Antigenic cartography using sera from sequence-confirmed SARS-CoV-2 variants of concern infections reveals antigenic divergence of Omicron

Graphical abstract

Highlights
- SARS-CoV-2 VOCs induce qualitatively different neutralizing antibody responses
- D614G and Alpha induce the strongest and broadest neutralizing antibody responses
- Omicron induces weaker neutralizing antibody responses
- Omicron BA.1 and BA.2 are antigenically distinct from the D614G strain

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In brief
Given the continued evolution of SARS-CoV-2, it is important to understand when and how to update vaccines to antigenically match circulating variants. van der Straten et al. demonstrate that infection with different SARS-CoV-2 variants leads to qualitatively different neutralizing antibody responses. Moreover, they show that Omicron represents a new cluster of antigenically distinct variants, which has implications for updating vaccines.
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SUMMARY

Large-scale vaccination campaigns have prevented countless hospitalizations and deaths due to COVID-19. However, the emergence of SARS-CoV-2 variants that escape from immunity challenges the effectiveness of current vaccines. Given this continuing evolution, an important question is when and how to update SARS-CoV-2 vaccines to antigenically match circulating variants, similarly to seasonal influenza viruses where antigenic drift necessitates periodic vaccine updates. Here, we studied SARS-CoV-2 antigenic drift by assessing neutralizing activity against variants of concern (VOCs) in a set of sera from patients infected with viral sequence-confirmed VOCs. Infections with D614G or Alpha strains induced the broadest immunity, whereas individuals infected with other VOCs had more strain-specific responses. Omicron BA.1 and BA.2 were substantially resistant to neutralization by sera elicited by all other variants. Antigenic cartography revealed that Omicron BA.1 and BA.2 were antigenically most distinct from D614G, associated with immune escape, and possibly will require vaccine updates to ensure vaccine effectiveness.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus), represents an enormous threat to human health and a burden to healthcare systems and economies worldwide. The unprecedented rapid development of efficacious vaccines fueled hope of curtailting this pandemic and permitting a return to a society without societal restrictions. However, genetic drift of SARS-CoV-2 resulted in the emergence of multiple variants of concern (VOCs) with a higher transmissibility compared with the ancestral strain, challenging the effectiveness of public health measures, vaccines, and/or therapeutics (World Health Organization, 2021). Based on this definition, the WHO designated the Alpha (Pango lineage B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529, including sublineages BA.1 and BA.2) variants as VOCs. The Alpha, Beta, Gamma, and Delta VOCs have approximately 7–12 mutations in the spike protein (S), whereas Omicron BA.1 with 34 mutations, of which 3 deletions, and BA.2 with 28 mutations differ substantially from the ancestral strain (Figure 1A) (World Health Organization, 2021). Approximately half of Omicron’s S mutations are located in the receptor binding domain (RBD) and eight mutations in the N-terminal domain (NTD), the two most
important antigenic sites of S. Indeed, sera from COVID-19 patients infected with the ancestral strain and sera from vaccinees show up to 7- and 4-fold reductions in neutralization activity against Beta and Gamma, whereas 20- to 40-fold reductions are observed against Omicron BA.1 (Canterino et al., 2021; García-Beltrán et al., 2021; van Gils et al., 2022; Wilhelm et al., 2021).

However, the precise antigenic relationships among these VOCs are only starting to become clear. Understanding the differences between the serological antibody responses elicited by these variants is important to assess the risk of re-infections after natural infection and breakthrough infections after vaccination. For seasonal influenza viruses, this type of antigenic data is combined with virus genetic and epidemiological data to quantify the evolution of the virus and guide annual updates of the seasonal influenza virus vaccines. Antigenic cartography can be used to visualize antigenic relationships among viral variants (Fonville et al., 2014; Smith et al., 2004) and is routinely used in influenza virus vaccine strain selection. Until recently, antigenic cartography for SARS-CoV-2 has only been applied to cohorts of COVID-19 patients with uncertainty about their history of previous SARS-CoV-2 exposure and COVID-19 vaccinations, and without the usage of Omicron-infected human data (Liu et al.,

Figure 1. SARS-CoV-2 VOCs elicit diverse serum responses against homologous and heterologous strains
(A) Molecular models of SARS-CoV-2 S, highlighting the locations of mutations in the D614G strain (blue) and Alpha (green), Beta (yellow), Gamma (orange), Delta (red), Omicron BA.1 (magenta), and Omicron BA.2 (pink) variants. Midpoint neutralization titers against the VOCs in international units per mL (IU/mL). The individuals are grouped per VOC and plotted accordingly. Median neutralization titers are indicated while the individual points are depicted with higher transparency. The light gray bar (10 IU/mL) indicates the neutralization cutoff for all strains except Omicron (cutoff 2 IU/mL, dark gray bar). Non-hospitalized patients are indicated with dots and hospitalized patients with triangles. The individuals who were infected with an Alpha strain that also included the E484K mutation are indicated in green squares. The homologous neutralization is highlighted using a light blue bar. The Wilcoxon signed rank test with Benjamini-Hochberg correction was used to compare cross-neutralization titers with the homologous neutralization (see Table S3A for exact p values). Only statistically significant differences are indicated. *p < 0.005, **p < 0.01, ***p < 0.001, ****p < 0.0001.
(B) Spider plot of the median neutralization titer (IU/mL) of each group against all VOCs. A cutoff of 10 IU/mL is used for all strains.

D614G-infected
Beta (B.1.351)
Alpha (B.1.1.7)
Gamma (P.1)
Alpha (B.1.1.7)
Delta (B.1.617.2)
Omicron BA.1-infected
Omicron BA.1-infected
Omicron BA.2-infected
Omicron BA.2-infected
Omicron BA.2-infected
Omicron BA.1-infected
Omicron BA.2-infected
Table 1. Sociodemographics and clinical characteristics

| Variant of the infection (of concern) | N = (%) | Age (years) median (range) | Male | Hospital admission | Sequence confirmed | Time since symptom onset (days) median (range) |
|--------------------------------------|---------|---------------------------|------|------------------|-------------------|-----------------------------------------------|
| D614G                                | 20 (30%)| 53 (22–74)                | 9 (45%) | 9 (45%)          | 0 (0%)            | 38 (30–45)                                   |
| Alpha                                | 11 (17%)| 48 (25–76)                | 6 (55%) | 9 (82%)          | 11 (100%)        | 39 (24–58)                                   |
| Alpha + E484K                        | 2/11    | 62 (48–76)                | 0     | 2/2              | 1/1              | 41 (24–58)                                   |
| Beta                                 | 8 (12%) | 41 (18–53)                | 3 (38%) | 0               | 3 (38%)          | 39 (30–63)                                   |
| Gamma                                | 4 (6%)  | 35 (27–10)                | 2 (50%) | 0               | 4 (100%)         | 40 (38–55)                                   |
| Delta                                | 11 (17%)| 31 (19–63)                | 6 (55%) | 1 (9%)           | 10 (91%)         | 46 (38–52)                                   |
| Omicron BA.1                         | 8 (12%) | 31 (21–45)                | 4 (50%)  | 0               | 3 (38%)          | 44 (25–75)                                   |
| Omicron BA.2                         | 4 (6%)  | 51 (31–55)                | 4 (100%) | 0               | 4 (100%)         | 40 (39–41)                                   |
| Total                                | 66      | 41 (18–76)                | 34 (52%) | 21 (32%)        | 39 (59%)         | 40 (24–75)                                   |

A summary of the convalescent SARS-CoV-2 patients included in this study. See also Table S1 for a more comprehensive overview per individual.

RESULTS

Study population

We collected and analyzed a set of serum samples from 66 COVID-19 patients with a PCR-confirmed primary SARS-CoV-2 infection who did not receive any COVID-19 vaccinations. Blood was drawn 3–11 weeks after symptom onset (median 40 days, range 24–75 days), which corresponds with the peak of the antibody response (Tables 1 and S1) (Long et al., 2020). In total, n = 20 D614G-infected, n = 11 Alpha-infected, n = 8 Beta-infected, n = 4 Gamma-infected, n = 11 Delta-infected, n = 8 Omicron BA.1-infected, and n = 4 Omicron BA.2-infected participants were included. Of these participants, 39 had a sequence-confirmed VOC infection. The other 27 participants met our inclusion criteria of a high likelihood of VOC infection (see STAR Methods section; Table S1), of which 20 participants were assumed to be infected with the D614G strain as they were sampled before the emergence of any VOC in the Netherlands, but after D614G became dominant (Korber et al., 2020).

Magnitude and breadth of serum neutralization depend on the infecting SARS-CoV-2 VOC

We assessed the neutralizing capacity of the convalescent sera in a lentiviral-based pseudovirus neutralization assay against the D614G strain and the Alpha, Beta, Gamma, Delta, or Omicron BA.1 or BA.2 variants and used this data as input for antigenic cartography to map the antigenic evolution of SARS-CoV-2. Here, we studied the (cross-)neutralizing antibody responses in sera from a well-defined population of convalescent individuals with a sequence-confirmed, or high likelihood of, primary infection by the D614G strain or Alpha, Beta, Gamma, Delta, or Omicron BA.1 or BA.2 variants and used this data as input for antigenic cartography to map the antigenic evolution of SARS-CoV-2. However, when comparing only non-hospitalized patients who generally have lower antibody levels compared with hospitalized patients, patients infected with the Alpha variant showed the strongest homologous neutralization (1881 IU/mL, range 1,658–2,103 IU/mL), followed by individuals infected with the Gamma variant (median of 156 IU/mL, range 22–761 IU/mL), Delta variant (median of 85 IU/mL, range 10–1,635 IU/mL), and the Omicron BA.2 (median of 64 IU/mL, range 10–95 IU/mL) and Omicron BA.1 variants (median of 23 IU/mL, range 10–90 IU/mL) (Figure 2A). By contrast, none of the Beta-infected participants showed substantial homologous neutralization against either Beta subvariants.

Overall, the VOCs differed in their capacity to induce cross-neutralizing antibodies. Individuals infected with the Alpha variant induced the broadest response, followed by D614G strain-infected, Gamma-infected, and Delta-infected patients (Figure 2B), although there was substantial heterogeneity within all groups (Figures 1A and 2B). Notably, none of the patients infected with the Beta, Omicron BA.1, or Omicron BA.2 variants showed substantial cross-neutralization activity.

Reductions in neutralizing activity against the two Omicron variants were substantial in all groups (Figures 1A and 1B). Omicron neutralization dropped below the limit of detection (10 IU/mL or an ID_{50} of 100) in 44/66 of the studied individuals for BA.1 and 37/66 for BA.2. The median fold reduction of Omicron BA.1 neutralization versus homologous neutralization was 9-fold (range 1- to 93-fold) when considering all patients, 10-fold (range 3- to 93-fold) for patients infected with a D614G strain, 52-fold (range 11- to 89-fold) for Alpha-infected, 6-fold (range 1- to 22-fold) for Gamma-infected, and 6-fold (range 1- to 51-fold) for Delta-infected patients. The median fold reduction of Omicron BA.2 neutralization versus homologous neutralization was 5-fold (range 1- to 134-fold) when considering all patients, 8-fold (range 3- to 47-fold) for patients infected with a D614G strain, 68-fold (range 18- to 134-fold) for Alpha-infected,
6-fold (range 1- to 22-fold) for Gamma-infected, and 6-fold (range 1- to 60-fold) for Delta-infected patients. Overall, we showed that exposure to different SARS-CoV-2 VOCs induces distinct serum responses, which differ in their abilities to neutralize homologous and heterologous virus strains.

**Omicron BA.1 and BA.2 are antigenically distinct from the D614G strain and other VOCs**

To explore the antigenic relationships among the VOCs, we used the neutralization data to construct a SARS-CoV-2 antigenic map (Figure 3A). In this map, homologous sera tend to cluster around the infecting strain, reflecting that homologous neutralization is dominant. The D614G and Alpha viruses cluster tightly together in the centre of the map, whereas the Beta (L242H, R246I), Gamma, and Delta variants all lie within 2 antigenic units (1 unit = 2-fold change in neutralization titer) of the D614G strain, suggesting a high degree of antigenic similarity. For influenza viruses, variants are considered to be antigenically similar in the case of antigenic distances below 3 antigenic units, i.e., an 8-fold change in neutralization titer, and different when above this threshold (Barr et al., 2014; Center for Disease Control and Prevention, 2021). By analogy, the D614G strain and Alpha, Beta (L242H, R246I), Gamma, and Delta variants belong to one antigenic cluster. Interestingly, the Beta (Δ242–244) subvariant is antigenically more distinct from the D614G strain, compared with Beta (L242H, R246I) (e.g., 3–4 units), implying that the deletion at region 242–244 has a substantial effect on antigenicity and illustrates the importance of the NTD as target of neutralizing antibodies and/or in modulating antigenicity of other domains by allosteric means. The distance between the main antigenic clusters and Omicron BA.1 and BA.2 variants is more than 4 antigenic units (>16-fold change in neutralization), implying that Omicron BA.1 and BA.2 are the antigenically most distinct SARS-CoV-2 variants (Figure 3A). One caveat is that it is unclear whether 2-fold changes in pseudovirus neutralization titers are directly comparable to 2-fold changes in hemagglutination inhibition assay titers used to define different antigenic clusters of influenza viruses. However, the change in neutralization between Omicron BA.1 and BA.2 and other variants of SARS-CoV-2, including the D614G strain, is striking.

We next used neutralization data from sera of 109 COVID-19 naive vaccinees receiving either two Moderna (mRNA-1273, n = 30), Pfizer/BioNTech (BNT162b2, n = 49), or AstraZeneca (AZD1222, n = 30) vaccines, which are all based on the ancestral S sequence to generate a second antigenic map (van Gils et al., 2022). This map (Figure 3B) agreed well with the convalescent sera map (Figure 3A) and corroborated that Omicron BA.1 represents a distinct antigenic variant from viruses currently included in vaccines. Interestingly, even though the distributions of sera from recipients of different vaccines overlap, there is a skew of sera of mRNA-1273 vaccinees toward Omicron BA.1, suggesting small differences in antigen stimulation among vaccine formulations considered here.

Taken together, these data indicate that whereas early variants belong to one antigenic cluster, the new Omicron BA.1 and BA.2 lineages are antigenically distinct from the D614G strain.

**DISCUSSION**

We have started to define the antigenic SARS-CoV-2 landscape after 2 years of antigenic drift, which should inform risk assessment of re-infections as well as strain selection for COVID-19 vaccine updates. We can draw several conclusions. First, homologous neutralization was usually stronger than heterologous neutralization. Second, heterologous responses were broadest and most potent in individuals infected with Alpha and D614G strains, whereas infection with Delta resulted in narrow-specificity responses. In addition, the individuals infected with the Beta and Omicron BA.1 variants, and to a lesser extent Omicron BA.2-infected individuals, developed weak neutralizing responses against any VOC, including the homologous strains,
suggested that the S proteins of the Beta and both Omicron variants could be less immunogenic, compared with the S of other VOCs. The weak homologous neutralization and cross-neutralization of the Omicron BA.1 variant-infected individuals are in line with other studies (Mykytyn et al., 2022). Third, the D614G and Alpha strains are at the center of our antigenic map, which supports the use of the current COVID-19 vaccines based on the ancestral strain, in the case of the circulation of the Alpha, Beta (L242H, R246I), Gamma and Delta variants. Our data suggest that updated vaccines based on the Beta (L242H, R246I) or Delta variants would not have been appreciably more effective than the ancestral virus-based vaccines. However, the substantial reduction of neutralization in all groups against the Beta variants, but especially against the Omicron variants, indicates a high risk of re-infections and vaccine breakthrough cases when exposed to these VOCs (Eggink et al., 2022). The long antigenic distance between Omicron variants and the preceding variants in the antigenic map indicates that the current high rates of Omicron infections are at least partially associated with immune escape and that a vaccine update is required. While finishing this study, several other efforts to antigenically characterize VOCs became available (Mykytyn et al., 2022; Wilks et al., 2022). Our antigenic cartography is largely in accordance with these other studies.

As in the case of seasonal influenza viruses, the prospect of SARS-CoV-2 becoming an endemic virus with recurring outbreaks implies the need for surveillance of antigenic drift and possibly yearly administration of updated vaccines, especially for individuals at risk of severe COVID-19. Antigenic cartography efforts, such as those presented here, can inform future vaccine updates.

Limitations of the study
The weak homologous neutralization and cross-neutralization levels in individuals infected with the Beta variant are in contrast with the higher titers found by others (Cele et al., 2022; Liu et al., 2021; Rössler et al., 2022; Wilks et al., 2022). It is unlikely that this conflicting weak homologous neutralization is caused by a sequence mismatch between the strains causing the infection and the sequence used in our pseudoviruses, as both Beta subvariants used in this study, Without the Beta (Δ242–244) subvariant. See Figure S1 for an antigenic map based on convalescent SARS-CoV-2 sera, including only one of the Beta subvariants (Beta L242H, R246I). 

Another limitation of this study includes the relatively small number of participants per group. For some VOCs, including the Beta and Gamma variants, the numbers of samples are limited due to the short period these variants circulated in the pandemic.
Netherlands. For the groups with Omicron BA.1- and BA.2-infected individuals, the justification of the low numbers is more divergent. First, as the pandemic advances, many Omicron-infected individuals have experienced a previous SARS-CoV-2 infection. Second, the high vaccination rate in the Netherlands further limited the available eligible participants. In addition, non-vaccinated individuals are often hesitant to participate in scientific studies that might inform vaccine research. Although the low numbers are a limitation, the main conclusions are nevertheless clear and consistent with those of other studies (Cele et al., 2022; Liu et al., 2021; Rössler et al., 2022; Wilks et al., 2022), showing that Omicron BA.1 and BA.2 are antigenically most distinct from D614G, associated with immune escape, and possibly requiring vaccine updates to ensure vaccine effectiveness.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Study population
  - Pseudovirus design
- **METHOD DETAILS**
  - SARS-CoV-2 pseudovirus neutralization assay
  - Antigenic cartography
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.immuni.2022.07.018.

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**AUTHOR CONTRIBUTIONS**

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**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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### STAR METHODS

#### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Media and buffers** |        |            |
| DMEM media | Gibco | Cat#: 11966025 |
| Opti-MEM I reduced serum media | Gibco | Cat#: 15392402 |
| Phosphate-buffered saline (PBS) | Gibco | Cat#: 15326239 |
| **Bacterial and virus strains** |        |            |
| Chemically competent DH5α *Escherichia coli* | Thermo Fisher Scientific | Cat#: 12879416 |
| **Biological samples** |        |            |
| Human sera, convalescent SARS-Cov-2 | This study | N/A |
| Human sera, post-COVID-19 vaccination | (van Gils et al., 2022) | N/A |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| FastDigest SacI | Thermo Fisher Scientific | Cat#: 10324720 |
| FastDigest Apal | Thermo Fisher Scientific | Cat#: 10450280 |
| Polyethyleneimine hydrochloride (PEI) MAX | PolySciences | Cat#: 24765-1 |
| Trypsin-EDTA | Gibco | Cat#: 25-200-056 |
| Glutamax | Gibco | Cat#: 35050061 |
| Glycyglycine, 99+% | Acros Organics/Thermo Scientific | Cat#: 120141000 |
| Magnesium sulfate heptahydrate, 99.5%, for analysis, Thermo ScientificTM | Acros Organics/Thermo Scientific | Cat#: AC21311500 |
| EGTA (ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid) ≥ 97%, Ultra Pure Grade | VWR | Cat#: 0732-100G |
| TritonTM X-100 (Electrophoresis), Fisher BioReagentsTM | Fisher BioReagents | Cat#: BP151-500 |
| Poly-L-Lysine Hydrobromide | Sigma-Aldrich | Cat#: P1399 |
| **Critical commercial assays** |        |            |
| Gibson Assembly | New England BioLabs | N/A |
| QuikChange Site-Directed Mutagenesis Kit | Agilent Technologies | Cat#: 200523 |
| **Experimental models: Cell lines** |        |            |
| HEK293T cells | American Type Culture Collection | CRL-11268 |
| HEK-293T-hACE2 | (Schmidt et al., 2020) | RRID:CVCL_A7UK |
| **Oligonucleotides** |        |            |
| SARS-CoV-2 D614G spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Alpha spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Beta spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Gamma spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Delta spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Omicron BA.1 spike gene fragment | Integrated DNA Technologies | GenBank:MT449663.1 |
| SARS-CoV-2 Omicron BA.2 spike gene fragments | Integrated DNA Technologies | GenBank:MT449663.1 |
| **Recombinant DNA** |        |            |
| pCR3 SARS-CoV-2–SΔ19 expression plasmid | GenBank | ID: MT449663.1 |
| pHIV-1NL4-3 ΔEnv-NanoLuc reporter virus plasmid | (Schmidt et al., 2020) | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rogier W. Sanders (r.w.sanders@amsterdamumc.nl).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- Patient characteristics and neutralization data have been deposited as supplemental information associated with this manuscript (Tables S1 and S2, respectively) and are publicly available as of the date of publication. Other references to data used in the study are listed in the key resources table. Additional supplemental information are available from Mendeley Data at https://doi.org/10.17632/58mjpm9mvb.1.
- This paper does not report original code.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study population
66 adults (aged 18 to 76) with a PCR proven primary SARS-CoV-2 infection were included in the COSCA-study (NL 73281.018.20) or the RECOVERED study (NL73759.018.20) between June 2020 and April 2022 at Amsterdam UMC and via the Dutch national SARS-CoV-2 sequence surveillance program as described previously (Grobben et al., 2021; Wynberg et al., 2021). In short, 3-11 weeks after symptom onset, blood, patient demographics, time between symptom onset and sampling, and admission status were collected (Tables 1 and S1). The diagnostic oropharyngeal swab was available for 39 participants and were used to determine the SARS-CoV-2 strain causing the infection. The remaining 27 SARS-CoV-2 infected participants fell within the following inclusion criteria: (1) ≥95% of circulating strains at time of symptom onset belonged the suspected VOC of infection or (2) ≥75% of circulating strains at the time of symptom onset belonged the suspected VOC of infection AND a household member had a concurrent sequence confirmed infection with that particular VOC. Prevalence data of CoVariants.org and the National Institute for Public Health and the Environment were used to determine the current prevalence of a VOC (CoVariants, 2022; Rijksinstituut voor Volksgezondheid en Milieu [RIVM]). Most individuals of which no sequence confirmation of the infected strain was available, were presumed to be infected with de D614G variant (n=20) as they were sampled before the emergence of any VOC in the Netherlands and after D614G became predominant in the Netherlands (Korber et al., 2020). More details about the remaining n=7 individuals can be found in the Table S1. The two Omicron individuals that may have been infected by either BA.1 or BA.2 are indicated as diamonds in all graphs. Two of the individuals infected with an Alpha strain harbouring the E484K mutation were excluded from analysis because they did not have enough non-threshold titres to be included in the map.

Neutralization data on COVID-19 naive vaccinee sera were kindly provided by the S3-study of the Amsterdam UMC, The Netherlands(NL73478.029.20) (van Gils et al., 2022). In short, post-vaccination sera was obtained approximately four weeks after the second doses of either Moderna (mRNA-1273), Pfizer/BioNTech (BNT162b2), or AstraZeneca (AZD1222). Post-vaccination serum after Janssen (Ad26.COV2.S) were excluded from analysis because they did not have enough non-threshold titres to be included in the map.

All above mentioned studies were conducted at the Amsterdam University Medical Centres, the Netherlands, and approved by the local ethical committees. All individuals provided written informed consent before participating.
Pseudovirus design

The D614G strain and the Alpha pseudovirus constructs contained the following mutations: D614G in D614G strain; deletion (Δ) of H69, V70 and Y144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H in Alpha. The two Beta subvariants differ from each other in the NTD region 242-246, where one Beta subvariant [L242H, R246I] is based on a very early available sequence while the other (Δ242-244) is retrospectively more representative for the predominant circulating strains. These two Beta pseudovirus constructs contain therefore the following mutations: L16F, D80A, D215G, L242H, R246I, K417N, E484K, N501Y, D614G, and A701V in Beta (L242H, R246I); L16F, D80A, D215G, Δ242-244, K417N, E484K, N501Y, D614G, and A701V in Beta (Δ242-244). Only the D614G infected individuals showed statistically significant reduced neutralization against the Beta (Δ242-244) subvariant compared to the Beta (L242H, R246I) subvariant (Figure S2, Table S3C for exact P-values). The Gamma pseudovirus constructs contained the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, and T1027I in Gamma; This Gamma pseudovirus construct differs from the predominant strain in that it lacks a V1176F back bone mutation. However, it is not likely that this mutation, positioned at the S2 domain of the S, will affect escape of neutralization substantially. The Delta and Omicron BA.1 and BA.2 pseudovirus constructs contained the following mutations: T19R, G142D, E156G, Δ157-158, L124S, G142D, P681H, N679K, and D950N in Delta; A67V, Δ69-70, T95I, G142D, A143-145, A211L, R246I, Δ214EPE, G339D, G356S, S371L, S373F, and K417N, L440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505F, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F in Omicron BA.1; and T19I, L24S, Δ125/127, G142D, V213G, G339D, S371F, S373F, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K in Omicron BA.2. The Omicron BA.1 strain used here harbors a Q493K mutation, while the predominant Omicron BA.1 harbors a Q493R mutation. This mutation did not impact neutralization of several monoclonal SARS-CoV-2 antibodies tested (data not shown). The spike constructs were ordered as globule block fragments (Integrated DNA Technologies) and cloned SacI and Apal in the pCR3 SARS-CoV-2–S_gRNA expression plasmid (GenBank: MT449663.1) using Gibson Assembly (Thermo Fisher Scientific). The pseudovirus constructs were made using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies) and verified by using Sanger sequencing. Pseudoviruses were procedures by cotransfecting HEK293T cells (American Type Culture Collection, CRL-11268) with the pCR3 SARS-CoV-2–S_gRNA expression plasmid and the pHIV-1NL43 ΔEnv-NanoLuc reporter virus plasmid. Transfection takes place in cell culture medium (DMEM), supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml). Medium is refreshed once 6-8 hours after transfection. 48 hours after the transfection, cell supernatants containing the pseudovirus were harvested and stored at -80 °C until further use.

METHOD DETAILS

SARS-CoV-2 pseudovirus neutralization assay

The pseudovirus neutralization assay was performed as described previously (Caniels et al., 2021). Shortly, HEK293T/ACE2 cells were kindly provided by P. Bieniasz (Schmidt et al., 2020) were seeded at a density of 20,000 cells per well in a 96-well plate coated with poly-lysine (50 μg/ml) 1 day before the start of the neutralization assay. The next day, heat-inactivated sera samples were in triplicate serial diluted in threefold steps, starting at 1:20 dilution to test for Omicron BA.1 and BA.2 pseudovirus neutralization and 1:100 for all the other variants. Sera was diluted in cell culture medium (DMEM), supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and GlutaMAX (Gibco), mixed in a 1:1 ratio with pseudovirus, and incubated for 1 hour at 37°C. These mixtures were then added to the cells in a 1:1 ratio and incubated for 48 hours at 37°C, and lysis buffer was added. The luciferase activity in cell lysates was measured using the Nano-Glo Luciferase Assay System (Promega) and GloMax system (Tuner BioSystems). Relative luminescence units were normalized to those from cells infected with SARS-CoV-2 pseudovirus in the absence of sera. The inhibitory neutralization titres (ID50) were determined as the serum dilution at which infectivity was inhibited by 50%, using a nonlinear regression curve fit (GraphPad Prism software version 8.3). The International Standard for anti-SARS-CoV-2 immunoglobulins provided by the WHO (Kristiansen et al., 2021) were used to convert the ID50 values into International Units per milliliters (IU/mL). Samples with IU/mL titres <10 were defined as having undetectable neutralization against the D614G, Alpha, Beta, Gamma and Delta variant. For Omicron BA.1 and BA.2 neutralization, the start-dilution of 1:20 enables a cut-off of <2 IU/mL for all samples except for some Alpha infected individuals. A limited amount of sera was available from the Alpha infected individuals, resulting in a start dilution of 1:100 of n=7 samples against all variants including Omicron BA.1 and BA.2. Neutralization data points of two Alpha infected individuals against BA.1 and BA.2 were excluded from Figure 1A because a neutralization titres <10IU/mL (Table S2). This exclusion did not impact the statistics as written below because a general cut-off of <10IU/mL were used for neutralization against any variant, including Omicron BA.1 and BA.2.

Antigenic cartography

Antigenic maps were constructed as previously described (Finnville et al., 2014; Smith et al., 2004) using the antigenic cartography software from https://acmacs-web.antigenic-cartography.org. In brief, this approach to antigenic mapping uses multidimensional scaling to position antigens (viruses) and sera in a map to represent their antigenic relationships. The maps here relied on the SARS-CoV-2 post-infection serology data and post-vaccination serology data shown in Figure 1A and Table S2. The positions of antigens and sera were optimized in the map to minimise the error between the observed pairwise virus-serum combinations in the pseudovirus assay described above and the resulting computationally derived map. Maps were constructed in 2, 3, 4, and 5 dimensions to investigate the dimensionality of the antigenic relationships. Both the convalescent (Figure 3A)
and post-vaccination datasets (Figure 3B) were strongly two-dimensional with only small improvements in residual mean squared error of the maps as map dimensionality increased.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Data visualization and statistical analyses were performed in GraphPad Prism software (version 8.3). Spider plots (Figure 1B) were made in Excel 2016. The antigenic maps were produced using the antigenic cartography software mentioned above. Wilcoxon signed rank test with Benjamini Hochberg correction was used to compare cross-neutralization titres with the homologous neutralization (Figure 1A). Mann-Whitney test was used for non-paired group comparisons (Figure 2). All statistics mentioned here were performed by using a general neutralization cut-off of 10IU/mL against any variant of SARS-CoV-2.
Supplemental information

Antigenic cartography using sera from sequence-confirmed SARS-CoV-2 variants of concern infections reveals antigenic divergence of Omicron

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This PDF file includes:
Supplementary Figure 1 (Figure S1)
Supplementary Figure 2 (Figure S2)
Supplementary Table 2 (Table S2)
Supplementary Table 3 (Table S3)

Included as a separate excel file:
Supplementary Table 1 and corresponding legend (Table S1)
Supplementary Figure 1. Antigenic cartography reveals antigenic diversification of SARS-CoV-2, related to Figure 3A

Antigenic map of SARS-CoV-2 VOCs based on convalescent SARS-CoV-2 infection sera. SARS-CoV-2 variants are shown as circles and sera are indicated as squares. Each square corresponds to sera of one individual and is coloured by the infecting SARS-CoV-2 variant. Both axes of the map are antigenic distance and each grid square (1 antigenic unit) represents a two-fold change in neutralization titre. The distance between points in the map can be interpreted as a measure of antigenic similarity, where the points more closely together show higher cross-neutralization and are therefore antigenically more similar. Compared to Figure 3A, this panel does not contain the Beta (Δ242-244) subvariant.
Supplementary Figure 2. Neutralization of convalescent VOC sera against two Beta subvariant pseudoviruses, related to Figure 1A

A. The left panel shows the correlation between the midpoint neutralization titres against both Beta subvariants used in this research, expressed in International Units per mL (IU/mL). In the right panel we studied the difference in neutralization titre against both Beta subvariants using a Wilcoxon signed rank test. Median neutralization titres are depicted as red bars. *** = p<0.001. B. Midpoint neutralization titres against both Beta subvariants in international Units per mL (IU/mL). The individuals are grouped per VOC they were infected with and plotted accordingly. Non-hospitalized patients are indicated with dots and hospitalized patients with triangles. The individuals that were infected with an Alpha variant that also included the E484K mutation are indicated in green squares. Median neutralization titres were compared using a Wilcoxon signed rank test. ns= non-significant, *** = p< 0.001.
Supplementary Table 2. Neutralization titres of convalescent SARS-CoV-2 sera against several VOCs, related to Figure 1-3.

Neutralization titres (ID50) were the serum dilution at which infectivity was inhibited 50% are converted to International Units per millilitre (IU/mL). Neutralization titers of post-vaccination sera can be found in Van Gils et al, Plos Medicine, 2022.

| Infected strain | Participant ID | D614G | Alpha | Beta (LZ42H, R246I) | Beta (Δ242-244) | Gamma | Delta | Omicron BA.1 | Omicron BA.2 |
|-----------------|----------------|-------|-------|-------------------|-----------------|-------|-------|--------------|-------------|
| D614G           | COSCA-020      | 233   | 37    | 109               | 22              | 72    | 54    | <2           | 81          |
| D614G           | COSCA-021      | 231   | 58    | 39                | <10             | 35    | 29    | <2           | 6           |
| D614G           | COSCA-022      | 28    | 11    | <10               | <10             | <10   | 23    | <2           | <2          |
| D614G           | COSCA-023      | 14    | 12    | 19                | <10             | 15    | 28    | <2           | 3           |
| D614G           | COSCA-024      | 90    | 106   | 31                | 13              | 61    | 61    | <2           | 7           |
| D614G           | COSCA-025      | 69    | 47    | 49                | 10              | 77    | 122   | <2           | 9           |
| D614G           | COSCA-026      | 101   | 83    | 83                | <10             | 38    | 97    | <2           | 4           |
| D614G           | COSCA-028      | 189   | 119   | 26                | 14              | 81    | 217   | 21           | 55          |
| D614G           | COSCA-033      | 48    | 29    | 16                | <10             | 28    | 36    | <2           | 4           |
| D614G           | COSCA-034      | 34    | 27    | <10               | <10             | <10   | <10   | <2           | 4           |
| D614G           | COSCA-112      | 929   | 1133  | 221               | 74              | 386   | 890   | 7            | 67          |
| D614G           | COSCA-113      | 122   | 97    | 31                | <10             | 89    | 134   | 3            | 24          |
| D614G           | COSCA-114      | 237   | 249   | 127               | 11              | 131   | 68    | 3            | 15          |
| D614G           | COSCA-115      | 601   | 1095  | 93                | 24              | 386   | 384   | 112          | 13          |
| D614G           | COSCA-116      | 510   | 1005  | 188               | 97              | 482   | 230   | 9            | 157         |
| D614G           | COSCA-117      | 223   | 206   | 27                | <10             | 92    | 48    | 5            | 7           |
| D614G           | COSCA-118      | 432   | 438   | 95                | 144             | 221   | 814   | 41           | 13          |
| D614G           | COSCA-119      | 430   | 220   | 30                | 20              | 186   | 326   | 11           | 19          |
| D614G           | COSCA-120      | 335   | 234   | 295               | 11              | 372   | 134   | 64           | 59          |
| D614G           | COSCA-123      | 852   | 463   | 54                | <10             | 393   | 586   | 8            | 18          |
| Alpha           | AMCVIS15       | 607   | 1366  | 194               | 71              | 436   | 344   | 23           | 13          |
| Alpha           | AMCVIS18       | 215   | 683   | 342               | 103             | 414   | 557   | 20           | <10         |
| Alpha           | AMCVIS20       | 291   | 891   | 158               | 182             | 171   | 482   | <10          | 11          |
|     |                  |     |     |     |     |     |     |     |
|-----|------------------|-----|-----|-----|-----|-----|-----|-----|
| Alpha| AMCVIS34 84      | 124 | 872 | 33  | 60  | 93  | 232 | 13  | <10 |
| Alpha| AMCVIS57 22      | 164 | 394 | 72  | 35  | 45  | 137 | <10 | <10 |
| Alpha| AMCVIS95 84      | 168 | 733 | 59  | 32  | 103 | 55  | 54  | 23  |
| Alpha| COSCA-303         | 229 | 2103| 341 | 56  | 122 | 108 | 40  | 16  |
| Alpha| GGDVIS33 43       | 1083| 1658| 458 | 89  | 1138| 159 | 148 | 92  |
| Alpha| VUVIS6992         | 263 | 788 | 81  | 138 | 149 | 252 | 4   | <10 |
| Alpha + E484K | COSCA-316       | 558 | 2091| 312 | 382 | 730 | 181 | 24  | 36  |
| Alpha + E484K | COSCA-320       | 59  | 213 | 38  | 32  | 82  | 16  | 9   | 6   |
| Beta | COSCA-331        | <10 | <10 | <10 | <10 | <10 | <10 | <2  | <2  |
| Beta | COSCA-336        | <10 | 16  | 15  | 17  | 34  | <10 | 5   | 3   |
| Beta | COSCA-337        | <10 | <10 | <10 | <10 | <10 | <10 | 2   | 5   |
| Gamma| COSCA-309        | 57  | 51  | 57  | 144 | 87  | <10 | 4   | 10  |
| Gamma| COSCA-310        | <10 | 28  | 93  | 63  | 224 | <10 | <2  | <2  |
| Gamma| COSCA-303        | <10 | <10 | 16  | <10 | <10 | <10 | <10 | <2  |
| Gamma| COSCA-334        | 537 | 326 | 726 | 357 | 761 | 403 | 798 | 725 |
| Delta| COSCA-321        | <10 | 14  | <10 | <10 | <10 | 217 | 10  | 8   |
| Delta| COSCA-322        | 11  | 10  | <10 | <10 | <10 | 159 | 3   | 2   |
| Delta| COSCA-323        | 50  | 54  | 12  | <10 | 16  | 110 | 5   | 12  |
| Delta| COSCA-325        | 105 | 37  | <10 | <10 | <10 | 439 | 5   | 9   |
| Delta| COSCA-327        | <10 | <10 | <10 | <10 | <10 | <10 | <2  | <2  |
| Delta| COSCA-328        | 72  | 38  | 32  | <10 | 21  | 150 | 70  | 30  |
| Delta| COSCA-329        | <10 | <10 | <10 | <10 | <10 | 19  | <2  | <2  |
| Delta| COSCA-330        | <10 | <10 | 17  | 22  | 12  | <10 | <2  | <2  |
| Delta| COSCA-332        | 10  | <10 | <10 | <10 | 10  | 59  | <2  | 4   |
| Delta| COSCA-333        | 114 | 78  | 57  | 61  | 121 | 1635| 32  | 27  |
| Delta| COSCA-335        | <10 | <10 | 12  | <10 | <10 | 27  | 8   | 7   |
| Beta | COSCA-301        | 17  | 12  | <10 | <10 | <10 | 11  | <2  | <2  |
| Beta | COSCA-305        | <10 | <10 | 24  | <10 | 67  | <10 | 8   | 36  |
|                   | COSCA-306 | <10 | <10 | 11  | <10 | 17  | <10 | <2 | <2 |
|-------------------|-----------|-----|-----|-----|-----|-----|-----|----|----|
| Beta              | COSCA-307 | <10 | <10 | 13  | 11  | 32  | <10 | 2  | 2  |
| Beta              | COSCA-308 | <10 | <10 | <10 | <10 | <10 | <10 | 2  | 2  |
| Omicron BA.1      | COSCA-348 | <10 | <10 | <10 | <10 | <10 | <10 | 5  | <2 |
| Omicron BA.1      | COSCA-349 | <10 | <10 | <10 | <10 | <10 | <10 | 24 | 15 |
| Omicron BA.1      | COSCA-350 | <10 | <10 | <10 | <10 | <10 | <10 | 61 | 21 |
| Omicron BA.1      | COSCA-351 | <10 | <10 | <10 | <10 | <10 | <10 | 22 | 2  |
| Omicron BA.1      | COSCA-352 | <10 | <10 | <10 | <10 | <10 | 12  | 23 | 5  |
| Omicron BA.1      | COSCA-353 | <10 | <10 | <10 | <10 | <10 | 11  | 90 | 22 |
| Omicron BA.1      | COSCA-354 | <10 | <10 | <10 | <10 | <10 | 10  | 4  | 9  |
| Omicron BA.2      | COSCA-356 | <10 | 16  | <10 | <10 | <10 | <10 | 2  | 2  |
| Omicron BA.2      | COSCA-357 | <10 | <10 | <10 | <10 | <10 | <10 | 15 | 95 |
| Omicron BA.2      | COSCA-358 | 14  | <10 | <10 | <10 | <10 | <10 | 16 | 95 |
| Omicron BA.1      | COSCA-360 | <10 | <10 | <10 | <10 | <10 | <10 | 3  | 37 |
| Omicron BA.2      | COSCA-361 | <10 | <10 | <10 | <10 | <10 | <10 | <2 | 7  |
Supplementary Table 3. Exact p-values of statistical analysis of Figure 1A, Figure 2A and Figure S1.

**Supplementary Table 3A. Statistical analysis of Figure 1A.**

|                   | Infected individuals |                   |                   |                   |                   |                   |                   |
|-------------------|----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   | Ancestral            | Alpha             | Beta              | Gamma             | Delta             | Omicron BA.1      | Omicron BA.2      |
| Ancestral         |                      |                   |                   |                   |                   |                   |                   |
| Alpha             | 0.1208               | 0.0010            | 0.6000            | 0.1750            | 0.0046            | 0.0729            | 0.25              |
| Beta (L242H, R246I) | 0.0002              | 0.0010            | 0.6000            | 0.1750            | 0.0046            | 0.0729            | 0.25              |
| Beta (Δ242-244)   | 0.0002               | 0.0010            |                   | 0.3750            | 0.0059            | 0.0729            | 0.25              |
| Gamma             | 0.0002               | 0.0010            | 0.3750            |                   | 0.0046            |                   | 0.0729            |
| Delta             | 0.0183               | 0.0010            | 0.3750            | 0.1750            |                   |                   |                   |
| Omicron BA.1      | 0.0001               | 0.0010            | 0.3750            | 0.3750            | 0.0046            |                   |                   |
| Omicron BA.2      | 0.0001               | 0.0010            | 0.8750            | 0.1750            | 0.0046            |                   | 0.2188            |

ns P > 0.05
* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001

**Supplementary Table 3B. Statistical analysis of Figure 2A.**

| P-values using Mann-Whitney U test         | P-value |
|------------------------------------------|---------|
| Hospitalized patients                    |         |
| D614G vs Alpha infected                  | 0.11    | ns      |
| Non-hospitalized                         |         |
| D614G vs Beta(Δ242-244) infected         | <0.0001 | ****    |
| D614G vs Delta infected                  | 0.46    | ns      |
| Beta (Δ242-244) vs Omicron BA.1 infected | 0.088   | ns      |

**Supplementary Table 3C. Statistical analysis of Figure S1.**

| P-values using Wilcoxon rank test         |       |       |       |       |       |       |       |
|------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Beta (L242H, R246I) vs Beta (Δ242-244)   | 0.0004| 0.21  | 0.875 | 0.625 | >0.99 | >0.99 | >0.99 |