

SARS-CoV-2 serological assay and viral testing: a report of professional football setting

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ABSTRACT

Purpose of the study PCR is the current standard test for the diagnosis of SARS-CoV-2 infection. However, due to its limitations, serological testing is considered an alternative method for detecting SARS-CoV-2 exposure. In this study, we measured the level of SARS-CoV-2 IgM and IgG antibodies of male professional football players and compared the results with the standard PCR test to investigate the association between the two tests.

Study design Participants were male professional football players and team officials. Nasopharyngeal swabs and peripheral blood samples were collected for the PCR and serological tests, respectively. Also, previous records of COVID-19 testing and symptoms were gathered. Those with previous positive PCR tests who tested negative for the second time were considered to be recovered patients.

Results Of the 1243 subjects, 222 (17.9%) were seropositive, while 29 (2.3%) tested positive for the SARS-CoV-2 PCR test. Sixty percent of symptomatic cases with a negative PCR were found to be seropositive. The mean level of IgM was significantly higher in PCR-positive and symptomatic subjects, whereas the recovered cases showed significantly higher levels of IgG.

Conclusion Our study revealed an inconsistency of results between the two tests; therefore, although application of serological assays alone seems insufficient in diagnosing COVID-19 disease, the findings are beneficial in the comprehension and the management of the disease.

INTRODUCTION

The novel coronavirus SARS-CoV-2 capable of interpersonal transmission has been the cause of the world's latest lethal outbreak.¹ An international collaborative effort was inevitable as the disease was spreading swiftly among nations; ergo, the WHO announced COVID-19 a pandemic in March 2020.²

Since the beginning, a search for an efficient test for case identification and case tracing has been initiated. To this date, PCR using nasopharyngeal swab samples has been identified as the gold-standard test for the diagnosis of COVID-19 infection.³ However, this method comes with some limitations; to remark, the nasopharyngeal sample-taking procedure is annoying and unpleasant for both the subjects and the examiners; it triggers sneeze or cough and can put the healthcare workers at risk of catching the disease. In addition, it requires appropriate sampling by trained staff and expensive equipment to successfully interpret the

test. Hence, they are complicated, pricey and relatively slow. Some studies even suggest a high rate of false-negative results for SARS-CoV-2 PCR tests. Besides, sample cross-contamination during collection and processing may rarely cause false-positive results.⁴⁻⁶

Furthermore, as the COVID-19 symptoms are mostly non-specific (especially compared with other upper respiratory viral infections), making a more precise diagnosis is crucial in managing the disease, especially in patients with a negative PCR.^{7 8}

On the contrary, the humoral response to the virus has been studied recently, proposing its application as an indicator to rule out infection, particularly in symptomatic subjects with a negative PCR test.⁹⁻¹¹ Nevertheless, to our knowledge, despite valuable efforts, a legitimate guideline for serological assay in the routine application is still lacking.

Although it has been reported that athletes are physically and physiologically superior to the normal population in the fight against viral respiratory infections,¹² competitive sport is considered to be a high-risk setting in COVID-19 era as interpersonal close contact is inevitable. Besides, data on SARS-CoV-2 seroprevalence and the comparison with PCR testing are still scarce. Therefore, we assessed the status of the SARS-CoV-2 antibody response and compared it with the standard PCR test results in a professional football setting to investigate the association between the two tests, aiming to contribute to the fast-growing evidence concerning the application of SARS-CoV-2 serological testing.

Design

To our knowledge, our exploratory study is the first to report of the status of SARS-CoV-2 viral and serological testing results and their association with Iranian professional football; therefore, we have studied our whole population. Data regarding PCR test results and serological assay were gathered between September 2020 and October 2020. We enrolled all football premier league (Persian Gulf league) and second division (Azadegan League) teams. Participants from the second division football league (Azadegan League) did not undergo any previous PCR test, whereas all the subjects from the premier league (Persian Gulf) had a previous certified PCR test result.

Symptom checker

We used two methods to monitor the symptoms: an online self-declaration form that was provided for



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the team members and the history-taking that was performed by the team physicians. The form was developed based on the latest report of the most common symptoms by the Ministry of Health and CORONA headquarters in sports. The most common symptoms of COVID-19 disease were explained to the participants by a medical expert, and each individual was asked to report any illness or suspicious symptoms through the form immediately. The symptoms were then verified by team physicians after a full examination.

COVID-19 diagnosis

Nasopharyngeal swabs for SARS-CoV-2 RNA PCR testing and peripheral venous blood samples for serological assays were collected for each subject and analysed immediately. Both viral specimen and blood samples were analysed in laboratories and by kits approved by the Iran Ministry of Health, the reference laboratories for COVID-19 identification. The assays were validated before routine use in line with the national standards. The average turnaround between PCR sampling and the result validation and announcement was approximately 24 hours, while serological results were ready to interpret about 6 hours after the sampling.

PCR testing

The Sansure SARS-CoV-2 RT-PCR kit (Hunan, China; target genes: ORF1ab/N) was used, and the reporting followed manufacturer's instructions based on the respective cycle threshold (cT) values of each gene target amplified. Results are reported as positive (cT < 40) and negative (cT > 40).

Serological testing

We used in direct ELISAs format to report the status of immunological response. The Pishtaz Teb Diagnostics SARS-CoV-2 Indirect ELISA kit (Iran) was used with a previously reported sensitivity of 79.4% and specificity of 97.3%. Tests were interpreted according to the manufacturer's recommendation, with cut-off indices 0.9 reported as negative and indices > 0.9 as positive for both IgM and IgG.

Statistical analysis

Kolmogorov-Smirnov test was used for distributional adequacy of our data set. We have used the data regarding immunoglobulin levels for testing of normality. For the age and the level of antibodies, data are presented as means, SD and CI. Kruskal-Wallis test, Mann-Whitney U test and Wilcoxon signed-rank test were performed to compare means in different groups. Also, binary logistic regression was used to predict the odds of being seropositive based on our predictors' values. The results with a p value of less than 0.05 were considered to be statistically significant. IBM SPSS V.25 was used to classify and analyse the data.

RESULTS

A total of 1243 individuals entered the study, of which 910 were players with the mean age of 25.6 ± 4.4 years and 333 were team officials with the mean age of 40.9 ± 8.6 years. Our report showed that 24 of 29 individuals with a positive PCR test and 158 of 222 who tested positive in the serological assay were players.

We included 650 individuals from the premier league (Persian Gulf) and 593 subjects from the second division league (Azadegan). As mentioned, there has been no previous PCR testing report for the second division league; however, individuals involving in the premier league were tested by PCR

Table 1 Seropositivity in symptomatic PCR-negative, PCR-positive and recovered subjects

Groups	IgM positive	IgG positive	Seropositive
Symptomatic PCR-negative cases (n=5)	2 (40%)	2 (40%)	3 (60%)
PCR-positive cases (n=29)	5 (17.2%)	6 (20.7%)	8 (27.6%)
Recovered cases (n=112)	1 (0.9%)	36 (32.1%)	36 (32.1%)

approximately 4 months ago. After comparing the previous results with the current PCR tests, it was revealed that a total of 112 individuals were infected by SARS-CoV-2 and all of them had negative PCR tests after 4 months. Therefore, we allocated the term 'recovered cases' to this group of subjects.

According to our results, 29 (2.3%, 95% CI=1.6% to 3.3%) participants tested positive based on PCR test, whereas 222 (17.9%, 95% CI=15.8% to 20.1%) were found to be positive when assessed by antibody levels. After comparing the results of the nasopharyngeal swab viral testing with the serum serological assay, we found that only eight subjects (0.64%, 95% CI=0.28% to 1.26%) tested positive for both tests. We also found that 214 (17.2%, 95% CI=15.2% to 19.4%) individuals were seropositive with a negative PCR test.

A total of seven subjects were symptomatic, of which three (42.9%) were seronegative and five (71.4%) were PCR-negative. According to table 1, 60% of symptomatic individuals with a negative PCR test were seropositive, while this rate was 27.6% and 32.1% for PCR-positive and recovered subjects, respectively.

Table 2 demonstrates that although compared with the level of IgM the mean level of IgG antibody is higher in PCR-positive, recovered and symptomatic subjects, these differences are not statistically significant in symptomatic cases (p=0.932).

The level of serum IgM and IgG antibody in PCR-positive, symptomatic and recovered subjects is compared between players and team officials in figure 1. It was revealed that in recovered subjects the mean levels of both IgM and IgG are significantly higher in officials (p=0.006 and p=0.025, respectively). The other differences between the groups were not statistically significant.

Binary logistic regressions were performed to identify predictive variables for the positive serological tests (table 3). A current positive PCR test and recovering from the disease were shown to be significantly correlated with seropositivity (OR=2.483, 95% CI=1.019 to 6.050, p=0.045; OR=3.534, 95% CI=2.190 to 5.703, p<0.001, respectively). Furthermore, the results showed that a current positive PCR test and expressing symptoms of the COVID-19 disease are significantly associated with IgM positivity (OR=5.519, 95% CI=1.708 to 17.828, p=0.004; OR=13.368, 95% CI=1.601 to 111.642, p=0.017, respectively), whereas recovery from the infection is significantly

Table 2 The mean level of antibodies in PCR-positive, recovered and symptomatic cases

Groups	IgM level*	IgG level*	P-Value
PCR-positive cases (n=29)	0.62 (1.2), 0.2 to 1.1	1.63 (3.8), 0.3 to 3.0	0.004
Recovered cases (n=112)	0.29 (0.3), 0.2 to 0.3	1.87 (3.4), 1.2 to 2.5	<0.001
Symptomatic cases (n=7)	1.10 (0.8), 0.5 to 1.7	1.14 (1.4), 0.1 to 2.2	0.932

*Data are presented as mean (SD), 95% CI.

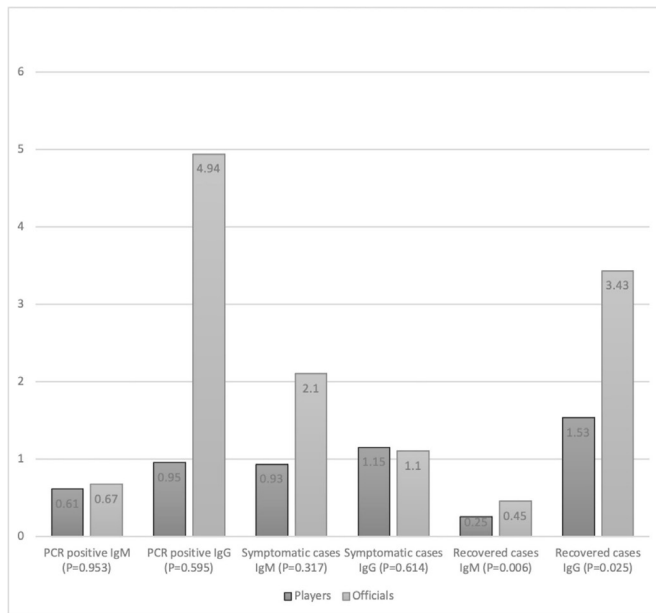


Figure 1 The comparison of antibody level in PCR-positive (n=29), symptomatic (n=7) and recovered (n=112) subjects between players and officials.

correlated with a positive IgG test (OR=4.063, 95% CI=2.501–6.601, $p < 0.001$).

DISCUSSION

Main outcomes

In this study, we have reported the status of humoral immunity of 1243 individuals from the professional football setting regarding the SARS-CoV-2 infection, and by comparing the results with the gold-standard PCR tests, our study revealed that in general, 2.3% of the participants were found to be PCR-positive, whereas 17.9% were found to be seropositive. We also found that 17.2% of the subjects were seropositive with a negative PCR test. This is relatively higher compared with a study conducted by Paradiso *et al*,¹³ which measured the antibody response using a rapid assay (6.8%). Furthermore, our results showed that of those with a positive PCR test, 27.6% also tested positive for serological assay. This insufficient synchrony between molecular testing and the immunological assay was not unexpected as each of the two tests is designed to provide information on a particular aspect of the disease.¹⁴ Nevertheless, the considerable inconsistency in results alongside higher proportion of seropositivity highlights the importance of investigating the serological testing in COVID-19 disease. Although the clinical application of serological assay for decision-making is yet to be verified,^{15 16} our study could reveal the concomitance of the SARS-CoV-2 antibody results with standard molecular testing alongside the association between the level of IgM/IgG and the occurrence of the symptoms.

Table 3 Odds of testing positive for serology

	OR	95% CI	P value
PCR-positive	2.483	1.019 to 6.050	0.045
Recovery	3.534	2.190 to 5.703	<0.001
Symptomatic	6.306	0.959 to 41.439	0.055

Serology and PCR

This study showed that in symptomatic cases with a negative PCR test, 60% of the subjects tested either IgM-positive or IgG-positive. Canetti *et al*¹⁷ reported that of the 43 symptomatic patients, 3 were PCR-negative, and their first serological assay returned IgG-positive, while the second test was inconclusive. These findings weigh on the limitations of the PCR tests and could justify the use of serological assays as a complementary test, especially in symptomatic patients. In addition, about one-third (32.1%) of our recovered cases were found to be seropositive, which can indicate that a significant immunity response is not always expected for the COVID-19 disease. Our results are not in accordance with the results of the study by Canetti *et al*,¹⁷ which reported that 100% of their recovered cases were seropositive. Larger sample size of our study (112 cases vs 6 cases) and an obvious difference in demographic characteristics of the two populations can justify this variation in results. In any case, these considerable positive immune responses in recovered cases suggest that the serological assay can provide assistance in case identification even after the diagnostic window of the PCR test.

Antibody level and PCR test

When comparing the level of IgM and IgG, we noticed that the mean level of IgG is significantly higher in patients with positive PCR test; this may indicate a higher anti-SARS-CoV-2 IgG activity even in the viral positivity window.

The results also showed that as expected the mean level of IgM is significantly lower in recovered subjects, which is in line with the studies conducted by Rode *et al*¹⁸ and Di Giambenedetto *et al*,¹⁹ stating that IgM is a more competent indicator of an acute immune response in patients infected by the SARS-CoV-2 virus, while IgG is formed in later stages of the disease. Although the difference between the level of IgM and IgG was not statistically significant in symptomatic subjects, the mean level of IgG was slightly higher than IgM. Our findings suggest that by the aid of serological assay, a current or past infection can be confirmed.

Players and team officials

In our study, we found that in those who were recovered from the COVID-19 disease, the mean level of both IgM and IgG was significantly higher in team officials compared with players, whereas the difference between the level of antibodies between players and team officials in subjects with positive PCR test and symptomatic cases was insignificant. Although the difference between the number of individuals in each group might have an impact on the results, further investigations are needed to elucidate the kinetics of SARS-CoV-2 antibodies in different populations regarding age and the level of physical activity.

Risk of testing positive for serology

We also calculated the odds of testing positive for both IgM and IgG. The association between the recovery and the serological assay in our study complies with the previous studies,^{20 21} stating that it takes time for the humoral immunity to react to the infection. In particular, the results showed that testing positive for PCR and having COVID-19 symptoms significantly increase the chance of finding higher levels of IgM in the serum, which again demonstrates the activity of IgM in earlier stages of the disease. On the contrary, only recovering from the disease was significantly associated with higher levels of IgG in the serum, which is the proof of IgG activity in the further phases of COVID-19.

Limitations

Besides, we compared the results of two principal COVID-19 diagnostic techniques in a football setting, which was more

equipped and was under more restricted hygiene protocols due to football resumptions, and these might limit our ability to implicate the results on the general population. Moreover, we only could perform viral testing during the last 4 months; therefore, we could not analyse the exact kinematics of the serological assay.

CONCLUSION

In conclusion, although serum antibody evaluation solely lacks a significant advantage over PCR testing, being a less time-consuming and cheaper test and requiring minimum professional training compared with viral testing could be an efficient method in the management, and referring to the COVID-19 centres, if needed, by confirming the diagnosis in symptomatic subjects can decrease the confusion in decision-making process for the healthcare staff. Consequently, our report revealed that serological assay as a complementary test could be useful in the comprehension of the disease and optimisation of the diagnosis in individuals. Further cohort researches are required to unfold additional information surrounding the application of serological assay in COVID-19 disease.

Main messages

⇒ Serological assay as a relatively cheaper and faster method compared with viral testing can be beneficial in the disease management. Approximately one-third of PCR-positive subjects express seropositivity. Anti-SARS-CoV-2 IgM can be a reasonable indicator of infection especially in symptomatic individuals. Anti-SARS-CoV-2 IgG seropositivity in PCR-negative patients can indicate a previous infection.

Current research questions

⇒ Both PCR testing and serological assay can provide valuable information surrounding the COVID-19 disease by helping in the diagnosis and management of the disease, especially in athletic population with high risks of close contact contamination. Further studies are required to evaluate the clinical value and significance of SARS-CoV-2 serological assay in different settings.

What is already known on this subject?

⇒ PCR is the gold-standard modality for diagnosing the COVID-19 disease. However, a variety of factors limit the efficacy of its application such as high costs and operator bias and false-negative results, especially in symptomatic patients.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. The study protocol was approved by the Ministry of Health and Iran Football Medical Assessment and Rehabilitation Center (IFMARC) Ethical Committee and Iran Football League Organization. Therefore, the number/ID of the approval was not applicable. IFMARC was responsible for conducting this study. The board members of all teams formally agreed to contribute to this study. Written informed consent was received from all teams on behalf of their members.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary data

Odds of testing positive for IgM.

Variables	OR	95% CI	P-value
PCR-positive	5.519	1.708-17.828	0.004
Recovery	0.289	0.037-2.241	0.235
Symptomatic	13.368	1.601-111.642	0.017

CI, confidence interval; OR, odds ratio; PCR, polymerase chain reaction

Odds of testing positive for IgG.

Variables	OR	95% CI	P-value
PCR-positive	2.227	0.858-5.780	0.100
Recovery	4.063	2.501-6.601	<0.001
Symptomatic	1.081	0.110-10.595	0.947

CI, confidence interval; OR, odds ratio; PCR, polymerase chain reaction

Figure 1. The comparison of antibody level in PCR-positive, symptomatic and recovered subjects between players and officials.

Variables	Groups	IgM level	P-Value	IgG level	P-Value
PCR-positive (n=29)	Player (n=24)	0.61 (1.4)	0.953	0.95 (1.9)	0.595
	Official (n=5)	0.67 (0.8)		4.94 (8.0)	
Recovered (n=112)	Player (n=92)	0.25 (0.2)	0.006	1.53 (3.0)	0.025
	Official (n=20)	0.45 (0.4)		3.43 (4.7)	
Symptomatic (n=7)	Player (n=6)	0.93 (0.8)	0.317	1.15 (1.6)	0.614
	Official (n=1)	2.10 (-)		1.10 (-)	

* Data are presented as: mean (SD)