

Supplementary Figures and Tables

The impact of first-time SARS-CoV-2 infection on human anelloviruses

Anne L. Timmerman ^{1,2}, Lisanne Commandeur ^{1,2}, Martin Deijs ^{1,2}, Maarten G. J. M. Burggraaff ^{1,2}, A. H. Ayesha Lavell ^{2,3}, Karlijn van der Straten ^{1,2,4}, Khadija Tejjani ^{1,2}, Jacqueline van Rijswijk ^{1,2}, Marit J. van Gils ^{1,2}, Jonne J. Sikkens ^{2,3}, Marije K. Bomers ^{2,3} and Lia van der Hoek ^{1,2,*}

¹ Laboratory of Experimental Virology, Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, Location University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

² Amsterdam Institute for Infection and Immunity, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

³ Department of Internal Medicine, Amsterdam UMC, Location Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

⁴ Department of Internal Medicine, Amsterdam UMC, Location University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

* Correspondence: c.m.vanderhoek@amsterdamumc.nl

Supplementary Figure S1, S2
Supplementary Table S1-S4 and S6

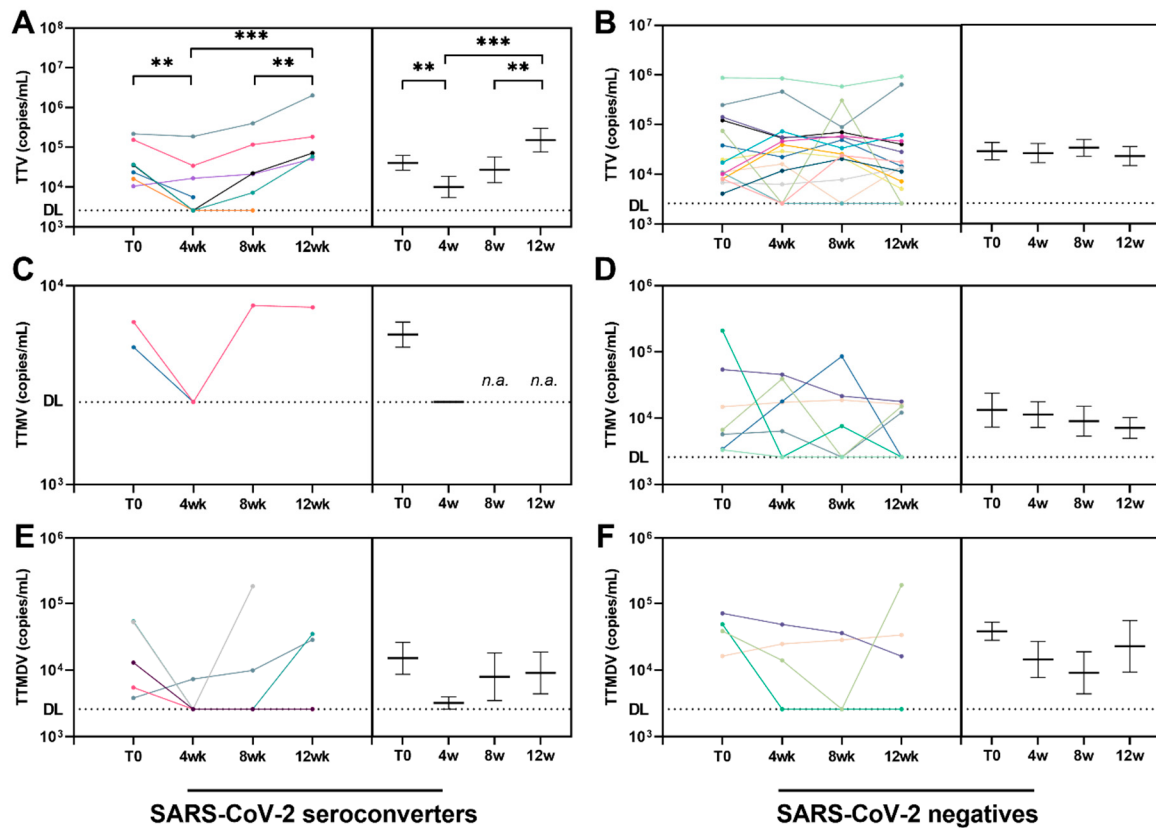


Figure S1. Anellovirus genus load in blood following SARS-CoV-2 infection. The anellovirus load (copies/mL), TTV (a+b), TTMV (c+d) and TTMDV (e+f), was determined in health care workers (HCWs) followed for three months (T0 – 12 weeks). The anellovirus load of each individual HCW (left), and mean with standard deviation (right) was shown. The anellovirus load was log transformed. Colors represent unique HCW. (a+c+e) SARS-CoV-2 seroconverters group. (b+d+f) SARS-CoV-2 negative group. Dashed line corresponds to the detection limit (DL).

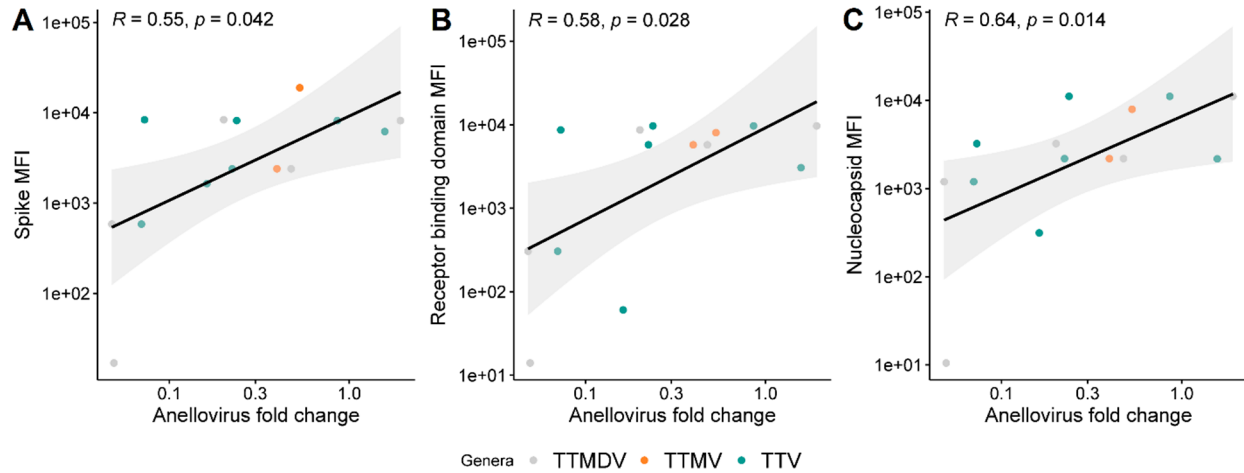


Figure S2. Correlation plots of anellovirus fold change and SARS-CoV-2 antibody MFI. The anellovirus concentration and antibodies against SARS-CoV-2 were determined in the SARS-CoV-2 seroconverters group. The anellovirus fold change was calculated between 4 weeks and T0 and antibody MFI represents highest value detected in the 3 month follow-up. Antibodies against the Spike (a), Receptor Binding Domain (b), and Nucleocapsid (c) of SARS-CoV-2 were measured. Black line and grey area represents the trend line and confidence interval (95%), respectively. The colors of the dots represent anellovirus genera (grey: TTMDV, orange: TTMV and blue: TTV).

Table S1. Association between anellovirus load at T0 and SARS-CoV-2 incidence. OR; odds ratio, CI; confidence interval.

	P-value	OR (95% CI)
All anelloviruses	0,398	0,755 (95% CI 0,373 to 1,433)
TTV	0,489	0,734 (95% CI 0,282 to 1,739)
TTMV	0,107	0,116 (95% CI 0,001 to 1,388)
TTMDV	0,829	1,165 (95% CI 0,270 to 4,760)

Table S2. Univariable linear mixed model analysis of anellovirus load. Geometric mean (95% CI), significance, and mean fold change (95% CI) of anellovirus load. SC = SARS-CoV-2 seroconverted group, NEG= SARS-CoV-2 negative group. CI = confidence interval.

Virus	Comparison 1, GM (95% CI)	Comparison 2, GM (95% CI)	P-value	Significance	Mean fold change (95% CI)
TTV	T0 (SC), 40579 (12708 to 129573)	4 weeks (SC), 10104 (4180 to 24429)	0,004	**	0,249 (0,103 to 0,602)
		8 weeks (SC), 25281 (9942 to 64480)	0,342	ns	0,623 (0,245 to 1,589)
		12 weeks (SC), 105749 (39199 to 285230)	0,074	ns	2,606 (0,966 to 7,029)
	4 weeks (SC)	8 weeks (SC)	0,068	ns	2,504 (0,984 to 6,385)
		12 weeks (SC)	0,000	***	10,486 (3,881 to 28,474)
	8 weeks (SC)	12 weeks (SC)	0,010	**	4,183 (1,519 to 11,577)
	T0 (NEG), 28854 (13056 to 63767)	4 weeks (NEG), 26459 (14456 to 48388)	0,786	ns	0,917 (0,501 to 1,677)
		8 weeks (NEG), 33903 (18524 to 62007)	0,616	ns	1,175 (0,642 to 2,149)
		12 weeks (NEG), 22997 (12580 to 42098)	0,481	ns	0,797 (0,436 to 1,459)
	4 weeks (NEG)	8 weeks (NEG)	0,443	ns	1,281 (0,697 to 2,356)
		12 weeks (NEG)	0,666	ns	0,87 (0,473 to 1,6)
	8 weeks (NEG)	12 weeks (NEG)	0,233	ns	0,679 (0,369 to 1,247)
	T0 (NEG)	4 weeks (SC)	0,025	*	0,271 (0,093 to 0,792)
ALL	T0 (SC), 21504 (9710 to 47619)	4 weeks (SC), 5505 (2688 to 11290)	0,000	***	0,256 (0,125 to 0,525)
		8 weeks (SC), 13784 (6473 to 29417)	0,262	ns	0,641 (0,301 to 1,368)
		12 weeks (SC), 33718 (15096 to 75501)	0,284	ns	1,568 (0,702 to 3,511)
	4 weeks (SC)	8 weeks (SC)	0,021	*	2,502 (1,175 to 5,333)
		12 weeks (SC)	0,000	***	6,117 (2,737 to 13,695)
	8 weeks (SC)	12 weeks (SC)	0,039	*	2,445 (1,076 to 5,562)
	T0 (NEG), 24465 (13657 to 43871)	4 weeks (NEG), 19181 (11327 to 32441)	0,375	ns	0,784 (0,463 to 1,326)
		8 weeks (NEG), 19352 (11450 to 32759)	0,394	ns	0,791 (0,468 to 1,339)
		12 weeks (NEG), 16759 (9908 to 28330)	0,168	ns	0,685 (0,405 to 1,158)
	4 weeks (NEG)	8 weeks (NEG)	0,973	ns	1,009 (0,596 to 1,709)
		12 weeks (NEG)	0,621	ns	0,874 (0,516 to 1,478)
	8 weeks (NEG)	12 weeks (NEG)	0,598	ns	0,865 (0,511 to 1,465)
	T0 (NEG)	4 weeks (SC)	0,017	*	0,327 (0,135 to 0,796)

Table S3. Anellovirus load after RCA in serum samples of individuals tested positive for SARS-CoV-2 infection. RCA; rolling circle amplification.

Serodate	Serodate	TTV (copies/mL)	TTMV (copies/mL)	TTMDV (copies/mL)
S3-02	T0	312917	2600	2600
S3-02	4 weeks	2600	2600	2600
S3-02	8 weeks	13978801324	2600	2600
S3-02	12 weeks	25093989902	2600	2600
S3-06	T0	2600	2600	3276573310
S3-06	4 weeks	2889370	7047	11853698843
S3-06	8 weeks	92964	15205	55514351777
S3-08	T0	77237533541	231456167	26502641513
S3-08	4 weeks	2600	2600	2600
S3-08	8 weeks	2600	2600	2600
S3-08	12 weeks	40618527	1092979844	9062855570
S3-11	T0	17414952917	133326597	19027197435
S3-11	4 weeks	31020567155	574511	2600
S3-11	8 weeks	30491016166	2049431619	339138238
S3-11	12 weeks	39918850957	44832122	2600
S3-15	T0	155728075848	217242987	8837610481
S3-15	4 weeks	70842290505	12826295291	5566940577
S3-15	8 weeks	201926938450	9375951065	10149647789
S3-15	12 weeks	635414067556	3531485194	13074209408

Table S4. Illumina results of the SARS-CoV-2 positive individuals. Reads and number of lineages present in the anellovirus. Trimmed illumina reads were aligned to SCANellome anellovirus database (Laubscher et al., 2023). A coverage of 75% was considered a hit.

sample	Serodate	Illumina output			Number of TTV lineages	Number of TTMV lineages	Number of TTMDV lineages	Total number of anellovirus lineages
		Total reads	Anellovirus reads	Anellovirus reads per million (rpm)				
S3-02	T0	2922528	83	28	1	0	0	1
S3-02	8 weeks	4090494	134507	32883	4	0	2	6
S3-02	12 weeks	3830600	179158	46770	1	0	0	1
S3-06	T0	2504924	91564	36554	1	0	1	2
S3-06	4 weeks	3759680	697773	185594	4	2	3	9
S3-06	8 weeks	4095328	55320	13508	2	0	2	4
S3-08	T0	3633222	171482	47198	1	0	2	3
S3-08	12 weeks	3424138	9150	2672	4	2	0	6
S3-11	T0	4169126	105234	25241	5	3	2	10
S3-11	4 weeks	3715018	81631	21973	3	1	0	4
S3-11	8 weeks	3260626	812607	249218	11	3	1	15
S3-11	12 weeks	4266356	600818	140827	6	1	0	7
S3-15	T0	3829454	765920	200008	8	0	1	9
S3-15	4 weeks	4142204	520831	125738	7	2	0	9
S3-15	8 weeks	4417964	959427	217165	10	1	1	12
S3-15	12 weeks	4274382	1832059	428614	13	2	0	15

Table S5: See Excel file

Table S6. Variants table of lineage MZ824892 infecting individual S3-15 in T0 and 12 weeks. Trimmed illumina reads were aligned to SCANellome anellovirus database (Laubscher et al., 2023). A coverage of 75% was considered a hit. Variants were called using Lofreq by using the positive strand (Timmerman *et al.*, 2022)

Serodate	Percentage of total Cs edited
T0	26
12 weeks	39