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COVID-19: Omicron – the latest, the least virulent, but probably not the last variant of concern of SARS-CoV-2

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Summary

The Omicron variant rapidly became the dominant SARS-CoV-2 strain in South Africa and elsewhere. This review explores whether this rise was due to an increased transmission of the variant or its escape from population immunity by an extensively mutated spike protein. The mutations affected the structure of the spike protein leading to the loss of neutralization by most, but not all, therapeutic monoclonal antibodies. Omicron also shows substantial immune escape from serum antibodies in convalescent patients and vaccinees. A booster immunization increased, however, the titre and breadth of antiviral antibody response. The cellular immune response against Omicron was largely preserved explaining a satisfying protection of boosted vaccinees against severe infections. Clinicians observed less severe infection with Omicron, but other scientists warned that this must not necessarily reflect less intrinsic virulence. However, in animal experiments with mice and hamsters, Omicron infections also displayed a lesser virulence than previous VOCs and lung functions were less compromised. Cell biologists demonstrated that Omicron differs from Delta by preferring the endocytic pathway for cell entry over fusion with the plasma membrane which might explain Omicron's distinct replication along the respiratory tract compared with Delta. Omicron represents a distinct evolutionary lineage that deviated from the mainstream of evolving SARS-CoV-2 already in mid-2020 raising questions about where it circulated before getting widespread in December 2021. The role of Omicron for the future trajectory of the COVID-19 pandemic is discussed.

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Variants of concern

Over the last two years, the world has seen a succession of variants replacing the original Wuhan SARS-CoV-2 virus isolate that circulated in the human population. A better understanding of the mechanisms underlying these viral successions would be of substantial help for projecting the future trajectory of the COVID-19 pandemic. Epidemiologists from Harvard University developed a mathematical model to investigate the dynamics of viral variation in face of vaccination and non-pharmaceutical mitigations (Bushman *et al.*, 2021). They considered variants with different intrinsic transmissibility and immune escape. Their model predicts that in a susceptible population, viral variants with increased transmissibility will easily invade the population, while variants with partial immune evasion will not. Viruses showing immune evasion might cause a wave of reinfection which should result in milder disease. If a variant shows both enhanced transmission and immune evasion, the model predicts an increased size of the epidemic and increased number of severe disease cases and deaths. However, the authors of this study warned that phenotypes of variants are frequently context dependent which makes predictions of population level outcomes difficult. It might even blur the definition what represents a variant of concern (VOC). Indeed, in 2020 the viral variants Alpha, Beta and Gamma cocirculated at least for some time and at certain places albeit Beta and Gamma never exceeded more than 10% of the circulating strains. Delta, which is 50% more transmissible than the previous variants, then rose quickly to dominance. As the fraction of immune subjects increases due to vaccination or infection, viruses showing immune escape should increase since they have a substantial selective advantage (Grubaugh and Cobey, 2021).

Epidemiology of Omicron infections

Just when the Harvard report went into press, a new VOC was reported in South Africa (November 25, 2021).

It was detected by a viral spike (S)-gene target amplification failure in PCR assays. Researchers described a sudden increase of cases in the Gauteng Province of South Africa, representing there the fourth wave of the COVID-19 pandemic. The Omicron (B.1.1.529) variant showed a shorter doubling time than Delta despite the fact that the latter displayed a higher viral load, longer duration of infectiousness and higher rates of reinfection than previous VOCs (Karim and Karim, 2021). Instead of thanking the South African virologists for this alert, many countries punished South Africa with useless travel bans (Mallapaty, 2021). In fact, 2 weeks later 55 countries reported the presence of Omicron (Maxmen, 2021) indicating that Omicron was already widespread. In South Africa, researchers had already documented a succession of variants: from the D614 Gvariant, an S mutant derivative of the original Wuhan-Hu-1 virus in the first infection wave (June/Aug 2020) to Beta (Nov 2020 to Feb 2021) and then to Delta (May to Sept 2021). After the sudden fall in Delta cases in mid-November 2021, Omicron cases increased in a population that according to seroprevalence studies showed antiviral antibodies in 70% of the population. The first cases were seen in education centres, but cases spread rapidly across South Africa. Estimates for the effective reproduction number R_e varied from 2.8 to 3.9. The viral genomes belonged to the B.1.1.529 lineage. The Omicron sequences were represented with three sub-lineages: BA.1 (the main clade) and BA.2 and BA.3. All three viruses were probably derived from a common ancestor that already circulated in October 2021. Omicron rose quickly in prevalence, doubling every 2 to 3 days and Omicron represented soon 90% of the circulating SARS-CoV-2 viruses. Omicron is phylogenetically distinct from any other known SARS-CoV-2 lineage and shows a broad mutation profile: 60 in total of which 50 are non-synonymous. It differs from the Wuhan-Hu-1 virus by 15 mutations alone in the receptor binding domain (RBD) of the spike protein S. In addition to mutations in the N- and C-terminal parts of the S protein, it has further three mutations adjacent to the S1/S2 furin cleavage site (involved in the fusion activity of the virus) of the S protein. Numerous mutations are also found in orf 1ab (encoding non-structural proteins), and the genes encoding the nucleocapsid and the membrane proteins. Evidence for positive selection was found in many Omicron genes suggesting that adaptive evolution played a significant role in the mutational divergence of Omicron. In Gauteng, Omicron had a growth advantage over Delta resulting in a 5-fold weekly increase of Omicron cases over Delta cases. The scientists concluded that partial immune evasion was a major driver for the observed dynamic of Omicron in South Africa (Viana *et al*., 2022).

Indeed, the Omicron infection wave occurred in face of substantial population immunity levels as revealed by a seroprevalence study conducted in the Gauteng province. Seven thousand participants provided serum samples in November 2021. The overall seroprevalence of antiviral IgG antibodies was 73%, it was lowest among children younger than 12 years of age (56%) and highest among adults older than 50 years of age (80%) and higher in females than in males (Madhi *et al*., 2022).

Omicron also showed a significant infection pressure in other countries. Since May 2020, the REACT-1 study followed monthly the spread of SARS-CoV-2 in England. The first Omicron infection was observed on 27 November 2021. A steep increase was seen in London and the South East with Omicron rising to a 6% prevalence by 14 December. The epidemiologists estimated a generation time of 4.6 days for Omicron infections and a reproduction number of 1.1 to 1.3. Older children eligible for vaccination showed a lower Omicron infection rate than younger children not yet eligible for vaccination. Also adults with a booster showed a lower Omicron infection rate than adults with only one or two immunizations. Both observations suggested some protection by vaccination. Omicron prevalence was 3-fold higher in larger than smaller households. Omicron infections were dominant among young adults which might explain the observed lower severity of Omicron infections. Nevertheless, in December 2021, the COVID-10-related hospitalization rate rose by 50% in London (Elliott *et al*., 2022).

The UK Health Security Agency evaluated 14 000 subjects infected with Omicron, 40 000 subjects infected with Delta and their 150 000 contacts (Allen *et al*., 2022). The serial interval was 4 days for Delta and 3 days for Omicron transmission. Among household contacts, the secondary attack rate was 15% for Omicron and 11% for Delta cases; for non-household contacts, these rates were 8% and 4%, respectively, indicating an enhanced transmission of Omicron. For Delta infections, the secondary attack rate was lower when household contacts were vaccinated (8% vs. 13% in unvaccinated contacts) and the transmission was also reduced when the index case was vaccinated (6% vs. 12% for unvaccinated index cases). Notably, the impact of vaccination on transmission rates for Omicron cases was considerably attenuated: boosted and unvaccinated index cases transmitted the Omicron infection to 12% and 16%, respectively, of household contacts. A household transmission study from Norway in 31 000 households with an index case revealed a secondary transmission rate of 25%, 19% and 18% if the index case was infected with Omicron, Delta or an unassigned SARS-CoV-2. Secondary attack rate of Omicron was only marginally higher for unvaccinated than vaccinated index cases (odds ratio

1.14) (Jørgensen *et al.*, 2022). All these data indicate a greater transmissibility of Omicron over the already highly transmissible Delta variant.

Omicron's origin

Sequencing of early Omicron isolates suggested that their genomes diverged already in mid-2020 from the lineages leading to the Alpha, Beta, Gamma and Delta VOCs (Mallapaty, 2022). Yet the earliest documented Omicron sequences date from 1 to 3 November 2021 in England, South Africa, Nigeria and the United States, raising the question where Omicron was hiding in the intervening time. Some of the Omicron mutations have also been observed in other VOCs, while others are exceeding rare in the database comprising more than 7 million SARS-CoV-2 sequences. One possibility is the silent spread of Omicron in a geographical area which lacks sequencing capacity such that it went unnoticed. Another possibility is that Omicron originated in immunocompromised patients where SARS-CoV-2 causes chronic infections (Choi *et al.*, 2020). However, the extent of viral genome mutations in those patients was much lower than that seen in Omicron. Another possibility is a type of "reverse zoonosis" where humans infected animals, where the virus experienced an evolution under constraints not encountered in the human population. The animal-adapted virus then crossed back into the human population. This hypothesis postulates two unlikely cross-species infections, but such an unlikely event has some support. For example, SARS-CoV-2 infections have been observed in zoo animals, in farm animals (minks), in wild animals (white-tailed deer) and in pet animals (ferrets, hamsters). Indeed, Omicron's spike protein binds the cellular receptor ACE2 protein from several animal species including mice (Cameroni *et al.*, 2022; Hoffmann *et al.*, 2022) facilitating infections of animals living close to humans. In addition, SARS-CoV-2 infections crossing from humans to animals and back from animals to humans have been documented for minks and workers on fur farms (Oude Munnink *et al.*, 2021) and from hamsters in pet shops to the shopkeeper followed by further onward infections to humans (Yen *et al.*, 2022).

Omicron's spike structure

Omicron has 37 mutations in the spike protein relative to the Wuhan-Hu-1 strain, with 15 mutations located in RBD (mediating receptor binding and a major target site for neutralizing antibodies). Omicron's S protein displays eight mutations (including deletions and insertions) in the N-terminal domain (NTD), four mutations in the region responsible for membrane fusion and six further amino acids replacements in the C-terminal half of the S protein. The mutations are distributed both on the surface and in the interior of the S protein trimer. Omicron showed a comparable affinity for the human ACE2 receptor as Delta. Chinese structural biologists resolved both the crystal and cryo-EM (electron microscopy) structures of the omicron RBD-ACE2 complex. The binding interface was more flexible in the cryo-EM structure than in the crystal structure, reflecting dynamic properties of the RBD-ACE2 interaction (Han *et al.*, 2022). Cryo-EM showed ACE2 bound to the RBD of one of the S protomers in the "up" position. The high number of amino acid (aa) replacements in the RBD poses a dilemma for Omicron with respect to ACE2 binding since some cause the loss of a salt bridge and are known to decrease the receptor binding. The structural analysis showed that Omicron has compensating mutations creating a new salt bridge, a hydrogen bond and π-stacking interactions (Mannar *et al.*, 2022). The mutations around the fusion SD2 region introduced inter-protomer electrostatic contacts between the S2 and S1 subunits and improved intra-protomer hydrophobic packing. Both effects might impair S1 shedding and the fusion activity of Omicron. The spike proteins from the original Wuhan isolate and Omicron can be largely superimposed except for a 10 aa region preventing the binding of site II neutralizing monoclonal antibodies in Omicron (McCallum *et al.*, 2022). Hotspot II mutations in Omicron notably affect epitopes which are the target of several therapeutic monoclonal antibodies. The RBD-RBD interaction in Omicron stabilizes the 'up' conformation of RBD and thereby ACE2 receptor binding. Overall, the Omicron RBD is more dynamic than the RBD of the Wuhan isolate (Yin *et al.*, 2022).

In the Omicron S trimer, the dominantly populated conformation is the closed state with all the RBDs buried, possibly leading to a conformational masking of sites which prevents antibody binding and virus neutralization. In Omicron, both S-close and S-open structures appear more compact than the spike structures in the Wuhan isolate, which may hinder its spike transformation towards the fusion-prone open state. Cryo-EM structural analysis of neutralizing monoclonal antibody S3H3 binding to S trimer of Omicron suggested that this antibody may function as a lock to block the release of S1 from S2, resulting in inhibition of virus entry. Since the target site of the S3H3 antibody in S1 is highly conserved among different VOCs, this antibody has not only therapeutic potential, but might point to broad-spectrum SARS-CoV-2 vaccines (Hong *et al.*, 2022).

The cryo-EM structures of the Omicron S-trimer in complex with human ACE2 showed two copies of ACE2 bound to two RBDs in the 'up' conformation. RBD-targeting neutralizing antibodies (NAb) can be

categorized into six classes (I to VI) based on epitope mapping from available RBD-NAb complex structures. The Chinese researchers observed for Omicron a positive correlation between hot immunogenic sites and areas with high mutation frequencies suggesting immune evasion. However, some sites involved in ACE2 binding were spared from mutations (Cui *et al*., 2022) raising the possibility that ACE2-mimic antibodies might have broadly cross-neutralizing activity against many VOCs of SARS-CoV-2 and further sarbecoviruses (a lineage in beta-coronaviruses) (Park *et al*., 2022).

Escape from therapeutic antibodies

The highly mutated Omicron spike protein presents a challenge for therapeutic monoclonal antibodies (mabs) in clinical development. An international consortium explored the neutralizing activity of 11 commercial mabs. Six lost activity against Omicron, four showed reduced activity and only one mab (S309) maintained neutralization. This mab binds a site on the viral spike distant from the other therapeutic antibodies which bound NTB or RBD (Dejnirattisai *et al*., 2022). S309 (sotrovimab) was also in other studies the only commercial mab which maintained its activity against Omicron (Cameroni *et al*., 2022; Hoffmann *et al*., 2022; VanBlargan *et al*., 2022). Another study noted that 17 of 19 neutralizing mabs lost activity against Omicron, which became thus the great escapee in comparison with prior VOCs. From the two mabs retaining activity, one lost activity against an Omicron variant that showed an addition mutation and represents currently 10% of the Omicron entries in the database. Only mab S309 remained active (Liu *et al*., 2022b,2022a). This observation is scientifically interesting and clinically important. It is scientifically interesting because S309 was isolated from memory B cells of a SARS patient 10 years after infection. It efficiently neutralizes SARS-CoV and SARS-CoV-2 in vitro. It recognizes a conserved glycan-containing epitope widely conserved across sabecoviruses, but does not interfere with receptor binding (Pinto *et al*., 2020). It is clinically important because sotrovimab has demonstrated efficacy in a controlled clinical trial with 583 patients. The patients showed early COVID-19 symptoms and were at risk of developing complications. Only 1% of patients treated with a single intravenous injection of sotrovimab compared to 7% of the controls treated with placebo showed disease progression with need for hospitalization. Only placebo patients needed intensive care treatment ($n = 5$) or died ($n = 1$) (Gupta *et al*., 2021).

Chinese scientists barcoded RBD variant gene segments that encoded single amino acid replacement mutations observed in VOCs and used them in high-throughput yeast display screening to determine the binding of 247 human anti-RBD neutralizing antibodies. Most of the neutralizing antibodies were isolated by using single-cell V(D)J sequencing of antigen-specific memory B cells from individuals who had been infected with SARS-CoV-2, with SARS-CoV-1 or were vaccinated. Yeast cells bound by antibodies were removed by magnetic-activated cell sorting, and the RBD mutants not bound by antibodies were identified by sequencing. This approach classified the antibodies into six epitope groups (A–F). Overall, 85% of the antibodies were unable to bind to mutations found in Omicron. This applies particularly to antibodies to sites on the spike protein that interact with the ACE2 receptor. Group E and F neutralizing antibodies were rarer, recognized a mixed protein and carbohydrate epitope (N343) outside of the ACE2 binding site and include sotrovimab. Since these antibodies are rare in the human population, there was apparently not enough selection pressure to mutate also this site in the Omicron spike protein (Cao *et al*., 2022).

Omicron's humoral immune escape

These data suggest that Omicron should display a substantial escape from neutralization by antibodies found in the sera from convalescent patients and vaccinees, which was indeed observed and documented in a flurry of research reports. An early report from the UK demonstrated that sera from subjects vaccinated with two doses of AstraZeneca's adenovirus-vectored vaccine displayed no neutralizing activity against Omicron 1 month after immunization. People vaccinated with Pfizer's mRNA vaccine showed higher neutralizing titres against VOCs and thus still some neutralizing activity against Omicron, but they were 30-fold lower than against Delta (Dejnirattisai *et al*., 2022b). South African subjects vaccinated with two doses of Pfizer's mRNA vaccine showed a 22-fold reduction of neutralizing antibody titres against Omicron compared with the early D614G virus variant, with half of the samples lacking any neutralizing activity against Omicron. Subjects who received two vaccine doses following a previous infection showed neutralizing activity against Omicron, but it was lower than against Delta (Cele *et al*., 2022). With a more systematic collection of sera from people with different exposure history to viral antigens, US researchers came to similar conclusions: 73% of convalescent patients lacked neutralizing antibodies to Omicron; double-vaccinated subjects receiving Pfizer or Moderna mRNA vaccines showed neutralizing activity to Omicron, but titres were 20- to 40-fold lower than against wildtype (WT) virus; boosted subjects suffered only an 8-fold reduction in neutralization of Omicron. Infected individuals receiving subsequently two mRNA doses showed

13-fold lower neutralization of Omicron compared to WT (Carreño *et al*., 2022).

UK scientists explored the neutralizing activity in convalescent sera from subjects from the early pandemic wave or from infections with Alpha, Beta, Gamma and Delta VOCs. In all cases – except for Gamma-infected subjects – the titre against Omicron was substantially lower than against the infecting strain. Also subjects immunized with either the AstraZeneca or the Pfizer vaccines showed lower neutralizing serum antibody titres against Omicron than against the WT virus. Neutralizing antibody titres against Omicron were substantially increased by a booster injection with the mRNA, but not with the adenovirus vaccine (Dejnirattisai *et al*., 2022a). Also, in a study from Austria, convalescent sera showed minimal neutralization of Omicron. AstraZeneca's vaccine induced lower titres against Omicron than Pfizer's vaccine, but heterologous adenovirus/ mRNA vaccination showed comparable titres as homologous vaccination with Pfizer's mRNA vaccine (Rössler *et al*., 2022). Five to 6 months after the second immunization with either AstraZeneca or Pfizer vaccines, no neutralizing titres against Omicron were detected. A booster increased the neutralization of Omicron substantially, but titres remained still 10-fold lower than against WT and Delta virus (Planas *et al*., 2022).

Booster vaccination restores neutralization of Omicron

A substantial literature documents that a booster immunization with vaccines based on the Wuhan virus sequence increased the neutralizing antibody titres against Omicron. A third dose of the Pfizer vaccine increased neutralizing titres against Omicron by a factor of 100 over the very low level after the second dose (Nemet *et al*., 2022). Anti-Omicron titres also increased substantially in convalescent subjects after vaccination (Schmidt *et al*., 2022). The US researchers compared neutralizing antibody titres in subjects that completed recently (< 3months) and longer ago (6–12 months) a two-dose mRNA vaccination scheme. Already recently vaccinated subjects showed low or no neutralizing titres against Omicron. With time, the titres decreased further. A booster injection increased the breadth of the immune response (Garcia-Beltran *et al*., 2022). After boosting, titres against Omicron corresponded to those measured against WT virus after two doses (Muik *et al*., 2022). One month after the booster, comparable neutralizing antibody titres were observed in people who received the original Wuhan isolate-specific mRNA, a Beta-specific or a Delta-specific mRNA vaccine. Six months after the booster, titres against Omicron decreased, but all vaccinees displayed Omicron-neutralizing activities (Pajon *et al*., 2022), which was also reported in an independent study (Xia *et al*., 2022). One might wonder whether a fourth dose immunization with mRNA vaccines (second booster) increases antibody titres and the breadth of the antibody response even further. Researchers in Israel explored this question in 270 health care workers receiving either the Pfizer or Moderna mRNA vaccine compared to 500 controls. The fourth dose induced a 10-fold neutralizing antibody titre increase against WT, Delta and Omicron compared to titres observed 5 months after the third dose, but the titres corresponded to those observed 1 month after the third dose. Titres against Omicron remained 5-fold and 10-fold lower than against Delta and WT virus (Regev-Yochay *et al*., 2022).

The efficiency of a boost 1 month after the second injection was also demonstrated for a protein subunit vaccine developed in China. In vaccinees, neutralizing antibody titres were observed against Omicron but were only a tenth of those against Delta, while convalescent sera showed no activity against Omicron. When the boost dose was given 4 months after the second dose, a 5- to 10-fold higher neutralizing antibody titre against VOCs including Omicron was observed. Half a year later, these titres dropped to levels seen after the short interval booster (Zhao *et al*., 2022). The observed effects may be explained by the longer evolution of memory B cells entering the germinal centres of the immune system where they experienced affinity enhancement of the antibodies (Wesemann, 2022; Willyard, 2022).

Cellular immunity

Researchers asked to what extent the cellular immunity arm of the antiviral immune response contributes to protection against SARS-CoV-2 infection or from COVID-19 disease. Apparently, $CD8^+$ T cells play an important role in the immune defence against SARS-CoV-2 infection. The expansion of the $CD8^+$ T cells in the bronchi and the lung was associated with rapid viral clearance and a mitigation of disease symptoms. Animal experiments in macaques showed that depletion of $CD8^+$ T cells resulted in reduced protective immunity. Researchers therefore asked whether the marked escape of the highly mutated Omicron variant from humoral immunity also extends to an escape from cellular immunity. To address this question, US researchers investigated the antiviral cellular immune response in 76 adults who differed in prior infection, vaccination and boosting history. The magnitude of effector T cell responses to the spike protein did not vary by variant virus and was not affected by age, sex and primary vaccine series. T cell response decreased only modestly with time after vaccination in contrast to the marked waning of the antibody response.

Twenty percent of the subjects showed a decreased T cell response to Omicron spike peptides compared to 10% after booster vaccination. The reduced T cell response to Omicron was explained by poor binding of Omicron peptides to the HLA haplotypes of these subjects. The researchers assessed the predicted binding affinity of all 8- to 11-mer peptides in the WT and Omicron spike proteins and only 7% of the Omicron peptides showed a loss of binding, explaining the largely preserved T cell response to Omicron. Interestingly, subjects with undetectable antibody response to Omicron had nevertheless measurable T cell responses against Omicron spike peptides (Naranbhai *et al*., 2022).

In a study from South Africa, more than 85% of vaccinees generated a T cell response to vaccination. Both vaccination and infection induced spike-specific $CD4^+$ T cell responses, while a $CD8^+$ response was less consistently detected. About a quarter of the investigated subjects showed a decreased $CD4^+$ and $CD8^+$ response to Omicron, while 15% lacked a $CD8^+$ response to Omicron. There were no significant differences in cross-reactive $CD4^+$ and $CD8^+$ T cell responses for Beta, Delta and Omicron VOCs. Epitope spanning revealed that Omicron spike mutations occurred in regions poorly targeted by $CD4^+$ T cells, but are more common in regions frequently targeted by $CD8^+$ T cells. However, most patients target conserved epitopes in the spike protein explaining why Omicron achieved a lesser, if any escape from cellular than from humoral immune response (Keeton *et al*., 2022). The US researchers compared the cellular immune response in subjects who received either the adenovirus-vectored or the mRNA vaccine. Both vaccines induced substantial spike-specific interferon gamma (IFNγ) responses in $CD8^+$ and $CD4^+$ T cell responses which showed similar reactivity against WT, Delta and Omicron when assessed 8 months after vaccination. Only 2 out of 47 vaccinees showed a cellular immune response only to the spike peptides of WT, but not of Omicron. Also, central and effector memory T cell subpopulations elicited by vaccination showed extensive cross-reactivity to Delta and Omicron variants (Liu *et al*., 2022b). Researchers from South Korea observed that substantial proportions of memory T cells elicited by vaccination or natural infection responded to Omicron spike peptides and concluded that VOCs cannot evade T cell responses because multiple T cell epitopes are scattered across structural and non-structural proteins (Choi *et al*., 2022).

Vaccine efficacy

While the loss of neutralizing activity in therapeutic antibodies is of direct clinical consequence, the consequence of a reduced neutralizing antibody response in vaccinated subjects is less clear since the cellular immune response to Omicron seems to be largely retained. Only vaccine efficacy (VE) studies against Omicron infection and disease can here provide clarity. A first estimate of VE came from South Africa where researchers calculated a VE of 70% against hospital admission for COVID-19 during the Omicron-dominated period (Nov-Dec 2021) compared to a VE of 93% in the prior Delta-dominated period (Sept-Oct 2021). The study subjects had received two doses of the Pfizer mRNA vaccine (Collie *et al*., 2022). A study from Southern California using electronic health records evaluated VE against infection and hospitalization with Omicron or Delta. Of 26 000 identified COVID-19 cases, 43% were unvaccinated and 57% had received the Moderna mRNA vaccine. VE against Omicron infection was 44% in the first 3 months after two vaccine doses and declined to 24% over the next 3 months; the corresponding VE against Delta was 80% and 69%, respectively. After booster vaccination, VE against Omicron infection was initially 72% and dropped to 47% after 2 months. After two and three vaccine doses, VE against hospitalization with Omicron was however with 85% and 99%, respectively, much higher. Only four boosted individuals were hospitalized with Omicron. They were older than 60 years and suffered from chronic diseases (Tseng *et al*., 2022). In England, 880 000 persons were infected with Omicron between Dec 2021 and mid-Jan 2022, while 200 000 persons experienced an infection with Delta. Data on disease and vaccination status were evaluated in a test-negative case–control design to estimate VE against symptomatic disease. A 50% VE efficacy against Omicron was observed 1 month after a 2-dose immunization with the adenovirus-vectored vaccine from AstraZeneca, while half a year after vaccination, no protection was observed. When these vaccinees received a booster injection with an mRNA vaccine, VE against disease increased to 70% and 60% with the Moderna or Pfizer vaccine, respectively. A two-dose scheme of mRNA immunization achieved a VE of about 70%, which also quickly dropped to 10% VE after half a year, but a booster increased VE again to 60% for both mRNA vaccines and was less quickly eroded over time. In contrast, VE against Delta was maintained at 65% to 80% half a year after two doses of Pfizer and Moderna vaccine, respectively, and a booster increased VE above 90%. The English researchers noted that VE against severe disease is likely to be substantially higher because only a small number of Omicron cases were hospitalized in their data set (Andrews *et al*., 2022).

A retrospective cohort study evaluated VE of booster vaccination (given 250 days after the second dose) in 280 000 Qataris compared to 1.2 million recipients of a two-dose Pfizer vaccine. After 1 month of follow-up

during an Omicron wave, the incidence of symptomatic infection was 2.4% in the booster and 4.5% in the non-booster cohort translating into a 49% VE of booster compared to 2-dose cohort. VE of a booster against severe COVID-19 leading to hospitalization was 76% compared to 2-dose recipients. For people receiving the Moderna vaccine, the incidence of symptomatic Omicron infection during the follow-up was 1.0% in the booster and 1.9% in the 2-dose group, yielding a similar VE of 47%. The number of severe COVID-19 was too low in the two Moderna vaccinee groups to calculate VE against severe infection (Abu-Raddad *et al*., 2022). Researchers from Israel analysed the effect of a second booster in health care workers. Compared to those receiving one booster, a second boost restored antibody titres and was associated with a moderate decrease in symptomatic infections (Regev-Yochay *et al*., 2022).

Reinfection protection

Researchers from Qatar analysed the national database for the effectiveness of previous SARS-CoV-2 infection in preventing reinfection. In a case-control design, they compared patients who experienced a prior PCR-proven infection with uninfected, unvaccinated subjects. Previous infection conferred a variable degree of protection against reinfection: 90% against Alpha, 86% against Beta, 92% against Delta, but only 56% protection against infection with Omicron. Only few severe reinfections were observed, and prior infection showed an 88% protection against severe Omicron disease (Altarawneh *et al*., 2022).

South Africa has seen 3.6 million confirmed COVID-19 cases in four infection waves. According to the national register, 105 000 individuals experienced two laboratory-confirmed infections and 1778 individuals experienced three infections. The time between the infections corresponded to intervals of 170, 350 and 520 days, reflecting infections occurring between sequential waves, or separated by one or two epidemic waves. When the reinfections were plotted according to calendar months, few reinfections occurred during the Beta wave, a small peak was seen with the surge of the Delta wave, while a major peak of reinfection was seen in December 2021 with the rise of the Omicron wave. A mathematical model showed that the reinfection risk rose constantly with time until the Delta wave while with the Omicron wave the reinfection risk increased suddenly, indicating that Omicron outcompeted the prior circulating viruses by immune evasion while Beta and Delta rose to dominance via increased transmissibility compared to prior variants (Pulliam *et al*., 2022). Somewhat related is the question what type of neutralizing antibody response is observed in subjects who experienced a primary Omicron infection or a reinfection with Omicron. Vaccinated subjects who experienced a primary Omicron infection as well as those with an Omicron reinfection showed moderate neutralizing antibody titres to Omicron and elevated titres to other VOCs. In contrast, unvaccinated subjects without a prior infection only mounted weak antibody titres against Omicron and very small titres against other VOCs. The authors of this Austrian study expressed the concern that unvaccinated Omicron-infected persons are only insufficiently protected against future infections (Rössler *et al*., 2022).

Clinical attenuation

According to the above-reported studies, Omicron is clearly a VOC with respect to transmission and immune escape, but is it also a VOC for its clinical severity? Does it even represent the long-awaited virus variant with an attenuated virulence phenotype, opening a pathway out of the pandemic? Early data from a private South African health care group that evaluated >10 000 patients presenting at emergency departments during the four infection waves provided first insights. During the third Delta wave, 69% of the presenting patients were hospitalized compared with 41% during the fourth Omicron wave; 74% of Delta vs. 18% of Omicron patients needed oxygen therapy and 30% vs. 19% needed intensive care and 29% vs. 3% died from Delta and Omicron, respectively (Maslo *et al*., 2022). Subsequently, South African epidemiologists reported clinical data for 11 000 cases with a likely Omicron infection. Compared to young adults, cases younger than 5 years and older than 60 years had an increased risk of hospitalization. After controlling for factors associated with severe disease, cases with Omicron infections during the fourth wave had a threefold lower odd of severe disease than cases with Delta infections during the third wave (Wolter *et al*., 2022). In a district of the Gauteng province in South Africa, 18% of the hospitalized COVID-19 patients were paediatric cases during the Omicron wave. While during the first three waves paediatric admissions lagged adult admissions, this pattern was reversed in the fourth wave. Paediatricians noted the clinical symptoms for 138 hospitalized, mostly <4 years old children: fever and cough were seen in the majority of cases, followed by shortness of breath, seizures, vomiting and diarrhoea (Cloete *et al*., 2022). An increased incidence of paediatric cases needing hospitalization was also reported for the Omicron infection wave in the United States (where children represented 5% of all hospitalizations) and in the United Kingdom (particularly in infants <1 year) when compared with the Delta wave (Kozlov, 2022). Public health scientists from Canada matched 9000 Omicron cases with Delta cases:

Omicron cases showed lower rates of hospitalization (0.6% vs. 1.4%) and death (0.03% vs. 0.3%) than Delta cases even when stratified for vaccination status (Ulloa *et al*., 2022).

In South Africa, the fourth Omicron infection wave showed the sharpest infection peak of all waves taking only 1 month from onset to maximum and was also the quickest to decrease. With respect to daily case numbers, the Delta wave was the highest and it showed also the highest weekly rate of hospitalization. The second highest case number was shown by the Omicron wave, but its weekly hospitalization rate was the lowest of all four waves. The first three waves were accompanied by substantial weekly excess death rates while only minimal excess death was seen during the Omicron wave. The Omicron wave contributed 11% and 3% of overall COVID-19 hospitalizations and excess deaths, respectively, much less than the Delta wave, which contributed 44% of the COVID-19 hospitalizations and 53% of excess deaths in Gauteng. The researchers suggested that the observed dramatic decoupling of hospitalizations and deaths from infections could indicate that Omicron may be less apt in causing serious illness (Madhi *et al*., 2022). Clinicians and epidemiologists from Boston warned that diagnosing a decreased virulence of Omicron might be wishful thinking of a scientific audience weary of the pandemic. They argued that viruses do not inevitably evolve towards being less virulent; evolution simply selects viruses that excel at multiplying. Since Omicron shows immune escape, it can infect subjects with an existing immune response acquired by prior SARS-CoV-2 infections. One might anticipate that reinfection or infection of subjects with a vaccination-primed immune response will necessarily lead to attenuated clinical severity and do not prove an intrinsically lower virulence of Omicron (Bhattacharyya and Hanage, 2022). To settle this controversy which is of substantial public health implication (potentially meaning that Omicron infections are dangerous for previously uninfected and unvaccinated subjects), it needs studies that investigate Omicron's clinical severity as a function of preexisting immunity or in the absence of immunity.

Animal experiments

Comparing the pathogenicity of different VOCs in animal experiments is one way to assess whether Omicron is really characterized by a lower intrinsic virulence. Chinese researchers infected mice transgenic for the human ACE2 receptor intranasally with WT, Delta and Omicron virus. WT and Delta replicated to high copy numbers along the respiratory tract while Omicron showed 1000-fold reduced viral copy numbers and 50-fold reduced infectious virus titres in both the nasal tract and the lung compared to WT and Delta. In parallel, Omicron induced both in the nose and lungs less proinflammatory cytokines than WT and Delta. Mice infected with Omicron showed only a mild body weight loss while mice infected with WT or prior VOCs – particularly those infected with Alpha – showed a marked weight decrease. A distinct virulence of VOCs was also observed with respect to survival of mice: Alpha-infected mice did not survive, mice infected with WT showed 20% and those infected with Delta 44% survival while 57% of Omicron-infected mice survived. The lungs of both WT- and Delta-infected mice revealed collapse of the alveoli wall, proteinaceous exudation in the alveoli cavity, epithelial damage in the small bronchioles and interstitial congestion while the lungs of Omicron-infected mice showed only weak pathological signs (Shuai *et al*., 2022). The US researchers working with four transgenic or non-transgenic mice (Omicron can use the mouse ACE2 receptor) confirmed the attenuated virulence of Omicron compared to previous viral variants. They documented reduced viral titres in the respiratory tract, and less weight loss, less lung function loss and less histopathological signs in Omicron-infected mice. They extended their observation to hamsters, another COVID-19 model. Omicron-infected hamsters showed comparable viral replication and titres in the upper respiratory tract, while in the lungs Omicron's viral copy numbers were 10-fold reduced compared to WT. Delta infection caused severe pathology in the lungs of hamsters which was also documented by microcomputed tomography abnormalities consistent with COVID-19 pneumonia. In contrast, only mild pathology was seen in Omicron-infected hamsters. The severe weight loss seen in WT-infected hamsters leading to the death of all animals was attenuated in Omicron-infected hamsters (no weight loss, 75% survival). The US consortium reproduced the results across different laboratories providing confidence in the results. However, the researchers noted that it still needs evaluation in non-human primates and unvaccinated, previously uninfected humans to conclude definitively on a lesser virulence of the Omicron variant (Halfmann *et al*., 2022). Japanese researchers quantitatively analysed the lung function and found that Omicron-infected hamsters were comparable to uninfected hamsters while Delta-infected hamsters exhibited respiratory disorders. In oral swabs, Delta showed peak viral RNA loads 1 day post infection which were maintained for a week, while Omicron titres peaked only at day 2 and then decreased rapidly. In contrast to Delta, Omicron showed only a slow spread along the bronchi and the animals showed only mild bronchitis. Again, in contrast to Delta, Omicron showed only limited inflammation and lower hyperplasia of type II pneumocytes in the lung (Suzuki *et al*., 2022).

Cell biology

German researchers showed that Omicron binds the cellular ACE2 receptor, infects ACE2 expressing cells and cell culture infection were inhibited by soluble ACE2. All observations demonstrate that Omicron uses the same ACE2 receptor as other SARS-CoV-2 viruses (Hoffmann *et al.*, 2022). However, in many established cell culture lines, Omicron replication was less efficient than that of Delta (Meng *et al.*, 2022). Chinese researchers also studied the replication competence and cellular tropism of Omicron in *ex vivo* explant cultures of human bronchus and lung (Hui *et al.*, 2022). Compared to WT and Delta virus, Omicron showed a higher replication in the bronchi – thus potentially enhancing transmission by the airborne route – but reduced replication in the lung. The different variants did not differ in cell tropism: ciliated epithelia, goblet cells and club cells were infected. Interestingly, Omicron viral particles were both seen in membrane-bound vesicles in the cytoplasm, as well as on the cell surface attached to microvilli of ciliated cells. This observation suggested a distinct entry mechanism for Omicron. The researchers conducted infections of cells differing in the expression of the transmembrane serine protease 2 (TMPRSS2). TMPRSS2 cleaves the spike S2 domain allowing the virus to enter the cell directly via cell fusion at the outer cell membrane as opposed to virus entry via the endocytic pathway. The success of the latter entry is dependent on cathepsins, intracellular proteases. Notably, enhanced infectivity was observed for WT and Delta when TMPRSS2 was overexpressed in cell cultures while Omicron is inefficient in utilizing TMPRSS2 for its entry. Omicron infection was however more sensitive to cathepsin inhibitors than Delta. This observation suggests that Omicron enters cells primarily via the endocytic pathway while Delta preferentially enters cells via fusion at the cell surface (Meng *et al.*, 2022; Shuai *et al.*, 2022). Other data concur with these observations. Compared with Delta, Omicron showed less cell fusion activity as demonstrated by smaller syncytia and smaller plaque size in cell culture infections, and less expression of the viral spike protein on the cell surface. In addition, the level of cleaved S2 of Omicron's spike protein was significantly lower than that of Delta virus (Meng *et al.*, 2022; Suzuki *et al.*, 2022). The animal and cell culture data do not explain why Omicron should outcompete Delta (Dance, 2022). Independence of Omicron from TMPRSS2 expression which is low in the nose combined with an immune escape phenotype might however at least partly explain its competitive edge.

The future

The emergence of Omicron and its rapid worldwide spread has alerted the public health community. Under the slogan 'no one is safe until everyone is safe', it was claimed that the whole world population should be vaccinated, starting with the most vulnerable populations. By 15 March 2022, an estimated 57% of the world population has been fully vaccinated, but in Africa this figure was only 13%. WHO proposed a treaty which temporarily waives intellectual property (IP) rights to COVID-19 vaccines and drugs (Anonymous, 2021). The EU resisted this IP plan, but donated substantial amounts of vaccine doses that many African countries were logistically unable to administer (Anonymous, 2022a). Omicron's immune escape capacity poses a new challenge for vaccines, and both Pfizer and Moderna have announced that they initiated clinical trials with Omicron sequence-based mRNA vaccines. Do we need an Omicron vaccine? Animal experiments might here provide some insight. When macaques were boosted with either the standard Moderna mRNA or an Omicron-adapted mRNA, comparable neutralizing antibody titres against WT and Omicron were observed in both cases. Both boosters increased the breadth of the antibody response (Gagne *et al.*, 2022). Macaques which were boosted with the Pfizer mRNA vaccine and were subsequently challenged with Omicron controlled rapidly viral replication in the lower respiratory tract. Most but not all boosted macaques also controlled viral replication in the nose. Failure to control nasal viral replication was correlated with low neutralizing antibody titres and an undetectable Omicron $CD8^+$ T cell response (Chandrashekar *et al.*, 2022).

Public health scientists asked whether such an adapted vaccine could come in time before the current Omicron wave has ended. Other scientists argue that the current vaccines protect against severe disease with Omicron while protection against infection is weak. However, as long as viral infection chains are maintained in the population, new variants can evolve leading to new infection waves (Waltz, 2022). In addition, Omicron has not run its full course – a problem are countries such as China where 65% of the over 80-year-old citizens had not been vaccinated. Recent mortality increases in Hong Kong indicate that also Omicron can lead to severe disease (Anonymous, 2022b).

In view of its widespread dissemination in the human population and the potential for animal reservoirs, many scientists think that SARS-CoV-2 is unlikely to disappear. The hope is that vaccination and natural infection will create an immunity level in the population preventing very deadly epidemics by future variants. Whether we will see in the near future a transition to a state of endemicity (which could take a decade) is difficult to predict by mathematical infection models that are mostly limited to forecasts over few weeks. Dropping restrictions will also depend on the tolerance of societies to accept

not only COVID-19 deaths but also the sequels of long covid (Adam, 2022).

Non-pharmaceutical interventions and vaccinations are not the only protective measures against COVID-19. Drugs preventing the transition from infection to severe disease and death will play an increasing role in the future to cope with COVID-19. Omicron also has mutations in the RNA-dependent RNA polymerase and the main protease which are the targets of the antiviral drugs remdesivir and molnupiravir, respectively. It is reassuring that direct-acting antivirals such as remdesivir and the active metabolite of molnupiravir displayed similar antiviral activity against Delta and Omicron (Meng *et al*., 2022; Takashita *et al*., 2022).

An important public health activity will remain the tracing of new variants. While the emergence of Delta was epidemiologically already apparent from overwhelmed hospitals in India, this was not the case for Omicron in South Africa. Here Omicron was detected by dedicated virologists analysing PCR tests and viral genome sequences. Such activities should be maintained in the future to allow the early detection of novel variants, increasing our understanding of SARS-CoV-2 evolutionary trajectories. On the practical side, when applied to sewage water, these activities could serve as early warning of impending new epidemic waves (Kucharski and Cohen, 2022). Some virologists think that new SARS-CoV-2 variant will sweep through the population every few months, modulated by climate factors. Notably, new VOCs have evolved not from the dominant preceding viral strains, but from separate lineages. That viruses evolve towards milder disease is frequently heard, but far from being clear. A variant that escapes from T cell immunity could cause major damage (Ledford, 2022) although current data suggest that such an escape will be difficult for the virus.

In fact, Omicron with its many mutations has already explored more evolutionary space than any of the prior variants (Dance, 2022). What viral evolution potentially has in its sleeves can also be investigated with repeated viral passages of SARS-CoV-2 in cell culture. Passage of SARS-CoV-2 in the presence of the antiviral remdesivir led to a mutant with reduced sensitivity to remdesivir. The phenotype could be traced back to a single amino acid replacement in the viral RNA-dependent RNA polymerase (Szemiel *et al*., 2021). Serial passage of SARS-CoV-2 with decreasing viral inoculum size as selection pressure led to a variant that could bind heparan sulfate on the cell surface for primary attachment before leading to interaction with the ACE2 receptor. Such a mutant showed increased viral spread and plaque size as well as higher infectivity titres. Mutations were located in the N-terminal part of the spike protein as well as in the furin cleavage site explaining the decreased syncytium formation by the mutated virus (Shiliaev *et al*., 2021).

Omicron comprises three subtypes: BA.1, BA.2 and BA.3. In Denmark, BA.2 replaced the previously dominant BA.1 within a single month (January 2022) indicating a higher transmission rate for BA.2 than BA.1 (Callaway, 2022). BA.2 is now also the dominant Omicron strain in India and South Africa. BA.2 contains 8 unique spike mutations and lacks 13 spike mutations found in BA.1 (Yu *et al*., 2022) and differs thus substantially from BA.1 in antigenic properties. Indeed, neutralizing antibody titres of convalescent and vaccinated subjects were even lower against BA.2 than against BA.1, but the difference did not reach statistical significance (Iketani *et al*., 2022). However, mRNA vaccinees showed after booster good neutralizing antibody titres to BA.1 (6-fold lower than to WT) and titres against BA.2 differed only by a factor of 1.4 from those against BA.1. Antigenic differences were detected: BA.2 resisted extensively therapeutical monoclonal antibodies, which also affected the efficacy of sotrovimab (Takashita *et al*., 2022). In addition, rare cases from Israel were reported of subjects who had recovered from a BA.1 infection and got reinfected with BA.2 (Callaway, 2022). A small study with 8 subjects infected with BA.1 showed poorly cross-reactive neutralizing antibody titres to prior VOCs and a 4-fold titre decrease to BA.2 (Richardson *et al*., 2022). In another study, subjects infected with Omicron mounted a comparable neutralizing antibody titre to WT, BA.1 and BA.2 (Yu *et al*., 2022). Whatever its antigenic relationship to BA.1, BA.2 is likely to extend the duration of the Omicron infection wave. Since BA.2, like BA.1, causes mostly mild disease in populations with high vaccine coverage, it will not raise much concern in the public. However, SARS-CoV-2 will evolve further since it finds enough susceptible subjects caused by vaccine hesitancy, problems of vaccine delivery in developing countries and viral immune escape mechanisms. Omicron has shown the great genetic plasticity of the coronavirus genome, and it would thus be very surprising if it will not evolve new variants that should occupy public health services in the near future. Maintaining restrictions that are an economic burden or that limit personal freedom are of course difficult to justify when confronting a relatively less virulent virus variant, but some cheap and easy interventions such as mask wearing should be kept to limit the viral spread in public gatherings. Research on viral evolution with advanced warning for newly emerging variants should also remain in place and go in parallel with influenza virus surveillance. A recent data analysis stressed that SARS-CoV-2 may still evolve over time in a direction that is not easily predicted and that we should not expect evolution towards lower virulence (Koelle *et al*., 2022). This analysis concurs with conclusions from mathematical models

where natural selection generally favours a lengthening of the pre-symptomatic transmission phase while selection tends to be weak for a decrease in infection-induced mortality. Pleiotropic mutations will strongly impact mortality, but pleiotropic effects are notoriously difficult to predict. The researchers predicted that mutations that increase viral replication rates will increase viral transmission with uncertain effects on mortality. Mutations that alter tissue tropism might generate indirect selection for lower mortality if it favours viral replication in the upper over the lower respiratory tract (Day *et al*., 2020). One gets the impression that this mid-2020 model predicted the Omicron variant. Complacency towards future variants is not indicated because even Omicron frequently fails to infect close contacts, suggesting that there is ample scope to increase viral transmission efficacy further. If combined with a moderate virulence phenotype in such a future variant, a 'flying infection' for SARS-CoV-2 as for measles or chickenpox virus could again become a public health challenge.

Conflict of Interest

None declared.

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