The effects of SARS-CoV-2 viral infection on elite athletes

Abstract of PhD Thesis

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Budapest
2026

1. Introduction

Pandemics have been recurring phenomena throughout history, continuously posing challenges to societies. Among the most devastating pandemics in human history are the plague between 1347–1352 (estimated 17–28 million deaths), the cholera outbreaks recurring since 1817 (approximately 40 million deaths in total), and the Spanish flu of 1918–1919 (around 50 million deaths). From the 21st century, the SARS-1 (774 deaths), Ebola (~11 000), and SARS-CoV-2 (>4 million) epidemics stand out.

Definition of a pandemic

The term *pandemic* was first recorded in 1666, describing an infectious disease that spreads across countries and continents, affecting large populations. The word originates from the Greek *pan* ("all") and *demos* ("people"). Since 1948, the World Health Organization (WHO) has been responsible for declaring pandemics. Such declarations usually lead to broad societal restrictions that affect all social groups; however, certain subpopulations – such as elite athletes – are particularly vulnerable both to the infection itself and to the consequences of these restrictions.

Background

The first major epidemic of the 21st century was the SARS outbreak originating from China in 2003, which spread to 33 countries within seven months, resulting in 8456 cases and 809 deaths. The clinical presentation included high fever, myalgia, dry cough, headache, and dyspnea. Laboratory findings frequently showed leukopenia with decreased CD4/CD8 ratios, thrombocytopenia, elevated D-dimer, and poor response to broad-spectrum antibiotics. In 2012, the Middle East Respiratory Syndrome (MERS) appeared – both SARS and MERS are zoonoses. The SARS-CoV-2 genome shares ~79% similarity with SARS-CoV and ~50% with MERS-CoV. Mortality rates are high for SARS (9.5%) and MERS (34.4%), while for SARS-CoV-2 it is approximately 2.13%.

Emergence and spread of SARS-CoV-2

The first cases were reported in Wuhan on December 12, 2019; by January 26, 2020, 2794 laboratory-confirmed cases and 80 deaths had been registered. On March 13, 2020, the WHO declared SARS-CoV-2 a pandemic, leading to global restrictions. In Hungary, the pandemic initially seemed distant, but its domestic impact soon became evident.

Sport and pandemics: a vulnerable subpopulation

In the spring of 2020, organized sports nearly came to a standstill. The Tokyo Olympic Games were postponed by one year and ultimately held without spectators under continuous medical supervision. The high exposure of elite athletes stems from frequent international travel, team-based training, close physical contact, and participation in early outbreak regions (e.g., Spain, Italy). At the beginning of the pandemic, little data were available regarding the characteristics of SARS-CoV-2 infection in elite athletes. Many major sporting events were postponed mainly due to socioeconomic pressure rather than well-controlled epidemiological assessments. In professional sports, financial and competitive demands often override medical caution; an extreme example was the 2022 Tour de France, where some cyclists continued competing despite positive test results.

Clinical course and inflammatory response

In young individuals, COVID-19 usually presents as a mild illness lasting 5–7 days; however, deterioration may occur on days 7–9, potentially leading to acute respiratory distress syndrome (ARDS) and multiorgan failure (MOF), marked by a “cytokine storm” (elevated IL-6, IL-8, TNF-α). Monitoring of inflammatory cytokines is therefore crucial. During intensive exercise, elite athletes experience transient increases in myokines such as IL-6, IL-8, and TNF-α, which may influence their immune response to infection. Consequently, their immunological reaction can differ significantly from that of the general population.

Convalescent plasma therapy

Early in the pandemic, plasma collected from recovered donors was introduced as a potential treatment for severe COVID-19. Its presumed benefits derive mainly from virus-specific neutralizing antibodies (IgG, IgM, and IgA for mucosal protection) and possibly from immunoregulatory and anti-inflammatory components. In Hungary, plasma donation campaigns were particularly successful among national-team athletes. Active elite athletes were excluded from donation due to WADA regulations but participated in data collection. Samples from five Olympic sports (swimming, kayaking, wrestling, fencing, cycling) provided a representative cohort for studying SARS-CoV-2-induced immune and inflammatory responses. COVID-19 causes acute viral pneumonia, requiring oxygen therapy in ~15% of cases, mechanical ventilation in ~5%, and has an estimated lethality of ~2.3%.

Immunological basis of vaccine development

The surface spike (S) protein of coronaviruses is responsible for receptor binding and membrane fusion. The SARS-CoV-2 S protein binds to the ACE2 receptor and enters the host cell via endocytosis, followed by membrane fusion and genome release. The S protein, particularly its receptor-binding domain (RBD), is the main target for neutralizing antibodies; antibodies binding here prevent viral entry. Consequently, the S protein rapidly became the principal target for vaccine development.

Vaccine efficacy and relevance to athletes

While convalescent plasma provided an early therapeutic option, vaccines became essential in curbing subsequent infection waves. Owing to the novel platforms – especially mRNA-based vaccines – direct comparisons with traditional inactivated vaccines are limited. Elite athletes and healthcare workers were among the first to receive vaccination both in Hungary and internationally, highlighting the importance of evaluating vaccine effects in athletic populations. The BNT162b2 (Pfizer-BioNTech) vaccine significantly reduced case numbers and disease severity, thereby decreasing hospitalization and mortality risks. Most studies compared individual vaccines with controls rather than performing multi-vaccine analyses. Doroftei et al. conducted a meta-analysis of 19 publications in an attempt to harmonize heterogeneous data; however, methodological variability limited the conclusions.

Vaccines used in Hungary

Hungary uniquely authorized eight COVID-19 vaccines within Europe, including EMA-approved (ChAdOx1/AstraZeneca, BNT162b2/Pfizer, mRNA-1273/Moderna) and Eastern vaccines (BBIBP-CorV/Sinopharm, Gam-COVID-Vac/Sputnik V). This diversity created a rare opportunity for head-to-head efficacy comparisons under identical epidemiological and laboratory conditions.

Immunological background

Vaccines activate both humoral (antibody-mediated) and cellular (cytotoxic T-cell) responses. Immune protection may wane over time, and some individuals fail to mount adequate responses. Since many countries used only 2–3 vaccine types, comprehensive cross-comparisons were difficult. Evaluating the five most frequently used vaccines under uniform conditions helps explain why some vaccinated individuals later tested positive or why symptom severity varied among elite athletes despite identical vaccination.

Conclusions and practical implications

The distinct exposure and immunophysiology of elite athletes justify the development of targeted epidemiological and medical protocols, including:
– regular health monitoring adjusted to training load (cytokines, cardiopulmonary function),
– flexible competition and training protocols adapted to local outbreak dynamics,
– individualized vaccination strategies (timing, boosters),
– early return-to-play algorithms tailored to specific sports, and
– continued research on passive immunization strategies (e.g., plasma therapy) in the context of elite sports.

2. Objectives

The global SARS-CoV-2 pandemic that began in 2020 not only challenged healthcare systems but also had a profound impact on almost every area of life, including sport.

Elite athletes, who constantly operate at the limits of their physical and mental performance capacity, represent a particularly interesting group for studying the effects of viral infections. How does an organism exposed to extreme physiological stress respond to an infection with severe global consequences?
Does the immune response of elite athletes differ from that of the general population? How do these differences influence their recovery, performance, and the effectiveness of various immunotherapies?

The aim of this research was to provide a comprehensive overview of the effects of SARS-CoV-2 infection on elite athletes, with special emphasis on the immunological mechanisms involved and the potential therapeutic implications.
The objectives of the thesis included gaining a deeper understanding of the immune response to SARS-CoV-2, identifying the role of inflammatory processes, and evaluating the effectiveness of different treatment approaches.

Beyond its focus on the health of elite athletes, this study also addresses questions that extend beyond the world of sport.
The findings contribute to the broader understanding of SARS-CoV-2 infection and its treatment and may serve as a foundation for future research.

Accordingly, the specific aims of the current research were as follows:

1. To investigate the **SARS-CoV-2-specific immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) responses** in elite athletes and to determine the level of **virus neutralisation** six to eight weeks after infection.
2. To analyse **inflammatory cytokines** that influence the cytokine storm in elite athletes using plasma samples.
3. To assess the **anti-inflammatory effects of convalescent plasma therapy** and its influence on the duration of hospitalisation among patients infected with SARS-CoV-2.

To evaluate the **immunological effects of the COVID-19 vaccines available in Hungary**, both in vaccinated individuals and in those who had recovered from infection.

3. Methods

Study methods in elite athletes

Hungarian national team athletes volunteered to participate in the study, representing the sports of swimming, fencing, wrestling, cycling, and canoe–kayak.
In March 2020, all of them had participated in international training camps or competitions, during which they experienced viral infections.
During the first wave of the pandemic, 97 athletes were tested, of whom 11 were PCR-positive.
Several athletes who showed typical symptoms and had known contact with positive individuals tested PCR-negative by the time of sampling.
Athletes with previous symptoms and/or confirmed infection (n = 29) also volunteered for blood sampling.
The cohort included Olympic, World, and European champions.

PCR testing

To detect viral RNA, nasopharyngeal and oropharyngeal samples were collected for PCR analysis.
Sampling was performed by a microbiologist, and measurements were carried out in an accredited laboratory (Neumann Laboratory).

Virus neutralisation

Neutralisation titres were determined at the National Virology Laboratory, University of Pécs, under the highest biosafety conditions.
The assays were conducted using the VeroE6 cell line (DMEM + 2% FBS, 1% penicillin–streptomycin).
The SARS-CoV-2 titre was established by $TCID_{50}$ assay.
Heat-inactivated serum samples (56 °C, 30 min) were serially diluted twofold and incubated with 100 $TCID_{50}$ virus before infecting confluent cell layers.
After adding maintenance medium, the cells were incubated for three days at 37 °C with 5% CO_2.

ELISA: IgG / IgM / IgA

In plasma samples, SARS-CoV-2-specific antibodies were quantified using semiquantitative ELISA assays:
– IgG and IgM antibodies against the nucleocapsid protein (Microgen/Genetics),
– IgG and IgA antibodies against the spike protein (Euroimmun).
Measurements were performed in the TE Laboratory.

Inflammatory cytokines

Twenty-seven cytokines were analysed from plasma using the Bio-Plex™ Human Cytokine Assay kit (#M500KCAF0Y, Bio-Rad).
These included IL-6, IL-8, TNF-α, IFN-γ, IL-1β, IL-10, MCP-1, IP-10, VEGF, PDGF-BB, and others.

Convalescent plasma therapy – clinical study

A prospective interventional clinical study was conducted with approval from the National Public Health Centre (NNK) and the Medical Research Council (TUKEB) (licence no. IV/3457-2-2020/EKU), and registered under NCT04345679.
For ethical reasons, no placebo or control group was used; the primary aim was to provide potentially life-saving therapy during the early phase of the pandemic, when specific antiviral drugs were unavailable.
The **primary endpoint** was patient survival; the **secondary endpoint** was the length of hospital stay.
Key laboratory parameters included white blood cell count, ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6).

Inclusion and exclusion criteria

Donor inclusion criteria:
– age between 18–65 years,
– full recovery from SARS-CoV-2 infection,
– laboratory-confirmed infection (PCR or nucleocapsid-specific IgG > 24 U/mL),
– meeting standard blood donation requirements.

Donor exclusion criteria:
– pregnancy,
– active SARS-CoV-2 infection.

All female donors underwent **HLA-antibody screening**; those with positive results were excluded to minimise the risk of transfusion-related acute lung injury (TRALI).

Recipient inclusion criteria:
– confirmed SARS-CoV-2 infection,
– hospitalisation.

Recipient exclusion criteria:
– previous transfusion reactions.

Plasma collection

From each donor, 400 mL of plasma was collected and divided into two 200 mL units. All plasma units were frozen within 360 minutes and underwent UVA irradiation and pathogen reduction treatment (nucleic acid inactivation and residual leukocyte elimination) to reduce infectious and transfusion-related risks.

Vaccinated individuals

Ninety-five participants provided written informed consent.
All were healthy adults aged 18–65 years without chronic illnesses (except for hypertension). Eighty-four participants had received two doses of vaccine (2–8 weeks prior to sampling). Eleven individuals had recovered from mild to moderate SARS-CoV-2 infection (without hospitalisation) and were unvaccinated at the time of sampling; all had negative PCR results. Ethical approvals were obtained from TUKEB and NNK (nos. 1943-6/2020/EÜIG; 38175-7/2021/EÜIG).

Venepuncture and sample processing

Whole blood samples were centrifuged at 1710 × g for 10 minutes at room temperature. Plasma was separated and stored at −20 °C until further analysis.

Antibodies and neutralisation (ELISA)

All tests were conducted according to the manufacturers' protocols.
– Nucleocapsid IgG: *recomWell SARS-CoV-2 IgG* (7304, Mikrogen, Germany)
– Spike IgG: *Anti-SARS-CoV-2 QuantiVac ELISA IgG* (EI 2606-9601-10G, Euroimmun)
– Neutralisation: *cPass™ SARS-CoV-2 Neutralisation Antibody Kit* (L00847-5, GenScript).
Readouts were performed using an LT-4000 LabTech device.

T-cell response (IFN-γ)

T-cell activation was evaluated with the *QuantiFERON SARS-CoV-2 test*.
One mL of whole blood was transferred into QFN tubes, shaken gently, and incubated overnight at 37 °C.
Samples were then centrifuged at 2000 × g for 15 minutes, and plasma was stored at 4 °C until IFN-γ measurement.
Data analysis was performed with *QuantiFERON R&D Analysis RUO* software.

Statistical analysis

Normality was assessed using the **D'Agostino–Pearson test**.
When data were normally distributed, **one-way ANOVA** was used for group comparisons, with **Tukey's post hoc test** for pairwise analyses.
Significance level: $p < 0.05$.
Data are presented as **mean ± SEM**.Correlations were assessed with **Pearson's correlation coefficient**.
Results with $r > 0.75$ and $p < 0.05$ were considered strong and significant.
Statistical and graphical analyses were performed with **GraphPad Prism 7**.

4. Results

Elite athletes

Direct viral neutralisation testing of plasma samples revealed that all athletes had neutralising titres below 1:10; neither strong nor moderate neutralisation was observed. This indicates that 4–6 weeks after infection, the level of highly neutralising antibodies was low.

IgG, IgM and IgA antibodies

ELISA testing within the same cohort detected SARS-CoV-2-specific IgG positivity in only one athlete, consistent with the neutralisation data. IgG levels against the spike and nucleocapsid proteins correlated with each other. No IgM positivity was detected. IgA, measured as a marker of mucosal immune response, was positive in 31 % of samples, suggesting a persistent mucosal-level immune activation even 4–6 weeks post-infection.

Inflammatory cytokines

Levels of IL-2, IL-4, IL-5, IL-6, IL-17, and MIP-1α were below the detection limit in all samples — no signs of a cytokine storm were observed. Nevertheless, several markers were elevated, forming a distinctive correlation pattern.

Two oppositely behaving cytokine clusters were identified:

1. the **TNF-related group** (MIP-1β, Eotaxin, RANTES, IP-10, IL-7, IL-9, MCP-1, IL-1β)
2. the **IFN-γ-related group** (IFN-γ, VEGF, IL-10, IL-15, G-CSF). The two clusters showed inverse correlations; in two wrestlers, however, both panels were elevated simultaneously.

Convalescent plasma therapy

A total of **267 patients** were treated (COVID wards = 202; Intensive Care Unit = 65). Median age: 67 years; mean hospital stay: 15 days.
More than 90 % had comorbidities, and inflammatory parameters were elevated before plasma therapy.
Seventy-six per cent were treated on COVID wards and 24 % in intensive care.

Comparisons between these subgroups revealed significant associations for **age, sex, duration of hospitalisation, mortality, white blood cell count (WBC), ferritin,** and **CRP**.
At baseline, WBC, ferritin, and CRP were higher among ICU patients.
Median time to plasma therapy was ~3 days in both groups.
No transfusion-related adverse events were observed.

CRP levels decreased significantly **one day after transfusion** (111.5 ± 73.45 → 86.2 ± 65.5 mg/L; $p = 0.0008$).
Patients who recovered had received plasma significantly earlier than those who died ($p = 0.0133$).
Plasma administration within ≤ 3 days of hospital admission was associated with **24 % mortality**, whereas later transfusion (> 3 days) resulted in **40 % mortality** ($p = 0.0226$).

Changes in cytokine and inflammatory parameters

In frozen serum samples from 21 patients, levels of IL-1β, IL-6, IL-8, and TNF-α were analysed.
IL-1β, IL-8, and TNF-α remained within physiological ranges and did not differ significantly between survivors and non-survivors.
By contrast, IL-6 was above normal before plasma transfusion and significantly higher among non-survivors (102.5 vs 28.9 pg/mL; $p = 0.0066$).

Ferritin ($p = 0.0025$), CRP, and IL-6 (both $p < 0.0001$) decreased significantly after plasma therapy.

Immune responses to SARS-CoV-2 vaccines available in Hungary

Nucleocapsid-specific IgG was highest in recovered patients, significantly exceeding levels seen in the BNT162b2, mRNA-1273, ChAdOx1, and Gam-COVID-Vac groups, though not significantly different from the BBIBP-CorV group ($p = 0.08$). Above-threshold nucleocapsid IgG was observed in 90.1 % of recovered individuals, 56.3 % of the BBIBP-CorV group, and 4.5 % of the Gam-COVID-Vac group; mean nucleocapsid IgG > 24 U/mL occurred only in the BBIBP-CorV cohort.

The highest spike-specific IgG titres and neutralisation activities were recorded in the **BNT162b2** and **mRNA-1273**groups ($p < 0.001$ vs recovered controls and vs vector/inactivated vaccines; multiple comparisons $p < 0.001–0.008$). The degree of signal inhibition in these two mRNA groups exceeded that of both recovered controls and the ChAdOx1 and BBIBP-CorV groups.

T-cell response

Overall, 28.42 % of participants showed no measurable T-cell reactivity. This was more frequent among recovered, vector-based, and inactivated-vaccine groups than among mRNA-vaccinated individuals.
In general, **mRNA vaccines induced stronger cellular responses.**

Correlation analyses revealed strong, significant associations between **spike-specific IgG** and **Ag1/Ag2 IFN-γ responses**($r_s \approx 0.54$ and 0.49; $p < 0.0001$), whereas no significant correlation was found for nucleocapsid IgG.
By vaccine group, the strongest correlation occurred in **mRNA-1273** (Ag2: $r_s = 0.83$; $p = 0.015$*) and the weakest in **ChAdOx1** ($r_s \approx 0.08$).
Radar-chart summaries indicated that **mRNA vaccines elicited robust IgG, neutralising, and T-cell responses.**

5. Conclusions

The research yielded several important findings regarding SARS-CoV-2 infection and its impact on elite athletes. Based on the results, the following conclusions can be drawn:

Immunological responses of elite athletes

It was observed that elite athletes recovered rapidly following SARS-CoV-2 infection and exhibited a strong **IgA-mediated immune response**, particularly through mucosal protection. This suggests that their specific immunological profile may provide more efficient defence against infection; however, this does not necessarily ensure long-term humoral immunity. IgG levels remained low, indicating that lasting immunity was not guaranteed and that the risk of reinfection persists.

Risk of cytokine storm

The cytokine analyses demonstrated that elite athletes had a **lower risk of developing cytokine storm** compared with the general population.
This may be related to their generally higher level of physical fitness and immune regulation. Nevertheless, their post-infection immunological status remained altered for several weeks, and **inflammatory markers**such as IL-6 and TNF-α remained elevated.
These findings highlight the importance of monitoring inflammatory and immunological changes even after clinical recovery in athletes.

Efficacy of vaccines

Comparative evaluation of different vaccines revealed that **mRNA-based vaccines** induced significantly stronger immune responses than vector-based formulations.
mRNA vaccines generated more pronounced T-cell activation and higher neutralising antibody titres, offering more robust protection against SARS-CoV-2 infection.
Nevertheless, **vector and inactivated virus vaccines** also provided measurable protection, though with milder post-vaccination immune responses.

Effectiveness of convalescent plasma therapy

Our results confirmed that **convalescent plasma transfusion** is a **safe and effective adjuvant therapy** that can be readily integrated into the complex treatment of severe COVID-19 cases. The therapy produced a marked decrease in inflammatory markers such as **CRP and IL-6**, and **earlier administration clearly improved outcomes**.
Patients who received plasma in the early phase of illness demonstrated higher survival rates and shorter hospital stays.
Therefore, early therapeutic timing is crucial to maximise the benefits of convalescent plasma treatment.

Long-term implications

The study revealed that the **immunological state of elite athletes remains altered for weeks after infection**, which may have long-term consequences.
While post-COVID syndrome and performance decline remain possible risks, our findings indicate that most athletes did not experience substantial performance deterioration after recovery.

General conclusions

Due to their **unique immune responses**, elite athletes require special attention both in infection prevention and in managing post-COVID effects.
These results may contribute to the development of more effective **treatment protocols** and **preventive strategies**tailored to elite athletes, optimising their health, recovery, and safe return to high-level competition.

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