 10, 2022 and June 30, 2022. ‡ Supplementary analysis 2 included BA.2, BA.5 and delta infections that occurred between January 1, 2022 and June 30, 2022; those with a previous infection prior to January 1, 2022 were excluded. ¤Includes a few cases hospitalised for other reasons. *Adjusted for age group, time (week number) of infection, sex, region, comorbidity, prior infection (except supplementary analysis 2) and vaccination status (except supplementary analysis 1). $ Three doses of mRNA-1273 or BNT162b2 before March 27, 2022.