An analysis of SARS-CoV-2 viral load by patient age

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Table 1: We added a column with the percentage of positively tested individuals with a viral load higher than one million viral copies and changed the table legend accordingly. We also made changes to the formatting of table A1.
Discussion, paragraph 2: In the second paragraph of the discussion, we changed the sentence *"An estimate based on the number of symptomatic admissions in a specialist pediatric hospital assumes that thousands of pediatric cases were missed during the early phase of the Wuhan outbreak, at a time at which only ca. 10,0000 adult patients were registered (13)."* to *"Further, an estimate based on the number of symptomatic admissions in a specialist pediatric hospital suggested approximately 1105 (95% CI: 592-1829) cumulative pediatric COVID-19 hospitalizations prior to the lockdown in Wuhan starting January 23rd, at which point only 425 confirmed cases had been reported across all age groups, none of which were under age 15 (13)."*

Abstract

Data on viral load, as estimated by real-time RT-PCR threshold cycle values from 3,712 COVID-19 patients were analysed to examine the relationship between patient age and SARS-CoV-2 viral load. Analysis of variance of viral loads in patients of different age categories found no significant difference between any pair of age categories including children. In particular, these data indicate that viral loads in the very young do not differ significantly from those of adults. Based on these results, we have to caution against an unlimited re-opening of schools and kindergartens in the present situation. Children may be as infectious as adults.

Introduction

The present measures to curb the spread of SARS-CoV-2 by non-pharmaceutical interventions are beginning to show effects in many countries. Along with the gradual lifting of measures of physical distancing, there is a growing discussion regarding the contribution of school- and kindergarten closures to the reduction of transmission rate (*1*) and to the expected rebound upon reopening. Studies to determine the contribution of children as sources of infection are complicated by the fact that non-pharmaceutical interventions including school- and kindergarten closures were in place before observational trials could begin. A household study in China and observations in a limited number of contact investigations in Germany suggest that children are infected by SARS-CoV-2 at a rate that may not be different from that of adults (*2*, *3*). However, the extent to which children can act as sources of infection remains unclear. A challenge when trying to address this question by epidemiological observation is posed by the present situation of physical distancing. Because kindergartens and schools are closed, it becomes less likely that children become index cases in households. During the early phase of the SARS-CoV-2 epidemic in many European countries, the seeding of cases by adult-aged travelers who visited early epidemic foci was an additional reason why children were under-represented in age-related incidence (*4*). It is therefore unlikely that epidemiological investigations undertaken under the present conditions can identify the actual risk of acquisition of infection from children by subjects of any age group.

An alternative way to achieve a correlate of infectivity is to directly analyze the virus concentration in the respiratory tract. We have previously shown that viral loads under a concentration of ca. 10^6 copies per mL of sputum or per entire throat swab are unlikely to yield infectious virus growth in cell culture (*5*). We also found that virus could not be isolated from respiratory samples after the first week of symptoms, which is highly concordant with transmission analyses based on actual transmission pairs, suggesting that infectivity ends by the end of the first week of symptoms (*6*). To enable an estimate of infectivity in children, we analyzed viral loads observed during routine testing at a large laboratory testing centre in Berlin (Charité Institute of Virology and Labor Berlin). Charité Institute of Virology was the first laboratory qualified to test for SARS-CoV-2 in Germany and until early February 2020 was the only SARS-CoV-2 testing facility in Berlin, a city of ca. 3.8 million inhabitants. Labor Berlin is a

large medical laboratory services provider in Berlin, owned by the senate of Berlin and serving Charité as well as other large hospitals in Berlin and beyond. Labor Berlin serves public testing centres that mainly see adult outpatients. It also tests out- and inpatients from several hospitals, and serves practitioners and public health agencies submitting samples taken during household-based contact tracing.

Results

From January to 26th April, 2020, virology laboratories at Charité and Labor Berlin screened 59,831 patients for COVID-19 infection, 3,712 (6.2%) with a positive real-time RT-PCR result. We divided patients according to two categorizations to investigate whether there is a relationship between patient age and viral load. The first categorization is based on ten-year brackets, ages 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, and 91-100. The second categorization is based on broad social strata: kindergarten (ages 0-6), grade school (ages 7-11), high school (ages 12-19), university (ages 20-25), adult (26-45 years), and mature (age over 45). Patient counts in each age group, and number and percentage of PCR positive patients are shown in **Table 1**. A comparison of age stratification in tested cases versus the Berlin population is shown in **Figure A1.** Of note, whereas younger age groups have lower detection rates (**Table 1**), this does not imply an age-based estimate of infection prevalence because of mostly symptoms-directed testing.

Due to the small sample sizes in the pediatric age groups, we examined diagnostic indications for 47 cases (1-11 years of age) for whom this information was available. Fifteen cases had indications pointing toward underlying disease or hospitalization. Average viral loads in these cases were lower than in children tested in outpatient departments, practices, or households **(Figure A2)**. This corresponds to the observation that hospitalization occurs after some days of symptoms, a time when viral loads in throat swabs are beginning to decline (*5*).

Viral load

The distribution of observed viral loads in a total of 3,712 cases are shown in **Figure 1**. The viral loads are not normally distributed but are skewed towards a mean (logarithm base 10) value of 5.19 (i.e., $10^{5.19}$ viral copies) per sample, with a median of 4.80, corresponding to threshold cycle (Ct) values of 30.01 and 31.23, respectively. The sharp drop on the left side of the distribution is due to the assay sensitivity limit. The viral load projection derived in our study is semi-quantitative, and projects viral load per mL of sputum or per entire swab sample, while only a fraction of the volume of both types of sample can actually reach the test tube. Also, quantification is based on a standard preparation tested once in multiple diluted replicates to generate a standard curve and derive a formula upon which Ct values are transformed into viral loads. This approach does not reflect inter-run variability or the variability between different RT-PCR setups and chemistries. However, these variabilities apply to all age groups and do not affect the interpretation of data for the purpose of the present study.

Analysis of variation in viral load between age groups

Viral loads are plotted according to categorization in **Figure 2** with per-group descriptive statistics shown in **Table 2**. Two key prior conditions for an analysis of variance are a) that the dependent variable is approximately normally distributed within each category and b) that the variance within each category is approximately equal. A Shapiro test for normal distribution in the first categorization (C1) has a value of 0.96 (p value 2.71^{-31}), and in the second categorization (C2) a value of 0.96 (p value 8.56^{-32}) (**Table A1**), strongly indicating that the $\log_{10}$ viral load numbers in both categories are not normally distributed, as is clear from **Figures A3** and **A4**. Regarding equality of variance, Levene's statistic (*7*) (using median values) in categorization C1 has value 1.80 (p value 0.063) while in categorization C2, the same statistic has value 2.30 (p value 0.042) (**Table A2**). Thus in C2 there is evidence that the viral load variance between the categories cannot be considered approximately equal. Given these results, we used the non-parametric Kruskal-Wallis H test (*8*), since it does not have pre-conditions of normality or equality of variance. The Kruskal-Wallis H statistic had value 22.39 (p value 0.008) for C1 and 14.97 (p value 0.011) for C2 (**Table A3**). Although the significant Kruskal-Wallis test indicates at least one significant pairwise difference exists between subgroups in both categorizations, due care must be exercised in the post hoc interpretation due to the influence of highly skewed distributions.

We performed pairwise post hoc analyses on both categorizations using three methods: the Tukey honestly significant difference (HSD) test (*9*) (**Table A4**), Bonferroni-adjusted pairwise T-test (*10*) (**Table A5**), and Dunn's test (**Table A6**) (*11*). For categorization C1, none of the three post hoc methods indicated a significant difference between any pair of the ten subgroups. For categorization C2 the situation was identical, apart from Dunn's test indicating a difference (p value 0.045) between the very youngest (kindergarten) and very oldest (Mature) subgroups. Thus the overwhelming conclusion from the three post hoc testing methods is that no significant differences in viral load exists between any subgroups in either categorization.

Discussion

Because of difficulties in conducting observational trials to investigate the infectivity of children as opposed to other age groups with SARS-CoV-2 infection, in this short study we attempt the provision of a direct measure of virus concentration from which one can extrapolate to infectivity.

Whereas the attack rate in children seems to correspond to that in adults (*2*), it is obvious that children are under-represented in clinical studies and less frequently diagnosed due to mild or absent symptoms. For instance, a recent systematic review identified only 1,065 pediatric SARS-CoV-2 cases in the medical literature as of April 2020 (*12*). Further, an estimate based on the number of symptomatic admissions in a specialist pediatric hospital suggested approximately 1105 (95% CI: 592-1829) cumulative pediatric COVID-19 hospitalizations prior to the

lockdown in Wuhan starting January 23rd, at which point only 425 confirmed cases had been reported across all age groups, none of which were under age 15 (*13*). Because they are mostly asymptomatic, children may not be presented at testing centers even if they belong to households with a confirmed index case. There are many other factors that complicate the determination of infection rates in, and transmission rates from children. For instance, the age profile during the early phase of the outbreak in many European countries makes it difficult to derive transmission rates from household contact studies. Early transmission clusters were started by travellers of adult age, making children less likely to be index cases in households (*4*). Another circumstance making children less likely to carry the virus into households is that kindergartens and schools were closed early in the outbreak in Germany. These combined effects will cause children to be more likely to receive rather than spread infections in households for purely circumstantial reasons. This observation may be misunderstood as an indication of children being less infectious. The determination of viral loads seems to provide an interesting means to achieve an indirect but robust estimate of infectivity in the present epidemiological circumstances. The correlation of RNA-based viral load in the respiratory tract with infectivity, as measured in cell culture, has been established (*5*, *14*).
In our study, the virus detection rate increased steadily with age of patients tested. As testing was predominantly directed by symptoms, this suggests that children with respiratory symptoms and fever are less likely than adults to suffer from acute SARS-CoV-2 infection. Many other respiratory viruses cause symptomatic disease in children, but less so in adults where endemic respiratory viruses often present as mild upper respiratory tract infection without fever. Our results should clearly not be taken as an indicator of age-specific prevalence in Germany. Rather, the low rate of SARS-CoV-2 detection in the tested children suggests that symptoms are not a good predictor of infection. At the same time, the absence of symptoms does not imply absence of virus excretion. In a study of people living in the Italian village of Vó, in which ca. 80% of the population were tested by RT-PCR twice within two weeks, about half the population were found to be asymptomatically infected, showing no symptoms over the observation period of two weeks, while viral loads were equivalent in symptomatic and asymptomatic patients (*15*).

It is a limitation that we have not generally discriminated the studied patients into sub-cohorts based on symptomatic status, underlying diseases, or other indications for diagnostic test application. At least for the children in the present study, we can say that hospitalized children with underlying disease were not found to have higher viral loads than children without known underlying disease tested in outpatient departments, practices, or households. The latter would represent children attending schools and kindergartens.

The viral loads observed in the present study, combined with earlier findings of similar attack rate between children and adults (*2*), suggest that transmission potential in schools and kindergartens should be evaluated using the same assumptions of infectivity as for adults. There are reasons to argue against the notion of adult-like infectivity in children, such as the fact that asymptomatic children do not spread the virus by coughing, and have smaller exhaled air volume than adults. However, there are other arguments that speak in favour of transmission, such as the greater physical activity and closer social engagement of children. We recommend

collecting and evaluating more viral load data from testing laboratories to achieve more robust statistical assessments and independent confirmation of the present results. Based on the absence of any statistical evidence for a different viral load profile in children found in the present study, we have to caution against an unlimited re-opening of schools and kindergartens in the present situation, with a widely susceptible population and the necessity to keep transmission rates low via non-pharmaceutical interventions. Children may be as infectious as adults.

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Categorization C1				
Group	**Count**	**+ve**	**% +ve**	**% +ve load >1M**
1-10	2,181	49	2.25	0.50
11-20	1,991	78	3.92	1.05
21-30	9,710	536	5.52	1.87
31-40	12,737	630	4.95	1.59
41-50	9,572	575	6.01	1.59
51-60	10,586	662	6.25	2.08
61-70	5,529	431	7.80	2.66
71-80	4,064	420	10.33	3.03
81-90	3,302	314	9.51	3.30
91-100	159	17	10.69	5.03

Categorization C2				
Group	**Count**	**+ve**	**% +ve**	**% +ve load >1M**
KG	1,759	37	2.10	0.40
GS	623	16	2.57	0.80
HS	1,790	74	4.13	1.12
Uni	4,587	267	5.82	2.05
Adult	23,665	1,247	5.27	1.63
Mature	27,407	2,071	7.56	2.42

Table 1: Categorization breakdown and positive PCR counts and percentages. The ‘Count’ column in each categorization gives the total number of patients tested. ‘+ve’ indicates a total number of positive RT-PCR results for the subgroup. ‘% +ve load >1M’ indicates the percentage of positively tested individuals with over one million viral copies. KG: kindergarten; GS: grade school; HS: high school; Uni: University, +ve: positive.

A) Category C1	N	Mean	SD	SE	95% Conf.	Interval
1	49	4.637858	1.826493	0.260928	4.121141	5.154576
2	78	4.798684	1.790027	0.202681	4.398859	5.198509
3	536	5.261825	1.93962	0.083779	5.097465	5.426185
4	630	5.213623	2.020657	0.080505	5.055708	5.371538
5	575	4.985018	1.87101	0.078027	4.831953	5.138083
6	662	5.258317	1.905385	0.074055	5.11306	5.403575
7	431	5.278967	1.872932	0.090216	5.101938	5.455996
8	420	5.174407	1.78352	0.087027	5.003631	5.345183
9	314	5.344452	1.899481	0.107194	5.134016	5.554887
10	17	5.609229	2.047993	0.496711	4.605712	6.612745
B) Category C2	**N**	**Mean**	**SD**	**SE**	**95% Conf.**	**Interval**
Adult	1247	5.15923	1.970687	0.055806	5.049806	5.268655
GS	16	5.364652	2.214843	0.553711	4.243786	6.485517
HS	74	4.783514	1.776356	0.206497	4.376017	5.191012
KG	37	4.371295	1.601139	0.263226	3.848256	4.894334
Mature	2071	5.229369	1.867447	0.041035	5.148921	5.309818
Uni	267	5.283627	1.946236	0.119108	5.049738	5.517517

Table 2: Statistics describing the viral load distributions in C1 and C2. The mean, standard deviation (SD), standard error (SE), 95% Confidence Interval (95% Conf.), and the interval are shown for the base-10 logarithm of viral load for **A)** categorization C1 (by age class), and **B)** categorization C2 (by schooling/social). KG: kindergarten; GS: grade school; HS: high school; Uni: University.

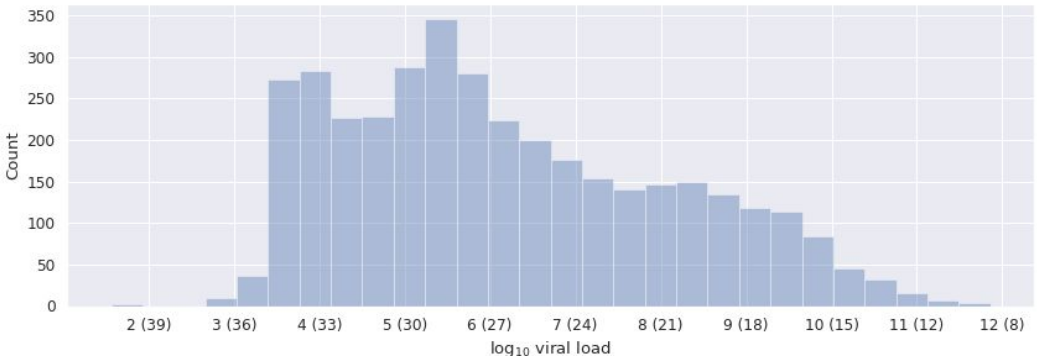

Figure 1: Histogram of viral loads: The plot shows the frequency distribution of 3,712 values of patient SARS-CoV-2 (logarithm base 10) viral load, estimated from real-time RT-PCR Ct values. The RT-PCR cycle corresponding to the logarithmic viral load is given in parentheses. The sharp drop on the left side of the distribution is due to RT-PCR sensitivity and the limit on cycles.

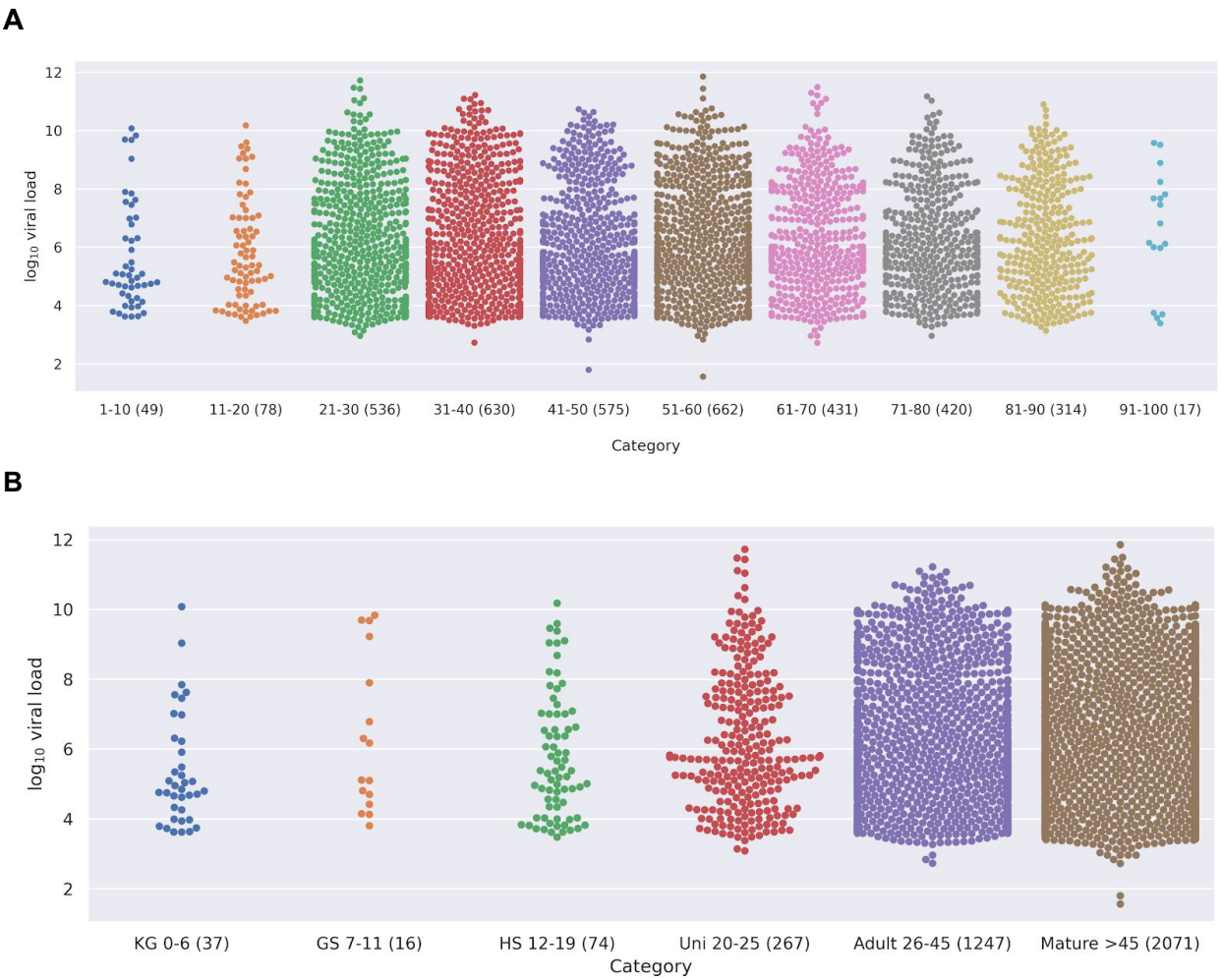

Figure 2: Viral load by patient category. A: categorization by 10-year age strata (C1): Patients were divided into categories based on age. The base 10 logarithm of viral load is estimated from the real-time PCR Ct value. Category counts are given in parentheses in the x-axis labels. **B: Categorization by schooling/social (C2):** Patients were divided into categories based schooling level, estimated on the basis of age. X-axis labels show the category (KG: kindergarten; GS: grade school; HS: high school; Uni: University), the age range in years, then the category count in parentheses.

Methods

Due to testing of some but not all positive cases by two RT-PCR targets, 3,712 of 59,831 (6.2%) patients had 5,285 positive results overall. In cases with more than one result, we selected the PCR result with the lowest Ct value. Results based on Light Cycler 480 PCR, as opposed to Roche 8800 or 6800, were chosen preferentially when results from more than one PCR system per patient was available (the latter systems were introduced in the laboratory during the observation period).

The following Python (version 3.8) software packages were used in the analysis and production of images: Scipy (version 1.4.1) (*16*), pandas (version 1.0.3) (*17*), researchpy (version 08/28/2018) (https://researchpy.readthedocs.io/en/latest/), statsmodels (version 0.11.1) (*18*), matplotlib (version 3.2.1) (*19*), numpy (1.18.3) (*20*), and seaborn (version 0.10.1) (*21*).

Viral load is estimated from Ct value based on the empirical formula $\log_{10}(8 * 10^{14} * e^{-0.745 * Ct})$. The formula is derived from testing a standard curve.

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Appendix

Categorization	Statistic	p value	Result
C1	0.959	2.713e-31	significant, not normally distributed
C2	0.957	8.563e-32	significant, not normally distributed

Table A1: Shapiro test for normal distribution.

Categorization	Statistic	p value	Result
C1	1.800	0.063	Not significant, equal variance
C2	2.302	0.042	Significant, unequal variance

Table A2: Levene's test for equality of variance.

Categorization	Statistic	p value	Result
C1	22.390	0.008	significant, a differing pair may exist
C2	14.969	0.010	significant, a differing pair may exist

Table A3: Kruskal-Wallis H test.

Categorization	Group 1	Group 2	Mean diff	p-adjusted	lower	upper	reject
C1	1	2	0.1608	0.9	-0.9385	1.2601	FALSE
	1	3	0.624	0.4633	-0.2761	1.524	FALSE
	1	4	0.5758	0.5617	-0.3186	1.4702	FALSE
	1	5	0.3472	0.9	-0.5503	1.2446	FALSE
	1	6	0.6205	0.4598	-0.2724	1.5133	FALSE
	1	7	0.6411	0.4379	-0.2681	1.5503	FALSE
	1	8	0.5365	0.6675	-0.3738	1.4469	FALSE
	1	9	0.7066	0.3171	-0.2197	1.6329	FALSE
	1	10	0.9714	0.7008	-0.7261	2.6689	FALSE

2	3	0.4631	0.5812	-0.2677	1.194	FALSE
2	4	0.4149	0.6989	-0.3089	1.1388	FALSE
2	5	0.1863	0.9	-0.5413	0.914	FALSE
2	6	0.4596	0.5755	-0.2623	1.1816	FALSE
2	7	0.4803	0.5549	-0.2618	1.2223	FALSE
2	8	0.3757	0.8309	-0.3678	1.1193	FALSE
2	9	0.5458	0.4159	-0.2172	1.3087	FALSE
2	10	0.8105	0.8371	-0.8036	2.4247	FALSE
3	4	-0.0482	0.9	-0.4026	0.3062	FALSE
3	5	-0.2768	0.314	-0.6389	0.0853	FALSE
3	6	-0.0035	0.9	-0.3539	0.3469	FALSE
3	7	0.0171	0.9	-0.373	0.4073	FALSE
3	8	-0.0874	0.9	-0.4804	0.3056	FALSE
3	9	0.0826	0.9	-0.3459	0.5112	FALSE
3	10	0.3474	0.9	-1.1382	1.833	FALSE
4	5	-0.2286	0.5354	-0.5764	0.1192	FALSE
4	6	0.0447	0.9	-0.291	0.3803	FALSE
4	7	0.0653	0.9	-0.3116	0.4423	FALSE
4	8	-0.0392	0.9	-0.4191	0.3407	FALSE
4	9	0.1308	0.9	-0.2858	0.5474	FALSE
4	10	0.3956	0.9	-1.0866	1.8778	FALSE
5	6	0.2733	0.2597	-0.0705	0.6171	FALSE
5	7	0.2939	0.313	-0.0903	0.6782	FALSE
5	8	0.1894	0.8622	-0.1977	0.5765	FALSE
5	9	0.3594	0.1781	-0.0637	0.7826	FALSE
5	10	0.6242	0.9	-0.8599	2.1083	FALSE
6	7	0.0206	0.9	-0.3526	0.3939	FALSE
6	8	-0.0839	0.9	-0.4601	0.2923	FALSE
6	9	0.0861	0.9	-0.3271	0.4994	FALSE
6	10	0.3509	0.9	-1.1304	1.8322	FALSE
7	8	-0.1046	0.9	-0.518	0.3089	FALSE
7	9	0.0655	0.9	-0.382	0.5129	FALSE

	7	10	0.3303	0.9	-1.1609	1.8215	FALSE
	8	9	0.17	0.9	-0.2799	0.6199	FALSE
	8	10	0.4348	0.9	-1.0571	1.9268	FALSE
	9	10	0.2648	0.9	-1.2369	1.7665	FALSE
C2	Adult	GS	0.2054	0.9	-1.1618	1.5726	FALSE
	Adult	HS	-0.3757	0.5574	-1.0259	0.2744	FALSE
	Adult	KG	-0.7879	0.1304	-1.6944	0.1186	FALSE
	Adult	Mature	0.0701	0.9	-0.1246	0.2649	FALSE
	Adult	Uni	0.1244	0.9	-0.242	0.4908	FALSE
	GS	HS	-0.5811	0.8701	-2.0793	0.917	FALSE
	GS	KG	-0.9934	0.503	-2.6193	0.6325	FALSE
	GS	Mature	-0.1353	0.9	-1.499	1.2285	FALSE
	GS	Uni	-0.081	0.9	-1.4796	1.3176	FALSE
	HS	KG	-0.4122	0.8885	-1.5063	0.6819	FALSE
	HS	Mature	0.4459	0.3561	-0.197	1.0887	FALSE
	HS	Uni	0.5001	0.3441	-0.2138	1.214	FALSE
	KG	Mature	0.8581	0.0728	-0.0432	1.7594	FALSE
	KG	Uni	0.9123	0.0701	-0.0409	1.8656	FALSE
	Mature	Uni	0.0543	0.9	-0.2991	0.4076	FALSE

Table A4: Tukey HSD post hoc analysis. No significant difference is found between any pair of subgroups in either of the two categorizations. KG: kindergarten; GS: grade school; HS: high school; Uni: University.

Categorization	Critical value	Result
C1	0.0011	No significant pairs
C2	0.0033	No significant pairs

Table A5: Bonferroni-adjusted pairwise post hoc T-tests. No significant difference is found between any pair of subgroups in either of the two categorizations.

	1	2	3	4	5	6	7	8	9	10
1	-1	1	0.588	1	1	0.499	0.425	0.829	0.301	1
2		-1	1	1	1	1	1	1	0.738	1
3			-1	1	0.589	1	1	1	1	1
4				-1	1	1	1	1	1	1
5					-1	0.322	0.335	1	0.216	1
6						-1	1	1	1	1
7							-1	1	1	1
8								-1	1	1
9									-1	1
10										-1

Table A6a: Dunn's post hoc test for categorization C1. No significant difference is found between any pair of subgroups in either of the two categorizations.

	Adult	GS	HS	KG	Mature	Uni
Adult	-1	1	0.996	0.128	0.847	1
GS		-1	1	0.847	1	1
HS			-1	1	0.455	0.549
KG				-1	0.045	0.056
Mature					-1	1
Uni						-1

Table A6b: Dunn's post hoc test for categorization C2. Just one inter-group comparison, Kindergarten vs Mature has a p value (0.045) less that the traditional 0.05 significance threshold. KG: kindergarten; GS: grade school; HS: high school; Uni: University.

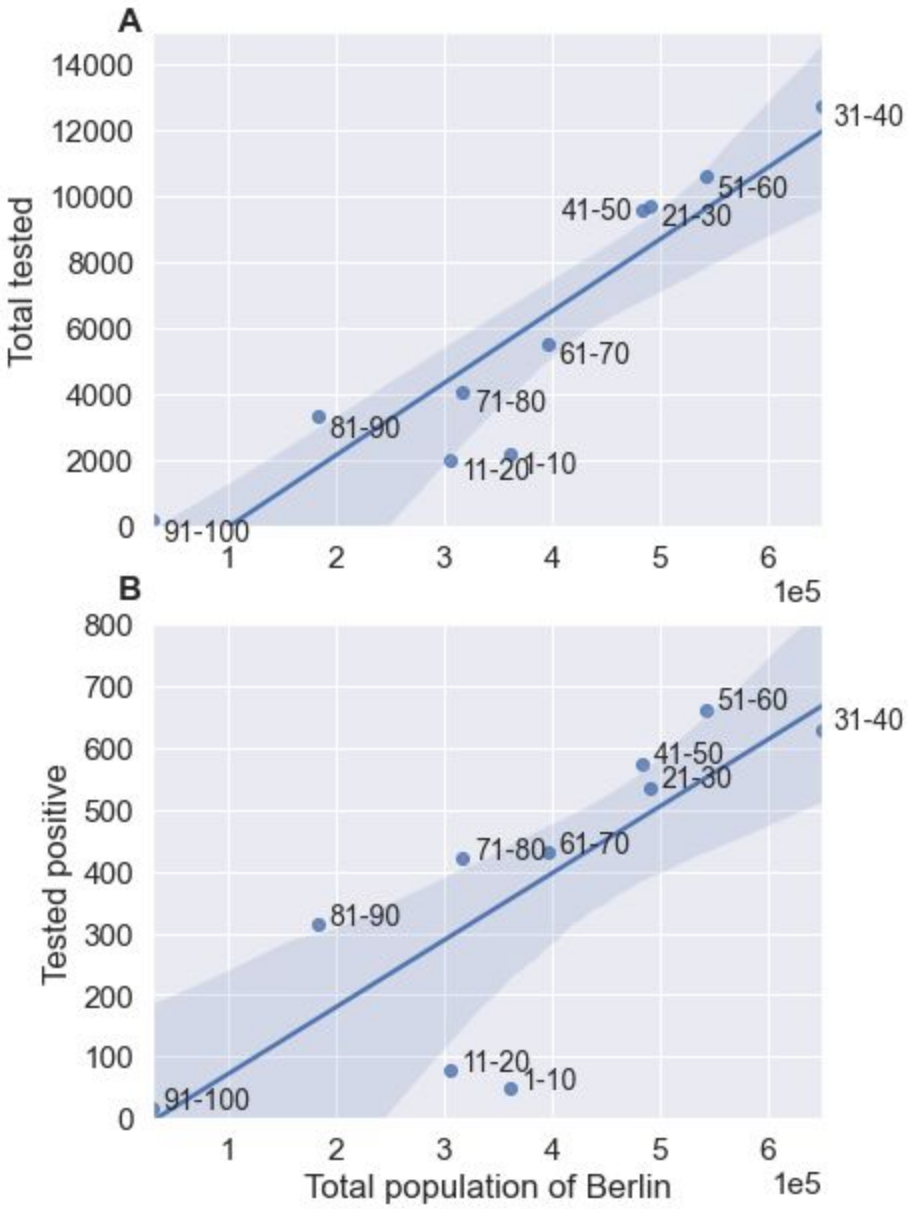

Figure A1: Positive age group counts versus population count. A) Total number of people tested for SARS-CoV-2 in each age group plotted against the total number of people in the corresponding age group in Berlin (acquired from Amt für Statistik Berlin-Brandenburg, https://www.statistik-berlin-brandenburg.de/, as of December 31, 2019). B) Number of people tested positive for SARS-CoV-2 plotted against the number of people in each age group in Berlin. Age categories 1-10 and 11-20 years have a relatively lower number of tested and positive cases. A linear regression is shown with the shaded area indicating the 95% confidence interval.

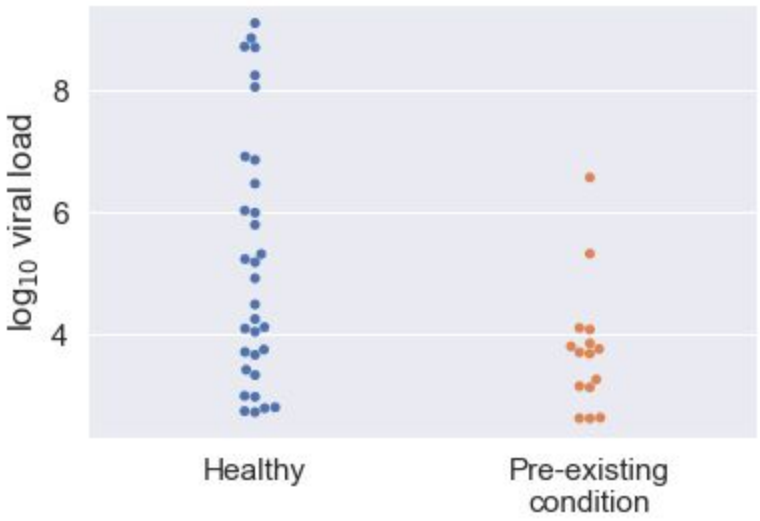

Figure A2: Differences in viral load in patients aged 1-11 years with and without a pre-existing condition. Wilcoxon rank-sum test indicates a significant difference between the two groups (p value 0.02).

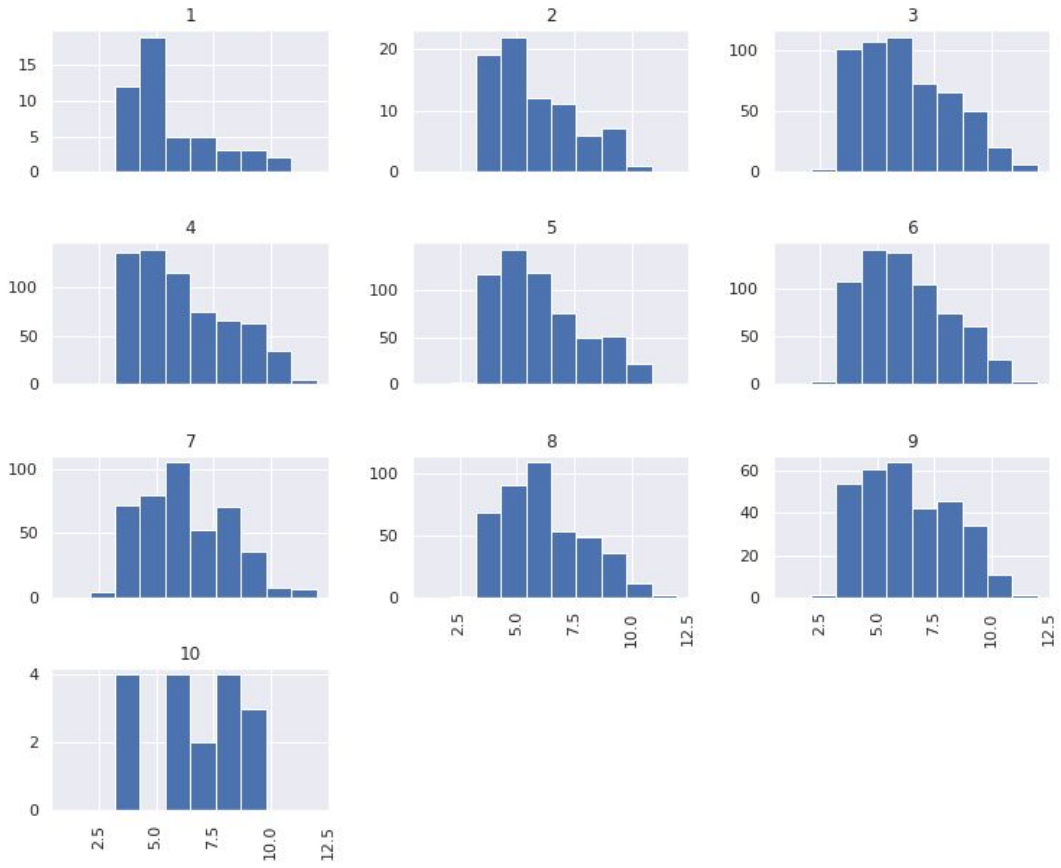

Figure A3: Per-group viral load histograms for categorization C1: The individual histograms for the ten groups of categorization C1 make it immediately clear that the underlying distribution of viral load for group 10 (91-100 years) is far from normal, and several other groups are clearly also not normally distributed. Note that the data above are also presented in Figure 2A, although there presented with viral load on the y-axis, with the distribution spreading horizontally in two directions, with added jitter for the spread visualization.

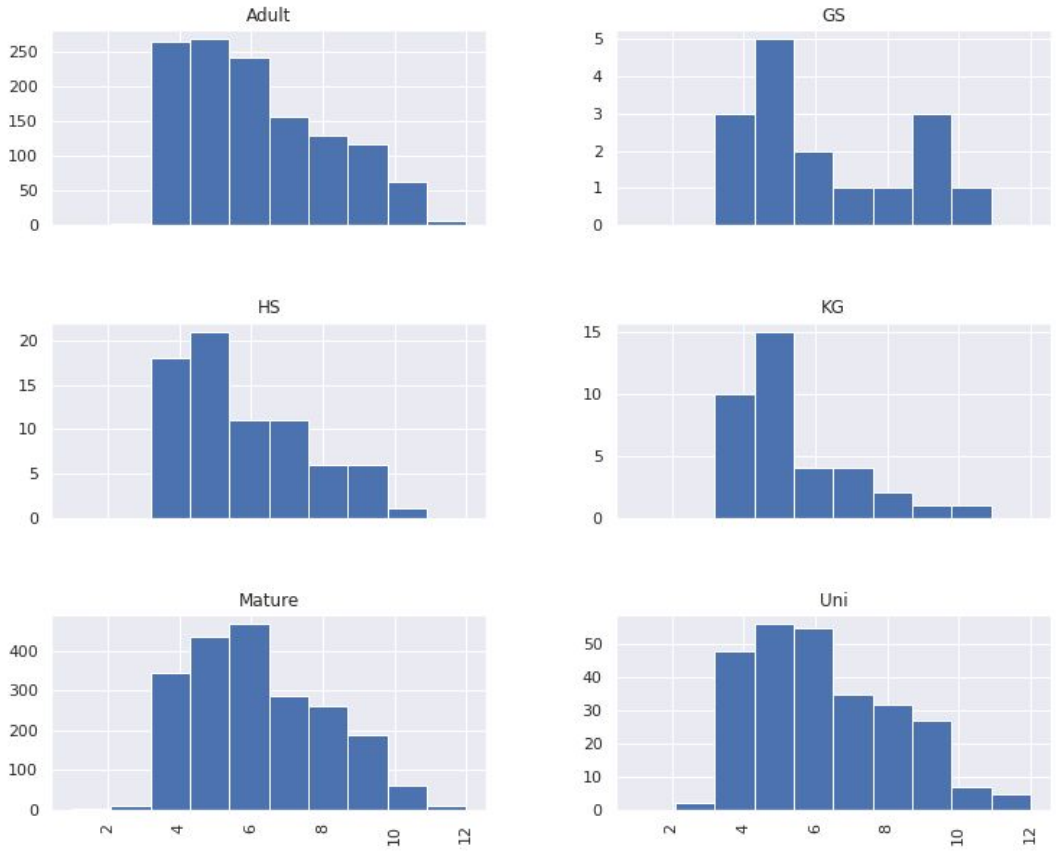

Figure A4: Per-group viral load histograms for categorization C2: The individual histograms for the six groups of categorization C2 make it immediately clear that the underlying distributions are not normal. Note that the data above are also presented in Figure 2B, although there presented with viral load on the y-axis, with the distribution spreading horizontally in two directions, with added jitter for the spread visualization.