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Supplementary appendix

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SUPPLEMENTAL FILE

Stability of hybrid vs. vaccine immunity against BA.5 infection over 8 months

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Methods

Participant selection

We followed an approach similar to what we reported in a previous registry-based study[1]. The population included in the study comprises: (1) All individuals resident in Portugal aged 12 years and older without a documented infection until the start of the follow-up period, which is June 1st to September 14th, 2022; and (2) All individuals resident in Portugal aged 12 years and older with a single documented infection between January 1st, 2022 (the initial period of dominance of BA.1) up to 90 days before the follow-up period and no other previous infection (see flowchart, Figure S1).

We used the national COVID-19 registry (*SINAVE*) to obtain information on all notified cases of infection, irrespective of clinical presentation. The "uninfected" population was defined as the population over 12 years of age without a documented infection in the registry at any time. The number of uninfected people on June 1st 2022 (the start of the BA.5 dominance period) was 5,325,097, representing 57% of the Portuguese population over 12 (data from the National Census 2021[2]).

The data available in *SINAVE* include cases of positive tests (PCR tests and rapid antigen tests) performed by healthcare workers in accredited diagnostic facilities. Testing by an accredited facility was a requisite for access to social security compensation for days of isolation – this is a reason for the comprehensiveness of the registry and the inclusion of only validated tests. Only tests performing above the EU-defined minimum for test sensitivity and specificity are used in Portugal. The testing policy officially changed on October 1st, 2022 but with some relaxation of the implementation in the period following their announcement just before the official date. Therefore, we considered infections until September 14th, 2022 as the period with consistent implementation of comprehensive testing policies.

We used the national SARS-CoV-2 genetic surveillance database[3] to identify periods when one variant represented >90% of the sample isolates, as also defined and used in other studies[1,4]. We assigned infected individuals to the variants' dominance periods and excluded all individuals who had more than one infection before the study period (Figure S1). We pooled BA.1 and BA.2 infections, given the slow transition between the period of dominance of these two subvariants. With this approach, we identified the periods of dominance of BA.1/BA.2 (January 1st to April 17th, 2022) and of BA.5 infections (June 1st to September 14th, 2022). We then divided those periods of dominance into approximately 15 days intervals (as seen in Figure 1A of the main

text). Coincidently, the period of BA.1/BA.2 dominance was divided into 7 sub-intervals, and the period of BA.5 was also divided into 7 sub-intervals (Tables S1 and S2).

Reinfection was defined as two positive tests in the same individual, at least 90 days apart[5]. Consequently, all cases of infection in the 90 days before the start of each sub-interval were not included, as these would not classify as "at risk of reinfection" for the entire duration of the sub-interval under the definition above.

Given the high vaccine coverage, we compared one population with "hybrid immunity" (vaccination + infection with BA.1/BA.2) with a group of vaccinated individuals without infection. In other words, we assessed the stability of hybrid immunity (induced with Omicron BA.1/BA.2 infection + vaccines) versus vaccine immunity. The change in the relative risk (RR) that we report, translates the waning of such "additional" protection afforded by natural infection of the vaccinated individuals.

It is possible that the population we classified as "uninfected" contains individuals with a prior unnoticed infection (i.e., asymptomatic infection). In a previous publication, we have shown that considering a proportion (20%-40%) of unreported infections within the "uninfected" group (in line with data from the national serologic survey[6]) only has the effect of decreasing slightly the relative risk of BA.5 re-infection in each sub-interval[1], without changing our overall results. This is intuitive because if more people were infected (and moved out of the "uninfected group"), that inflates the absolute risk of first infection, and thus the relative risk of a second infection with BA.5 decreases.

In conclusion, the study design is similar to a prospective study but taking place in the past: the groups of interest were selected (i.e., individuals with no recorded infection or individuals with one infection in a defined period of time and without any additional infection reported until the start of the study period); and afterwards the individuals from the different groups were followed, under the same epidemiological conditions, for a pre-defined (and equal) number of days and their infections were recorded. We considered other study designs, such as test-negative study[4,7,8], but our registry-based dataset only includes information on positive tests, thus precluding the use of a test-negative design.

Vaccination coverage

The vaccine coverage with the primary vaccination series in the Portuguese residents over 12 years was >98% by the end of 2021[9]. The primary series of the vaccination campaign used

EU/EMA-authorized vaccines: Comirnaty (Pfizer/BioNTech), 69%; Spikevax (Moderna), 12%; Vaxzevria (AstraZeneca), 13%; and Janssen 6%.

While at the start of the BA.1/BA.2 period of dominance (January 1st, 2022), the coverage with the first booster was residual (mostly long-term care facility residents), at the start of the BA.5 period of dominance (June 1st), the coverage with the first booster was 82%. The vaccine boosters relied exclusively on mRNA vaccines (77% Comirnaty and 23% Spikevax). At the start of the BA.5 period, a second booster was not yet in use except for a highly specific (and very small) population of patients with severe immunosuppression.

Statistics

We estimated the relative risk of BA.5 reinfection in each sub-interval using the modified Poisson regression method with a robust sandwich estimator for the variance as described previously[10]. We compared the risk of BA.5 infection for people with a previous single infection at different intervals, with the same risk for people without any previously recorded infection in the same interval periods (Table S2). Protection efficacy was estimated, in percentage, as (1-RR) x 100%. Confidence intervals for the RR were calculated using the Wald normal approximation method, with the *epitools* R package[11].

To ascertain the change in relative risk over time, we considered the sub-intervals of BA.5 infection as a blocking factor and used a mixed-effect approach to estimate the change in risk over time, where "sub-interval" was the random effect[12]. We fitted the increase in risk with the following saturating function:

$$RR(t \geq 90) = rr_0 + (rr_A - rr_0)\frac{(t-90)^n}{T_M^n + (t-90)^n} \qquad \text{Eq. (1)}$$

In this equation, t represents time in days, which is larger than 90, since re-infections can only occur after that time, rr_0 is the initial relative risk when t = 90, and rr_A is the asymptotic risk when time is very large, T_M is the time, after 90 days, at which the relative risk is approximately 1/2 of the asymptotic risk. Finally, n allows for different steepness in the increase of relative risk. This is a general saturation function, and it allowed us to test simpler versions, such as fixing rr_A=1 or n=1, which did not describe the data as well. We also tested other saturation functions, such as logistic or generalized logistics, but the results were similar (i.e., the initial and the asymptotic relative risk). We used the software Monolix 2021R1 (Lixoft SAS, Antony, France) to fit this model using each sub-interval of BA.5 as the random effect (Figures 1B and S2). The best fit included a random effect for T_M and n, but only fixed effects for rr_0 and rr_A. The population parameters for the best mixed-effect fit are rr_0=0.064 (95% CI: 0.056-0.074), rr_A=0.368 (95% CI: 0.321-0.424),

T_M=65.7 (95% CI: 52.8-81.9), n=3.2 (95% CI: 2.3-4.4). The stability of the estimated parameters for initial and asymptotic relative risk (with no random effect supported by the data) over the different time intervals strengthens our conclusions, as biases due to, for example, undocumented infections are unlikely to be important for the periods studied.

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Declaration of interests: The authors have no competing interests to declare.

Data availability. Data from the Portuguese COVID-19 registry (SINAVE) is available upon reasonable request from Direcao Geral de Saude Portugal which curates the registry.

Ethics statement. The study design was approved by the Ethics Review Board of the Centro Academico de Medicina de Lisboa.

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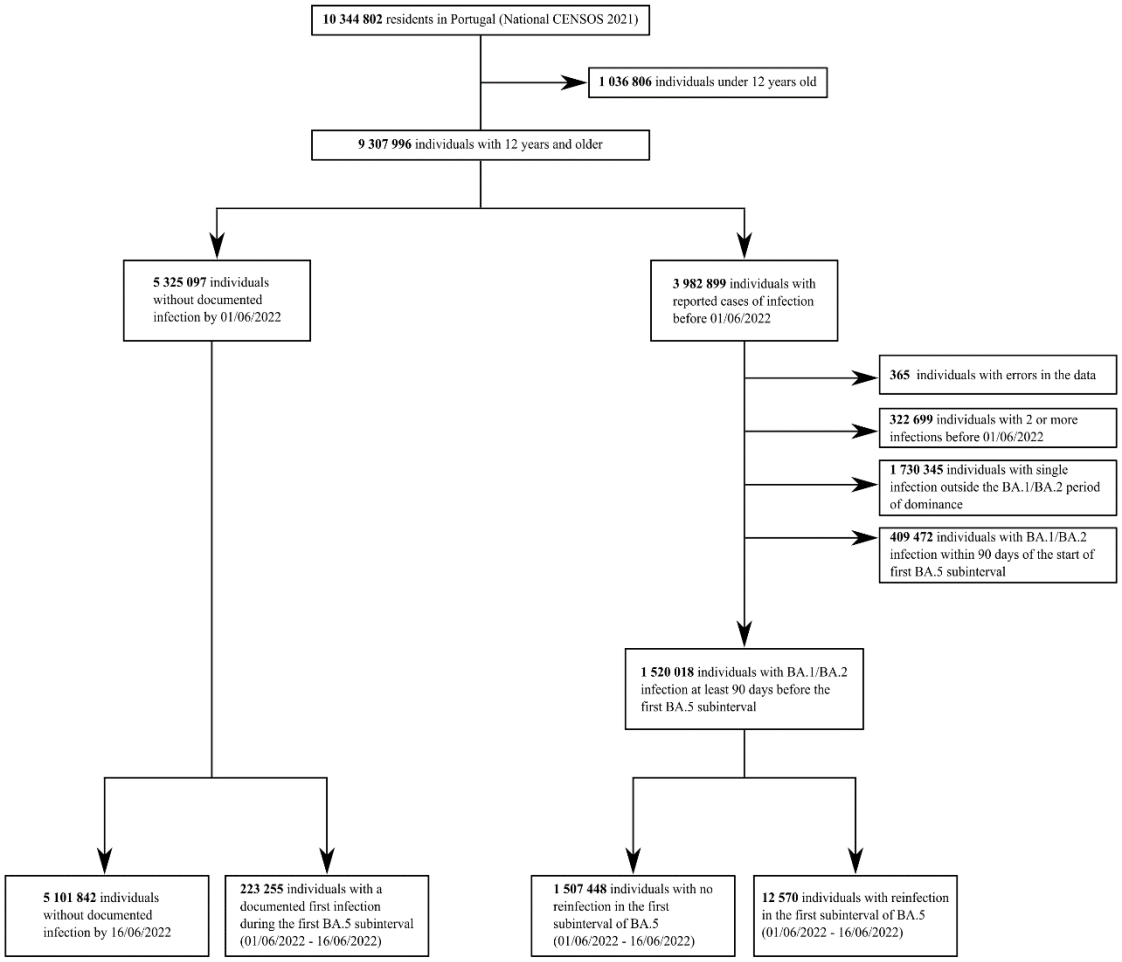

Figure S1. *Flowchart describing the population selection.* Representative flowchart representing the selection for the first subinterval of BA.5 reinfection. For later BA.5 intervals, the 90-day period prior to the start of the interval allowed inclusion of a further subperiod of BA.1/BA.2 (see Figure 1A in the main document). Note: the date format is day/month/year.

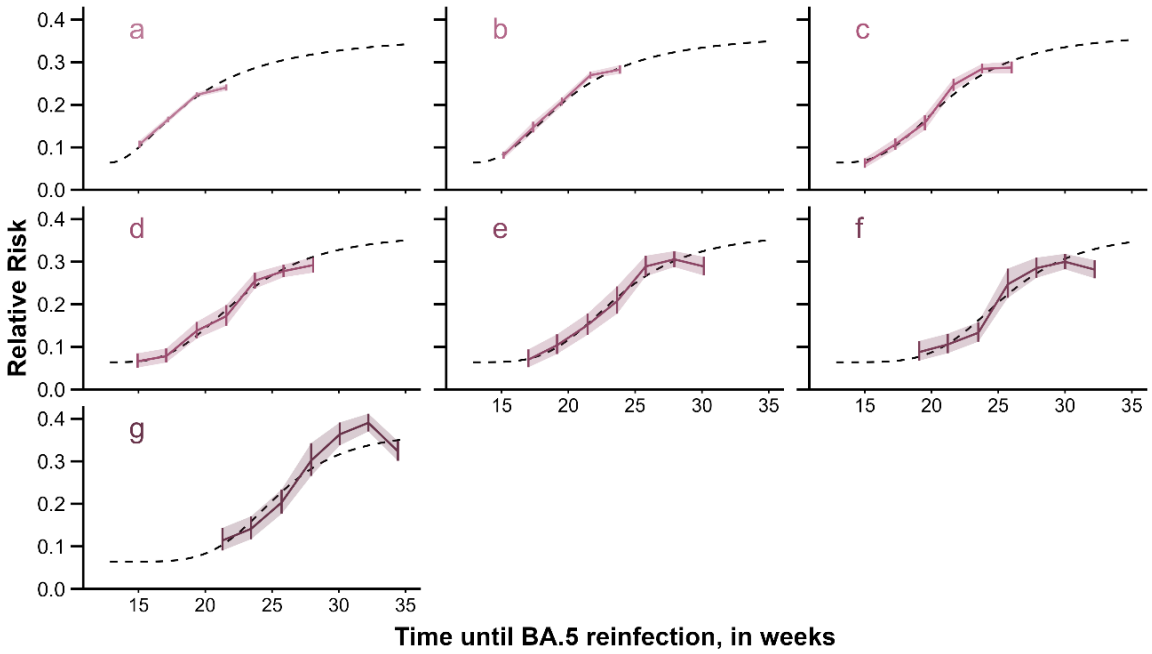

Figure S2. *Variation of RR of protection against BA.5 infection over time since BA.1/BA.2 infection.* Individual sub-interval fits of equation (1) (dashed lines) to the different periods of BA.5 dominance (intervals *a* to *g*), corresponding to the population fit presented in figure 1B of the main text. The RR calculated from the data is indicated in the solid lines with corresponding 95% confidence intervals (shaded area).

Variant	Interval	Start date	End date	Days
BA.1/BA	A	01/01/22	16/01/22	15
	B	17/01/22	31/01/22	14
	C	01/02/22	15/02/22	14
	D	16/02/22	02/03/22	14
	E	03/03/22	18/03/22	15
	F	19/03/22	03/04/22	15
	G	04/04/22	17/04/22	13
BA.5	a	01/06/22	16/06/22	15
	b	17/06/22	02/07/22	15
	c	03/07/22	16/07/22	13
	d	17/07/22	31/07/22	14
	e	01/08/22	14/08/22	13
	f	15/08/22	29/08/22	14
	g	30/08/22	14/09/22	15

Table S1. *Subintervals of BA.1/BA.2 and BA.5 dominance used in the study*. Both periods of BA.1/BA.2 and BA.5 dominance were split in seven periods with approximately 2 weeks each. The fact that the two subvariants have the same number of intervals is a coincidence.

BA.5 (a)		Date interval	Uninfected on Jun 1st 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		5325097	–	223255	0.042	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	421200	4130	0.010	0.240 (0.233, 0.248)	75.97 (75.22, 76.68)
	B	17/01/22 – 31/01/22	–	620102	5488	0.009	0.223 (0.217, 0.229)	77.69 (77.09, 78.26)
	C	01/02/22 – 15/02/22	–	341779	2328	0.007	0.165 (0.159, 0.172)	83.46 (82.77, 84.11)
	D	16/02/22 – 02/03/22	–	136937	624	0.005	0.107 (0.099, 0.116)	89.29 (88.41, 90.10)
BA.5 (b)		Date interval	Uninfected on Jun 17th 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		5101842	–	135093	0.026	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	417070	3004	0.007	0.283 (0.273, 0.293)	71.73 (70.71, 72.72)
	B	17/01/22 – 31/01/22	–	614614	4103	0.007	0.269 (0.261, 0.278)	73.07 (72.25, 73.87)
	C	01/02/22 – 15/02/22	–	339451	1803	0.005	0.207 (0.198, 0.217)	79.31 (78.34, 80.24)
	D	16/02/22 – 02/03/22	–	136313	530	0.004	0.147 (0.135, 0.160)	85.31 (84.01, 86.51)
	E	03/03/22 – 18/03/22	–	166811	356	0.002	0.081 (0.073, 0.090)	91.89 (91.01, 92.69)
BA.5 (c)		Date interval	Uninfected on Jul 3rd 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		4966749	–	70757	0.014	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	414066	1616	0.004	0.287 (0.274, 0.302)	71.26 (69.84, 72.62)
	B	17/01/22 – 31/01/22	–	610511	2295	0.004	0.284 (0.273, 0.296)	71.57 (70.40, 72.69)
	C	01/02/22 – 15/02/22	–	337648	1140	0.003	0.247 (0.233, 0.261)	75.34 (73.88, 76.73)
	D	16/02/22 – 02/03/22	–	135783	301	0.002	0.157 (0.141, 0.176)	84.27 (82.39, 85.95)
	E	03/03/22 – 18/03/22	–	166455	249	0.001	0.107 (0.094, 0.121)	89.32 (87.91, 90.57)
	F	19/03/22 – 03/04/22	–	133119	116	0.001	0.062 (0.052, 0.074)	93.81 (92.58, 94.84)
BA.5 (d)		Date interval	Uninfected on Jul 17th 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		4895992	–	41767	0.009	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	412450	975	0.002	0.292 (0.274, 0.311)	70.81 (68.94, 72.57)
	B	17/01/22 – 31/01/22	–	608216	1331	0.002	0.278 (0.264, 0.293)	72.21 (70.70, 73.64)
	C	01/02/22 – 15/02/22	–	336508	701	0.002	0.255 (0.237, 0.275)	74.49 (72.54, 76.30)
	D	16/02/22 – 02/03/22	–	135482	196	0.001	0.172 (0.150, 0.198)	82.77 (80.19, 85.02)
	E	03/03/22 – 18/03/22	–	166206	191	0.001	0.138 (0.119, 0.159)	86.23 (84.14, 88.05)
	F	19/03/22 – 03/04/22	–	133003	87	0.001	0.078 (0.063, 0.096)	92.20 (90.38, 93.68)
	G	04/04/22 – 17/04/22	–	109542	61	0.001	0.066 (0.051, 0.085)	93.39 (91.50, 94.85)
BA.5 (e)		Date interval	Uninfected on Aug 1st 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		4854225	–	28337	0.006	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	411475	657	0.002	0.289 (0.268, 0.312)	71.12 (68.85, 73.22)
	B	17/01/22 – 31/01/22	–	606885	1000	0.002	0.306 (0.288, 0.325)	69.44 (67.52, 71.25)
	C	01/02/22 – 15/02/22	–	335807	542	0.002	0.289 (0.266, 0.314)	71.11 (68.59, 73.42)
	D	16/02/22 – 02/03/22	–	135286	161	0.001	0.207 (0.178, 0.242)	79.26 (75.80, 82.22)
	E	03/03/22 – 18/03/22	–	166015	143	0.001	0.151 (0.128, 0.178)	84.89 (82.20, 87.17)
	F	19/03/22 – 03/04/22	–	132916	79	0.001	0.104 (0.083, 0.129)	89.62 (87.06, 91.67)
	G	04/04/22 – 17/04/22	–	109481	44	0.0004	0.070 (0.052, 0.094)	93.01 (90.61, 94.80)
BA.5 (f)		Date interval	Uninfected on Aug 15th 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		4825888	–	29100	0.006	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	410818	660	0.002	0.282 (0.261, 0.304)	71.85 (69.64, 73.89)
	B	17/01/22 – 31/01/22	–	605885	1011	0.002	0.300 (0.282, 0.319)	70.02 (68.14, 71.78)
	C	01/02/22 – 15/02/22	–	335265	551	0.002	0.285 (0.262, 0.309)	71.51 (69.05, 73.77)
	D	16/02/22 – 02/03/22	–	135125	198	0.001	0.247 (0.215, 0.284)	75.30 (71.62, 78.50)
	E	03/03/22 – 18/03/22	–	165872	130	0.001	0.133 (0.112, 0.158)	86.68 (84.19, 88.78)
	F	19/03/22 – 03/04/22	–	132837	83	0.001	0.106 (0.085, 0.131)	89.44 (86.91, 91.48)
	G	04/04/22 – 17/04/22	–	109437	57	0.001	0.088 (0.068, 0.114)	91.23 (88.63, 93.23)
BA.5 (g)		Date interval	Uninfected on Aug 30th 2022	1st infection	BA.5 infection	Absolute Risk	RR (95% CI)	Protection Efficacy, % (95% CI)
	Uninfected		4796788	–	28125	0.006	–	–
	BA.1/BA.2 A	01/01/22 – 16/01/22	–	410158	738	0.002	0.323 (0.301, 0.347)	67.66 (65.27, 69.89)
	B	17/01/22 – 31/01/22	–	604874	1290	0.002	0.390 (0.370, 0.412)	60.97 (58.82, 63.00)
	C	01/02/22 – 15/02/22	–	334714	685	0.002	0.363 (0.337, 0.391)	63.68 (60.89, 66.27)
	D	16/02/22 – 02/03/22	–	134927	235	0.002	0.302 (0.266, 0.343)	69.83 (65.73, 73.44)
	E	03/03/22 – 18/03/22	–	165742	193	0.001	0.203 (0.176, 0.234)	79.69 (76.62, 82.35)
	F	19/03/22 – 03/04/22	–	132754	108	0.001	0.141 (0.117, 0.171)	85.87 (82.94, 88.29)
	G	04/04/22 – 17/04/22	–	109380	72	0.001	0.114 (0.090, 0.144)	88.60 (85.65, 90.95)

Table S2. *Risk of omicron BA.5 infection at different intervals for individuals infected with BA.1/BA.2 in specific periods*. We included in the study the population 12 years and older. Under "1st infection" is the number of individuals at risk for a second infection by BA.5 in the respective interval (i.e., without a second infection until that time). Note that the risk may depend on the epidemic situation and may differ in the BA.5 periods. RR, relative risk; CI, confidence interval.