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Research note

Prevalence of SARS-CoV-2 IgG antibodies in a large prospective cohort study of elite football players in Germany (May–June 2020): implications for a testing protocol in asymptomatic individuals and estimation of the rate of undetected cases

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ABSTRACT

Objectives: Elite professional football players and staff are a unique group that might give insight into the epidemiology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in Germany and thus can serve as a model for geographical distribution and an estimation of undetected infections.
Methods: In this prospective cohort study seroprevalence was determined twice in May and June 2020 in players and staff from the German Bundesliga. As screening assays, a commercial ELISA (Euroimmun) and a chemiluminescent immunoassay (CLIA) (Roche) were used, and an in-house neutralization assay (NT) was used as reference standard. Participants were tested twice weekly using PCR from nasopharyngeal and/or oropharyngeal swabs.
Results: Seroprevalence (NT used as confirmation) in 2164 samples from 1184 players and staff was rather similar in May (23/1157, 1.99%) and June (21/1007, 2.09%). All participants were PCR-negative during the study period. Significant regional differences in seroprevalence were not observed. When comparing seroprevalence with the cumulative incidence of infections derived from the German notification system (subgroup matching to cohort; men, age 20–69 years), IgG was found eight to ten times more frequently, pointing to a high rate of undetected infection. ELISA and CLIA correlated only moderately (κ 0.52).
Conclusions: Seroprevalence with a high-quality diagnostic in Germany seemed to be around 2%. The number of undetected infections seems to be eight to ten times higher than in notification data. The quality of antibody assays is rather variable, thus results should ideally be confirmed at least by a second assay to prove IgG positivity. **Dietrich Mack, Clin Microbiol Infect 2021;27:473.e1–473.e4**

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pandemic virus causing mainly a respiratory disorder (coronavirus disease 2019, COVID-19). Prevalence of infection, its local distribution, and the number of undetected infections compared to notification data are important factors in epidemiology. One shutdown measure was to suspend German football. However, the Bundesliga and Bundesliga 2, with more than 1700 players and staff, established a special hygiene concept with matches behind closed doors in mid-May [1,2].

In this study we determined the prevalence of SARS-CoV-2 IgG in more than 1000 players and staff of the Bundesliga at two time points with two different screening assays and a neutralization

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assay (NT) as confirmation. Comparison with notification data enabled us to estimate the number of undetected infections.

Methods

Individuals

In this 2-month prospective observational study all 36 professional soccer teams in Germany—players and staff in direct contact with players (e.g. coaches, physiotherapists)— were invited. Exclusion criteria were lack of written informed consent. Primary outcome was IgG positivity. All participants were tested twice weekly using nasopharyngeal and/or oropharyngeal swabs by PCR starting at least 2 weeks prior to the first serum sampling [2].

Ethical approval

Ethical approval was obtained from the Landesärztekammer Rheinland-Pfalz, Germany (Registration 2020-15023_2). All participants provided written informed consent.

Assays

Samples were tested using two screening assays. The EUROImmun anti-SARS-CoV-2 ELISA (IgG) (EUROimmun, Lübeck, Germany) and the Cobas Elecsys Anti-SARS-CoV-2 chemiluminescent immunoassay (CLIA) (Roche, Mannheim, Germany) were processed according to the manufacturers' instruction.

An NT was employed for confirmation in all samples that tested equivocal or positive in at least one of the two screening assays. In the case of paired samples with reactivity in only one of them, both samples were retested by NT. Details of the NT are described elsewhere [2]. Samples with titre ≥1:16 were considered positive.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8.0 including Cohen's κ coefficient (agreement between assays) and χ^2 test (prevalence in geographical regions). Receiver operating characteristic (ROC) curves were calculated (method: http://www.jrocfit.org).

Results

Three out of 36 teams refused to take part since players had already been tested earlier. One team provided sera only during the first testing for logistic reasons. Some individuals took part only once for personal reasons. Altogether, 812 players (sample pair 694, single sample 118) and 372 staff members (sample pair 291, single sample 81) participated (Supplementary Material Table S1 and Fig. S1). During the study period (14 days prior to first sampling until last sampling) none had a positive PCR despite twice weekly testing, thus seroconversions were not expected [2].

When using the NT as confirmation, 23/1157 samples (1.99%) taken in May and 21/1007 (2.09%) in June tested positive. Teams were located over the whole of Germany (Supplementary Material Fig. S2). Prevalence was rather similar between the different regions and time points (p 0.78) (Table 1).

When comparing the IgG prevalence with the regional notification data (irrespective of age and sex) the number of undetected infections could be estimated to be nine to ten times higher (range 4–21 times, Table 1) [3]. When using notification data from a subgroup that matches the cohort most closely (males, age group 20–69) the cumulative incidence according to notification data was 217/100 000 and 247/100 000 for calendar weeks 18 and 24, respectively [4,5]. This corresponds to a number of undetected infections of factor 8 or 9 compared to notified infections, respectively.

Using ELISA, 36/59 positive samples and 5/33 equivocal samples were positive on NT. On CLIA, 36/38 samples were confirmed. Only 33 samples were congruently positive with both screening assays, and all tested positive on NT (Table 2; Supplementary Material Table S2 and Fig. S3).

In paired sera, the NT showed one reversion (1:16 to negative). On CLIA, results were identical in pairs. In contrast, with ELISA 28 seroconversions and two reversions occurred (Supplementary Material Table S3). Not surprisingly, the screening assays correlated only moderately (κ 0.52, CI 0.41–0.63).

When using the NT as confirmation these data translate into the test characteristics displayed in Supplementary Material Table S4 and to the ROC analysis in Supplementary Material Fig. S4.

Discussion

The prevalence was around 2% in this unique cohort with more than 2000 samples from elite football players and staff in mid-May and end of June, and no confirmed seroconversions (with NT) were found. This is in line with the fact that participants had to adhere to a strict hygiene concept (e.g. home quarantine, distancing and masks whenever possible) during that time, and none had a positive PCR despite twice-weekly PCR testing [1,2]. Moreover, the incidence during that lockdown period was rather low (7-day incidence in Germany <10/100 000 inhabitants) [5]. The prevalence in Germany found by other studies ranged between 0.91% in

Table 1
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG prevalence according to the region in Germany and the time point. Estimation of a factor of unreported infection

	IgG prevalence first sample (first 2 weeks of May)	IgG-prevalence second sample (last week of June 2020)	Cumulative incidence per 100 000 inhabitants[a] 1st May/15th June	Estimated factor of unreported infections [b] 1st May/15th June
Region North	4/222 (1.8% (0.7–4.5))	4/217 (1.8% (0.7–4.6))	136/170	14/11
Region West	11/430 (2.6% (1.4–4.3))	10/368 (2.7% (1.5–4.9))	168/199	19/16
Region East	3/155 (1.9% (0.7–5.5))	3/99 (3.0% (1.0–8.5))	125/164	14/21
Region South	5/350 (1.4% (0.6–3.4))	4/323 (1.2 % (0.5–3.1))	307/342	5/4
Germany	**23/1157 (1.99% (1.33–2.97))**	**21/1007 (2.09% (1.37–3.17))**	**204/237**	10/9

Samples were considered positive when the neutralization assay (NT) revealed a positive titre (≥1:16). For the regions and the location of the teams see Supplementary Material Fig. S2.

[a] Notification data (irrespective of age and sex) by the Robert Koch Institute from federal states of the respective region [3]. Data from federal states without teams were excluded (see Supplementary Material Fig. S2). Dates used for calculation were 14 days prior to first and second sampling, since IgG production should be expected after this period. Thus, IgG prevalence on 15th May corresponds to the cumulative incidence in notification data of 1st May.

[b] Factor of unreported infections was calculated using the cumulative incidence compared to the respective IgG prevalence.

Table 2
Results of the confirmatory neutralization assay in relation to both screening assays. Samples that tested negative in screening assays were included since they belong to serum pairs (see Methods)

Results of pretesting			Results of confirmation			
ELISA	CLIA	Number of samples	NT	Number of samples	Minimum titre	Maximum titre
Negative	Negative	23	Negative	22		
			Positive	1[a]	1:16	1:16
Equivocal	Negative	33	Negative	28		
			Positive	5	1:16	1:16
Positive	Negative	26	Negative	23		
			Positive	3	1:16	1:16
Negative	Positive	5	Negative	3		
			Positive	2	1:64	1:64
Positive	Positive	33	Negative	0		
			Positive	33	1:64	1:≥ 1024

[a] This sample was the first of a pair with an identical neutralization assay titre in the second sample and a conversion from negative to equivocal in ELISA, whereas in chemiluminescent immunoassay (CLIA) both samples tested negative.

blood donors (March–June) [6] and 1.2% in asymptomatic outpatients (26th March to 4th June) [7] to 15.5% (31st March to 6th April) in a hotspot area [8].

No profound geographical differences were found in seroprevalence, indicating a rather equal distribution. Using the overall cumulative incidence defined by regional notification data (no differentiation between age and sex) a rate of undetected infections of around factor 9–10 (range 4–21) was found. When using the notification data from only men of the age group 20–69, an almost similar factor of 8–9 could be calculated for the entire cohort. It should be kept in mind that this factor is influenced not only by the rate of asymptomatic infection but also by the availability of the test capacities, which was problematic at the beginning of the pandemic. Estimations of the number of undetected infections have rarely been done so far. A study using data from Austria and Iceland found an almost similar factor of 8.35 [9], whereas a factor of 5 was determined in a hotspot area [8].

The correlation between the screening assays was rather low (κ 0.52). CLIA results seemed to correlate better with NT and to be more plausible than ELISA since ELISA had a high rate of unexpected seroconversions. Our data suggest that the screening assays must not be used as a standalone diagnostic tool for defining IgG positivity.

One limitation of this study is that our cohort did not match the German population in all aspects. Women were under-represented; however, the rate of infection is rather similar between the sexes [5]. Moreover, the age group does not match completely. This is why notification data of a closely matching group were used to determine the rate of undetected infections.

The NT was not applied in all sera but only to a selection of mainly positive sera. The rate of false and true negatives might have been different had all sera been tested; however, the rates of true and false positives are not affected by this approach. In addition, we assumed that only persons with detectable neutralizing antibodies were true positives, possibly underestimating the performance of the screening assays.

In conclusion, prevalence of SARS-CoV-2 IgG in the cohort seemed to be around 2% (May and June 2020) without significant regional differences. Importantly, the number of undetected infections seems to be eight to ten times higher than in notification data. Positive IgG results should ideally be confirmed by at least another assay when used as a marker for immunity.

Transparency declaration

DM, KD, and OH are employees of Bioscientia Labor Ingelheim, which performed antibody testing, and received a grant for this study. AR, JK and DL have no conflicts of interest to declare.TM, WK, BG were members of the DFL task force and received a fee from the DFL for their work in the task force. BG received lecture fees from Roche, and DM from DiaSorin. The study was funded by the DFL (Deutsche Fußball Liga, Germany). The DFL had no access to the data and had no influence on analysis or the manuscript.

Access to data

The authors have full access to the anonymized data.

Author contributions

DM, BG, OH, WK, and TM designed the study. TM and WK recruited the subjects. KD, AR and JK performed experiments. All authors performed analysis. BG, DM and TM wrote the manuscript. All authors reviewed the manuscript and approved the final version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.11.033.

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