Further humoral immunity evasion of emerging SARS-CoV-2 BA.4 and BA.5 subvariants

SARS-CoV-2 BA.4 and BA.5 lineages have been the dominant strains in most regions worldwide and are continuously gaining mutations in the receptor-binding domain.[1,2] Multiple BA.4 and BA.5 subvariants with Arg346 mutations in the spike glycoprotein have been identified in various countries, such as BA.4.6, BF.7, BA.5.2.6, BA.4.1.9, and BE.1.2 harbouring Arg346Thr; BA.4.7 and BF.13 harbouring Arg346Ser; and BA.5.9 with Arg346Ile mutations (appendix p 4). These subvariants, especially BA.4.6, exhibit growth advantages compared with other variants including the original BA.4 and BA.5 strains.[3] Previous studies have identified Arg346 as an important immunogenic residue because Arg346 mutations would allow the virus to escape neutralisation by a large group of neutralising antibodies.[2] Unlike Arg346Lys carried by BA.1.1, which maintained a similar chemical property, mutations from Arg to either Thr, Ser, or Ile correspond to a much stronger shift in antibody recognition.[4,5] The efficacy of vaccines and neutralising antibody drugs against these BA.4 and BA.5 sublineages needs immediate evaluation.

In this study, we measured the neutralising titres of plasma samples against the SARS-CoV-2 BA.4 and BA.5 subvariants with Arg346 mutations. The plasma samples were obtained from vaccinated individuals that received three doses of an inactivated vaccine (CoronaVac) without SARS-CoV-2 infection or with BA.1, BA.2, or BA.5 breakthrough infection (appendix pp 7–9). Plasma from breakthrough infections were obtained 3 to 5 weeks after a positive PCR test for SARS-CoV-2. Vesicular stomatitis virus-based pseudoviruses were used in the neutralisation assays.

Plasma samples from individuals who received three doses of CoronaVac without infection showed a 1·5–1·7-fold decrease in 50% neutralisation titres (NT_{50}) against BA.4 or BA.5 sublineages with Arg346Ile (BA.5.9), Arg346Thr (BA.4.6), and Arg346Ser (BA.4.7), compared with the NT_{50} against BA.4 or BA.5 (figure A). A similar reduction in neutralisation titres was also observed in plasma from BA.1 or BA.2 breakthrough infection convalescents (figures B and C). Importantly, BA.4 or BA.5 sublineages with Arg346Ile, Arg346Thr, or Arg346Ser mutations could significantly evade neutralisation by plasma samples from BA.5 breakthrough infection, exhibiting a 2·4–2·6-fold decrease in NT_{50} (figure D). In contrast, the antibody-escaping capability of BA.1.1 that harbours a Arg346Lys mutation is similar to BA.1, as expected (appendix p 5). These results indicate the strong humoral immunity evasion capability of BA.4 and BA.5 sublineages with
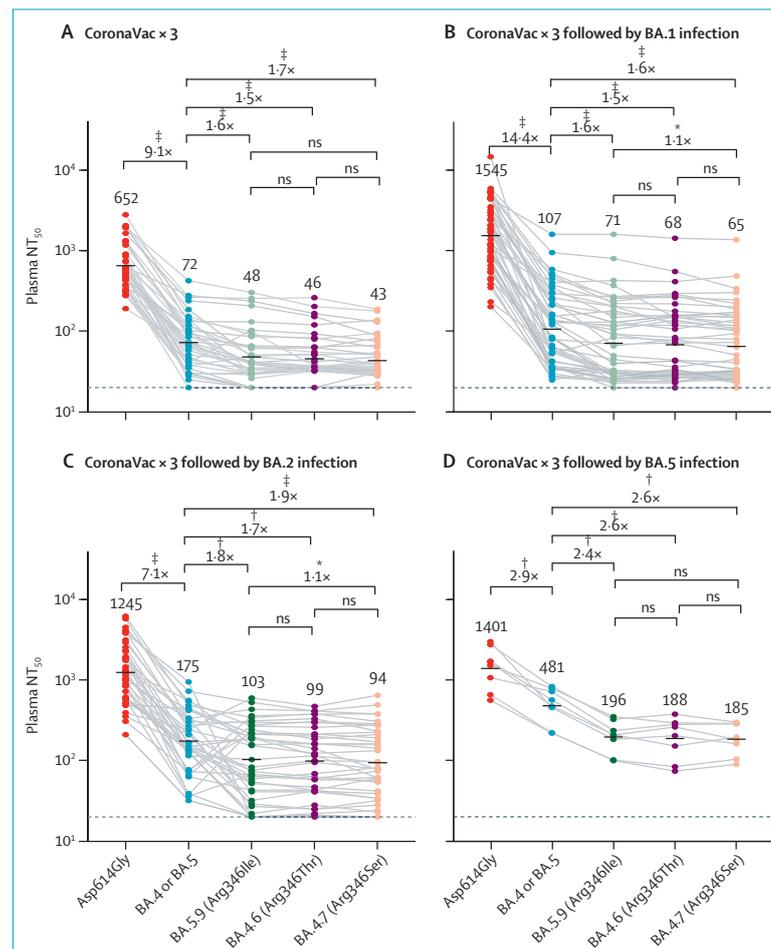
See Online for appendix

Figure: **Efficacy of convalescent plasma against BA.4 and BA.5 subvariants with mutations on spike Arg346**

NT_{50} against SARS-CoV-2 Asp614Gly, BA.4 or BA.5, BA.5.9 (BA.4 or BA.5 + Arg346Ile), BA.4.6 (BA.4 or BA.5 + Arg346Thr), BA.4.7 (BA.4 or BA.5 + Arg346Ser) pseudovirus by plasma samples from individuals who received three doses of CoronaVac (N=40; A), and those who received three doses CoronaVac followed by BA.1 breakthrough infection (N=50; B), BA.2 breakthrough infection (N=39; C), or BA.5 breakthrough infection (N=8; D). Geometric mean titres are annotated above each group. NT_{50}=50% neutralisation titres. ns=not significant. *p<0·05; †p<0·01; ‡p<0·001. P-values are calculated by two-tailed Wilcoxon singed-rank test of paired samples.

Arg346 mutations, suggesting that these sublineages, including BA.4.6, BA.4.7, BA.5.9, BF.7, BA.5.2.6, BA.4.1.9, BE.1.2, and BF.13 might gain an advantage in transmissibility under the global background of the pandemic caused by BA.4 and BA.5 sublineages. Of note, BA.5 convalescent plasma shows higher neutralisation titres against BA.5 than BA.1 and BA.1.1, but due to immune imprinting, or so-called original antigenic sin, convalescent plasma from omicron (including BA.1, BA.2, and BA.5) breakthrough infection is more effective against the ancestral strain with Asp614Gly compared with the respective infected strain.[2,6]

We then evaluated the pseudovirus-neutralising activities of the approved neutralising antibody drugs, including 11 monoclonal antibodies and four cocktails, against the Arg346-mutated BA.4 and BA.5 sublineages (appendix p 6). Cilgavimab did not affect BA.4 and BA.5 sublineages with Arg346Ile, Arg346Thr, or Arg346Ser mutations, resulting in the complete loss of efficacy of Evusheld (tixagevimab with cilgavimab)[7] against BA.4.6, BA.4.7, BA.5.2.6, and BA.5.9 sublineages. The neutralising activity of REGEN-COV (casirivimab with imdevimab)[8] was also reduced due to decreased reactivity of imdevimab against Arg346-mutated sublineages. Furthermore, the potency of sotrovimab[9] was further reduced. Of note, bebtelovimab[10] remained highly potent and was the only neutralising antibody drug approved by the US Food and Drug Administration.

Together, our findings suggest that significant humoral immune evasion, especially against convalescents from BA.4 and BA.5 breakthrough infection, contributes to the emergence and rapid spread of multiple Arg346-mutated BA.4 and BA.5 sublineages. The decreased neutralisation titres of plasma samples from BA.5 breakthrough-infection convalescents indicate worrisome potential reinfection of BA.4.6 after the recovery from BA.4 or BA.5 infection. Importantly, individuals that received Evusheld as long-term prophylaxis, especially those that are immunodeficient or exhibit high-risk comorbidities, are at particular risk of those subvariants. Also, BA.4 and BA.5-based vaccine boosting strategies should be evaluated in light of the prevalence of these BA.4 and BA.5 subvariants.

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