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A refined analysis on SARS-CoV-2 Omicron variability in infected immunocompromised individuals

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Introduction

Different SARS-CoV-2 variants of Concern (VOCs) emerged during the pandemic. This study aims to characterize SARS-CoV-2 Omicron variability with a focus in immunocompromised people (IPs).

Methods

This retrospective study includes SARS-CoV-2 infected hospitalized and non-hospitalized IPs (HIP and NHIP) and hospitalized and non-hospitalized non-IPs (HP and NHP).

For each subject, nasopharyngeal swabs (NS) were collected from January 2022 to May 2023.

SARS-CoV-2 whole-genome sequencing was performed by Miseq-platform.

Δ days from day of first COVID-19 symptoms to day of NS sampling was calculated.

Additional mutations (AMs) (intra-host prevalence >20%), not present in each Omicron sublineage consensus, were characterized.

Among structural and non-structural proteins, 8 genes were investigated: spike, nucleocapisd and nonstructural proteins (RNA-dependent RNA polymerase, main-protease, papain-like-protease, helicase, Orf6 and Orf9b).

Results

Characteristics of study population

Sequences were obtained for 249 individuals, 157 IPs and 92 non-IPs.

Regarding hospitalization, our population was divided into: 37 Hospitalized and 212 Not Hospitalized (Table 1) and into 18 HIP,19 HP, 139 NHIP and 73 NHP (Table 2).

IPs were younger than non-IPs and different rate of pneumonia was observed, particularly higher in HP. Higher frequency of no-vaccination was observed in Hospitalized patients, particularly in group HP (Tables 1-2).

Table 1	Overall N=249	IPs N=157	Non-IPs N=92	Hospitalized N=37	Not Hospitalized N=212	P-value[a] IPs *vs* Non-IPs	P-value[a] Hosp *vs* Not Hosp
Female, n (%)	126 (50.6)	83 (52.9)	43 (46.7)	13 (35.1)	113 (53.3)	0.351	0.041
Age, years, median (IQR)	63 (50-73)	59 (47-70)	70 (58-78)	70 (60-76)	61 (49-73)	0.00001	0.009
No Vaccinated, n (%)	21 (8.4)	11 (7.0)	10 (10.9)	8 (21.6)	13 (6.1)	0.292	0.002
Δ day, median (IQR)	4 (3-6)	4 (3-5)	4 (3-6)	7 (4-9)	4 (3-5)	0.027	<0.001
Pneumoniae, n (%)	23 (9.2)	8 (5.1)	15 (16.3)	23 (62.2)	0(0)	0.003	0.00001
Immunocompromised, n (%)	157 (63.1)	157 (100)	-	18 (48.6)	139 (65.6)	-	0.049
Onco-haematological	84 (33.7)	84 (53.5)	-	13 (35.1)	71 (33.5)	-	0.845
Other reason[b]	73 (29.3)	73 (46.5)	-	5 (13.5)	68 (32.1)	-	0.022
Cycle-Threshold (Ct):							
E, median (IQR)	22 (19-25)	22 (19-24)	22 (19-25)	23 (20-28)	21 (19-24)	0.811	0.019
N, median (IQR)	20 (18-24)	20 (18-23)	21 (18-24	23 (19-27)	20 (18-24)	0.652	0.036
RdRp/S, median (IQR)	23 (20-26)	23 (20-26)	23 (20-23)	24 (22-28)	23 (20-25)	0.773	0.032

Table 2	HIP N=18	HP N=19	NHIP N=139	NHP N=73	P-value[a]
Female, n (%)	5 (27.8)	8 (42.1)	78 (56.1)	35 (47.9)	0.102
Age, years, median (IQR)	69 (49-74)	71 (65-79)	58 (46-68)	69 (53-78)	0.00003
Month of sample collection, median (IQR)	Sept 22 (Feb 22-Jan 23)	Feb 22 (Feb 22-Nov 22)	Mar 22 (Feb 22-Jun 22)	Apr 22 (Jan 22-Sept 22)	0.395
No Vaccinated, n (%)	3 (16.7)	5 (26.3)	8 (5.8)	5 (6.9)	0.011
Δ day, median (IQR)	5 (3-8)	8 (6-11)	4 (3-5)	4 (3-5)	0.00012
Pneumoniae, n (%)	8 (44.4)	15 (78.9)	0(0)	0(0)	0.00001
Immunocompromised, n (%)	18 (100)	-	139 (100)	-	
Onco-haematological	13 (72.2)	-	71 (51.4)	-	0.131
Other reason[b]	5 (27.8)	-	68 (48.9)	-	0.131
Cycle-Threshold (Ct):					
E, median (IQR)	23 (21-27)	23 (20-29)	22 (19-24)	20 (19-24)	0.128
N, median (IQR)	22 (20-27)	23 (19-28)	20 (18-23)	20 (18-24)	0.216
RdRp/S, median (IQR)	24 (22-28)	24 (20-29)	23 (20-25)	22 (20-25)	0.203

Tables. Clinical Characteristics of individuals
[a] P-values were calculated by Kruskal-Wallis tests and Chi Square for Trend as appropriate.
[b] Other reason: after organ transplant, multiple sclerosis, HIV, autoimmune disease.

Overall, 51 different Omicron sublineages were identified and according to the temporal collection of samples, BA.1.1 was the most prevalent (18.1%) followed by BA.1 and BA.2 (both 14.9%) during the first Omicron wave, and followed by BA.2.9 (6.4%) and BQ.1.1 (5.2%), until March 2023.

Conclusions

- Overall, 51 different Omicron sublineages were identified, with a prevalence changing mainly according to the time of infection.
- Some sublineages (XBB.1, BQ.1.10 and BA.1.15) were found more prevalent in Hospitalized people, with BQ.1.10 with a significant higher prevalence in HIP.
- Around 60% of individuals showed at least 1 AM in at least one of the analysed gene, with only 8% of people showing ≥ 2 AMs, confirming a low intrinsic intra-host variability of SARS-CoV-2.
- A higher variability with statistical significance was found for Spike and RBD in hospitalized individuals, particularly when immunocompromised. A similar trend was observed also for the helicase.
- For all other genes, no significant differences were observed on the presence of AMs between the different groups of individuals.
- The increase in genetic variability in Hospitalized patients correlated with the Δ days from first COVID-19 symptoms, that were significantly longer than in the other individuals.
- All these results deserve further investigation in a larger population, specifically to better investigating the variability in N, Orf6 and Orf9b, genes related to the innate antiviral response.

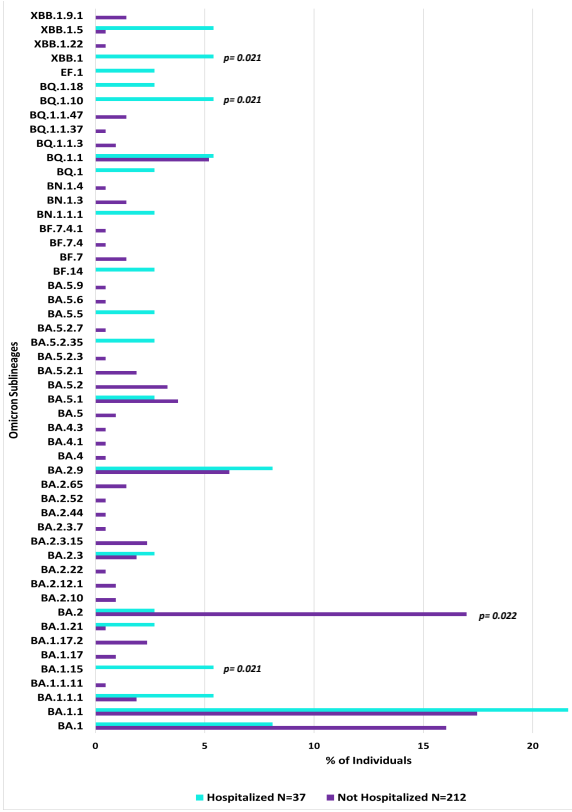
Different lineages distribution

Significantly higher prevalence was found for XBB.1, BQ.1.10 and BA.1.15 Omicron sublineages in Hospitalized people than Not Hospitalized, while BA.2 was higher in Not Hospitalized group (Figure 1).

BQ.1.10 was with a significant higher prevalence in HIP (Figure 2).

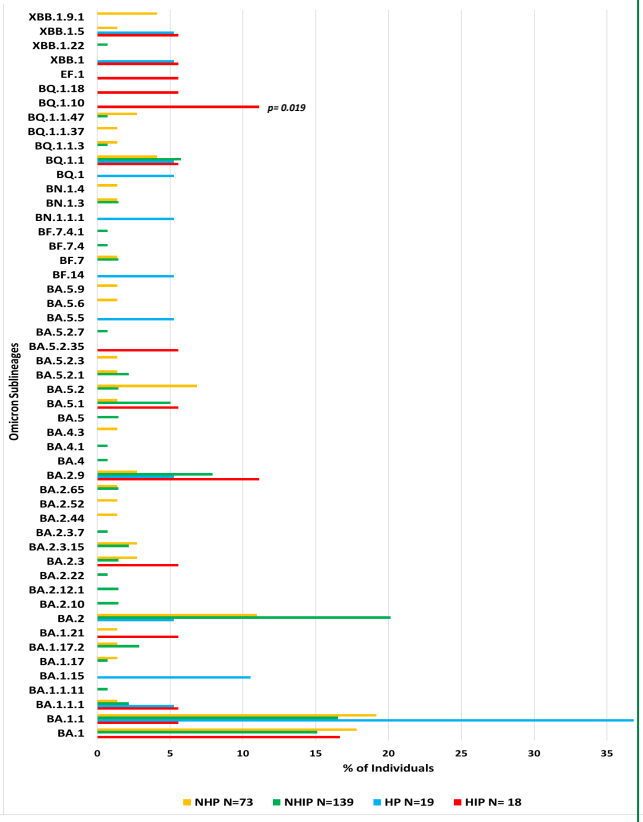
Distribution of Omicron sublineages in Hospitalized & Not Hospitalized individuals

Fig. 1



Distribution of Omicron sublineages in HIP, NHIP, HP and NHP

Fig. 2



Higher variability in hospitalized and in immunocompromised people

Overall, 61.4% of individuals showed ≥ 1 AM in at least one of the analysed gene, with 8% of people showing ≥ 2 AMs in ≥ 1 gene.

A significant higher prevalence of AMs was observed in Hospitalized vs not Hospitalized and in Immunocompromised Hospitalized (HIP) vs Immunocompromised Not Hospitalized (NHIP) individuals in the overall genes, and in Spike and RBD (p values <0.05) (Table 3).

A higher prevalence of ≥ 2 AMs was observed in HIP and HP vs NHIP and NHP groups in Spike (11.1% and 15.8% vs 1.4% and 2.7%, p=0.0045) and in RBD (11.1% and 5.3% vs 0% and 0%, p=0.018).

Among the non structural genes, in Helicase a higher prevalence of AMs was observed in the group of Hospitalized vs Non-Hospitalized (p=0.038, Table 3).

These results correlate with the Δ days from first COVID-19 symptoms to NS sampling, that were significantly longer in Hospitalized individuals (median [IQR] days: 8 [6-11] in HP vs 5 [3-8] in HIP vs 4 [3-5] in NHIP vs 4 [3-5] in NHP p<0.00012) (Table 2).

None of AMs associated with resistance to molnupiravir and nirmatrelvir was observed in the cohort.

Table 3

Additional Mutations		Overall N=249	Hospitalized N=37	Not Hospitalized N=212	IPs N=157	Non-IPs N=92	HIP N=18	NHIP N=139	HIP N=18	HP N=19	NHIP N=139	NHP N=73	P values
N=			*a*		*b*		*c*		*d*				
Overall n(%)	0	96 (38.6)	**14 (37.8)**	**82 (38.7)**	61 (38.9)	35 (38.0)	**7 (38.9)**	**54 (38.8)**	7 (38.9)	7 (36.8)	54 (38.8)	28 (38.4)	***a* 0.026**
	1	133 (53.4)	**16 (43.2)**	**117 (55.2)**	85 (54.1)	48 (52.2)	**7 (38.9)**	**78 (56.1)**	7 (38.9)	9 (47.4)	78 (56.1)	39 (53.4)	*b* 0.740
	>1	20 (8.0)	**7 (18.9)**	**13 (6.1)**	11 (7.0)	9 (9.8)	**4 (22.2)**	**7 (5.0)**	4 (22.2)	3 (15.8)	7 (5.0)	6 (8.2)	***c* 0.036**
													d 0.201
Spike n(%) 1273aa	0	202 (81.1)	**25 (67.6)**	**177 (83.5)**	**122 (77.7)**	**80 (87.0)**	**11 (61.1)**	**111 (79.9)**	**11 (61.1)**	**14 (73.7)**	**111 (79.9)**	**66 (90.4)**	***a* 0.005**
	1	38 (15.3)	**7 (18.9)**	**31 (14.6)**	**31 (19.7)**	**7 (7.6)**	**5 (27.8)**	**26 (18.7)**	**5 (27.8)**	**2 (10.5)**	**26 (18.7)**	**5 (6.8)**	***b* 0.017**
	>1	9 (3.6)	**5 (13.5)**	**4 (1.9)**	**4 (2.5)**	**5 (5.4)**	**2 (11.1)**	**2 (1.4)**	**2 (11.1)**	**3 (15.8)**	**2 (1.4)**	**2 (2.7)**	***c* 0.039**
													***d* 0.002**
RBD n(%) 223aa	0	239 (96.0)	**32 (86.5)**	**207 (97.6)**	149 (94.9)	90 (97.8)	**15 (83.3)**	**134 (96.4)**	**15 (83.3)**	**17 (89.5)**	**134 (96.4)**	**73 (100)**	***a* 0.002**
	1	7 (2.8)	**2 (5.4)**	**5 (2.4)**	6 (3.8)	1 (1.1)	**1 (5.6)**	**5 (3.6)**	**1 (5.6)**	**1 (5.3)**	**5 (3.6)**	**0 (0)**	*b* 0.446
	>1	3 (1.2)	**3 (8.1)**	**0 (0)**	2 (1.3)	1 (1.1)	**2 (11.1)**	**0 (0)**	**2 (11.1)**	**1 (5.3)**	**0 (0)**	**0 (0)**	***c* 0.011**
													***d* 0.002**
ORF6 n(%) 61aa	0	246 (98.8)	37 (100)	209 (98.6)	156 (99.4)	90 (97.8)	18 (100)	138 (99.3)	18 (100)	19 (100)	138 (99.3)	71 (97.3)	*a* 1
	1	2 (0.8)	0 (0)	2 (0.9)	1 (0.6)	1 (1.1)	0 (0)	1 (0.7)	0 (0)	0 (0)	1 (0.7)	1 (1.4)	*b* 0.393
	>1	1 (0.4)	0 (0)	1 (0.5)	0 (0)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)	*c* 1
													d 0.804
N n(%) 419aa	0	218 (87.6)	30 (81.1)	188 (88.7)	138 (87.9)	80 (87.0)	15 (83.3)	123 (88.5)	15 (83.3)	15 (78.9)	123 (88.5)	65 (89.0)	*a* 0.081
	1	30 (12.0)	6 (16.2)	24 (11.3)	18 (11.5)	12 (13.0)	2 (11.1)	16 (11.5)	2 (11.1)	4 (21.1)	16 (11.5)	8 (11.0)	*b* 0.700
	>1	1 (0.4)	1 (2.7)	0 (0)	1 (0.6)	0 (0)	1 (5.6)	0 (0)	1 (5.6)	0 (0)	0 (0)	0 (0)	*c* 0.148
													d 0.203
ORF9b n(%) 98aa	0	242 (97.2)	35 (94.6)	206 (97.2)	151 (96.2)	90 (97.8)	16 (88.9)	135 (97.1)	16 (88.9)	19 (100)	135 (97.1)	71 (97.3)	*a* 0.280
	1	7 (2.8)	2 (5.4)	5 (2.4)	5 (3.2)	2 (2.2)	2 (11.1)	3 (2.2)	2 (11.1)	0 (0)	3 (2.2)	2 (2.7)	*b* 1
	>1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	*c* 0.102
													d 0.224
NSP3-PLpro n(%) 1945aa	0	179 (71.9)	23 (62.2)	156 (73.6)	114 (72.6)	65 (70.7)	13 (72.2)	101 (72.7)	13 (72.2)	10 (52.6)	101 (72.7)	55 (75.3)	*a* 0.213
	1	59 (23.7)	11 (29.7)	48 (22.6)	35 (22.3)	24 (26.1)	3 (16.7)	32 (23)	3 (16.7)	8 (42.1)	32 (23)	16 (21.9)	*b* 0.662
	>1	11 (4.4)	3 (8.1)	8 (3.8)	8 (5.1)	3 (3.3)	2 (11.1)	6 (4.3)	2 (11.1)	1 (5.3)	6 (4.3)	2 (2.7)	*c* 0.327
													d 0.354
NSP5-3CLpro n(%) 306aa	0	236 (94.8)	35 (94.6)	201 (94.8)	152 (96.8)	84 (91.3)	18 (100)	134 (96.4)	18 (100)	17 (89.5)	134 (96.4)	67 (91.8)	*a* 0.741
	1	12 (4.8)	2 (5.4)	10 (4.7)	5 (3.2)	7 (7.6)	0 (0)	5 (3.6)	0 (0)	2 (10.5)	5 (3.6)	5 (6.8)	*b* 0.119
	>1	1 (0.4)	0 (0)	1 (0.5)	0 (0)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)	*c* 1
													d 0.442
NSP12-RdRp n(%) 932aa	0	218 (87.6)	32 (86.5)	186 (87.7)	134 (85.4)	84 (91.3)	16 (88.9)	118 (84.9)	16 (88.9)	16 (84.2)	118 (84.9)	68 (93.2)	*a* 0.790
	1	31 (12.4)	5 (13.5)	26 (12.3)	23 (14.6)	8 (8.7)	2 (11.1)	21 (15.1)	2 (11.1)	3 (15.8)	21 (15.1)	5 (6.8)	*b* 0.232
	>1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	*c* 1
													d 0.332
NSP13-Helicase n(%) 601aa	0	220 (88.4)	**29 (78.4)**	**191 (90.1)**	138 (87.9)	82 (89.1)	**14 (77.8)**	**124 (89.2)**	14 (77.8)	15 (78.9)	124 (89.2)	67 (91.8)	***a* 0.038**
	1	28 (11.2)	**7 (18.9)**	**21 (9.9)**	18 (11.5)	10 (10.9)	**3 (16.7)**	**15 (10.8)**	3 (16.7)	4 (21.1)	15 (10.8)	6 (8.2)	*b* 0.735
	>1	1 (0.4)	**1 (2.7)**	**0 (0)**	1 (0.6)	0 (0)	**1 (5.6)**	**0 (0)**	1 (5.6)	0 (0)	0 (0)	0 (0)	***c* 0.055**
													d 0.085

The p values were calculated among these categories: a: Hospitalized *vs* Not Hospitalized; b: IPs *vs* Non-Ips; c: HIP *vs* NHIP; d: HIP *vs* HP vs NHIP vs NHP

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