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Original Scientific Paper

RAPID ANTIGEN TESTING FOR COVID-19 SHOULD BE AVOIDED AS SCREENING TEST AT LARGE SPORTING EVENTS WITH CLOSE PHYSICAL CONTACT BETWEEN COMPETITORS

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SUMMARY – Large sporting events during the pandemic are organized as 'COVID-19 free zones', function under quarantine measures, and are regularly testing all participants. Recently, point-of-care tests (POCT) for rapid detection of COVID-19 antigen were presented as a cheaper, faster, and easier-to-perform option than reverse transcription polymerase chain reaction (RT-PCR) testing. However, no study confirmed the efficacy of POCT used at a large sporting event that includes close physical contact, such as judo. An observational study was conducted that included 670 participants from 36 countries attending two consecutive judo competitions. In total, 839 RT-PCR tests and 839 POCT tests for COVID-19 were performed. There were 508 participants tested at one time point when entering the venue, while 162 were tested at two time points in order to participate in both competitions. Seven subjects had positive RT-PCR results, while 4 had positive POCT results, meaning the prevalence of COVID-19 at the competitions was 1.04% according to the RT-PCR results. The main finding of this study was that POCT testing proved to be an effective method in detecting true-negative results. However, due to sensitivity that is insufficient to detect all true-positive cases, POCT should always be used in combination with RT-PCR at large sporting events with close physical contact between competitors in order to avoid COVID-19 outbreak.

Keywords: *COVID-19; Point-of-care testing; Reverse transcription polymerase chain reaction; Sports; Martial arts; Mass gatherings*

Introduction

In January 2020, the World Health Organization (WHO) declared that the outbreak of the coronavirus disease 2019 (COVID-19) was a public health emergency of international concern[1]. During the first wave

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of the pandemic, it was speculated that large sporting events (LSEs) facilitated the spread of COVID-19[2]. Many LSEs were either canceled or postponed since then, culminating in the rescheduling of the Tokyo 2020 Olympic Games[3]. However, sporting events have a considerable impact on broader community, both financial and psychosocial, and may provide unity and hope if held[4,5]. Moreover, some authors describe that the absence of LSEs severely affects athletes' physical and mental health[6]. Therefore, as the pandemic persisted, LSEs were introduced again, under strict control measures, such as the 'bubble' organized by the National Basketball Association in the USA and the Ultimate Fighting Championship Fight Island in United Arab Emirates[7,8]. Both acted like 'COVID-19 free zones', function under quarantine measures, regularly testing all participants by reverse transcription-polymerase chain reaction (RT-PCR). The specificity of the RT-PCR is possibly higher than 99%[9]. Recently, point-of-care tests (POCT) for rapid detection of COVID-19 antigen have been introduced as a cheaper, faster, and easier-to-perform option than RT-PCR, yet effective in the detection of infected individuals[10]. The specificity of POCT in a study was estimated to be around 98% in asymptomatic patients, also in line with the U. S. Food and Drug Administration estimate[11,12]. However, to the best of our knowledge, no study confirmed the efficiency of POCT used in a LSE that includes close physical contact, such as martial arts, i.e., judo. The aim of this article is to present results of an observational study of COVID-19 prevalence in pretested participants of a LSE, as well as to compare POCT to RT-PCR testing.

Materials and Methods

Large sporting event general information

The European Judo Junior Championship with 356 competitors from 37 countries and U23 European Judo Championship with 327 competitors from 36 countries were held at the same venue, on November 4-6 and November 9-11, 2020, respectively. In total, 971 people were involved in either organization or participation in these competitions. All of them had to obtain a negative RT-PCR test for COVID-19 not older than 72 hours to enter the venue of the event. On entrance, all the participants were tested for COVID-19 by RT-PCR. Nasopharyngeal swabs were taken in front of the venue by educated personnel appointed by the Public Health Institute of the Istrian County. In brief, samples were obtained by insertion of a swab into the nostril, holding it parallel to the chin, until resistance is felt. The swab is then rotated five to six times while held in place and is afterwards carefully removed. The participants were isolated in hotel rooms until results came negative. Afterward, they were able to enter the 'COVID-19 free zone' for the competition. Those participants involved in both competitions had to be tested again by RT-PCR before the start of the second competition.

Study design

An observational study was conducted that included 670 participants of both competitions. All study participants were older than 18 years, gave a written informed consent, and took a RT-PCR test for COVID-19 at the venue. The study was approved by the Public Health Institute of the Istrian County Ethics Committee and by the School of Medicine, University of Zagreb Ethics Committee. Participants of the study underwent an additional nasopharyngeal swab in the same manner immediately after being swabbed for RT-PCR testing for POCT testing. Swabbing was performed by the same educated personnel appointed to perform RT-PCR testing. Participants waited for POCT results in front of the venue. If the results of POCT came positive, they were immediately retested by both RT-PCR and POCT. If the results of POCT came negative, they were isolated in hotel rooms. Upon arrival of RT-PCR results, if they were positive, they were retested in their rooms by both methods, and swabs were taken by the same educated personnel. This approach had the goal to lower the possibility of a false-positive result.

RT-PCR COVID-19 testing

After the nasopharyngeal swab was taken from the participant, the sample was transferred within six hours to the Teaching Public Health Institute of the Istrian County, Pula, Croatia, to the laboratory for molecular diagnostics. All RT-PCR tests for

COVID-19 were conducted following the manufacturer's instructions.

The QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) was used for RNA extraction. Detection of COVID-19 through RT-PCR was confirmed with the GeneFinder™ COVID-19 PLUS RealAmp Kit (OSANG Healthcare Co., Ltd., Gyeonggi-do, Korea) by amplification of RdRp, E gene and N gene on two ROTOR GENE Q 5 Plex HRM (Qiagen GmbH, Hilden, Germany), each 72 specimens *per* run in 90 minutes.

POCT COVID-19 testing

After the nasopharyngeal swab was taken from the participant, an immediate POCT was performed. NADAL® COVID-19 Antigen Rapid Test (Nal Von Minden GmbH, Moers, Germany) was used for testing of all study participants. The test is a chromatographic lateral flow immunoassay used to detect protein fragments of COVID-19 from oral or nasopharyngeal swabs. The swab is extracted to a buffer solution and transferred onto a cassette, with results ready in approximately 20 minutes, according to the manufacturer's instructions.

Statistical analysis

For numerical data, we report using whole numbers, mean and standard deviation, as well as percentages. We tested two hypotheses in order to provide appropriate conclusions. Firstly, we tested the hypothesis H_0 that POCT has a specificity of 98% or less. Such a test was performed to see whether the specificity described in parts of previous literature also held in our study. Secondly, we tested the hypothesis H_0 that POCT has a specificity of 99% or less. This hypothesis was intended to test whether the specificity of POCT was lower than that of RT-PCR. We note that there are two ways of testing the described hypotheses, both of which are used. One way is to assume all positive POCT results are false positives. The alternative is to assume all positive POCT results which also have a positive RT-PCR are in fact true-positives. We reported p-values of all the tests performed, which were obtained using parametric bootstrap. Statistical analysis was performed using R Statistical Software Version 4.0.2[13].

Results

A total of 670 (455 male) participants from 37 countries were included in the study. Of these, 437 were competitors, 141 were coaches, 17 were referees, and 75 were members of the organizing committee and staff. The mean age of study participants was 27.2±12.2 years. In total, 839 RT-PCR tests and 839 POCT tests for COVID-19 were performed. There were 508 participants tested at one time point when entering the venue, while 162 were tested at two time points in order to participate in both competitions.

Out of 670 participants, seven had positive RT-PCR results, while four had positive POCT results. For all participants whose results came positive, either on POCT or RT-PCR, testing was repeated by both methods, as described in the study design section. The second round of testing confirmed the results of the first testing in all cases. The prevalence of COVID-19 at the competitions was 1.04%, according to the RT-PCR results. All of the participants were asymptomatic at the time when they were tested. Also, all of the participants that had positive POCT had positive RT-PCR tests as well. Of the four participants positive on POCT, two had negative POCT and RT-PCR results when entering the first competition. They were positive on both tests when retested to enter the second competition. Out of the seven participants who tested positive on RT-PCR, three were competitors, two were coaches, one was physiotherapist, and one was member of the organizing committee. Out of the four participants who tested positive on POCT, one was competitor, two were coaches, and one was member of the organizing committee.

The sensitivity of the POCT used was 57%, while the specificity of the test was 100% compared with RT-PCR as reference testing. Therefore, POCT proved to be as efficient in detecting true-negative results as the RT-PCR. On the other hand, comparison of POCT and RT-PCR testing for detection of true-positive results did not show a statistically significant difference.

The hypothesis that the specificity of POCT is 98% or lower was rejected at 5% significance level, even when assuming that all POCT positive results are false-positives (p=0.003). Under the assumption that both positive RT-PCR and POCT results in fact

correspond to a true-positive case, the significance of the result was even stronger ($p<10^{-5}$), indicating that POCT had higher specificity than previously thought in the literature. Further, using the same assumption, the hypothesis that POCT had a specificity of 99% or lower was rejected (p=0.001), indicating that the specificity of POCT might lie in the same range as that of RT-PCR. However, under the very conservative assumption that all POCT positive results are false-positives, the hypothesis cannot be rejected (p=0.223).

Discussion

The main finding of this study is that POCT testing proved to be an efficient method in detecting true-negative results. However, due to sensitivity that is insufficient to detect all true-positive cases, POCT should always be used in combination with RT-PCR in LSEs with close physical contact between competitors in order to avoid COVID-19 outbreak.

This LSE included many participants from numerous countries with different epidemiological status of COVID-19, thus presenting an interesting epidemiological 'melting pot'. Nevertheless, COVID-19 prevalence of 1.04% made this LSE a low prevalence setting. Such a result was facilitated by a mandatory pre-entrance negative RT-PCR test not older than 72 hours. Moreover, the specific value of the study is indeed the insight in the subset of already tested individuals. As Mooney *et al*. suggest, the only way to efficiently detect asymptomatic and presymptomatic COVID-19 patients is by RT-PCR testing[14]. They further emphasize that such patients are responsible for up to 55% of COVID-19 transmissions[14]. Some studies suggest RT-PCR has a sensitivity and specificity greater than 95%[15,16]. On the other hand, some studies suggest that chest CT or serology testing provides more accurate results[17,18]. Ai *et al*. state RT-PCR should be treated with caution as false-negative results may occur in up to 34% of tests[19]. According to Mooney *et al*., the probability of a false-positive RT-PCR for COVID-19 has not yet been described[14]. However, they highlight that false-positive results in RT-PCR testing for most respiratory viruses tend to be low[14]. Nevertheless, RT-PCR is currently accepted as the gold standard for diagnosing COVID-19[9]. Therefore, it is considered as a reference test in numerous studies[20]. Even so, one should always keep its limitations in mind, always staying cautious.

Recommendations for the use of POCT issued by the WHO state that this type of test should not be used for screening in a low prevalence setting, at least until high-quality studies confirm the high specificity of POCT[21]. A meta-analysis reports that the mean sensitivity of POCT is 56.2%, while the mean specificity is 99.5%, both consistent with our results[20]. According to Healy *et al*., false-positive results are proportionally more of an issue in a low prevalence setting[22]. A warning was issued from the Food and Drugs Administration to clinical laboratory staff and physicians, highlighting that a POCT with a 98% specificity in a prevalence of 1% would have up to 70% of false-positive results[12]. Dinnes *et al*. suggest that POCT may have a triage role for RT-PCR, allowing earlier detection and management of COVID-19 positive patients[20]. However, they believe this could only be performed in a high prevalence setting because of the relative risk of false-positive results in a low prevalence setting, suggesting a mandatory follow-up RT-PCR in such a setting[20]. As Mann *et al*. report, elite athletes will periodize their training and form toward LSEs[4]. Therefore, it is of utmost importance to lower the possibility of false-positive testing, thus not allowing an athlete to be banned from the competition without a valid reason. Surprisingly, our study results show POCT did not lead to more false-positive results than RT-PCR, thus proving to be at least as efficient as RT-PCR in finding true-negative results in a low prevalence setting.

When considering the false-negative rate of POCT with the RT-PCR as reference testing, several studies were conducted. As stated earlier, POCTs seem to have inferior sensitivity and specificity in comparison to RT-PCR[23,24]. In general, these tests seem to be efficient in symptomatic patients and those with higher viral loads[25-30]. The POCT we used was analyzed in a study, showing 100% sensitivity in samples with high viral load, 61.85% in medium viral load samples, while all samples with a low viral load came out negative[10]. The participants of our study were all asymptomatic, thus probably having a low viral load[24]. Therefore, if a different POCT was used in this study,

it may have had a lesser rate of false-negative results. There are numerous POCTs available on the market of European Union[31]. The POCT used in our study was validated by an independent prospective clinical study, therefore it has a higher level of evidence to validate the product in comparison with most other registered POCTs[31]. Moreover, a significant number of products have a higher sensitivity and specificity value reported by manufacturers than by independent studies[31]. Therefore, it is questionable would a different POCT provide better results. Nevertheless, as studies suggest, due to the lesser sensitivity in comparison to RT-PCR, POCTs are likely to miss some COVID-19 patients with low viral load, thus allowing the spread of the disease[24,26,32,33]. Therefore, a whole LSE may be compromised by such individuals. Pray *et al*. propose that a confirmatory RT-PCR test should be considered in symptomatic patients with negative POCT and in asymptomatic subjects with positive POCT[11].

No studies confirmed transmission of COVID-19 caused by a judo match. However, there are several outbreaks of COVID-19 associated with recreational hockey games, fitness dance classes, squash, curling, etc.[34-37]. Wong *et al*. report that in 90 minutes of a professional football match, the average duration of close contact between players was 19 minutes, and each player had an average of 52 episodes of behavior that is considered a risk for acquiring COVID-19[3]. Moreover, a massive team-to-team transmission of COVID-19 was reported in the U20 Finnish National Hockey League, caused by an asymptomatic player[38].

By enrolling in a judo match, one cannot avoid close physical contact, thus being in a significant threat of COVID-19 transmission if fighting an infected individual. Moreover, Ahmetov *et al*. found that combat athletes possessed a higher genetic risk of COVID-19 severity than untrained subjects or endurance athletes[39]. Moreno *et al*. report on two COVID-19 outbreaks among intercollegiate university athletic programs, despite mandatory daily POCT in a congregate setting[40]. Although considered as incidental findings by statistical analysis, positive cases by RT-PCR and negative by POCT in our study were two competitors and a physiotherapist. As they have multiple close physical contacts, we believe that if we used POCT exclusively, it would put the whole LSE to a danger of a COVID-19 outbreak. Therefore, we would highly recommend using RT-PCR and POCT, or at least RT-PCR, but not POCT alone. We believe testing should be done before the start of the competition and during the competition at different time points, with a rational consideration of disease spread risk and financial aspect of testing.

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Sažetak

BRZO ANTIGENSKO TESTIRANJE NA COVID-19 TREBA IZBJEGAVATI KAO TEST PROBIRA NA VELIKIM NATJECANJIMA SPORTOVA KOJI UKLJUČUJU BLISKI FIZIČKI KONTAKT IZMEĐU NATJECATELJA

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Veliki sportski događaji se tijekom pandemije organiziraju kao „zone slobodne od COVID-19“, poštivajući mjere izolacije i provodeći redovita testiranja svih sudionika. Nedavno su u primjenu ušli brzi antigenski testovi za COVID-19 kao jednostavnija, jeftinija i brža metoda za testiranje nego što je RT-PCR. Dosad nije objavljeno istraživanje koje bi potvrdilo učinkovitost brzih antigenskih testova u svrhu prevencije zaraze na velikim sportskim događajima koji uključuju bliski fizički kontakt. Ovo istraživanje provedeno je na 670 sudionika iz 36 zemalja koji su sudjelovali na dva uzastopna judo natjecanja. Ukupno je provedeno 839 RT-PCR i 839 brzih antigenskih testiranja na COVID-19. Jednom je testirano 508 sudionika, dok je njih 162 testirano u dva navrata kako bi mogli sudjelovati na oba natjecanja. Sedam testiranih imalo je pozitivan RT-PCR, dok ih je 4 imalo pozitivan brzi antigenski test, što bi značilo da je učestalost COVID-19 na natjecanjima iznosila 1,04% prema RT-PCR rezultatima. Ovo istraživanje pokazalo je kako je brzo antigensko testiranje učinkovita metoda za otkrivanje stvarno negativnih ispitanika. No, zbog nedovoljne osjetljivosti brzo antigensko testiranje nije u mogućnosti identificirati sve stvarno pozitivne ispitanike, stoga bi se uvijek trebalo koristiti u kombinaciji s RT-PCR testiranjem na velikim sportskim događajima koji uključuju bliski fizički kontakt, s ciljem izbjegavanja širenja zaraze virusom COVID-19.

Ključne riječi: *COVID-19; Brzo antigensko testiranje; Testiranje lančanom reakcijom polimeraze nakon reverzne transkripcije; Sport; Borilačke vještine; Veliki sportski događaji*