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## HIV and SARS-CoV-2 coinfection - cross-sectional findings from a German 'hotspot' --Manuscript Draft--

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<b>Abstract:</b>	<p><b>Background:</b> The prevalence of SARS-CoV-2 coinfection in people living with HIV has not been investigated systematically. Based on several considerations it is, however, unclear if data from general populations can serve as a reference. This study aimed to determine the proportion of people living with HIV with anti-SARS-CoV-2 IgG-antibodies in a sample from a large single HIV center in Munich, Germany, after the first phase of the coronavirus pandemic and to infer about the prevalence in people living with HIV.</p> <p><b>Methods:</b> Prospective sub-study of the ongoing ArchIV cohort between May and July 2020. Anti-SARSCoV-2 IgG-antibodies were measured using the recomWell SARS-CoV-2 IgG ELISA (Mikrogen, Neuried, Germany). Demographic and medical data were extracted from the electronic patient files.</p> <p><b>Findings:</b> Overall, 500 people living with HIV were included in the study (83% male, median age: 51 years). Of those, 22 were found to be positive for SARS-CoV-2 IgG antibodies (0.044), resulting in an estimated seroprevalence (considering sensitivity and specificity of the test) of 5.1 % (CI95%: 3.17; 7.02) for the entire study sample, and 5.2 % (CI95%: 2.62; 7.69) for the subset of Munich citizens. Only two out of 22 PLWH (9.1%) with positive SARS-CoV-2 serology had previously been diagnosed with SARS-CoV-2 coinfection. The proportion of Caucasian people living with HIV was significantly smaller in the group with positive when compared to the group of negative test results (68.2% vs. 88.1%, p=0.021).</p> <p><b>Interpretation:</b> Anti-SARS-CoV-2 antibodies are frequently found in people living with HIV. Many people are olig- or asymptomatic but probably still able to pass the infection to others. Racial disparities seem to play a role in the risk of exposure to SARS-CoV-2 even in high-income areas.</p> <p><b>Funding:</b> Mikrogen GmbH (Neuried, Germany) provided the test kits used in this study. The funding source was not involved in any aspects of the study.</p>

# HIV and SARS-CoV-2 coinfection - cross-sectional findings from a German ‘hotspot’

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**Background:** The prevalence of SARS-CoV-2 coinfection in people living with HIV has not been investigated systematically. Based on several considerations it is, however, unclear if data from general populations can serve as a reference. This study aimed to determine the proportion of people living with HIV with anti-SARS-CoV-2 IgG-antibodies in a sample from a large single HIV center in Munich, Germany, after the first phase of the coronavirus pandemic and to infer about the prevalence in people living with HIV.

**Methods:** Prospective sub-study of the ongoing ArchHIV cohort between May and July 2020. Anti-SARS-CoV-2 IgG-antibodies were measured using the recomWell SARS-CoV-2 IgG ELISA (Mikrogen, Neuried, Germany). Demographic and medical data were extracted from the electronic patient files.

**Findings:** Overall, 500 people living with HIV were included in the study (83% male, median age: 51 years). Of those, 22 were found to be positive for SARS-CoV-2 IgG antibodies (0.044), resulting in an estimated seroprevalence (considering sensitivity and specificity of the test) of 5.1 % (CI95%: 3.17; 7.02) for the entire study sample, and 5.2 % (CI95%: 2.62; 7.69) for the subset of Munich citizens. Only two out of 22 PLWH (9.1%) with positive SARS-CoV-2 serology had previously been diagnosed with SARS-CoV-2 coinfection. The proportion of Caucasian people living with HIV was significantly smaller in the group with positive when compared to the group of negative test results (68.2% vs. 88.1%,  $p=0.021$ ).

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**Keywords:** COVID-19, antiretroviral therapy, Munich

# 1 Introduction

The global spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) beginning in December 2019 was declared a pandemic by the world health organization (WHO) in early 2020 and has infected more than 16,000,000 people worldwide and caused more than 650,000 deaths as of July 2020 [1]. The Munich area played a particular role in the global spread of the disease, with the first patient - the first in Germany - being diagnosed with the coronavirus disease 2019 (COVID-19) as early as January 2020, therefore becoming an early ‘hotspot’ of the disease outside China. It was also here that first evidence for the transmission of the disease via asymptomatic carriers emerged [2], which had not been considered before. While the risk of transmission was generally assumed to be high in SARS-CoV-2, it became clear that certain risk factors might contribute to acquisition or a more severe course of the disease: male sex, age, pulmonary and cardiovascular comorbidities have been identified as potential risk factors [3], among others. In contrast, the role of HIV-1 infection remains controversial due to the lack of data on this subject. As an immunodeficiency disease, HIV infection could be associated with a higher risk of acquiring COVID-19 and/or worse outcomes. However, due to the high efficacy of current antiretroviral therapies (ART), most people living with HIV (PLWH) are virologically suppressed and often show normal or only slightly diminished CD4 cell counts. Furthermore, several antiretroviral agents (ARVs) directed against the reverse transcriptase (RT), exhibit structural analogy to the novel nucleotide analogue remdesivir that has demonstrated moderate positive effects on the course of COVID-19 [4]. However, data on potential effects of modern ART-regimens (excluding lopinavir) on the course of COVID-19 is inconclusive [5, 6, 7]. Based on current knowledge, it is hence unclear if data from general populations can be extrapolated to PLWH.

The seroprevalence, defined as a prevalence derived from the number of positive serologic tests in a representative study sample after accounting for sensitivity and specificity of the test used, can give a deeper insight into the spread of SARS-CoV-2 between the outbreak and a defined time during the pandemic.

Therefore, the objective of our study was to determine the fraction of PLWH with anti-SARS-CoV-2 IgG-antibodies in a sample from a large single HIV-center in Munich after the first phase of the corona pandemic in Germany. We aimed to infer about the prevalence of SARS-CoV-2 coinfection in a population of PLWH and sought to investigate into factors associated with positive SARS-CoV-2 serology.

# 2 Methods

This study was performed as a prospective, cross-sectional sub-study of the Munich ArchHIV cohort, an ongoing cohort in PLWH in Munich, Germany, with approval of the local ethic’s committee dating May 28<sup>th</sup>, 2020. Patients attending the center for routine laboratory control for chronic HIV-1 infection (with or without treatment) between May 29<sup>th</sup> and July 15<sup>th</sup>, 2020 were consecutively asked to participate in the study. There were no exclusion criteria except for the unwillingness or inability to give written informed consent. After obtaining informed consent, an additional 8 ml serum blood sample was drawn. Patients were asked if they had been diagnosed with SARS-CoV-2 infection before, and if they were in contact with a person with confirmed SARS-CoV-2 infection. Physicians were asked to determine the ethnicity of each participating patient. Data on demographics, laboratory results, and ART were obtained from the electronic patient files. The primary outcome was the seroprevalence of SARS-CoV-2 coinfection in PLWH, approximated by the number of PLWH tested positive for anti-SARS-CoV-2 IgG antibodies:

Assuming that the number of PLWH tested positive is the sum of ‘true’ positive ( $T_+|D_+$ ) and ‘false’ positive ( $T_+|D_-$ ) results, with conditional probabilities  $p_{(T_+|D_+)} = \text{sensitivity}$  and  $p_{(T_+|D_-)} = 1 - \text{specificity}$ , the seroprevalence was estimated using the following equation derived from Bayes’ theorem:

$$SP = \frac{\frac{n_{pos}}{n} + spec - 1}{sens + spec - 1}$$

where SP = seroprevalence, n = number of patients tested,  $n_{pos}$  = number of patients tested positive, sens = sensitivity of the test, and spec = specificity of the test. As many PLWH at the study site are living outside Munich, the analyses were performed for the overall study sample as well as only those participants living in

Munich. Other predefined variables of interest were age, sex, ethnicity, viral load, and CD4 cell count. Anti-SARS-CoV-2 IgG-antibodies were determined using the recomWell SARS-CoV-2 IgG ELISA (Mikrogen, Neuried, Germany), that was found to have a sensitivity and specificity of 86.4% and 100%, respectively [8]. We performed a sensitivity analysis assuming higher sensitivities between 88.2% and 96.8% at a specificity of 99.0%, as reported in a preprint of a recent analysis [9]. Results of the ELISA were reported as ‘positive’, ‘negative’, or ‘intermediate’, where ‘intermediate’ results were considered ‘negative’ for the purpose of this study. To account for the influence of these results, a sensitivity analysis was performed, assuming different fractions of these tests to be actually ‘positive’ in order to investigate the consecutive change of the seroprevalence. To investigate possible (selection) bias due to the study design, a comparison to the overall population of PLWH under permanent medical care of the study site (referred to as PLWH population subsequently) was performed, including all patients attending the study site within the last year prior to termination of the current study. To assess the geographic representativeness of the study sample, the fraction of PLWH (with 95% confidence intervals [CI95%]) living in each of 26 districts of Munich was determined and compared to the fraction of the overall population for each district. As enrollment of 500 PLWH would allow for detection of a seroprevalence of up to 15% with a precision of a maximum of about 3%. We therefore planned to enroll 500 PLWH. Descriptive statistics were performed using means with standard deviations (SD) (for the comparisons of the study sample with the PLWH population) or medians with interquartile ranges (IQR) (for the comparison of PLWH with positive and negative SARS-CoV-2 serology) for continuous variables; frequencies (absolute and relative) were used for categorical variables. Likewise, t-test and Mann-Whitney test were used for comparison of continuous variables, and Fisher’s exact test for categorical variables. Unless otherwise declared, p-values <0.05 were considered to be statistically significant. Statistical analysis was performed using R 4.0.0. The manuscript was written in accordance with the ‘Strengthening the Reporting of Observational Studies in Epidemiology’ (STROBE) statement [10].

### 3 Results

In total, 500 PLWH were included in this study. The baseline characteristics of the study sample together with a comparison to the overall PLWH population under regular medical care at the study site can be found in table 1. 75.2%, 6.6%, 29.4%, and 10% were on an integrase inhibitor-, protease inhibitor-, non-nucleoside reverse transcriptase inhibitor-, and TDF-containing regimen, respectively; 172 (34.4%) were on a two-drug regimen.

In the study sample, 437 (87.4%) and 28 (5.6%) were of Caucasian and African ethnicity, respectively. 3 (0.6%) reported having been tested positive for SARS-CoV-2 before and 29 (5.8%) reported prior contact to a person with confirmed COVID-19. Overall, 22 PLWH were tested positive for anti-SARS-CoV-2 IgG-antibodies, corresponding to a fraction of 0.044. Assuming a sensitivity of 86.4% and a specificity of 100% [8], the estimated seroprevalence in a PLWH population as represented in our study was 5.1 % (CI95%: 3.2; 7). From the 22 PLWH tested positive, only two had been diagnosed with COVID-19 before (9.1%).

A subgroup analysis was performed for those participants with residency in Munich (n = 292). Within Munich, 13 PLWH (0.045) were tested positive. Therefore, the prevalence for the Munich PLWH population was estimated to be 5.2 % (CI95%: 2.6; 7.7). The results of the analysis of the geographic representativeness of the Munich study sample when compared to all PLWH in clinical care at the study site is shown in figure 1 and table 2.

Sensitivity analysis on the impact of changes of sensitivity and specificity on the PLWH population prevalence of SARS-CoV-2 coinfection was performed, assuming a sensitivity ranging between 88.2% and 96.8%, with a specificity of 99.0% [9], resulting in an estimated prevalence between 3.6% (CI95%: 1.9; 5.2) and 3.9% (CI95%: 2.2; 5.6).

Results of our sensitivity analysis for different fractions of ‘intermediate’ results being actually ‘positive’ in the overall study sample is displayed in figure 2. Assuming an equal distribution of ‘truly’ ‘positive’ and ‘negative’ among the ‘intermediate’ test results, the prevalence on the PLWH population level was found to be 6% (CI95%: 3.9; 8.1).

## 4 Discussion

To the best of our knowledge, we herein present the first data on the seroprevalence of SARS-CoV-2 coinfection in a representative sample of PLWH, derived from a large single HIV-center cohort in Munich, Germany, one of the first European ‘hotspots’ in the SARS-CoV-2 pandemic outside China. Based on the number of positive test results, after accounting for sensitivity and specificity of the test used, the seroprevalence of SARS-CoV-2 coinfection in a population of PLWH as represented by our sample is estimated to be 5.1% (CI95%: 3.2; 7). The ‘true’ prevalence could be even higher, depending on the meaning of the ‘intermediate’ test results and be as high as 7.2% (2).

As many of the participating PLWH were not from Munich, the subset with residency in Munich was analyzed separately for a more homogeneous baseline risk of acquiring SARS-CoV-2 coinfection. From the Munich-only study sample, the PLWH population seroprevalence was estimated to be, similar to the overall seroprevalence, 5.2 % (CI95%: 2.6; 7.7). This exceeds the cumulative prevalence derived from the number of reported cases for the city substantially: in Munich, at the time of the end of our study, overall 6,964 people out of a population of 1,561,720 had been tested positive, resulting in a cumulative prevalence of only 0.45% [11]. Therefore, the estimated prevalence derived from our study was about 12-fold higher. Yet, these findings are most likely not attributable to HIV-infection but represent a discrepancy that has been found before, with the seroprevalence being more than ten-fold higher than estimated by the reported [12, 13]. Also, within our sample, among 22 subjects with positive serologic results, only two PLWH reported having been tested positive for COVID-19 by swabs before (both in the presence of symptoms), which could be proven from the electronic patient files. One additional patient with negative IgG-antibodies reported having been tested positive for SARS-CoV-2 during an episode of chills, fever, and coughing about 15 weeks prior to enrollment in the study, which could also be confirmed from the electronic patient file. These results are pointing at a relevant proportion of PLWH with seemingly subclinical SARS-CoV-2 coinfection or at least exposure, that might be unaware of the infection but still be able to spread it.

When comparing findings from PLWH with positive and negative SARS-CoV-2 serology, ethnicity was the most significant difference between both groups, with a higher proportion of people with non-Caucasian ethnicity in the ‘positive’ group. This might have been driven by the higher frequency of African-descent PLWH in the group tested positive for anti-SARS-CoV-2 IgG-antibodies. While resulting from an overall low number of positive tests, our findings are, however, in line with the ethnic disparity in the burden of SARS-CoV-2 infection that has been addressed before [14]. Of interest, the four African-descent PLWH that were tested positive for SARS-CoV-2 have only been living in Germany in the first generation and have come to Germany as refugees. This could have had implications on the access to medical care, and housing conditions.

An alternative explanation for the higher proportion of non-Caucasian PLWH in the group of subjects tested positive might be racial differences in the humoral immune response to SARS-CoV-2 exposure. However, there is not enough data to support this at this very moment. Interestingly, we also observed a trend for a higher proportion of PLWH with detectable viral load in the anti-SARS-CoV-2 positive sample, although this for sure does not allow to conclude on causality. While, based on what was said before, we also observed a trend towards a higher likelihood of having detectable viral load for African PLWH (data not shown), too few events (positive test results) made further adjusting unreliable.

As every observational study, ours might have been prone to unmeasured confounders. Our study was limited to PLWH attending the clinic for routine clinical care during a short period of time, which might have resulted in a selection bias. However, comparing the study sample with the overall PLWH population yielded in very similar characteristics, and we therefore assume no major bias in our sample. This was also true for the geographic representativeness, at least in the Munich study subset (figure 1, table 2). We were not able to adequately compare ART status and the distribution of different ART regimens within the PLWH population, as the documentation of those parameters for the last year has not been finished yet (as evident from the high and equal numbers of missing data for transmission risk and being ART naive). Also, ethnicity was not available for the whole PLWH population. It is reasonable to assume overdispersion for our data due to the partly clustered nature (on several levels, from couples and people tested from the same household, to similar exposures within the MSM community or probably within certain ethnic groups) that we did not account for. Although this might not have a large impact on the point estimates, it is likely that the confidence intervals might have been underestimated. We do, however, consider few tests to derive from clusters and

therefore assume no relevant influence. It is important to keep in mind, that the sole use of IgG-antibodies could have missed COVID-19 infections in their early phases. However, at the time the study was carried out, infection rates were low and therefore this should not have been of substantial impact on the overall results. Additionally, we did not perform a confirmation test for the positive results (available as recomLine SARS-CoV-2 IgG), which might also have led to a slight overestimation of the prevalence of SARS-CoV-2 coinfection.

On the other hand, not every subject with a history of SARS-CoV-2 infection seems to develop specific antibodies and also at least in some subjects, specific antibodies could disappear as early as three months after an infection [15]. This might also explain the negative serostatus of one of our study participants 15 weeks after confirmed COVID-19. Therefore, the prevalence derived from the data of this study might slightly underestimate the ‘true’ prevalence. This could particularly be true, as a fraction of ‘intermediate’ test results might ‘truly’ be ‘positive’ and therefore add to the PLWH population prevalence, as demonstrated in our sensitivity analysis. The generalizability of our data might be limited by a different epidemiology of SARS-CoV-2 infection in different areas in the world, but also within Germany itself. However, it might offer the possibility to compare data from PLWH to a representative sample from Munich as a whole with the results from the prospective Munich COVID-19 cohort [16], expected to be published soon. This comparison, in turn, could serve as the first of its kind and help us to understand whether or not PLWH are at excess risk of (co-)infection with SARS-CoV-2 and to compare their manifestation index.

## 5 Conclusion

The seroprevalence of SARS-CoV-2 coinfection in our sample of PLWH was much higher than expected from confirmed cases in Munich, hinting at a low manifestation index of SARS-CoV-2 infection in PLWH (and probably also in general). We observed ethnic disparities also among PLWH in Germany, that it seems important to address in order to reduce harm and minimize the spread of COVID-19, particularly in vulnerable populations. Furthermore, detectable viral load could serve as a surrogate marker for risk of SARS-CoV-2 infection. These findings require further investigations. With the prospect of data on the seroprevalence of SARS-CoV-2 infection from over 3,000 random Munich households being available soon from the prospective Munich COVID-19 cohort [16], our data will hopefully offer the first possibility for a head-to-head comparison of people living with and without HIV from representative samples of the same city.

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### Authors’ contributions:

SN was engaged in developing the study idea and protocol, recruiting patients, data collection and organization, statistical analysis, writing, and revising the manuscript.

FS, SH, KR, AK, and CW were engaged in patient recruitment and revising the manuscript.

WM and FT were responsible for laboratory analysis and revising the manuscript.

AB and EG was engaged in data collection, organization, preparation, and analysis.

CJ and HJ were engaged in developing the study idea and protocol, recruiting patients, writing, and revising the manuscript.

EW was engaged in developing the study idea and protocol, data collection and organization, statistical analysis, writing and revising the manuscript.

## **Declaration of interest:**

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SN reports honoraria and/or grants for research or travel from Gilead Sciences, GSK, MSD Sharp & Dohme, Hexal, Hormosan Pharma, HRA Pharma, Ipsen Pharma, Janssen-Cilag, Shire Pharmaceuticals, and ViiV Healthcare.

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KR reports grants for travel from Abbvie and Gilead Sciences.

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HJ reports honoraria and/or grants for research or travel from Abbvie, Gilead Sciences, GSK, Janssen-Cilag, and ViiV Healthcare.

EW reports honoraria and/or grants for research or travel from Abbvie, Gilead Sciences, GSK, Janssen-Cilag, and ViiV Healthcare.

WM, FT, AK, CW, AB, and EG have nothing else to disclose.

Table 1: Demographic data of 500 PLWH included in this study in comparison to the PLWH population, defined as all PLWH attending the study site within the previous year. Asterisks marking population parameters differing significantly from the sample estimates on an  $\alpha=0.05$  level.

	Study sample (n =500)			PLWH population (n = 2728)		
			[CI 95%]			
<b>Age [years], mean (SD)</b>	50	(11)	[49-51]	48	(13)	*
missing, n	0			0		
<b>Male, n (%)</b>	415	(83)	[79.4-86.1]	2173	(79.7)	
missing, n	0			0		
<b>Munich citizens, n (%)</b>	292	(58.4)	[53.7-62.5]	1595	(58.5)	
missing, n	0			0		
<b>Homosexual transmission, n (%)</b>	232	(46.4)	[49.1-58.0]	997	(50.2)	
missing, n	0			741		
<b>ART naive, n (%)</b>	7	(1.4)	[0.6-3.0]	15	(0.8)	
missing, n	0			741		
<b>Patients with viral load &lt;50 copies/mL, n (%)</b>	466	( 93.2 )	[90.5-95.2]	2499	(91.6)	
missing, n	0			0		
<b>CD4 cells [cells/<math>\mu</math>L], mean (SD)</b>	752	(295)	[745-759]	731	(315)	*
missing, n	0			0		



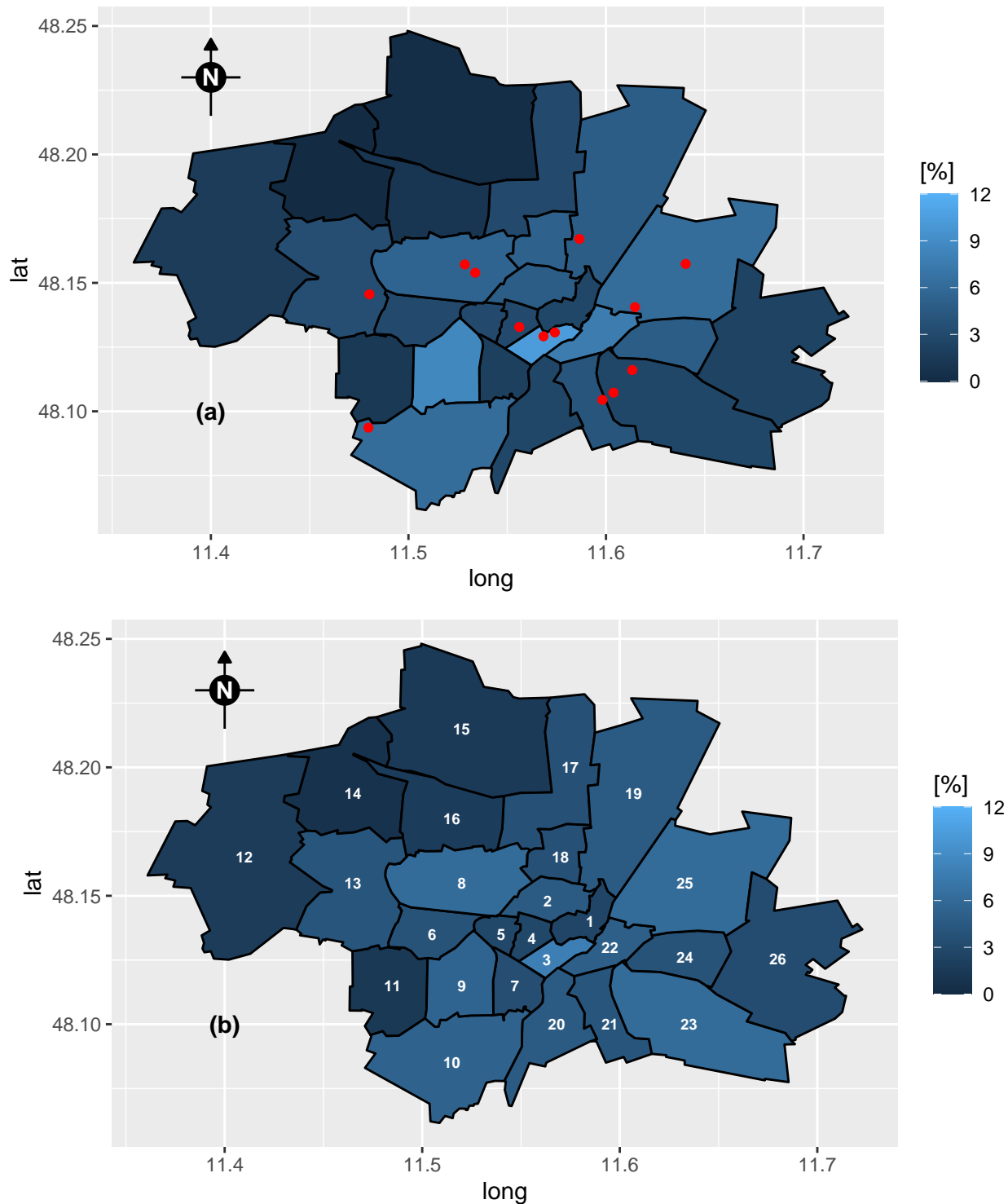


Figure 1: Comparison of the percentage of PLWH in each district from (a) the study sample and (b) the overall PLWH population from the study site in Munich. Red dots in (a) are indicating the residence of PLWH tested positive for anti-SARS-CoV-2 antibodies in the study sample. Numbers in (b) are referring to the identification (numbering) of the districts, corresponding to the district number in table 2.

Table 2: Percentages of PLWH living in each of 26 districts of Munich in the study sample (with CI 95%) as well as the overall PLWH population in medical care at the study site. Asterisks marking population parameters differing significantly from the sample estimates on an  $\alpha=0.05$  level.

District	Study sample		PLWH population
	%	[CI 95%]	%
01	2.7	[ 1.2 - 5.3 ]	2.6
02	4.8	[ 2.6 - 7.9 ]	4.6
03	10.3	[ 7.0 - 14.3 ]	7.9
04	3.1	[ 1.4 - 5.8 ]	2.8
05	3.1	[ 1.4 - 5.8 ]	2.8
06	3.1	[ 1.4 - 5.8 ]	3.9
07	2.1	[ 0.8 - 4.4 ]	3.4
08	5.5	[ 3.2 - 8.7 ]	6.0
09	8.6	[ 5.6 - 12.4 ]	5.5 *
10	6.2	[ 3.7 - 9.6 ]	5.3
11	1.4	[ 0.4 - 3.5 ]	1.4
12	1.7	[ 0.6 - 4.0 ]	1.7
13	3.4	[ 1.7 - 6.2 ]	4.1
14	0.0	[ 0.0 - 1.3 ]	0.7
15	0.3	[ 0.0 - 1.9 ]	1.6
16	1.0	[ 0.2 - 3.0 ]	1.8
17	3.1	[ 1.4 - 5.8 ]	3.6
18	5.1	[ 2.9 - 8.3 ]	3.6
19	4.8	[ 2.6 - 7.9 ]	4.6
20	2.7	[ 1.2 - 5.3 ]	4.6
21	4.1	[ 2.1 - 7.1 ]	4.1
22	7.5	[ 4.8 - 11.2 ]	5.0
23	2.7	[ 1.2 - 5.3 ]	6.0 *
24	4.8	[ 2.6 - 7.9 ]	3.9
25	6.2	[ 3.7 - 9.6 ]	6.0
26	2.4	[ 1.0 - 4.9 ]	3.3

Table 3: Comparison of characteristics between the groups of PLWH with positive and negative anti-SARS-CoV-2 serostatus. Due to the marked differences in the sizes of both groups, frequencies are only displayed as percentages instead of absolute numbers. Asterisks marking p-values <0.05.

	Serostatus		p-value
	positive (n=22)	negative (n=478)	
age, years, median (IQR)	46 (37;56)	51 (43;57)	0.163
male sex, %	86.4	82.7	1.000
Munich citizens, %	59.1	58.2	1.000
Homosexual transmission risk, %	45.5	53.7	0.576
Caucasian ethnicity, %	68.2	88.1	0.021 *
African ethnicity, %	18.2	5.1	0.045 *
Viral load below detection, %	81.8	93.8	0.060
CD4 cells [cells/ $\mu$ L], median (IQR)	657.5 (457;922)	719.0 (559;923)	0.546

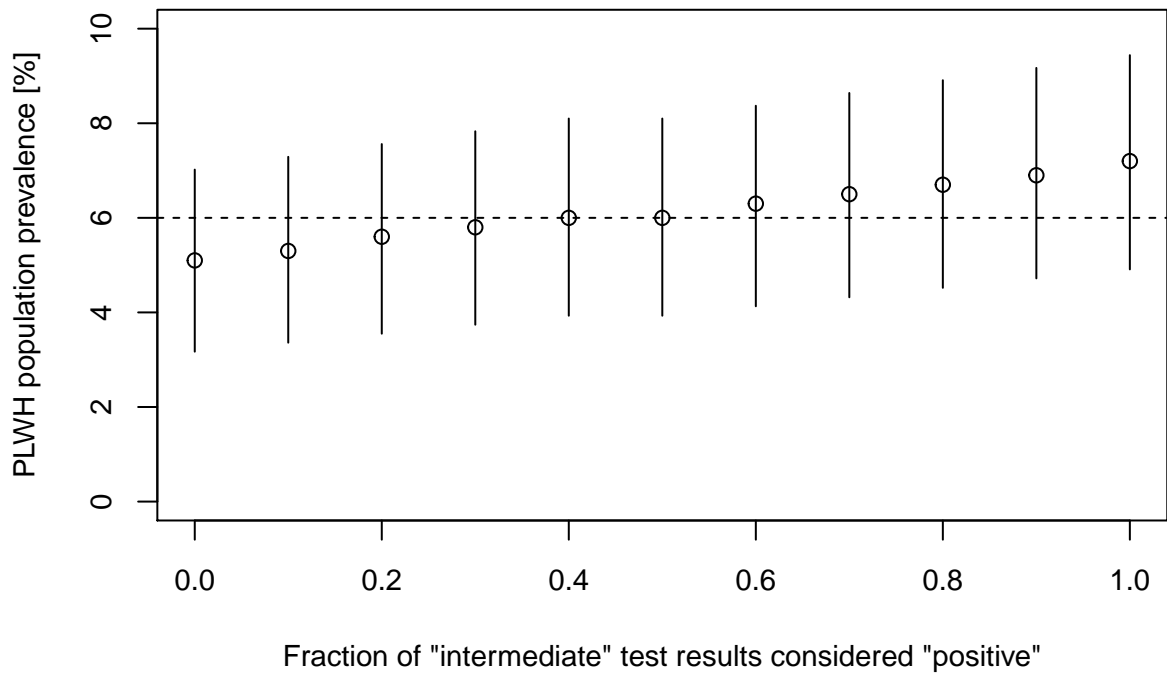


Figure 2: Sensitivity analysis of the impact of different fractions of ‘intermediate’ test results considered ‘truly’ positive on the estimated PLWH population prevalence (with CI 95%). The dashed line indicates the estimated PLWH population prevalence for equal distribution between being ‘truly’ positive and ‘truly’ negative within the group of ‘intermediate’ test results.

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