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Immune Response after COVID-19 Vaccination in Elite Athletes

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COVID-19	Coronavirus disease 2019
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
mRNA	messenger ribonucleic acid
PCR	Polymerase chain reaction
ACE-2	Angiotensin-converting-enzyme-2
RTP	Return-to-play
EU	European Union
STIKO	Ständige Impfkomission
DOSB	Deutscher Olympischer Sportbund
CD	Cluster of differentiation
MHC	Major histocompatibility complex
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgD	Immunoglobulin D
mm	Millimeter
ELISA	enzyme-linked immunosorbent assay
IQR	Interquartile Range
BMI	Body mass index
RKI	Robert-Koch-Institut

1 Summary in English and German

1.1 Summary

The COVID-19 pandemic led to the development of new and different types of vaccines. These vaccines differ in mechanism, immunogenicity and reactogenicity and have been widely used in the adult population. However, there were concerns about their efficacy and the acceptability of their side effects in athletes. Therefore, this study investigates the immunogenicity and reactogenicity in elite athletes after COVID-19 vaccination and compares the responses between a double-dosed mRNA and a single-dosed vector vaccine.

The immune response was analysed in 72 athletes (56 mRNA (BNT162b2/mRNA-1273), 16 vector (Ad26.COV2.S) vaccinations). Blood samples were taken before the first vaccination, 14 days after the second mRNA vaccination and 21 days after the single Ad26.COV2.S vaccination. The long-term immune response was analysed 6 months after the last vaccination. The vaccine-induced immunoglobulin G antibody response, its neutralizing activity, CD 4 T-cells and CD 8 T-cells were assessed. Side effects, including time loss in training, were self-reported by the athletes.

Overall, the induction of immunoglobulin G antibodies was significantly greater with the double-dosed mRNA vaccines (5702 BAU/ml, $p<0.001$) compared to a single dose of Ad26.COV2.S (61 BAU/ml). In addition, the median neutralizing activity after mRNA vaccination was significantly greater (99.7%, $p<0.001$) than after the single-dosed Ad26.COV2.S vaccination (11%). This was also observed for CD 4 T-cells, which were induced significantly stronger by the mRNA vaccines (0.13%, $p<0.001$) than by the Ad26.COV2.S vaccine (0.05%), while the opposite was true for CD 8 T-cells (mRNA: 0.02%, Ad26.COV2.S (0.15%, $p<0.001$).

After reviewing the initial results, a booster immunisation was indicated for the Ad26.COV2.S sample. This was done with the BNT162b2 vaccine in 11 athletes. Two weeks after this booster immunisation IgG antibodies increased significantly to 3456 BAU/ml ($p<0.001$), as did the neutralizing activity of the antibodies (100%, $p<0.001$), CD 4 T-cells (0.13%, $p<0.001$) and CD 8 T-cells (0.43%, $p<0.001$).

The cumulative median time loss in training after the double-dosed mRNA vaccines was 2 days. Initially, the single-dosed Ad26.COV2.S vaccine also resulted in a median time loss of 2 days. The cumulative median time loss after Ad26.COV2.S and mRNA boost vaccination was 3 days.

Our results indicate that the immune response in competitive athletes after vaccination with Ad26.COV2.S results in a poorer immune response than after vaccination with mRNA vaccines. A booster vaccination after Ad26.COV2.S vaccination leads to a significant increase in immune parameters comparable to the initial immune response after mRNA vaccination. The effects of vaccination on training, as measured by the duration of training restrictions and the incidence of side effects, were comparable.

1.2 Zusammenfassung

Die COVID-19 Pandemie hat zu einer schnellen Entwicklung von neuen Impfstoffen geführt, die sich in Bezug auf ihren Mechanismus, ihre Immunogenität und die Reaktogenität unterscheiden. Die verschiedenen Impfstoffe wurden in der Erwachsenenbevölkerung in großem Umfang eingesetzt. Dennoch gab es Bedenken hinsichtlich ihrer Wirksamkeit und der Akzeptanz ihrer Nebenwirkungen bei Sportlern. In der vorliegenden Studie werden daher die Immunogenität und die Reaktogenität bei Leistungssportlern nach einer COVID-19-Impfung untersucht und die Reaktionen zwischen einer doppeldosierten mRNA-Impfung und einer einfachdosierten Vektor-Impfung (Ad26.COV2.S -Impfung) verglichen.

Die Immunreaktion wurde bei 72 Sportlern (56 mRNA- (BNT162b2/ mRNA-1273), 16 Vektor- (Ad26.COV2.S) Impfungen) analysiert. Die Blutproben wurden vor der Impfung, 14 Tage nach der zweiten mRNA- und 21 Tage nach der einmaligen Ad26.COV2.S-Impfung genommen. Zur Beobachtung der Langzeitimmunität wurde 6 Monate nach der letzten Impfung erneut Blut abgenommen. Anhand der entnommenen Blutproben wurden die Immunglobulin G (IgG) Antikörperreaktion, die neutralisierende Aktivität der Antikörper, CD 4 T-Zellen und CD 8 T-Zellen analysiert. Die Nebenwirkungen, einschließlich des Zeitverlusts beim Training, wurden von den Sportlern in einem Tagebuch dokumentiert. Insgesamt war die Induktion von IgG-Antikörpern bei einer doppelten Dosis mRNA-Impfstoff signifikant größer (5702 BAU/ml, $p<0.001$) als bei einer einzelnen Dosis Ad26.COV2.S (61 BAU/ml). Darüber hinaus war die

mediane neutralisierende Aktivität nach mRNA-Impfstoffen signifikant höher (99,7 %, p<0.001) als nach der Ad26.COV2.S-Einzelimpfung (11 %). Dies wurde auch für die CD 4 T-Zellen beobachtet, die durch die mRNA-Impfstoffe signifikant stärker induziert wurden (0.13%, p<0.001) als durch den Ad26.COV2.S-Impfstoff (0.05%). Die CD 8 T-Zellen wurden durch die Ad26.COV2.S Impfung signifikant mehr induziert (0.15%) als durch die mRNA-Impfung (0.02%, p<0.001).

Nach einer vorläufigen Analyse wurde eine Auffrischungsimpfung für die Ad26.COV2.S-Stichprobe empfohlen. Diese wurde mit dem BNT162b2-Impfstoff bei 11 Sportlern durchgeführt. 2 Wochen nach der Auffrischungsimpfung stiegen die IgG-Antikörper signifikant auf 3456 BAU/ml an (p<0.001), ebenso die neutralisierende Aktivität der Antikörper (100%, p<0.001), die CD 4 T-Zellen (0,13%, p<0.001) und die CD 8 T-Zellen (0.43%, p<0.001).

Der kumulative mediane Zeitverlust beim Training nach den mRNA-Impfungen betrug 2 Tage. Auch die Einzeldosis des Ad26.COV2.S -Impfstoffs führte zunächst zu einem medianen Zeitverlust von 2 Tagen. Der kumulierte mediane Zeitverlust durch die Nebenwirkungen der Impfung betrug nach der heterologen Ad26.COV2.S und mRNA-Impfung 3 Tage.

Unsere Ergebnisse deuten darauf hin, dass die Immunantwort bei Leistungssportlern nach der Impfung mit Ad26.COV2.S schlechter ausfällt als nach der Impfung mit mRNA-Impfstoffen. Eine Auffrischungsimpfung nach der Ad26.COV2.S-Impfung führt zu einem signifikanten Anstieg der Immunparameter, der mit der anfänglichen Immunantwort nach der mRNA-Impfung vergleichbar ist. Die Auswirkungen der Impfung auf das Training, gemessen an der Dauer der Trainingseinschränkungen und dem Auftreten von Nebenwirkungen, waren vergleichbar.

2 Introduction

2.1 COVID-19

2.1.1 Onset of the disease

The global pandemic known as Coronavirus disease 2019 pandemic (COVID-19) was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was identified in late 2019. The pandemic posed many political, social, and health care system related challenges due to its novelty. The virus is a positive-sense single-stranded RNA virus, it belongs to the coronavirus group and is transmitted by aerosols, with the risk of infection arising from speaking, singing, coughing, or sneezing and is spread indoors and by close body contact [70]. Different incubation times of virus variants [21] and contagious but asymptomatic people [18] make the new virus difficult to manage. Because of its ability to bind to the angiotensin-converting-enzyme-2 (ACE-2) receptor [71] the manifestation of the virus depends on the receptor frequency in the organs [54]. The ACE-2 receptor is expressed in many tissues in the body especially in the lungs, but also in heart, intestine, kidney and endothelium [71]. The ubiquitous presence of the ACE-2 receptor is one of the reasons for the wide range of symptom complexes that can be caused by SARS-CoV-2. Common serious health problems caused by SARS-CoV-2 include pneumonia and acute respiratory distress syndrome, common symptoms are fever, coughing or dyspnea [40]. Neurologic complications [3], olfactory and gustatory dysfunctions [30], and cardiac manifestations [65] are also risks of a COVID-19 infection. In addition, older age (>60 years), hypertension, diabetes, and coronary heart disease are risk factors for severe disease course [73].

The first case of COVID-19 in Germany was identified on the 27th of January, 2020 [43]. Schilling et al. divide the first year of the COVID-19 pandemic until February 2021 into 4 distinct phases [55]. While only sporadic cases occurred in the first 4 weeks (phase 0), an increase in cases occurred in the first wave from calendar week 10/2020 to 20/2020. In the following phase, the number of cases decreased to a summer plateau (calendar week 21/2020 to 39/2020). The second wave of COVID-19 was defined from calendar week 40/2020 to 8/2021. During these four phases, 2,444,983 people in Germany were identified as having confirmed COVID-19. Of these, 1,337,428 people became mildly ill (77%), while 192,191 (10%) required

hospitalisation. Within the first year, 75,402 people (3.1%) died in Germany because of COVID-19 [56].

2.1.2 COVID-19 in elite athletes

Overall, professional athletes do have a lower risk of developing severe disease from COVID-19 than the general population [31]. Hull et al. observed British athletes with confirmed or probable infection due to their clinical presentation and time loss in training and states that the athletes' range of symptoms during a COVID-19 infection is like that of non-athletes [22]. However, it must be considered that they may also suffer from contracting long-COVID [31] and myocarditis, although myocarditis is more common in non-athletes [28]. Furthermore, Hull et al. found that COVID-19 was worse than other respiratory diseases in athletes because it led to longer courses of the disease and more recovery time after it [22]. Secondly, COVID-19 resulted in a median time loss of training of 18 days whereas other respiratory illnesses resulted in a loss of training of only 6 days [22] – though it needs to be mentioned that return-to-play (RTP) recommendation were set generously long at 7 days minimum due to lacking data about COVID-19 [31]. Hull et al. also found that 25% of the examined athletes needed more than 28 days to recover from COVID-19 and return to training [22].

In addition to the impact of COVID-19 on the health of athletes, it is important to mention the impact it has on their careers. A study of the prevalence of IgG antibodies in professional football players in Germany showed that the number of unrecognised infections in this study population appears to be 8 to 10 times higher than the reported data in Germany [34]. Even if the primary infection does not lead to symptomatic disease in the athlete, it may be the starting point for transmission to other athletes or long-term consequences. Long-term health problems such as long-COVID and myocarditis have a negative impact not only on the athlete's body, but also on their mental health and their career due to the loss of training. Eighteen days of training loss can be devastating for athletes preparing for major competitions [22]. Neil et al. reported that even a few days of quarantine due to a coronavirus infection can severely affect an athlete's training schedule [38]. Additionally, at the outset of the pandemic, a mandatory isolation quarantine was implemented in Germany following a positive polymerase chain

reaction (PCR) test. This regulation meant that individuals were unable to participate in training prior to the completion of the quarantine, irrespective of any symptoms.

Training schedules are rigorous, and a major loss of training time can disrupt preparations and threaten athletic performance and earnings [22]. In addition, return-to-play after recovery is not easy to manage when dealing with a new virus. Given the paucity of data on the virus and the conditions of recovery in athletes, no data were provided on the safe return-to-play right at the beginning of the pandemic. As a result, recommendations for RTP were set generously long to ensure that athletes were not put at unnecessary risk. Initially, RTP was not recommended before 7 days of asymptomatic recovery [31]. Over time, RTP-recommendation changed and started to depend on the severity of the symptoms, their duration and the kind of symptom itself, which also determined if RTP needed to be medically monitored or not [64]. Still, in the beginning of the pandemic the total number of training days missed due to the generally increased duration of COVID-19 and the recommended 7-day symptom-free period before RTP results in a relevant loss of training for competitive athletes with a tight training schedule.

2.1.3 Preventive measures

As no specific treatments or vaccines were available at the start of the pandemic, it was difficult to recommend key preventive measures because little was known about their effectiveness in the context of COVID-19. Wearing a face mask, social distancing, and special hygiene were suggested to help stop the spread of the virus [44]. In early 2020, a COVID-19 specific PCR test was developed to detect a COVID-19 infection [44] and allow isolation of infected people. In addition, quarantine for people who have been in contact with an infected person has been recommended to stop the spread [44] as well as isolation for people living in retirement homes [45]. Because isolation is difficult to manage in sport, special preventive measures were needed. The German Bundesliga kicked off again in the summer of 2020 with a very strict hygiene policy, including symptom monitoring, regular PCR testing and antibody testing. An accompanying study showed that no athlete or official was infected as a result of this hygiene concept, and strict hygiene policies are therefore sensible [35]. The development of specific treatments and prophylactic vaccines has been difficult given the time constraints, but the need for safety and efficacy has been even greater.

2.2 COVID-19 vaccines

2.2.1 Different types of vaccines

The emergence of SARS-CoV-2 led to a fast development of vaccines in the world, to help control the virus and end the acute phase of the pandemic more quickly. Considering that “the most effective means of avoiding infections are vaccinations” [37] the fast development of vaccines was meaningful to have a more specific tool against SARS-CoV-2 than through the general hygiene measures alone, which, however, remained important. The first licensed vaccine in the European Union (EU) was set for the new technology-based mRNA vaccine BNT162b2 (Comirnaty) from BioNTech Manufacturing GmbH on the 21st of December 2020. This vaccine is administered as a two-dose vaccine, with a second dose recommended after three weeks [5] and 95% vaccine efficacy reported [6]. The second vaccine admission was given for mRNA-1273 (Spikevax) by Moderna Biotech Spain, S.L. on the 6th of January 2021. This vaccination is an mRNA-based vaccine, and a second dose is recommended 28 days after the first dose [7]. Vaccine efficacy is reported to be 94% [8]. The EU approved the vector-based vaccine ChAdOx1 (Vaxzevria) from AstraZeneca AB, Sweden on the 29th of January 2021 with a vaccine efficacy of 74% [9]. A second dose of the vaccine should be given between 4 and 12 weeks after the first dose [10].In spring 2021 unexpected issues of life-threatening cerebral venous thrombosis and thrombocytopenia occurred, which led to the recommendation of using this vaccine for people aged 60 years or older only [32] On 13th of March 2021 Ad26.COV.2 (Jcovden) from Janssen-Cilag International NV was approved. It is a single-dosed vector vaccine [11] and its vaccine efficacy was reported to be 67% [12]. By summer 2021 the mRNA-1273 and the BNT162b2 vaccines had been licensed for people aged 12 years and older, while the Ad26.COV.2 vaccine had been licensed for people aged 18 years and older. As of 30 March 2021, ChAdOx1 was only recommended for people over 60 years of age [46]. As of 10 November 2021, the STIKO in Germany has recommended that people under 30 years of age should only be vaccinated against COVID-19 using BNT162b2 [47].

The following Figure 1 shows the temporal relationships between the COVID-19 pandemic, our study, and the vaccines and their recommendations. As the main group of our study participants were between 18 and 30 years old, the figure refers to this age group.

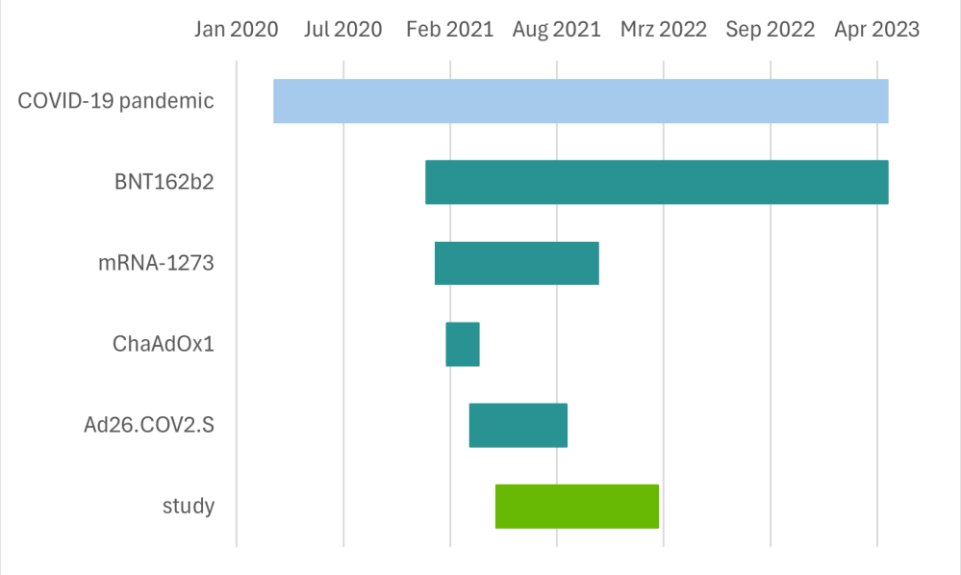

Figure 1: Timeline of the COVID-19 pandemic, the vaccines with their recommendations for the age group between 18 and 30 years, and our study

2.2.2 Vaccination prioritisation and adverse vaccine reactions

The responsible institution in Germany ("Ständige Impfkommission", STIKO) recommended an immunization for all people older than 18 years in early 2021. The introduction of new vaccines without a substantial infrastructure and the within a pandemic, which resulted in a high demand for these vaccines, initially hampered the ability to produce them at the pace required to vaccinate as many people as possible. As a result, the STIKO in Germany established vaccination priorities to ensure a safe and equitable distribution. In general, the distribution was organised according to age with the oldest receiving the vaccine offer first. In addition, health care workers with close contact to high-risk patients, such as doctors and nurses in intensive care units, also received an early offer [48]. The special needs of people with immunodeficiencies or specific diseases, geriatric nurses and public health workers were also considered for early prioritisation. In June 2021, the STIKO released a statement regarding the vaccination of adolescents between the ages of 12 and 17 years. This statement indicated that vaccination for adolescents was not generally recommended at that time; however, it was justifiable for specific indications and following consultation with a doctor [49].

In August 2021, vaccination against SARS-CoV-2 was recommended for all adolescents between the ages of 12 and 17 with BNT162b2 or mRNA-1273 [50].

With BNT162b2 and mRNA-1273, two double-dosed vaccines were available in early 2021. This somehow doubled the shortage since one person needed two doses. To stagger the shortage and provide a basic immunisation with one dose already, the second dose was planned as late as possible [48].

In addition to the aspect that common vaccines should offer a good prevention of infection and severe disease progression, adverse effects can occur after any vaccination, as has been observed with the COVID-19 vaccines. The side effects varied from vaccine to vaccine but were generally comparable. Overall, all side effects were recorded less frequent and milder in older people (>65 years). For the mRNA-1273 vaccine the most common adverse event was pain at the injection site (reported by 92%), followed by fatigue (70%), headache (65%), myalgia (62%), arthralgia (46%), chills (45%), nausea (23%), swollen lymph nodes (20%), fever (16%), swollen injection site (15%) and redness at the injection site (10%). These side effects occurred in more than 1/10 of people vaccinated [8]. For the BNT162b2 vaccine the most common adverse events were similar with pain at the injection site being the most common (>80%) followed by fatigue (>60%), headache (>50%), myalgia (>40%), chills (>30%), arthralgia (>20%), fever (>10%) and a swollen injection point (>10%) [6]. These side effects were more common after the second vaccination. For the Ad26.COV2.S vaccine the most common adverse event was pain at the injection site as well (49%). Headache was reported by 39%, fatigue by 39%, myalgia by 33%, nausea by 14% and 9% recorded fever [12]. The ChAdOx1-S vaccine led to pressure pain at the injection point in 68%, followed by pain at the injection side (58%), headache (53%), fatigue (53%), myalgia (44%), chills (32%), arthralgia (27%), nausea (22%) and fever (8%) [9]. The first vaccination resulted in more side effects than the second dose. These side effects can be distressing but are usually self-limited and harmless. However, serious side effects have been observed, too. Myocarditis and pericarditis are rare side effects, particularly in young, healthy males vaccinated with BNT162b2 [6]. Myocarditis has also been observed after vaccination with mRNA-1273 [8]. Very rare adverse reactions in ChAdOx1-S were anaphylaxis, sinus and cavernous thrombosis and thrombocytopenia [9]. Thrombocytopenia and venous thrombosis were observed after vaccination with the Ad26.COV2.S vaccine, although the incidence was very low (>1/10.000).

Some of the side effects of the various COVID-19 vaccines have also been seen with other vaccines before and are therefore not vaccine-specific [62].

2.2.3 Vaccination in elite athletes

The vaccine recommendation for the general population cannot be easily applied to elite athletes, who have specific needs and time schedules due to training and competitions [36]. It has been shown that SARS-CoV-2 transmission in playing football is very unlikely [17], but especially in very close contact sport the risk of infection with a respiratory transmitted disease due to close body contact is present [36]. In addition, travelling to different countries for international competitions and trainings camps increases the risk of infection [36]. While it is reasonable to vaccinate athletes due to the increased risk of infection and subsequent disease and transmission, there are sport-specific issues that need to be considered.

For COVID-19 vaccination, a period of two to three days of reduced training or no training is recommended [36]. For athletes with very tight competition and training schedules this is a difficult issue, as three days of no training can dramatically affect the outcome. In addition to the recommended period of no training after vaccination, adverse reactions to the vaccine may also lead to a break in training due to side effects that affect training intensity and schedule. With given advantages and disadvantages, it has been generally recommended in the literature to vaccinate athletes generously, as COVID-19 is a major health issue in the world, affecting the lifes of athletes as much as the general population [36]. Furthermore, it has been shown that the COVID-19 vaccination in athletes had less impact on training loss than the disease itself [29].

2.2.4 Special needs for Olympians 2021

The 2020 Summer Olympic Games were postponed due to the COVID-19 pandemic, as were many other national and international events [23]. During the first year of the COVID-19 pandemic contact reduction, no large events and preventive hygiene measures were important rules that were followed in many parts of the world to stop the spread of the virus. With about 11.200 athletes, the Olympics are the biggest sports event in the world [63], which makes it more difficult to follow these aspects. This is caused by the Olympic Village itself which houses

all of the athletes who compete in different sports, eat in the same dining area, take the same buses to the sports facilities and celebrate their victories together.

The rapid development of vaccines provided new means of protection. Given the shortage of vaccines, there was no prioritisation of vaccines for athletes, as they are less likely to become seriously ill than older people. As time until the Games became shorter in spring 2021, the German government decided to offer vaccination to Olympic candidates in time to ensure a safer sport [25]. The choice of vaccine for athletes was difficult because all the vaccines were new and specific data about vaccinating athletes was lacking. Vaccination of Olympic aspirants was organized by 10 medical centres in Germany [26] and a single-dosed vaccine was recommended to simplify the process of vaccinating the few athletes (relative to the vaccinated population). The single-dosed vaccine Ad26.COV2.S was considered as a “pragmatic choice” to use as a vaccine for Olympic aspirants [36]. With different vaccines available, the Ad26.COV2.S vaccine was chosen referring to its efficacy against virus variants and its benefit of only having one vaccine shot required [16]. Vaccine reactions and training restrictions were expected to be lower, and the basic immunisation was expected to be achieved more quickly with a single vaccination [36]. The lower efficacy of the vaccine was considered sufficiently high, with reported efficacy of 80% for the reduction of severe cases [16]. The DOSB decided not to compel their athletes to get vaccinated, but they strongly recommended it, while the medical staff such as doctors and physiotherapists had to be vaccinated to be nominated for the Olympic Games [16].

2.3 Immune system

2.3.1 Body’s defences

To gain a better understanding of the influence of vaccines on the immune system and its protective function, the main components of the immune system are explained below. These characteristics are important for studying the immune response after COVID-19 vaccination.

Firstly, the immune system consists of two distinct parts producing an innate, non-specific immunity and an acquired, specific immunity, both working in close interaction. Innate immunity consists of mechanisms that fight pathogens immediately after they enter the body on a humoral and cellular basis. The innate immune system cannot easily differentiate between the

pathogens but reacts quickly and non-specifically between the disease pathogens. It consists of granulocytes, macrophages, and epithelial cells. They are activated by the pathogen and secrete mediators, which help to regulate the immune response to fight the pathogen [56]. Granulocytes contain potent chemicals that can destroy the pathogens. Macrophages phagocytize the pathogen by engulfing and digesting it before releasing in small harmless pieces so that the body can eliminate it [69] . Epithelial cells are the first barrier to microbes trying to enter the body. For example, the nasal surface can produce protective mucus once being entered by a pathogen [69].

The specific part of the immune system is made up of B- and T-lymphocytes. It can be activated by many different agents that the body does not recognize as its own. These are called antigens. These antigens get bound by the antibodies and lymphocytes of the specific immune system resulting in an immune response. Lymphocytes are produced by the stem cells of the bone marrow and are formed by the bone marrow itself (B-lymphocytes) or the thymus (T-lymphocytes) [56].

All T-cells have a T-cell receptor on their surface and a protein named cluster of differentiation (CD). The type of the CD protein identifies the T-cells as CD 4 and CD 8 T-cells. CD 4 T-cells are also called helper cells because of their ability to release cytokines, which are activators of various immune cells. The activated cells are used to fight pathogens, activate other T-cells and help in the production of antibodies by interacting with B-lymphocytes. The CD 8 T-cells are cytotoxic and help destroy virus-infected cells. T-cells are unable to detect antigens themselves. Macrophages digest foreign proteins from pathogens and attach short pieces of these proteins to Major Histocompatibility Complex (MHC)-molecules. These MHC molecules are presented by the macrophages, and, because of their endogenous nature, the T-cells recognise the potential threat and mount an immune response. MHC molecules can be divided into MHC I and MHC II. While MHC I is expressed on all body cells and is recognised by CD 8 T-cells, MHC II proteins are only expressed on immune cells and are recognised by CD 4 T-cells [56].

Another part of the specific immune system are the B-lymphocytes. These lymphocytes produce antibodies once they have been activated. These antibodies are capable to neutralize or destroy pathogens after binding to the antibody itself. There are five different types of antibodies: immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA),

immunoglobulin E (IgE) and immunoglobulin D (IgD). All these antibodies have a very high range of differentiation because of the many different pathogens that have been presented to the immune system. This diversity is generated by somatic recombination. After exposure to antigens, activated B-lymphocytes produce IgM-molecules. IgM are constructed as pentamers, which optimizes their effectiveness due to their size. Elapsing time leads to remodelling of the immunoglobulin; parts are changed, and the IgG antibody develops. When antibodies bind to pathogens, these pathogens can be recognised more easily by the body and attacked directly by phagocytes. These antibody-pathogen bindings also help to activate the complement system and to neutralize pathogens [56].

2.3.2 Immune system and the coronavirus

Paces et al. state that the SARS-Co-Virus triggers various pathways of the innate immune system and has a major impact on the adaptive immune responses [39]. The humoral immune response plays an important role in preventing severe COVID-19 infections and stimulates the immune system to produce neutralizing antibodies helping to prevent the virus from entering cells. These antibodies therefore play an important role in virus clearance [39]. This not only highlights the importance of antibodies during the early phase of infection but also demonstrates a key aspect of vaccine efficacy control. Knowing antibodies and their neutralizing activity are an important preventive feature of the humoral immune system to control a viral infection, the induction of an antibody response may not only be helpful to maximize vaccine efficacy. By analysing the induction of the antibody response after vaccination it may also help to assess the ability of vaccines of inducing this important preventive parameter [39]. In addition, it has been shown that the CD 4 T-cells have high response rates of 100% and CD 8 T-cells of 70% in patients following COVID-19 infection, whereas CD 4 T-cell induction without infection was observed in 50% of the cases and CD 8 T-cell induction in only 20% of cases [39]. Knowing that T-cells are important for the immune system and that they decrease after COVID-19 infection, an increase in T-cells is helpful in preventing severe cases because a larger pool of cells are able to fight the virus. The immune system of SARS-CoV-2 unexposed patients may have problems mobilizing T-cells to prevent severe cases. Therefore, examining T-cells after COVID-19 vaccination is helpful to better understand the efficacy of a vaccination [39]. Concluding, antibodies are an important

parameter of the body to prevent initial infection of the body by the virus. T-cells are crucial in the prevention of severe courses of infections that have already occurred.

2.4 Aim of the study

Data on vaccination of athletes against SARS-CoV-2 were lacking and had to be collected. Given the challenges of vaccinating athletes and the specificities of different vaccines, monitoring of vaccinating athletes was obviously of particular importance. In front of this background, the aim of the present study was to analyse the humoral and cellular immune response after COVID-19 vaccination in elite athletes. At the start of our study, the ChAdOx1 vaccine was no longer recommended for individuals under the age of 60 years. The available vaccines for our study population were BNT162b2, mRNA-1273 and Ad26.COV2.S. Given the two different mechanisms of the vaccines, our objective was twofold: to investigate their efficacy in athletes and to compare the double-dosed mRNA and the one-dosed vector based vaccines.

Because of the demanding training and competition schedules of athletes, it is crucial to investigate the potential of side effects of the vaccinations and the resulting impact on their training. This was therefore also a focus of our study.

The aim of the study was to analyse the immune response and the side effects including training restrictions of the single-dosed vector vaccine and the double-dosed mRNA vaccines and compare them.

We hypothesized that

(1) mRNA and vector vaccines would lead to a relevant immune response and disease prevention with a slightly higher protection by the mRNA vaccines and
(2) the one-dosed vaccine would result in fewer adverse vaccine reactions and therefore fewer training restrictions.

3 Methods

3.1 Study design

This was a prospective study in professional athletes from different types of sport performing on international, national and high regional level. To describe the immune response after COVID-vaccination, blood samples were taken before and after their vaccination – the exact time points depended on the vaccine scheme. For all vaccination programmes, blood samples were taken before the first vaccination to ensure that no one had been previously infected or vaccinated and to allow comparisons before versus after vaccination. In total, 3 athletes were vaccinated with mRNA-1273 (*Spikevax*), 53 athletes were vaccinated with BNT162b2 (*Comirnaty*) and 16 athletes received Ad26.COV.2 (*Jcovden*) as a vaccine. The distribution was not part of the study as the shortage of vaccines resulted in distribution which was out of control for the study conductors. Choice of vaccine types was not possible for all people in Germany at that point in time. For mRNA-1273 and BNT162b2, the second vaccination was administered 4-6 weeks after the first one for all participants, depending on the different vaccination schedules of different medical practices and personal preferences of the athletes. A further blood sample was taken two weeks after the second vaccination. Due to similar vaccination schemes and type of vaccine, mRNA-1273 and BNT162b2 are combined into one group called mRNA. For the single-dosed Ad26.COV2.S vaccine, the second blood sample was taken three weeks after the vaccination due to known differences between the vaccine-induced peak of the immune response after mRNA and Ad26.COV2.S vaccination [57]. To monitor the long-term efficacy of the vaccination, another blood sample was taken 26 weeks after the last vaccination. All participants recorded any side effects by completing a standardized paper diary over one week.

This study was carried out in accordance with the Declaration of Helsinki. The local ethics committee approved the study (133/21, Ärztekammer des Saarlandes, Saarbrücken, Germany). It was financially supported by the German Federal Institute of Sport Sciences (Bundesinstitut für Sportwissenschaften; reference: 2521BI0106) and part of a larger study being registered in the German Clinical Trials register (DRKS00023717). Participants were informed about the study design, the risks of blood sampling and the possibility of withdrawing

from the study at any time without giving a reason and without any personal disadvantage. Afterwards the participants or their parents (for minor participants) gave written informed consent after being informed about the study procedures.

3.2 Participants and recruitment

Seventy-two athletes (38 men, 34 women) with an age range from 16 to 49 years participated in this study. The athletes engaged in their field of sports on high regional, national, or international level. Anthropometric data is shown in Table 1.

Vaccine	N	W	M	Age*)
mRNA	56	29	27	21y ± 6y
Ad26.COV2.S	16	5	11	28y ± 5y

Table 1: Anthropometric data
**) Age: mean ± standard deviation*

Recruitment was managed by the Olympic Training Centre and the Institute of Sports and Preventive Medicine in Saarbrücken, the Institute of Applied Training Science (IAT) in Leipzig and the Charité Berlin. Personal communication with athletes and coaches from May 2021 to August 2021 led to the recruitment of 72 athletes. Inclusion criterion was training at a high-performance level in their sport defined as at least 5 days of training each week and participating on international, national or high regional level. Athletes from 17 different sports participated with the number of athletes per sport varying (badminton: 14, swimming: 10, water diving: 9, athletics: 7, triathlon: 7 fencing: 5, soccer: 4, handball: 3, horse riding: 3, mountain biking: 3, gymnastics: 1, tennis: 1, canoe: 1, wrestling: 1, shooting: 1, hockey: 1, cycling: 1).

Previous COVID-19 vaccination, acute illness with fever or pregnancy were exclusion criteria for this study.

Initially 78 athletes participated in this study. Four athletes dropped out before the second blood sample was taken and 2 blood samples could not be analysed due to time and transport issues. At the 6-months follow-up, 8 individuals from the mRNA group did not return for blood sampling appointment due to concurrent COVID-19 infections, personal issues or booster vaccinations before the 6 months follow-up was due.

3.3 Implementation

3.3.1 Process of implementation

The first athletes got vaccinated in early May 2021 and the last athletes in early August 2021. Blood samples were taken from the athletes just before the vaccination or less than one day prior. To record the side effects, all athletes were given a paper diary to fill in every day for one week after each vaccination. Venous blood was collected from an antecubital vein in a supine position (9ml, lithium-heparin tubes, serum tubes). The blood samples were stored and then analysed at the Department of Transplant and Infection Immunology, Saarland University, Homburg, Germany.

According to public regulations at the time, all athletes were vaccinated by external physicians with either BNT162b2, mRNA-1273 or Ad26.COV2.S. One week after vaccination, the athletes had to return their adverse event diaries. The second vaccination of the mRNA group took place after an average of 38 days (± 7days). After the second vaccination, another side effect diary was completed according to the same scheme. In the mRNA group, the second blood sample was taken an average 16 days (± 3 days) after the second vaccination. For the Ad26.COV2.S group the second blood sample was taken an average of 23 days (± 3 days) after the first vaccination.

The vaccine adverse event diary was completed by 49 athletes after the first mRNA vaccination which equals 87.5% and 40 athletes after the second vaccination (71.4%). Sixteen athletes returned the completed diary after the Ad26.COV2.S vaccination (100%).

Implementation is shown in Figure 2.

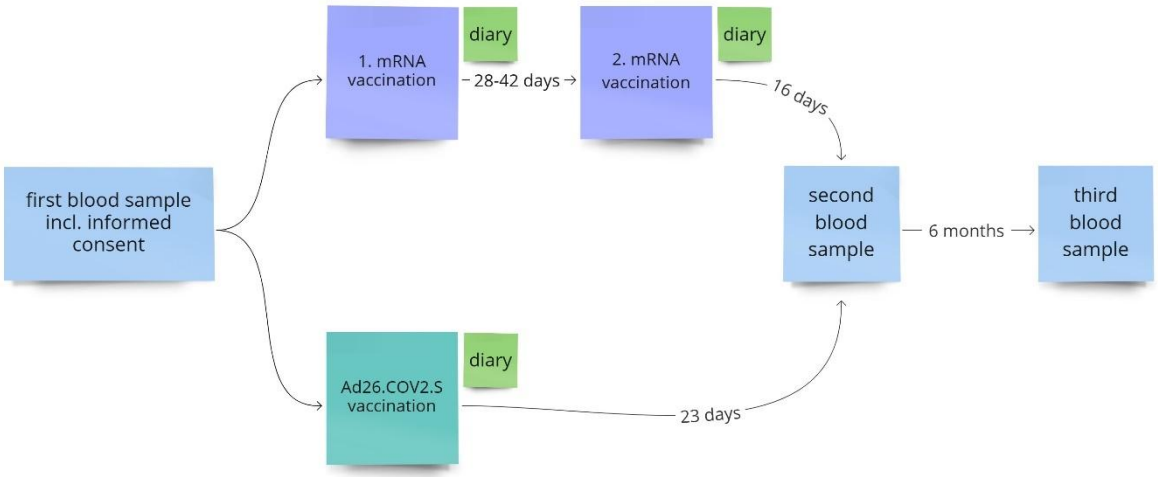

Figure 2: Process of implementation

3.3.2 Filling in the diary

All participants received an information sheet with details of the study and their tasks, and the informed consent form to sign before participating (see Appendix 8.1). They were also given the adverse event diary to complete daily after each vaccination for one week. The “Brighton Collaboration Case Definition” [19] was used as a template for creating the diary. The diary is attached (see Appendix 8.2).

Overall, the diary was divided into two different tables to distinguish between local and systemic side effects. Local side effects included pain at the injection point, redness and swelling. Systemic side effects were subdivided into body temperature, headache, muscle pain, chills, nausea, and fatigue. In addition, there was space to add side effects not mentioned in a free text box. The occurrence of side effects could be specified for each day over 7 days after vaccination. If symptoms had not disappeared after 7 days, there was space to mention the last day of occurrence. Each side effect had to be rated according to four different levels of severity. No occurrence of side effect were reported with 0, whereas 1 meant mild, 2 meant moderate and 3 equaled severe side effects. Mild side effects were defined as those that did not interfere with daily routine and practice, moderate side effects interfered with daily routine and practice and severe side effects did not allow practice or daily routine. For redness and

swelling the diameter in millimetre (mm) was used as a parameter, body temperature had to be reported in degrees Celsius. There was also space for people to list any medication they had to take because of side effects.

3.4 Procedure

Quantification of lymphocyte populations and plasma blasts has been described in detail elsewhere [58]. After a 6 h stimulation with SARS-CoV-2 spike-derived overlapping peptides (each peptide 2 µg/ml, JPT, Berlin, Germany) COVID-19 specific CD 4 and CD 8 T-cells were quantified as described before [59]. The experiment was performed using 0.64% dimethyl sulfoxide (DMSO) and 2.5 µg/ml of Staphylococcus aureus Enterotoxin B as negative and positive controls, respectively, in order to ensure cell specificity. Immunostaining was then carried out with anti-CD4 (clone SK3, 1:33.3), anti-CD8 (clone SK1, 1:12.5), anti-CD69 (clone L78, 1:33.3) and anti-IFNγ (clone 4S.B3, 1:100) and analysed by flow-cytometry (BD FACS Canto II including BD FACSDiva software 6.1.3) [58]. SARS-CoV-2-reactive CD 4 or CD 8 T-cells were characterized as IFNγ producing activated CD69-positive T-cells. The percentage of specific T-cells was determined by calculating the difference between the percentage of T-cells after negative control stimulation and that after spike-specific stimulation. The detection limit was set at 0.03% as described elsewhere [41].

To analyse the humoral immune response, enzyme-linked immunosorbent assay (ELISA) assays from Euroimmun (Lübeck, Germany) were used to detect the IgG antibodies and their neutralizing activity according to the manufacturer's instructions. To quantify SARS-CoV-2 specific IgG antibodies against the receptor binding domain an ELISA (SARS-CoV-2-QuantiVac) was used. The manufacturer's cut-off values were set as <25.2 BAU/ml for being negative, ≥25.2 to < 35.2 BAU/ml for being intermediate and ≥35.2 BAU/ml for being positive. To quantify SARS-CoV-2 specific IgG towards the nucleocapsid (N) protein an anti-SARS-CoV.2 NCP-ELISA was used. A surrogate neutralization assay (SARS-CoV-2-NeutraLISA) was also performed, which quantifies the antibody-mediated inhibition of soluble ACE2 binding to the plate-bound S1 receptor-binding domain. This assay utilised a single serum dilution. The surrogate neutralizing capacity was determined as the percentage of inhibition (IH), calculated by subtracting the ration of the sample absorbance to the blank value absorbance from 1 [58].

As set by the manufacturer`s instructions the stimulus threshold was set with IH being negative below 20%, intermediate between 20 and 35%, and positive above 35%.

The Department of Transplant and Infection Immunology, Saarland University, Homburg/Saar, Germany, determined the immunological parameters IgG antibodies, the neutralizing activity, CD 4 and CD 8 T-cells.

3.5 Statistical methods

Data analysis was performed using R statistical software in R studio (version 4.0.5). After data collection, the Shapiro-Wilk test was used to assess whether the data were normally distributed. The parameters IgG antibodies, antibody neutralizing activity, CD 4 T-cells and CD 8 T-cells were not normally distributed. Therefore, to analyse the immune response before versus after vaccination, the non-parametric Wilcoxon test was used. The six-months follow-up was also analysed using the Wilcoxon test, but only within the group of participants who showed up for the third blood sample. The Mann-Whitney-U test was used to compare the different vaccines and their efficacy.

To analyse the occurrence of side effects, the results were expressed as percentages for each side effect and vaccine. The training restrictions were analysed using the Mann-Whitney-U test, as these data were found to be not normally distributed. The duration of training restrictions was defined by the duration of the longest lasting adverse event. As side effects rated 2 were defined as moderate side effects with restrictions on daily routine and training, only side effects rated 2 or 3 were included in the calculation of training restrictions. As the mRNA vaccinations include two shots with possible side effects and training restrictions, but Ad26.COV2.S consists of only one vaccination, the training restrictions after the first and second mRNA vaccination were added together to compare the total number of days with training restrictions.

The significance level was set at $p < 0.05$ for the α error. The effect size for the Wilcoxon text and the Mann-Whitney-U-test was calculated with $|Z| / \sqrt{n}$ with Z being the standardised value and n the number of cases. Z was calculated with $x - \mu / \delta$. The effect size was defined with r being small > 0.10, medium > 0.30 and large > 0.50 [14].

The problem of multiple comparisons was addressed as follows: For the baseline comparison 6 months after vaccination no correction was needed since the two groups were only compared to each other with respect to the primary outcome signals of interest. Multiple comparison problems arose with respect to the longitudinal analyses which we solved employing the Bonferroni correction method. The correction is made by dividing the significance level (here: $p < 0.05$) by the number of tests. Since we introduced only one additional group comparison in extent to the baseline calculations, we had to adjust the Bonferroni-corrected significance level for the post-hoc analyses to $p < 0.025$.

4 Study adjustment

4.1 Motivation of study adjustment

At the end of 2021, analysis of the first data was possible. Due to the low and certainly in parts insufficient immune response after Ad26.COV2.S vaccination in terms of neutralizing antibody production, it was decided to adjust the study to ensure a presumably more adequate immune response in all participants. The 16 athletes who had been vaccinated with Ad26.COV2.S were informed of their inadequate immune response. Data on heterologous vaccination after Ad26.COV2.S vaccination was lacking but based on the successful heterologous booster vaccination for the ChAdOx1-S vaccine [58] it was expected to be sufficient and safe. As ChAdOx1-S is a vector vaccine like Ad26.COV2.S and the optimization after ChAdOx1-S vaccination was done with an mRNA vaccine, our study group recommended an mRNA vaccine as well. The adjustment was approved by the regional ethics committee on September 6th (Ärztekammer des Saarlandes, Saarbrücken, Germany).

4.2 Implementation adjustment

Eleven participants decided to continue their study participation with a change in their vaccination scheme, all of whom were vaccinated with BNT162b2 as the booster vaccination. As the first data analysis was performed approximately 2 months after vaccination, the second vaccination with Ad26.COV2.S was performed 119 days (mean ± 22 days) after the first vaccination. Another blood sample was taken after 19 days (mean ± 9 days) to determine the immune response after the heterologous boost. This limits the comparison between the different vaccine regimes. After this booster vaccination, all 11 participants completed another diary. Due to the delayed vaccination schedule for these athletes, a long-term follow-up at 6 months could not be realized. The adjusted study design for this subsample is shown in Figure 3.

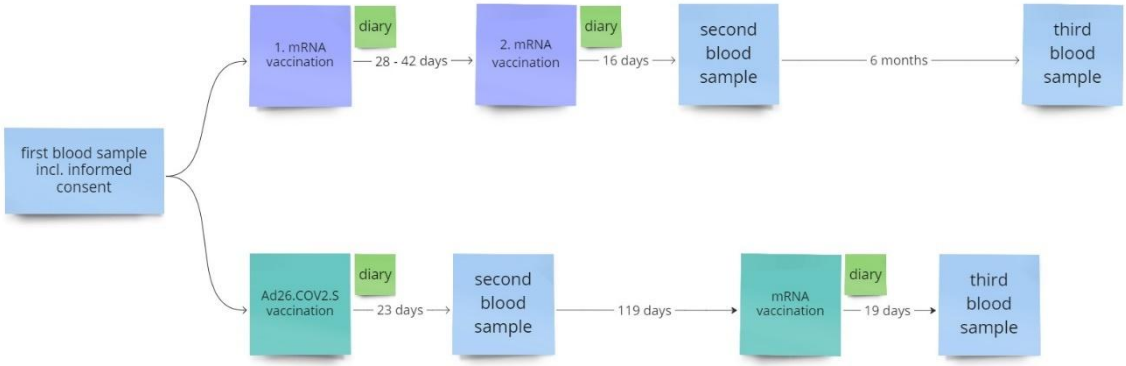

Figure 3: Process of adjusted implementation

5 Results

5.1 Immune response after vaccination

5.1.1 IgG antibodies

Compared to the Ad26.COV2.S vaccine the mRNA vaccines induced significantly more IgG antibodies (z = - 6.1, p<0.001, r = 0.71). The IgG antibodies before and after vaccination as well as their comparison is shown in Table 2. IgG antibodies are shown in Figure 4.

Vaccine	Parameter	Before vaccination		After vaccination				Comparison before and after vaccination		
		Median	IQR	Median	IQR	Min	Max	z	p	r
mRNA	IgG antibodies	5	4	5703	4343	677	79946	-4.2	<0.001	0.87
Ad26.COV2.S	IgG antibodies	4	2	61	52	23	245	-6.1	<0.001	0.71

Table 2: The IgG antibodies of the mRNA and Ad26.COV2.S vaccines before and after vaccination (threshold value for IgG antibodies: ≥35.2 BAU/ml; threshold value marks the level of the blood parameters leading to a positive result; Median, Interquartile Range (=IQR), Minimum (=Min) and Maximum (=Max) are given in BAU/ml.)

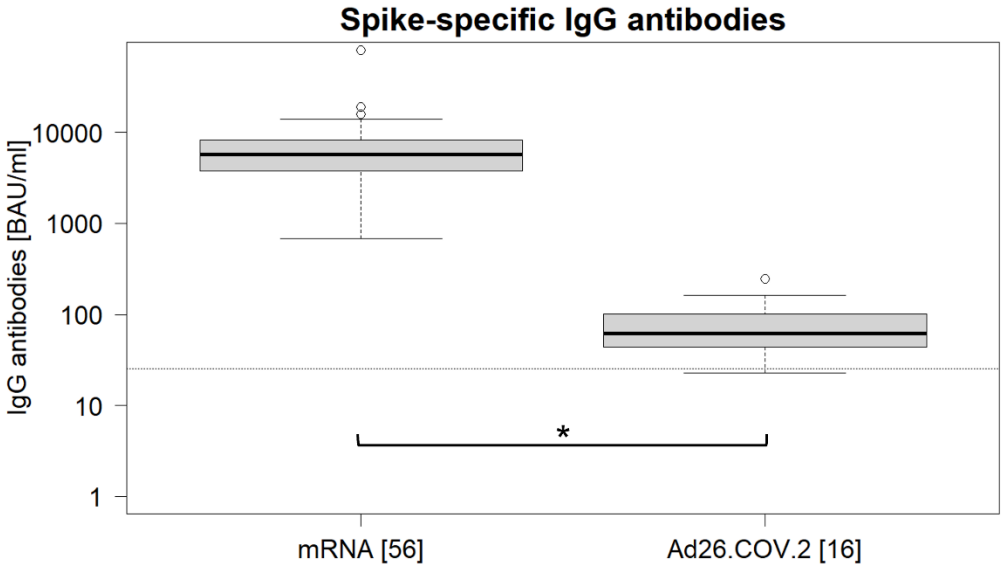

*Figure 4: Spike-specific IgG antibodies after mRNA and Ad26.COV2.S vaccination (bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

5.1.2 Neutralizing activity

The neutralizing activity was induced significantly stronger after mRNA vaccination than after Ad26.COV2.S vaccination (z = -6.1, p <0.001, r = 0.71). The neutralizing activity of the antibodies before and after vaccination as well as their comparison is shown in Table 3:

The graphic illustration of neutralizing activity is shown in the appendix A.3.1.

Vaccine	Parameter	Before vaccination		After vaccination				Comparison before and after vaccination		
		Median	IQR	Median	IQR	Min	Max	z	p	r
mRNA	Neutralizing activity	0	5.77	99.7	0.5	91	100	-6.5	<0.001	0.87
Ad26.COV2.S	Neutralizing activity	0	0	11	24	0	48	-3.4	<0.001	0.88

Table 3: Neutralizing activity of mRNA and Ad26.COV2.S vaccines before and after vaccination (threshold value for neutralizing activity: ≥35%; Median, Interquartile Range (=IQR), Minimum (=Min) and Maximum (=Max) are given in %)

5.1.3 CD 4 T-cells

The mRNA vaccines induced significantly more CD 4 T-cells than the Ad26.COV2.S vaccine (z = -4.4, p < 0.001, r = 0.52). The CD 4 T-cells before and after vaccination and its comparison is shown in Table 4. Graphic illustration of the CD 4 T-cells after mRNA and Ad26.COV2.S vaccination is shown in the appendix A.3.2.

Vaccine	Parameter	Before vaccination		After vaccination				Comparison before and after vaccination		
		Median	IQR	Median	IQR	Min	Max	z	p	r
mRNA	CD 4 T-cells	0	0.01	0.13	0.12	0.02	0.68	-6.5	<0.001	0.87
Ad26.COV2.S	CD 4 T-cells	0.01	0.01	0.05	0.05	0.05	0.17	-3.4	<0.001	0.87

Table 4: CD 4 T-cells of mRNA and Ad26.COV2.S vaccines before and after vaccination (threshold value for CD 4 T-cells: ≥0.03% ; Median, Interquartile Range (=IQR), Minimum (=Min) and Maximum (=Max) are given in %)

5.1.4 CD 8 T-cells

Overall, the Ad26.COV2.S vaccine induced significantly more CD 8 T-cells than the mRNA vaccine (z = - 4.1, p<0.001, r = 0.48). The CD 8 T-cells before and after vaccination and its comparison is shown in Table 5. CD 8 T-cell induction after mRNA and Ad26.COV2.S vaccination can be seen in a graphic in the appendix A.3.3.

Vaccine	Parameter	Before vaccination		After vaccination				Comparison before and after vaccination		
		Median	IQR	Median	IQR	Min	Max	z	p	r
mRNA	CD 8 T-cells	0	0.01	0.02	0.06	0	0.84	-4.9	<0.001	0.7
Ad26.COV2.S	CD 8 T-cells	0	0.01	0.15	0.19	0.02	1.3	-4.2	<0.001	0.88

Table 5: CD 8 T-cells of mRNA and Ad26.COV2.S vaccines before and after vaccination (threshold value for CD 8 T-cells: ≥0.03% ; Median, Interquartile Range (= IQR), Minimum (=Min) and Maximum (=Max) are given in %)

5.2 Reactogenicity after first and second vaccination

5.2.1 Occurrence of side effects

The occurrence of side effects was defined as the presence of a side effect with a severity of 1, 2 or 3 for at least one day.

After the first mRNA vaccination, all athletes reported pain at the injection site. After the second vaccination, only 76% reported the occurrence of pain at the injection site. Redness and swelling were rare side effects, with 15% of athletes reporting redness and 17% swelling after the first vaccination and 16% (redness) and 14% (swelling) after the second vaccination.

Systemic adverse events also occurred after the mRNA vaccines. Headache was reported by 45% of the participants after the first vaccination and 60% after the second vaccination, while fatigue was reported by 70% of the participants after the first vaccination and 71% after the second mRNA vaccination. Muscle pain was reported by 38% after the first vaccination and 43% after the second vaccination. Chills were reported less frequently with 6% after the first

vaccination and 24% after the second vaccination. Nausea was reported by 10% of the athletes after the first vaccination and 29% after the second vaccination.

After the first mRNA vaccination one athlete had to take an analgesic (1x ibuprofen 600 mg) and one athlete needed an ointment (heparin). After the second vaccination, two athletes needed to take an analgesic (1x voltaren dolo 25 mg, 1x ibuprofen 400 mg).

For the Ad26.COV2.S vaccine, the local side effect of pain at the injection point was 100%. One third of the athletes reported redness and 20% reported swelling of the injection point. The systemic adverse event of headache occurred in 87% and fatigue in 93%. Eighty-seven % of the athletes reported muscle pain and 67% reported chills. 20% of the athletes complained about nausea after the Ad26.COV2.S vaccination.

One athlete had to take three different painkillers after the first Ad26.COV2.S vaccination (aspirin 400mg, ibuprofen 600 mg, paracetamol 500 mg) and one athlete had to take one painkiller (ibuprofen 400mg).

Distribution of side effects is shown in appendix A.3.4.

5.2.2 Training restrictions due to side effects

Training Restrictions due to side effects were defined as 2 on the side effect severity scale. The double-dosed mRNA vaccine regime resulted in a median time of training restrictions of 2 days with an IQR of 1 day. The minimum time of training restrictions was 0 days, and the maximum time of training restrictions after the first and second mRNA vaccines was 8 days. The first Ad26.COV2.S vaccination also resulted in 2 days of training restrictions with an IQR of 1 day. The minimum value was 1 day, and the maximum value was 5 days of training restrictions. Comparison of the one-dosed Ad26.COV2.S vaccination and the double-dosed mRNA vaccine scheme showed that there was no significant difference in training restrictions between those vaccination schemes ($z = - 0.09$, $p=0.92$, $r=0.01$) – see appendix A.3.5.

5.3 Immune response after six months

The immune response was analysed again after six months. IgG antibodies, neutralizing activity, CD 4 T-cells and CD 8 T-cells 6 months after vaccination and in addition the median value directly after vaccination as well as their comparison is shown in Table 6. Graphic presentation of the data is shown in appendix A.3.6, A.3.7, A.3.8, and A.3.9.

Vaccine	Parameter	Directly after vaccination	6 months after vaccination				Comparison directly and 6 months after vaccination		
		Median	Median	IQR	Min	Max	z	p	r
mRNA	IgG antibodies	5703	1043	1112	125	6399	-7.7	<0.001	0.87
mRNA	Neutralizing activity	99.7	98.6	6	62	100	-4.8	<0.001	0.70
mRNA	CD 4 T cells	0.13	0.03	0.03	0	0.13	-5.9	<0.001	0.86
mRNA	CD 8 T cells	0.02	0.01	0.02	0	0.29	-3.0	0.003	0.45

Table 6: Immune response after six months of mRNA vaccine (threshold value IgG antibodies: ≥35.2 BAU/ml, neutralizing activity: ≥35%, CD 4 T-cells: ≥ 0.03%; CD 8 T-cells: ≥0.03%. Median, Interquartile Range (=IQR), Minimum (=Min) and Maximum (=Max) are given in % for neutralizing activity, CD 4 T-cells and CD 8 T-cells; for IgG antibodies they are given in BAU/ml)

5.4 Results after the study adjustment

5.4.1 Immune response

Following the mRNA boost of the Ad26.COV2.S vaccine, all parameters increased significantly. IgG antibodies, neutralizing activity, CD 4 T-cells and CD 8 T-cells after the mRNA boost vaccination and in addition the median value directly after vaccination as well as their comparison is shown in Table 7. Compared to the double-dosed mRNA vaccine scheme, Ad26.COV2.S + mRNA induced more IgG antibodies ($z = -2.6$, $p = 0.009$, $r = 0.32$). An overview with comparison of IgG antibodies after mRNA, Ad26.COV2.S and Ad26.COV2.S + mRNA is shown in Figure 5. In contrast, the Ad26.COV2.S + mRNA vaccine scheme induced significantly more neutralizing antibodies than the double-dosed mRNA vaccine regimen (z =

- 3.6, p <0.001, r = 0.45). There was no significant difference compared to the mRNA vaccination scheme in order of the CD 4 T-cell induction (z = - 0.6, p = 0.54, r = 0.08). Compared to the mRNA double-dosed vaccine scheme, Ad26.COV2.S + mRNA resulted in a significantly higher percentage of CD 8 T-cells (z = - 4.8, p <0.001, r = 0.58). Graphic illustration of neutralizing activity, CD 4 T-cells and CD 8 T-cells is added in the appendix A.3.10, A.3.11, and A.3.12

Vaccine	**Parameter**	**Directly after vaccination**	**After the boost vaccination**				**Comparison directly after vaccination and after boost vaccination**		
		Median	**Median**	**IQR**	**Min**	**Max**	**z**	**p**	**r**
Ad26.COV2.S	IgG antibodies	61	3456	2209	1069	6829	-3.3	<0.001	0.88
Ad26.COV2.S	Neutralizing activity	11	100	0.24	99.8	100	-3.3	<0.001	0.88
Ad26.COV2.S	CD 4 T cells	0.05	0.13	0.11	0.06	0.36	-2.6	<0.001	0.75
Ad26.COV2.S	CD 8 T cells	0.15	0.43	1	0.1	4.55	-2.6	<0.001	0.75

Table 7: Immune response of Ad26.COV2.S vaccine after study adjustment (threshold value IgG antibodies: ≥35.2 BAU/ml, neutralizing activity: ≥35%, CD 4 T-cells: ≥ 0.03%; CD 8 T-cells: ≥0.03%. Median, IQR, Minimum (=Min) and Maximum (=Max) are given in % for neutralizing activity, CD 4 T-cells and CD 8 T-cells; for IgG antibodies they are given in BAU/ml)

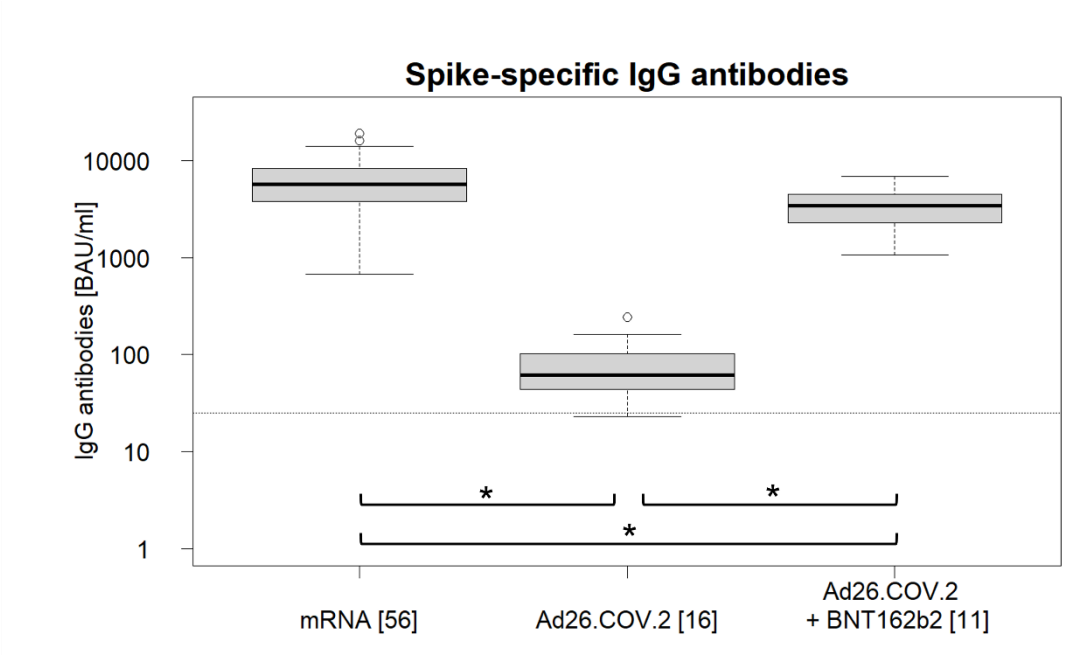

Figure 5: Spike-specific IgG antibodies after mRNA, Ad26.COV2.S and Ad26.COV2.S + BNT162b2 vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

5.4.2 Reactogenicity and training restrictions

The heterologous boost vaccination resulted in pain at the injection site in 92% of athletes. 8% reported swelling at the injection site and 8% reported redness as a side effect. Headache was reported by 75% of the cases, and fatigue by 83%. Muscle pains was reported by 67% and chills by 33%. 25% of the athletes experienced nausea following the mRNA boost after Ad26.COV2.S vaccination.

Three athletes took an analgesic (2x 400mg ibuprofen, 1x 600mg ibuprofen).

The combination of the Ad26.COV2.S and mRNA vaccination resulted in a median of 3 days of training restriction (IQR 1 day). The minimum value of training restriction was 2 days, and the maximum value was 5 days. The comparison between the double-dosed mRNA vaccine scheme and the double-dosed Ad26.COV2.S + mRNA vaccine scheme showed no significant difference ($z = -0.73$, $p = 0.46$, $r = 0.1$). Data of training restriction is shown in the appendix A.3.13.

6 Discussion

6.1 Discussion of the results

The aim of our study was to evaluate the humoral and cellular immune response after COVID-19 vaccination in elite athletes as well as training restrictions due to vaccine-induced adverse events. Different vaccines (mRNA based, vector based) with different schemes (double-dosed, single-dosed) were considered, and their immunogenicity and reactogenicity were analysed and compared. Due to the lack of data on the immune response in elite athletes and their reactogenicity after COVID-19 vaccination, this study was important to analyse the consequences of specific circumstances of vaccinating athletes against COVID-19. The main findings were that (i) the humoral and cellular immune response was evident after a single-dosed vector vaccine and double-dosed mRNA vaccine in elite athletes, (ii) there was a difference between the immunogenicity induced by the double-dosed mRNA and the single-dosed vector vaccine with Ad26.COV2.S being less potent and insufficient in terms of increasing IgG antibodies, neutralizing activity and CD 4 T-cells, but more potent in inducing CD 8 T-cell responses, (iii) training restrictions did not differ between the vaccine schemes, while side effects did not lead to substantial training loss, (iv) a heterologous boost vaccination after Ad26.COV2.S prime increased the humoral and cellular immune response in all investigated parameters, and (v) 6 months after vaccination the humoral and cellular immune response of mRNA vaccinated individuals decreased when compared to the initial response.

6.1.1 Immune response after vaccination

In the present study, we were able to show that the induction of IgG antibodies, their neutralizing activity, CD 4 and CD 8 T-cells was significant in both the double-dosed mRNA and the single-dosed vector vaccine group. According to Io Sasso et al. [33] antibodies help to block the entry of the virus into the cell and thus prevent an infection. An increase in these parameters would therefore be expected to reduce the risk of an infection with COVID-19.

In addition, the double-dosed mRNA vaccination induced three out of four parameters significantly more than the single-dosed vector vaccination, only CD 8 T-cells were higher after the single-dosed vector vaccination. The neutralizing activity of the antibodies for the single-

dosed vector vaccine was below the threshold for this target - as defined by the manufacturer – although it increased significantly from a statistical perspective. With the lower number of antibodies after the Ad26.COV2.S vaccine compared to the double-dosed mRNA vaccine and the lower percentage of neutralizing activity, the overall neutralizing capacity induced by the single-dosed vector vaccine is likely much lower, when looking at the absolute numbers of the induced blood parameters.

Initial studies about the Ad26.COV2.S vaccine showed an adequate induction of neutralizing antibody titres after a single dose of the vaccine, and a sufficient protection against asymptomatic or symptomatic infection with COVID-19 as well as against hospitalization, severe-critical disease and death [53]. Protection against severe-critical cases (defined as occurring after more than 28 days) was reported to be sufficiently high at 85%. Self et al. [60] also investigated IgG antibody induction and vaccine efficacy of preventing hospitalization following vaccination – he compared Ad26.COV2.S, BNT162b2 and mRNA-1273. The Ad26.COV2.S vaccine showed lower antibody responses – comparable to our results. Self et al. [60] also showed that the efficacy of preventing hospitalization after the single-dosed vector vaccination was lower at 71% compared to the double-dosed BNT162b2 (88%) and mRNA-1273 (93%) vaccine. This observation supports the notion that the number of antibodies correlates with the level of clinical protection, even though not in a linear manner. Although these results do not allow a precise modelling of the relationship between antibody titre and protection against infection, it is expected that a higher antibody titre will result in greater protection.

Over time, new virus variants emerged, challenging the efficacy of the vaccines and prompting more studies on antibodies and how they change with virus variants, as well as more answers on the efficacy of antibodies in general. Jongeneelen et al. [27] reported that the neutralization activity of antibodies after a single dose of Ad26.COV2.S vaccine differed depending on the virus variant infected with. Although some neutralizing antibody activities are lower than others, Jongeneelen et al. [27] claimed that these variations do not relevantly affect the efficacy of the vaccine. This conclusion was drawn because the B1.351 (Beta variant) had lower antibody activity but still protected against hospitalisation in 95% of the cases in this study population. Jongeneelen et al. [27] summarize that Ad26.COV2.S is still strong in the protection of severe courses of the disease. This was also observed and concluded by Alter et al. [1] who showed

5.0-fold lower neutralizing antibody titres against B.1.351 (“beta variant”) induced by Ad26.COV2.S vaccine and 3.3-fold lower neutralizing antibody titres against P.1 (“gamma variant”). Despite the lower neutralizing antibody titres, the CD 8 T-cell and CD 4 T-cell responses were largely preserved and the protective efficacy of Ad26.COV2.S was similar in all the geographical locations compared – regardless of the predominant virus variant. These two studies show that protection against severe disease can be possible even with low levels of neutralizing antibodies. However, the role of antibodies is to prevent infection, whereas T-cells play a more important role in preventing severe disease progression. Therefore, it cannot be assumed that low neutralizing antibody activity will still result in good protection against infection.

A good way to study the clinical efficacy of a vaccine is to detect breakthrough infections. Data from the RKI show that there were more breakthrough reactions after vaccination with Ad26.COV2.S than after vaccination with mRNA vaccine in Germany [51]. Those breakthrough infections were mostly seen in people between 18 and 59 years of age, which overlaps with the main age group in our study population. This also shows that breakthrough infections are not unusual for both vaccine groups, but mRNA is more beneficial in disease prevention than Ad26.COV2.S, which is consistent with the result of our study when looking at IgG antibodies and their neutralizing activity. This difference of induction of neutralizing activity for the mRNA vaccines and the Ad26.COV2.S vaccine was also seen in the study of Tada et al. [66] which investigated the neutralization activity of antibodies depending on the virus variant. While the BNT162b2 and mRNA-1273 vaccines had modest neutralization resistance against different virus variants, the Ad26.COV2.S vaccination showed lower neutralizing activity for virus variants but also for the wild type in general.

After evaluating various studies on Ad26.COV2.S, it has been shown that an adequate increase in antibodies and neutralizing antibody activity is important for an adequate protection of infection and therefore indirectly for prevention of hospitalisation and severe courses of the disease. Given the lack of adequate immune responses to the vaccine in our study and the percentagewise higher breakthrough infections in this age group in Germany, it must be said that the Ad26.COV2.S vaccine is not as sufficient as originally specified and its ability to prevent infection does not seem to be as strong as expected.

In addition to antibodies and their neutralizing activity, it is important to monitor and compare T-cells. Overall, T-cells are important for the prevention of severe cases of COVID-19 [20]. The results of our study showed that the induction of CD 8 T-cells was significantly higher after vaccination with Ad26.COV2.S compared to mRNA, while the opposite was observed for CD 4 T-cells. It is crucial to gain insight into the underlying mechanisms and implications of this difference. Rydyznski Moderbacher et al. [52] have shown that higher CD 8 T-cells are associated with a better outcome after COVID-19 infection, referring to its ability to exert cytotoxicity against infected cells. Thus, the Ad26.COV2.S vaccine can protect the body from severe cases even if it does not protect the body from an infection in the first place due to an inappropriate antibody response. On the other hand, CD 8 T-cells were observed in fewer patients than CD 4 T-cells after native infection [20] , showing that the body does not frequently build CD 8 T-cells when exposed to the virus. The role of CD 4 T-cells is also important to understand the differences in vaccine efficacy. First, the induction of antibody production against an infectious agent depends on the CD 4 T-cells [15] and the T-cells are required to produce high-quality neutralizing antibodies [52]. CD 4 T-cells are therefore important for inducing protection against the virus entering the body and its cells. Second, Rydyznski Moderbacher et al. [52] found that CD 4 T-cells are associated with less COVID-19 disease severity in a more prominent way than CD 8 T-cells or antibodies [52]. In our study, the CD 4 T-cells were induced more by the double-dosed mRNA vaccine than by the single-dosed vector vaccine. The importance of the adaptive immune responses was demonstrated by Sette et al. [61] who claimed that severe SARS-CoV-2 infections are associated with a late and inadequate adaptive immune response, including antibodies and T-cell responses. In contrast, individuals with a moderate SARS-CoV-2 infection have been shown to have a robust adaptive immune response.

In summary, it can be assumed that the mRNA vaccinated athletes may have a better protection against severe COVID-19 disease and an infection in the first place due to higher CD 4 T-cells, antibodies and neutralizing activity. With a higher CD 8 T-cell induction by the Ad26.COV2.S vaccine, very severe disease is less likely in athletes vaccinated with this vaccine. However, athletes cannot be considered typical candidates for such a severe course. It is not easy to compare the different mechanisms of the immune system and their significance, as the exact relationship between an absolute number and its importance for the immune system cannot easily be defined or measured. However, it is reasonable to assume

that the protection provided by the mRNA vaccine is higher because of better protection against infection in the first place and good protection against severe disease. This does not seem to be equalised by the higher protection against very severe courses after Ad26.COV2.S vaccination.

6.1.2 Reactogenicity after vaccination

As hesitation regarding vaccination of athletes due to side effects and associated training loss [23] is an issue, consideration and classification of the side effects and associated limitations is very important. It has to be considered that there may be additional reasons for athletes not to train besides side effects, such as general caution after vaccination or official recommendations or restrictions from governing bodies. However, in our study only training restrictions that were caused by side effects with a score greater than 1 were considered, meaning that they either limited practice or made it impossible.

In our study, the overall incidence of side effects differed between vaccines and vaccine schemes. Percentagewise the first mRNA vaccine resulted in fewer systemic side effects than the second one. This is consistent with the results of several studies that found systemic adverse events being more frequent after the second vaccination [36],[42]. For the Ad26.COV2.S vaccine, headache and fatigue were the most common side effects in our athletes. In a study by Krzywanski et al. [29] side effects were also observed and analysed according to their cumulative occurrence. It was shown that the Ad26.COV2.S vaccine led to more side effects overall, regardless of whether it also led to longer training absence.

In our study population of athletes, the average number of days of training restrictions was 2 days – regardless of which vaccine was given (for the double-dosed mRNA vaccines the training restrictions were cumulative). Training restrictions were defined as moderate side effects that interfered with daily routine and practice. This means that limited training was still possible, but either the training plan had to be changed, or the training content had to be reduced. Comparison with other studies is difficult because of different methodological approaches. For example, Hull et al. [24] reported the percentage of athletes who were able to train without problems (73%) and the percentage who were unable to train at all (6%) with the rest being in between. It was shown that athletes with moderate side effects also had one day of restrictions after the first and the second vaccination. Another study that analysed similar

restrictions due to vaccination in athletes was conducted by Krzywanski et al. [29]. Their study showed slightly higher proportion of athletes experiencing side effects and related trainings restrictions. While 28% felt that their training was affected, 19% had to stop training for at least one day. It is not easy to compare these studies because of the different study designs, but also because of national requirements and different national practices in dealing with COVID-19 in each country. Nevertheless, they all show that training restrictions due to the vaccines are limited and that vaccination does not necessarily interfere with the entire training schedule. Thus, on average adverse events in elite athletes appear to have a limited impact on training. However, individual athletes may be affected for considerably longer (up to 9 days; [24]), leading to the recommendation that vaccinations should be scheduled as far away from the next competition as possible to minimise the impact on training processes. Furthermore, Krzywanski et al. were able to show that a COVID-19 infection causes more time loss in practice than the side effects of vaccination against it [29]. It is evident that, despite the inconvenience of training restrictions after vaccination, vaccination is the superior option in terms of training restrictions compared to an infection. This is due to the shorter duration of training restrictions following vaccination, as well as the greater flexibility of a planned vaccination. While a vaccination can be scheduled and integrated into a daily training regimen, an infection can occur at any time, potentially disrupting a phase of high-intensity training or competitions.

6.1.3 Vaccination of Olympic Games candidates

In spring of 2021, the Ad26.COV2.S vaccine was considered “as a pragmatic choice” [36] for Olympic aspirants to ensure good protection during the Olympic Games, due to its characteristics of only one vaccine shot needed and fewer expected side effects and training restrictions. This procedure was not only used in Germany, for example the Polish Olympic participants were also vaccinated with Ad26.COV2.S [29].

The present study showed that – retrospectively - the immune response after the Ad26.COV2.S vaccination was not sufficient to justify any prioritisation over the double-dosed mRNA vaccination, even when the supposed faster attainment of immunity was taken into account. It is not rationale strive for “quick” immunity after three weeks instead of 6-8 weeks (depending on the interval between the first and second mRNA vaccine) if the immunity is not good enough to protect athletes from infection and disease. On the one hand, it is not

unimportant that the Ad26.COV2.S vaccine protects athletes from severe cases of infection but on the other hand a positive PCR test at the Olympic Games already led to exclusion from any competition – regardless of whether the athletes were sick or not. It was therefore very important for Olympic athletes to be protected from infection to be allowed to travel, compete and perform at the Olympics. Another advantage of the Ad26.COV2.S vaccine appeared to be fewer side effects and training restrictions due to its property of being single-dosed. The current study showed that training restrictions did not differ between the single-dosed vector vaccine and double-dosed mRNA vaccines, leading to the conclusion that there is no relevant advantage of the single-dosed vector vaccine in terms of training restrictions and impact on the preparations for the Olympic Games. Additionally, despite comparable training restrictions between the vaccinations, the mRNA vaccination resulted in a sufficient immune response, in contrast to the Ad26.COV2.S vaccination. In addition to the lower protection of the athletes, the loss of training due to an insufficient vaccination led to an increase in the negative attitude of athletes towards vaccinations. Sufficient vaccination is therefore not only important to protect athletes, but also to strengthen compliance for subsequent vaccinations.

6.1.4 Immune response after study adjustment

The vaccination with Ad26.COV2.S was not satisfactory in terms of immune response parameters in our study population of athletes, which led to the decision to change the study protocol for ethical reasons. In August 2021, there was a lack of information on heterologous boost vaccination after Ad26.COV2.S vaccination. But in spring 2021, the recommendation for the vector vaccine ChAdOx1-S was revised due to unexpected issues of life-threatening cerebral venous thrombosis and thrombocytopenia [32], which led to the use of heterologous vaccination schemes with a vector vaccine followed by an mRNA vaccine. Schmidt et al. [57] analysed the different types of vaccine schemes and compared heterologous and homologous schemes [57]. They were also able to show that a heterologous mRNA vaccine regimen following a ChAdOx1-S vaccination was more effective in stimulating the immune system than a ChAdOx1-S homologous scheme. In addition, they demonstrated that the heterologous scheme was as effective as a homologous mRNA scheme in terms of immune stimulation. This knowledge was taken into consideration when the decision was made about how to proceed with our study.

Other studies also decided in favour of heterologous boost vaccinations, which aligns with our findings that the Ad26.COV2.S vaccine does not produce sufficient results. Atmar et al. [2] analysed the boost vaccination after the single shot vector vaccine Ad26.COV2.S for its ability to induce antibodies and their neutralizing activity. It was shown that the induction of the comparably low antibody titres after Ad26.COV2.S vaccination increased significantly after a second vaccination with mRNA-1273 as well as after BNT162b2. The same increase in neutralizing antibodies was observed after the heterologous vaccination scheme. These results are in line with the results of our study and show that a second vaccination with an mRNA vaccine – in our case the BNT162b2 vaccine – is effective. It helps to increase the observed parameters and - given the discussed importance of IgG antibodies, their neutralizing activity and T-cells - most likely the protection of the athletes. To ensure the health of athletes, the decision to optimize their immune response with a second vaccination (in our case: BNT162b2) was reasonable and sensible at the time.

In line with the results of our study, the vaccination recommendations for people vaccinated with Ad26.COV2.S in Germany were changed in October 2021 [51]. The recommendation was aimed at people who had received a Ad26.COV2.S vaccine and subsequently had no confirmed COVID-19 infection. For them, a booster vaccination with an mRNA vaccine according to a heterologous vaccination scheme was recommended at least 4 weeks after the Ad26.COV2.S vaccination. As mentioned before, the Ad26.COV2.S vaccine had the highest number of breakthrough infections after vaccination at this time, with an efficacy against symptomatic infections of 2-36%, whereas BNT162b2 (83%), mRNA-1273 (83%) and ChAdOx1-S (61%) were more effective in preventing symptomatic infections [51].

Overall, the adaptation of the study protocol was justified on scientific and ethical grounds. Our results confirm this adjustment and its justification based on the improved immune response and the associated better protection against infection and severe disease progression. The same procedure was subsequently applied in other studies at the similar time.

6.1.5 Reactogenicity after study adjustment

The reactogenicity after the study adjustment was observed as well. Occurrence and prevalence of the side effects were slightly different to the first vaccine shot with Ad26.COV2.S and slightly different to the second mRNA vaccine shot. Still, side effects were well tolerated

and showed acceptable occurrence. In their study Atmar et al. [2] compared different booster vaccination schemes including the vaccines Ad26.COV2.S, mRNA-1273 and BNT162b2. In comparison to the results of this study, the prevalence of headache and fatigue was lower. Overall, their study showed that it was all well tolerated and that there was no difference to their primary series of vaccination.

The training restrictions due to side effects cumulated after the first Ad26.COV2.S and the followed BNT162b2 vaccine led to a median restriction of 3 days. There was no significant difference to the double-dosed mRNA vaccines. As previous information about reactogenicity of athletes after a heterologous boost vaccination with BNT162b2 was not given, our study showed that training restrictions due to heterologous boost vaccination are comparable to homologous double-dosed scheme.

6.1.6 Immune response after 6 months

Six months after the last vaccination, all tested blood parameters significantly decreased. While the number of antibodies and the neutralizing antibodies were still above the threshold value (which marks the level of the blood parameters leading to a positive result), the CD 4 T-cells were close to the threshold value and the CD 8 T-cells were below the threshold value. The study by Choi et al. [13] also observed the course of the antibodies and their neutralizing activity after mRNA-1273 vaccination. After six months the amount of antibodies decreased significantly by 58%. A significant decrease in neutralizing activity was also observed. These results correlate with our data. The study also analysed these parameters of interest before the second vaccination, and the antibodies and their neutralizing activity were higher after six months than immediately before the second vaccination. Tre-Hardy et al. [68] analysed not only the time course of antibodies and their decline after mRNA-1273 vaccination, but also possible association with age, sex, and body mass index (BMI) of the vaccinated individuals. While they did not find an association between these parameters and the decrease in antibodies, the study by Terpos et al. [67] claimed something different. In their cohort, younger age was associated with higher antibody titres 3 months after BNT162b2 vaccination. Although the comparison is limited due to the different timing and vaccines, this is an interesting finding, especially for athletes who tend to be younger.

In addition to the development of antibodies and neutralizing activity, it is also important to understand the time course of T-cell responses. As in the previously named studies, Zhang et al. [72] observed antibodies and their tendency to decrease after six months. However, they also investigated T-cells and their development over time. In contrast to our results, they found a relatively stable number of T-cells over time, which they associate with a lower number of hospitalizations, as T-cells can prevent severe courses of the disease. A similar conclusion was reached by Chemaitelly et al. [4] who found no evidence of decreased efficacy in protecting against severe courses of COVID-19 – although the overall efficacy in preventing infections decreased by more than 50% after seven months. Overall, Chemaitelly et al. [4] conclude that symptomatic courses of COVID-19 were prevented more effectively than asymptomatic ones. Although the importance of preventing asymptomatic cases decreased over time, it was important at the beginning of the pandemic and during the Olympic Games, where exclusion criteria was a positive PCR test that was not related to symptoms and therefore athletes with asymptomatic courses were not allowed to participate. In summary, it can be assumed that declining immune parameters over time lead to poorer protection against disease and severe courses of disease. In Germany, this has led to a STIKO recommendation that a booster should be given 6 months after the initial vaccination. For persons under 30 years of age vaccination with the BNT162b2 vaccine was recommended.

6.2 Limitations and Outlook

Due to the vaccine shortage at the start of this study, it was not possible to randomly allocate the different vaccines to the athletes. Therefore, the number of athletes within the different vaccine groups varied, limiting the comparability between these groups. However, still vaccine assignment followed something like chance (as opposed to preference). Another limitation is the unexpected adjustment of the study due to an insufficient immune response after vaccination with Ad26.COV2.S. The interval between the Ad26.COV2.S vaccination and the newly added booster vaccination with BNT162b2 was longer than between the two planned mRNA vaccinations. This limits the comparability between groups. Unpredictable changes in the national COVID-19 policy had a relevant impact on our study protocol without invalidating the measurements per se but weakening the conclusions. In addition, we assessed surrogate markers of immunity in terms of antibody concentrations and T-cell counts, but not the clinical

phenomena of breakthrough infection and clinical disease. Furthermore, it is beyond the scope of this study to differentiate between different viral variants that have emerged over time like alpha (B.1.1.7), beta (B.1.351), gamma (P.1) or delta (B.1.617.2) or to differentiate between different sports disciplines.

For further studies, it would be interesting to investigate the relationship between the analysed immune response and the number of people infected after vaccination, as well as the severity of the infection. This could provide a better understanding of the true risk of infection after vaccination and the protection provided by the immune response. However, much larger samples would be necessary. In addition, it would be beneficial to determine the attitudes of athletes towards vaccination in general to derive better vaccination recommendations specifically for athletes and to increase the willingness of athletes to be vaccinated by addressing their concerns.

Another new issue that can be explored in the future is a more detailed analysis of the side effects. In our study, side effects and training limitations were recorded using paper-based questionnaires. It would also be interesting to investigate limitations using objective measuring devices. Nowadays, there are many possibilities for this, such as fitness watches or similar devices. These can record values such as heart rate, heart rate variability, sleep phases and skin temperature and correlate them with the vaccination and existing side effects.

In addition, it may be interesting to analyse the long-term immune response after the mRNA boost vaccination which was not possible in our study due to the unforeseeable study adjustment. The focus of our study was laid on the immune response in athletes. Another question was the difference in the immune response and reactogenicity between athletes and non-athletes to understand the differences in how their bodies deal with the COVID-19 vaccination, which was also performed in our study group and has already been submitted for publication.

6.3 Conclusion

Overall, the COVID-19 vaccinations were well tolerated by the athletes and induced an immune response. However, contrary to our hypothesis, there were differences between the vaccine schemes. While the repeated application of mRNA vaccines (mRNA-1273 and BNT162b2)

elicited a sufficient immune response, indicating a high level of protection against both infection and a severe course of the disease, a single vaccination with Ad26.COV2.S was unsatisfactory. Antibody and neutralizing antibody production were insufficient and protection against infection did not appear to be provided. The well-developed CD 8 T-cells and the associated protection against severe courses and hospitalization may not compensate for this disadvantage.

The side effect profiles of the different vaccines and vaccine schemes were similar. Training restrictions due to side effects did not differ between the different vaccines, although Ad26.COV2.S was administered as a single-dosed and mRNA as a double-dosed vaccine Overall, training restrictions were acceptable for all vaccines, and the potential side effects of vaccination do not significantly impact the long-term training plan if appropriate precautions are taken.

The boost immunization with BNT162b2 after the Ad26.COV2.S vaccination was a reasonable choice to optimize the immune response of the athletes and to ensure lower infection rates, less severe courses of disease and guarantee a safer sport. For the athletes, the booster vaccination did not cause any disadvantage in terms of immune response and protection against disease and severe progression. In addition, the side effects and training limitations were also comparable to those of homologous vaccination regimens.

Vaccinating athletes in times of emerging health concerns was difficult, as brand-new vaccines that had not been extensively tested in humans were needed to ensure safer Olympic Games and other sport events. So, a balance had to be found between different risks. This study does not provide any evidence against vaccinating athletes against COVID-19, but it does show that it is important to monitor new vaccines to respond to unforeseen challenges, in this case insufficient protection.

7 Literature

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A. Appendix

A.1 Written informed consent

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Universität des Saarlandes | Campus Homburg
Abteilung für Transplantations- und Infektionsimmunologie | 66421 Homburg
Probandenaufklärung

Aktenzeichen
Datum 14.09.23
Projekt **Analyse der SARS-CoV-2-spezifischen Immunantwort bei Leistungssportlerinnen und Leistungssportlern nach COVID-19 Impfung**

Sehr geehrte Probandin, sehr geehrter Proband,

Das erstmals Ende 2019 nachgewiesene neue Coronavirus (SARS-CoV-2) hat sich mittlerweile weltweit verbreitet und bei vielen Menschen die Krankheit COVID-19 verursacht. Seit Ende 2020 ist nun eine Impfung gegen SARS-CoV-2 verfügbar. Durch die Impfung, bestehend aus einer zweimaligen Gabe des Impfstoffs im Abstand von mindestens 3 Wochen, wird eine Abwehrkraft gegen das Virus in Form von sogenannten Antikörpern und T-Zellen gebildet. Es ist davon auszugehen, dass die Impfung auch bei Personen mit regelmäßigem leistungssportlichen Training einen Schutz vor einer Erkrankung vermittelt. Bislang liegen keine Erkenntnisse vor, in welchem Maße Antikörper und T-Zellen bei Menschen unter leistungssportlichem Training gebildet werden.

Unsere Arbeitsgruppen beschäftigen sich schon seit längerer Zeit mit der menschlichen Immunantwort auf bestimmte Krankheitserreger sowie deren Beeinflussbarkeit. Zu den zu berücksichtigenden Einflussfaktoren zählt auch die leistungssportliche Aktivität. Die für Leistungssportlerinnen und -sportler besonders bedeutsamen Schutzimpfungen erfordern eine adäquate Immunantwort, um voll wirksam zu sein. Daher befasst sich diese wissenschaftliche Studie mit den Auswirkungen einer COVID-19 Impfung bei Leistungssportlerinnen und -sportlern.

Studienleitung

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Transplantations- und Infektionsimmunologie

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Die Vermehrung eines Erregers wird in erster Linie von sogenannten T-Zellen kontrolliert. Des Weiteren wird die Infektion durch Antikörper kontrolliert, die ihrerseits von B-Zellen gebildet werden. In diesem Projekt soll die Immunreaktion auf eine COVID-19 Impfung untersucht werden. Das Ziel einer Impfung ist es, den Körper zur Bildung dieser spezifisch gegen einen bestimmten Erreger wirkenden Immunzellen und Antikörper anzuregen. Sowohl T- als auch B-Zellen gehören den weißen Blutkörperchen an und können nach einer einfachen Blutentnahme rasch analysiert werden. Wir möchten in diesem Projekt im Kontext der COVID-19 Impfung die gegen das SARS-CoV-2 gerichtete Abwehrkraft untersuchen. Aus dieser Analyse können sich wertvolle, klinisch relevante Rückschlüsse über die Stärke der durch die Impfung hervorgerufenen Abwehrkraft ergeben, die unter anderem die Frage klären können, ob und wie lange die Impf-induzierte Immunabwehr vor einer SARS-CoV-2 Infektion schützen kann. Ein weiteres Ziel dieser Analyse ist es, herauszufinden, inwieweit Leistungssportlerinnen und –sportler in ihrer Immunfunktion beeinträchtigt sind bzw. inwieweit leistungssportliches Training zu einer Beeinträchtigung der Impfantwort oder der Impfverträglichkeit führt. Zur besseren Beurteilung der Ergebnisse sind auch Vergleichsmessungen an nicht leistungssportlich aktiven Kontrollpersonen notwendig.

Wir sprechen Sie an als eine Person, die gemäß der vom Robert Koch Institut empfohlenen Priorisierung eine COVID-19 Impfung erhält. Bei Teilnahme an der Studie zur Analyse der Immunität benötigen wir von Ihnen je eine Blutprobe von 5 ml (ca. 1 Teelöffel) vor der Impfung sowie 2-3 Wochen nach dem kompletten Impfzyklus (2 Wochen nach der 2. Impfung bzw. 3 Wochen nach der 1. Impfung bei Impfstoffen, die lediglich einmalig verabreicht werden). Eine weitere Messung erfolgt 6 Monate später, um die Langlebigkeit der SARS-CoV-2-spezifischen Immunabwehr zu untersuchen. Die Entnahme dieser Blutproben ist grundsätzlich nur mit einem sehr geringen Risiko verbunden. An der Einstichstelle kann es zu leichten Schmerzen kommen oder es kann ein "blauer Fleck" (Bluterguss) entstehen, der eventuell einige Tage sichtbar ist. In äußerst seltenen Fällen kann nach Blutentnahme auch die Bildung eines Blutgerinnsels (Thrombose), eine örtlich begrenzte Entzündung oder eine Infektion an der Einstichstelle auftreten oder es kann zu Schädigungen von Blutgefäßen oder Nerven kommen.

Im Nachgang einer Impfung kann es zu Schmerz an der Impfstelle und/oder zu allgemeinen Nebenwirkungen wie leichtes Fieber, Erschöpfung, Kopfschmerz, Muskelschmerzen oder Lymphknotenschwellungen kommen. Etwaige Nebenwirkungen werden in Form eines Fragebogens erfasst.

Im Rahmen der Studie werden von Ihnen am Lehrstuhl für Transplantations- und Infektionsimmunologie sowie am Institut für Sport- und Präventivmedizin der Universität des Saarlandes Daten erhoben. Diese Datenerhebung sowie die Auswertung der Daten in pseudonymisierter Form erfolgt unter der Verantwortung der Studienleitung Prof. Dr. Tim Meyer und Prof. Dr. Martina Sester. Pseudonymisiert bedeutet, dass Ihre Daten nicht mit Ihrem Namen oder Ihren Initialen, sondern

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nur mit einem Buchstabencode gekennzeichnet werden. Diese Datenerhebung, Speicherung und Verarbeitung erfolgt nach den Vorgaben der DSGVO (Datenschutz-Grundverordnung).

Sie haben jederzeit das Recht, Auskunft über die von Ihnen gespeicherten Daten zu erhalten und eine kostenlose Kopie dieser Daten anzufordern. Im Falle dass Daten fehlerhaft erhoben wurden, können Sie jederzeit eine Berichtigung verlangen.

Die Bestimmungen der ärztlichen Schweigepflicht und des Datenschutzes sind gewährleistet. Wir weisen jedoch darauf hin, dass zu Kontrollzwecken den Überwachungsbehörden bzw. speziell autorisierten Personen eine Einsichtnahme in Ihre Krankenakte gestattet wird. Mit Ihrer Einwilligung zur Teilnahme an der Studie willigen Sie auch in diese Offenlegung ein. Sollten Sie weitere Fragen zu dem Thema Datenschutz haben, so beantwortet Ihnen diese der/die Datenschutzbeauftragte der Universität des Saarlandes unter:

Meerwiesertalweg 15, 66123 Saarbrücken

Telefon: 0681 302-2813/Fax: 0681 302-2687

E-Mail: datenschutz@uni-saarland.de

Wir versichern Ihnen, dass Ihre personenbezogenen Daten absolut vertraulich behandelt werden und nicht an die Öffentlichkeit gelangen. Wenn die Ergebnisse der Studie veröffentlicht werden, bleibt Ihre Identität natürlich geheim. Sollte dennoch ein Anlass zu Beschwerden bestehen, so können Sie sich jederzeit an die zuständige Datenschutz-Aufsichtsbehörde wenden:

Unabhängiges Datenschutzzentrum Saarland

Fritz-Dobisch-Straße 12, 66111 Saarbrücken

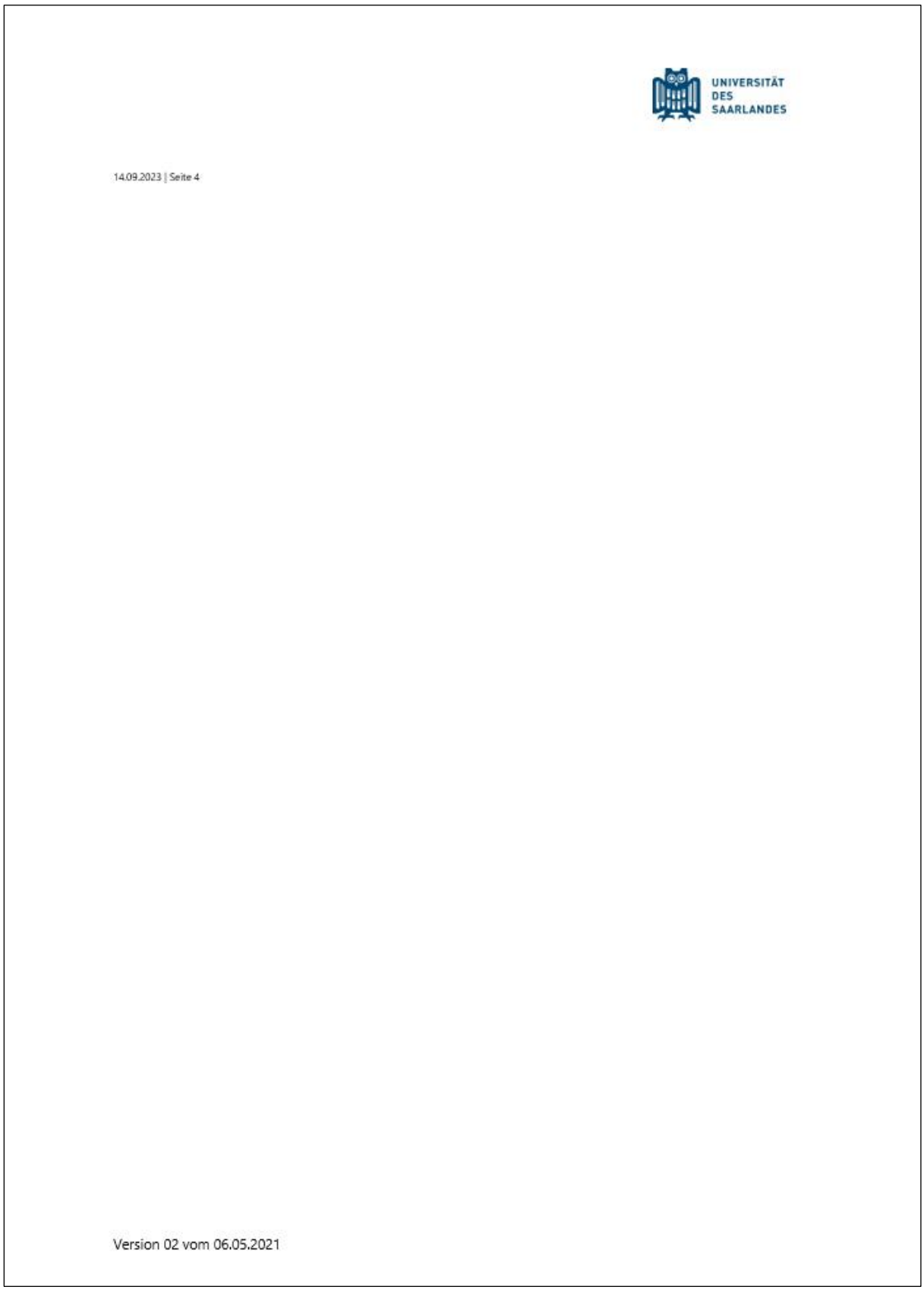
Telefon: 0681 94781-0/Telefax: 0681 94781-29

Email: poststelle@datenschutz.saarland.de

Die Teilnahme an der Studie ist freiwillig. Die Ablehnung ebenso wie der Widerruf der Einwilligung ist ohne Nachteile möglich. Im letzteren Falle besteht das Recht auf Löschung der bis dahin erhobenen Daten.

Für Ihre Mithilfe möchten wir uns herzlich bedanken!

Version 02 vom 06.05.2021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Analyse der SARS-CoV-2-spezifischen Immunantwort bei Leistungssportlerinnen und Leistungssportlern nach COVID-19 Impfung

Einwilligungserklärung

Ich, ..., geb. am ....., habe die umseitig wiedergegebenen Informationen über die Analyse der Impf-induzierten Immunantwort nach SARS-CoV-2 Impfung gelesen. Ich willige in die Blutentnahmen zu diesem Zweck ein. Zudem erkläre ich meine Einwilligung zur Verwendung der daraus ermittelten Daten.

Ich habe die Information gelesen und verstanden und alle Fragen sind ausreichend beantwortet worden. Ich weiß, dass ich jederzeit meine Einwilligung ohne Angabe von Gründen zurückziehen kann. In diesem Falle werden auch sämtliche Ergebnisse und Materialien vernichtet.

Ich wurde darüber aufgeklärt, dass meine persönlichen Daten im wissenschaftlichen Institut verbleiben bzw. an das Institut für Sport- und Präventivmedizin in Saarbrücken weitergegeben werden; ihre Archivierung erfolgt nur in verschlüsselter Form, damit sich für Dritte kein Hinweis auf meine Identität ergeben kann. Ich erhalte eine Kopie der Informationsschrift und der Einwilligung.

_____ _____

Datum, Ort Unterschrift des Probanden/der Probandin

_____ _____

Datum, Ort Unterschrift der Ärztin/des Arztes

_____

Name des Arztes/der Ärztin [in Druckbuchstaben]

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Analyse der SARS-CoV-2-spezifischen Immunantwort bei Leistungssportlerinnen und Leistungssportlern nach COVID-19 Impfung

Einwilligungserklärung

Ich, ..., geb. am ....., habe die umseitig wiedergegebenen Informationen über die Analyse der Impf-induzierten nach SARS-CoV-2 Impfung gelesen. Ich willige in die Blutentnahmen zu diesem Zweck ein. Zudem erkläre ich meine Einwilligung zur Verwendung der daraus ermittelten Daten. Ich erkläre mich mit einer Blutentnahme bei mir bzw. meinem Kind bzw. der von mir betreuten Person (Name des Kindes/der betreuten Person: .....) zu diesem Zweck einverstanden.

Ich habe die Information gelesen und verstanden und alle Fragen sind ausreichend beantwortet worden. Ich weiß, dass ich jederzeit meine Einwilligung ohne Angabe von Gründen zurückziehen kann. In diesem Falle werden auch sämtliche Ergebnisse und Materialien vernichtet.

Ich wurde darüber aufgeklärt, dass meine persönlichen Daten im wissenschaftlichen Institut verbleiben bzw. an das Institut für Sport- und Präventivmedizin weitergegeben werden; ihre Archivierung erfolgt nur in verschlüsselter Form, damit sich für Dritte kein Hinweis auf meine Identität ergeben kann. Ich erhalte eine Kopie der Informationsschrift und der Einwilligung.

_____	_____
Datum, Ort	Unterschrift des Probanden/der Probandin des Erziehungsberechtigten/der Erziehungsberechtigten des gesetzlichen Betreuers/der gesetzlichen Betreuerin
_____	_____
Datum, Ort	Unterschrift der Ärztin/des Arztes
	_____
	Name des Arztes/der Ärztin [in Druckbuchstaben]

Version 02 vom 06.05.2021

Medikamente und behandelte Beschwerden bei Nebenwirkungen				
Substanz	**Dosis**	**Indikation**	**Datum Beginn**	**Datum Ende**

Bemerkungen

Tagebuch

Vertraulich

Name:

Geburtsdatum:

Sportart:

Datum der Impfung:

Liebe/r Teilnehmer/in,

wir freuen uns, dass Sie an unserer Studie teilnehmen, in der wir untersuchen wollen, ob und ggf. wie sich körperliches leistungssportliches Training die Wirksamkeit und Nebenwirkungen einer COVID-Impfung beeinflusst. Daher ist es erforderlich, dass Sie beiliegendes Nebenwirkungstagebuch ausfüllen. In diesem Tagebuch werden einerseits die Lokalreaktionen erfasst. Dazu gehört möglicherweise ein Schmerz, eine Rötung oder eine Schwellung an der Injektionsstelle. Im zweiten Teil des Tagebuches werden sog. „Allgemeinreaktionen" erfasst. Hierzu gehören Kopfschmerzen, Muskelschmerzen, Schüttelfrost, Übelkeit und Müdigkeit. Wenn weitere Symptome auftreten sollten, können Sie diese unter „Sonstige" ebenfalls eintragen. Bitte tragen Sie alle Beschwerden ein, auch wenn Sie denken, dass sie nicht im Zusammenhang mit der Impfung stehen. Wenn Sie die Körpertemperatur bestimmen, dann messen Sie die Temperatur bitte abends. Wenn Sie mehrmals am Tag messen, tragen Sie bitte die höchste Temperatur ein. Wenn Sie den Durchmesser einer Rötung oder einer Schwellung messen, tragen Sie bitte auch dann den größten Durchmesser ein.

Bitte tragen Sie auch ein, wenn Sie Medikamente benötigen, um Nebenwirkungen zu behandeln. Wenn Sie keine Symptome haben, tragen Sie bitte eine „0" in die Felder ein.

Bitte bringen Sie dieses Tagebuch bei Ihrem nächsten Besuch mit, oder schicken Sie es an folgende Adresse:

Lea.halmans@t-online.de **Ihre nächste Blutabnahme ist am:**

Wenn schwere oder unerwartete Reaktionen auftreten, kontaktieren Sie uns bitte sofort:
Telefonnummer: **01520-7605612** (Herr Dr. med. **Andreas Venhorst**)

Vielen Dank für Ihre Hilfe.

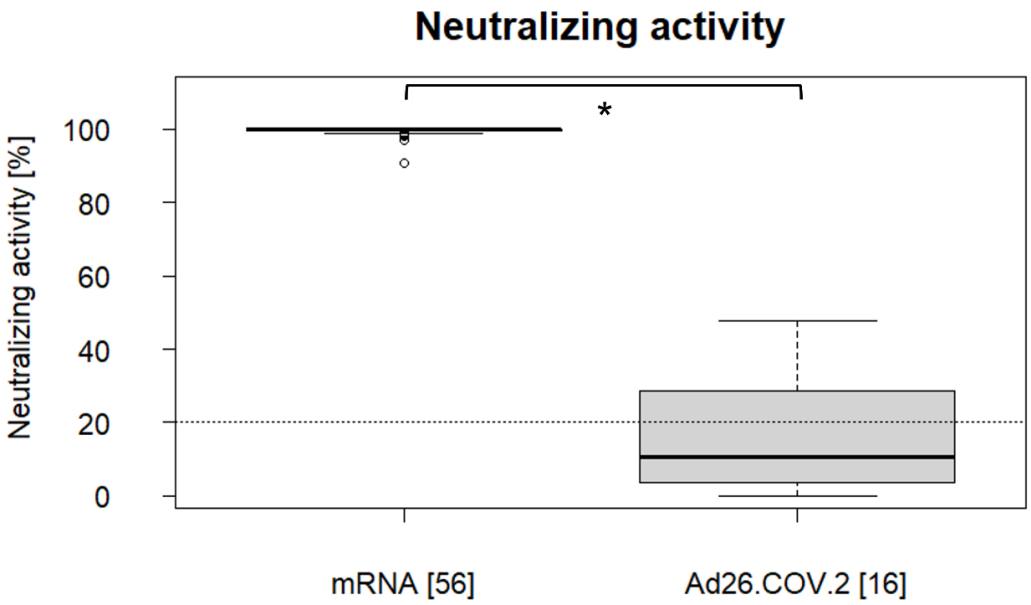
Bitte füllen Sie die Tabelle aus

Lokale Beschwerden an der Einstichstelle		Tag der Impfung	Tag 1	Tag 2	Tag 3	Tag 4	Tag 5	Tag 6	Tag 7	Symptome nach Tag 7		
										Hält noch nach 7 Tagen an?	Datum Letzter Tag mit Beschwerden	Maximaler Schweregrad
Schmerzen	0 = Keine Schmerzen 1 = Schmerz bei Berührung 2 = Schmerz bei Bewegung des Armes 3 = Spontan schmerzhaft									❒ nein ❒ ja	dd mmm yyyy	
Rötung	Größe in mm Bitte geben Sie den größten Durchmesser an, wenn sie mehrmals am Tag nachmessen	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	❒ nein ❒ ja	dd mmm yyyy	____ mm
Schwellung		____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	❒ nein ❒ ja	dd mmm yyyy	____ mm
Andere (z.B. Stechen) _____	0 = Keine Symptome 1 = Mild: Tägliche/sportliche Aktivitäten nicht beeinträchtigt 2 = Moderat: Tägliche/sportliche Aktivitäten beeinträchtigt 3 =Schwer: Tägliche/sportliche Aktivitäten können nicht mehr durchgeführt werden.									❒ nein ❒ ja	dd mmm yyyy	
Andere _____										❒ nein ❒ ja	dd mmm yyyy	

Allgemeinreaktionen	Schweregrad	Tag der Impfung	Tag 1	Tag 2	Tag 3	Tag 4	Tag 5	Tag 6	Tag 7	Hält noch nach 7 Tagen an?	Datum Letzter Tag mit Beschwerden	Maximaler Schweregrad
Körpertemperatur	Bitte geben Sie die höchste Temperatur an, wenn sie mehrmals am Tag nachmessen									❒ nein ❒ ja	dd mmm yyyy	
Kopfschmerzen	0 = Keine Symptome 1 = Mild: Tägliche/sportliche Aktivitäten nicht beeinträchtigt 2 = Moderat: Tägliche/sportliche Aktivitäten beeinträchtigt 3 =Schwer: Tägliche/sportliche Aktivitäten können nicht mehr durchgeführt werden.									❒ nein ❒ ja	dd mmm yyyy	
Muskleschmerzen										❒ nein ❒ ja	dd mmm yyyy	
Schüttelfrost										❒ nein ❒ ja	dd mmm yyyy	
Übelkeit										❒ nein ❒ ja	dd mmm yyyy	
Müdigkeit										❒ nein ❒ ja	dd mmm yyyy	
Andere _____										❒ nein ❒ ja	dd mmm yyyy	
Andere _____										❒ nein ❒ ja	dd mmm yyyy	

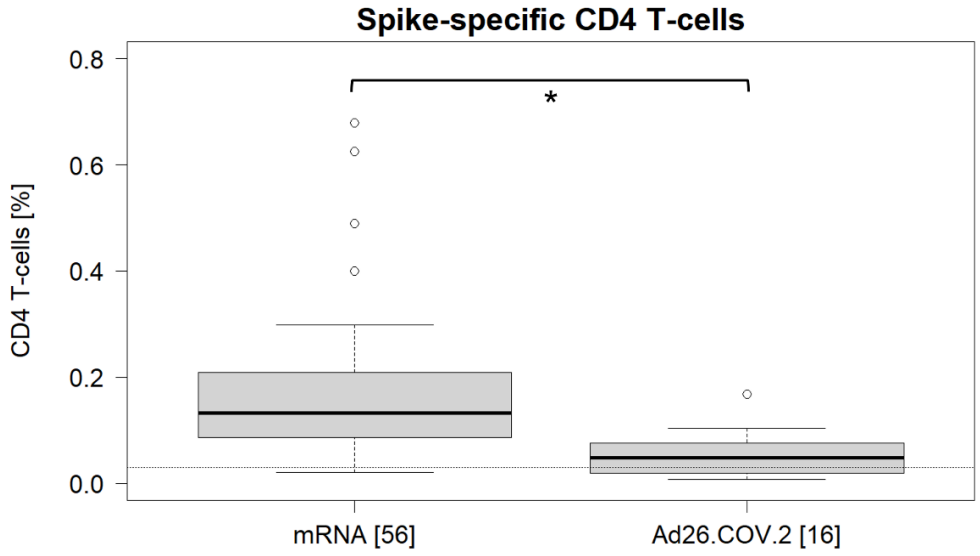
A.3 Additional figures

A.3.1 Neutralizing activity after vaccination



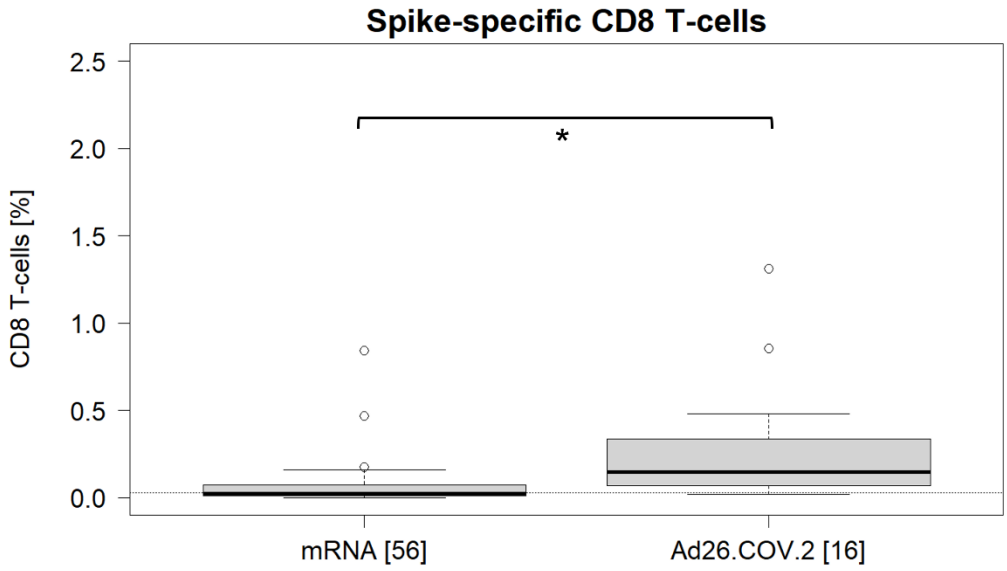
Neutralizing activity of antibodies after vaccination with mRNA and Ad26.COV2.S
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.2 CD 4 T-cells after vaccination



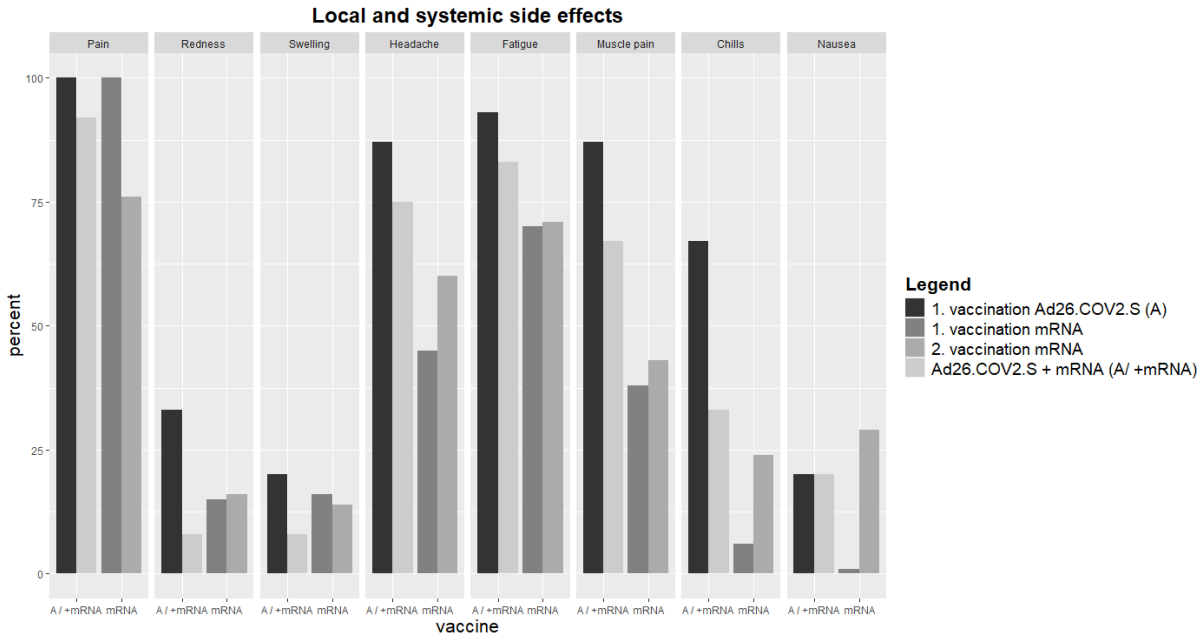
Spike-specific CD 4 T-cells after vaccination with mRNA and Ad26.COV2.S
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.3 CD 8 T-cells after vaccination



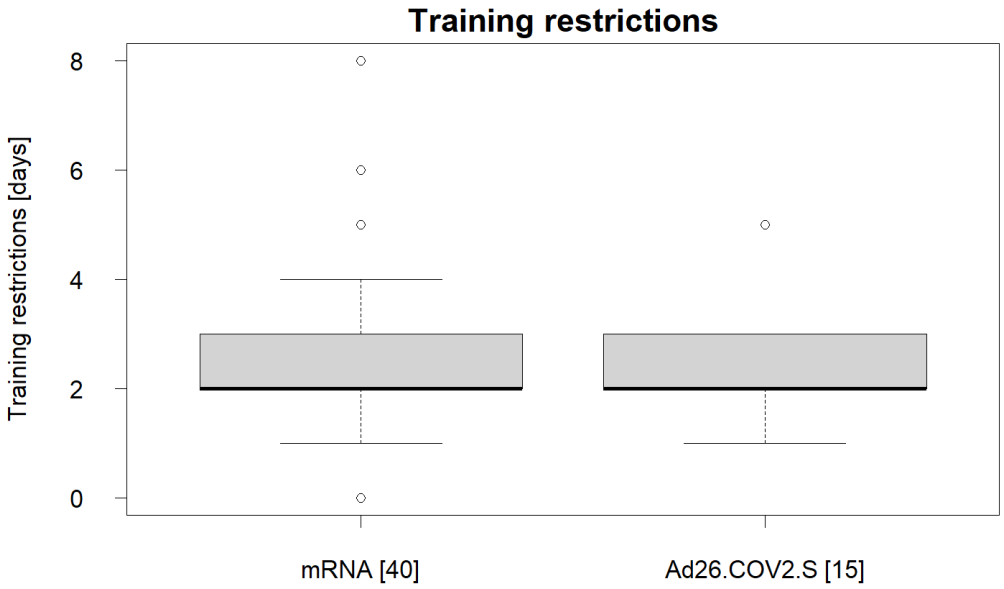
Spike-specific CD 8 T-cells after vaccination with mRNA and Ad26.COV2.S
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.4 Local and systemic side effects



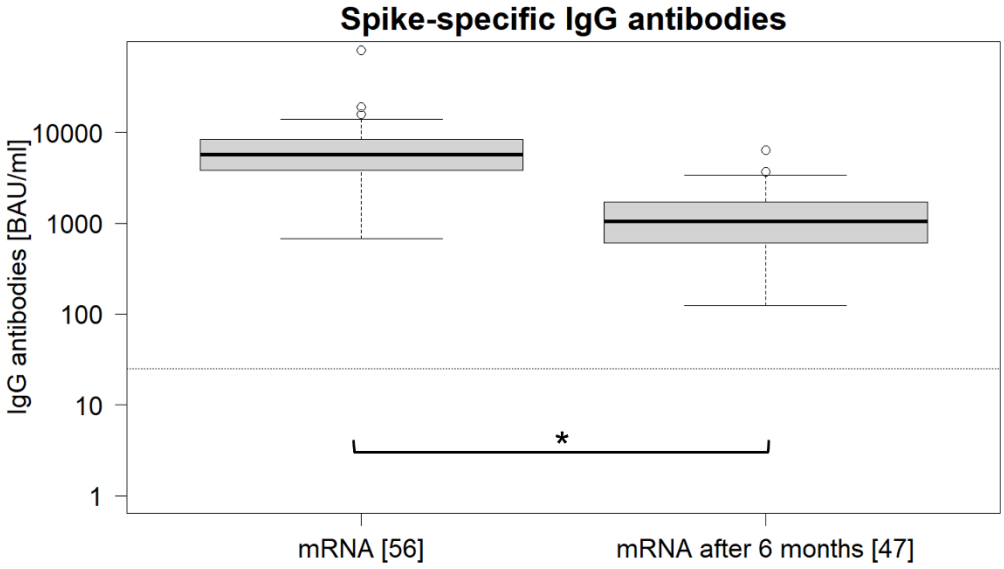
Local and systemic side effects after mRNA and Ad26.COV2.S + mRNA vaccination

A.3.5 Training Restrictions



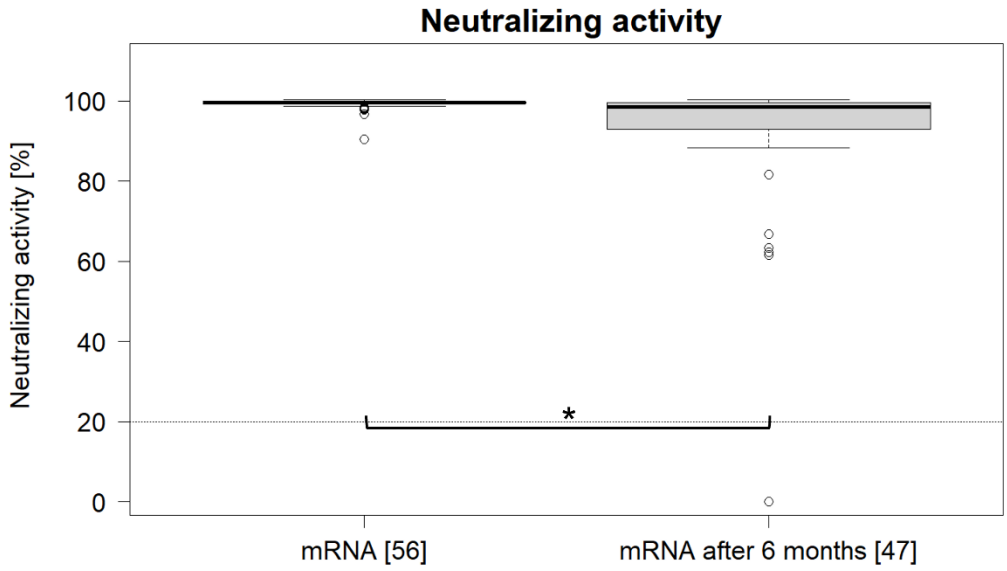
Cumulative training restrictions after mRNA and Ad26.COV2.S vaccination
(bold bar = median, box= interquartile range)

A.3.6 IgG antibodies mRNA 6 months



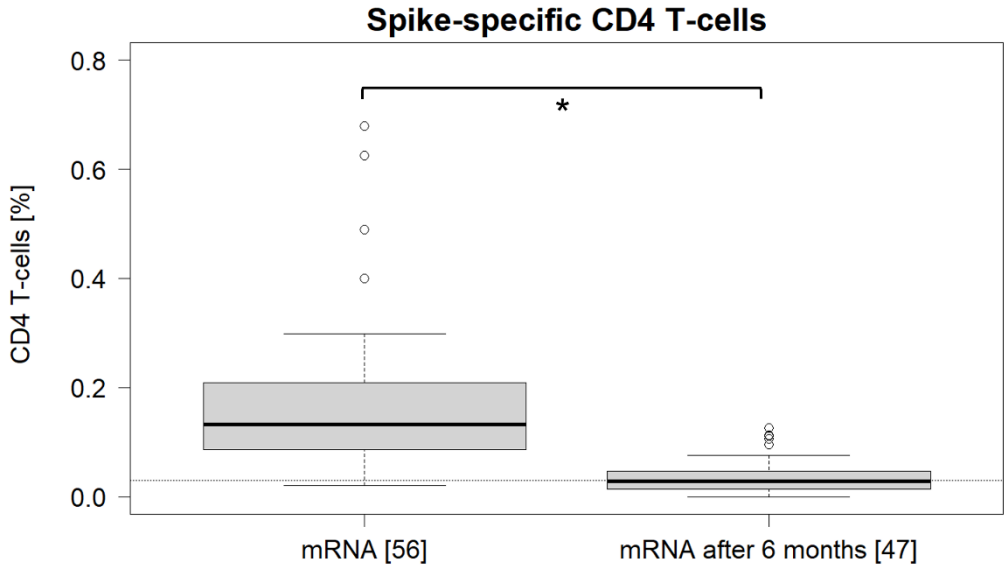
Spike-specific IgG antibodies 6 months after mRNA vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.7 Neutralizing activity mRNA 6 months



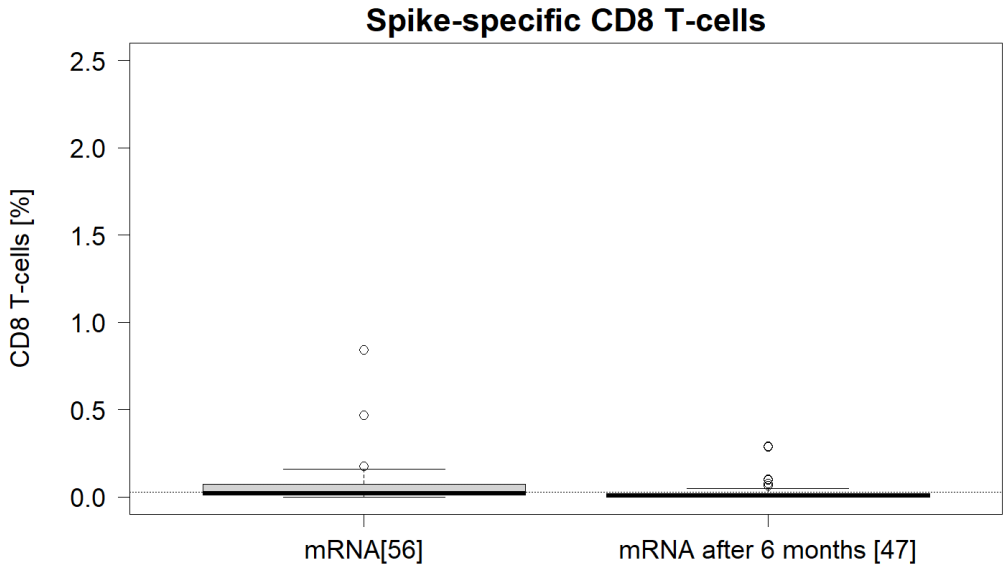
Neutralizing activity 6 months after mRNA vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.8 CD 4 T-cells mRNA 6 months



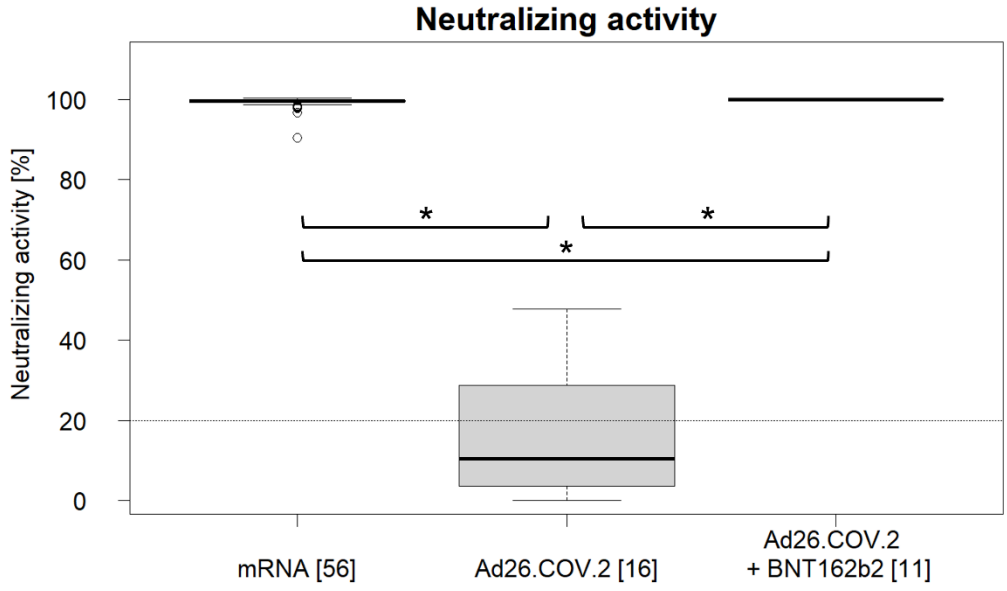
Spike-specific CD 4 T-cells 6 months after mRNA vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.9 CD 8 T-cells mRNA 6 months



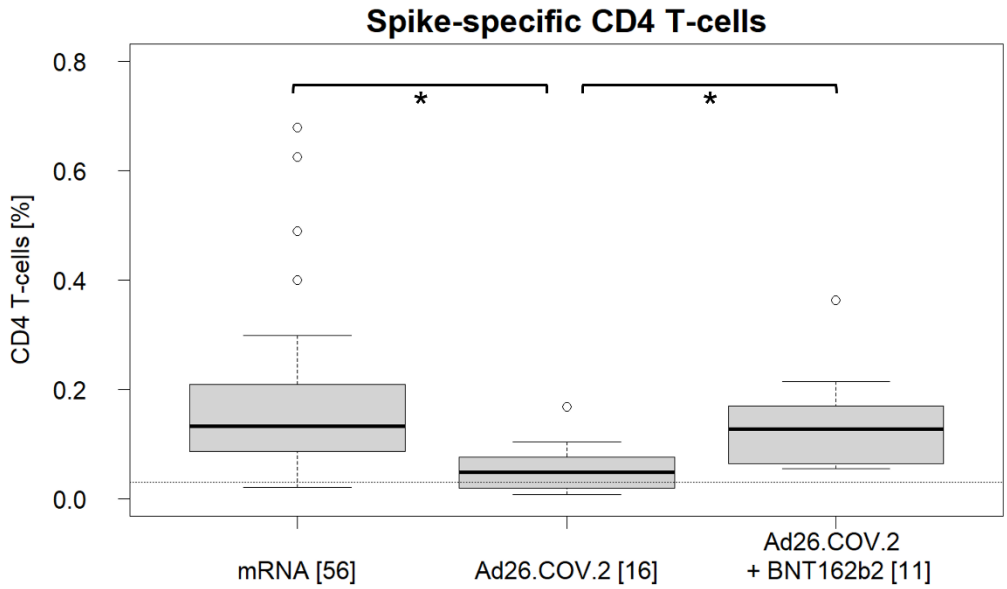
Spike-specific CD 8 T-cells 6 months after mRNA vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.10 Neutralizing activity after the study adjustment



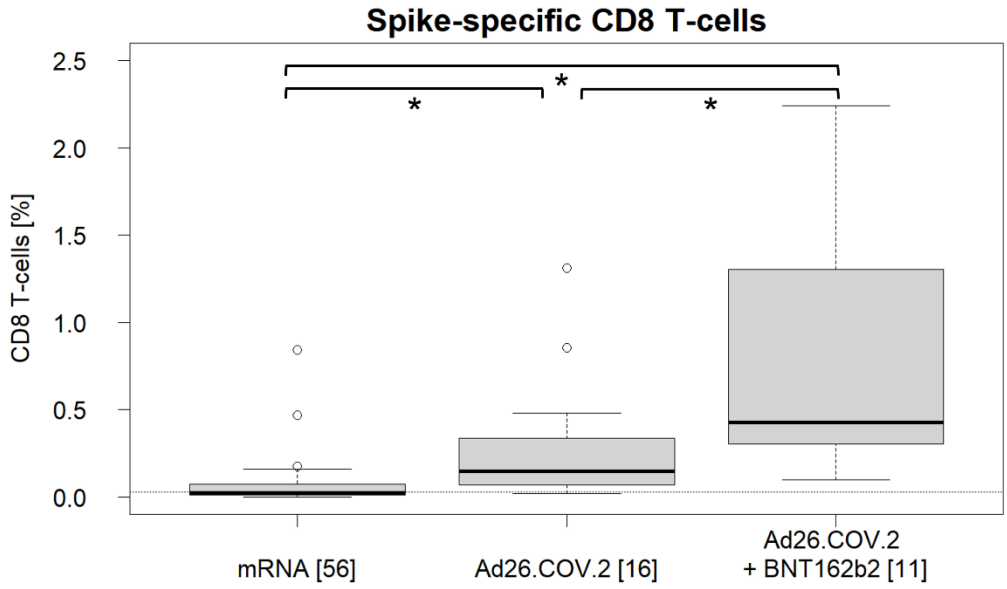
Neutralizing activity after mRNA, Ad26.COV2.S and Ad26.COV2.S + BNT162b2 vaccination
*(bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.11 CD 4 T-cells after the study adjustment



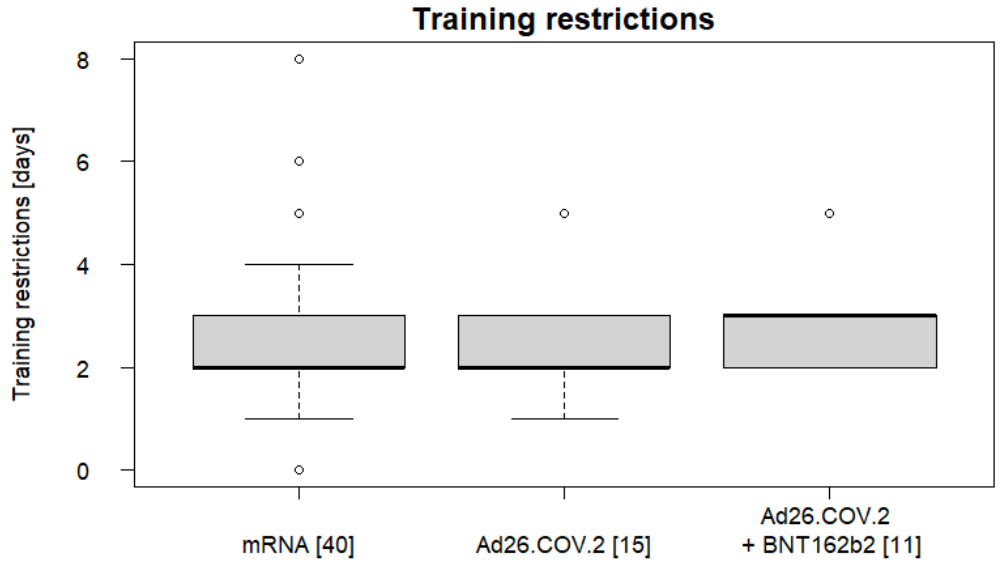
*Spike-specific CD 4 T-cells after mRNA, Ad26.COV2.S and Ad26.COV2.S + BNT162b2 vaccination (bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.12 CD 8 T-cells after the study adjustment



*Spike-specific CD 8 T-cells after mRNA, Ad26.COV2.S and Ad26.COV2.S + BNT162b2 vaccination (bold bar = median, box= interquartile range, dotted line = threshold, * = significant difference)*

A.3.13 Training restrictions including the study adjustment



Training restrictions after mRNA, Ad26.COV2.S and Ad26.COV2.S + BNT162b2 vaccination (bold bar = median, box= interquartile range)

A.4 Publication

73 Analysis of immune response and vaccine reactions

Immune Response to COVID-19 Vaccination in Elite Athletes

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ABSTRACT

Purpose: This study analyses the immune response of elite athletes after COVID-19 vaccination with double-dose mRNA and a single-dose vector vaccine.

Methods: Immunoglobulin G (IgG) antibody titers, neutralizing activity, CD4 and CD8 T-cells were examined in blood samples from 72 athletes before and after vaccination against COVID-19 (56 mRNA (BNT162b2 / mRNA-1273), 16 vector (Ad26.COV.2) vaccines). Side effects and training time loss was also recorded.

Results: Induction of IgG antibodies (mRNA: 5702 BAU/ml; 4343 BAU/ml (hereafter: median), vector: 61 BAU/ml; 52 BAU/ml, p<0.01), their neutralizing activity (99.7%; 10.6%, p<0.01), and SARS-CoV-2 spike-specific CD4 T-cells (0.13%; 0.05%; p<0.01) after mRNA double-dose vaccines was significantly more pronounced than after a single-dose vector vaccine. SARS-CoV-2 spike-specific CD8 T-cell levels after a vector vaccine (0.15%) were significantly higher than after mRNA vaccines (0.02%; p<0.01). When athletes who had initially received the vector vaccine were boostered with an mRNA vaccine, IgG antibodies (to 3456 BAU/ml; p<0.01), neutralizing activity (to 100%; p<0.01), CD4 (to 0.13%; p<0.01) and CD8 T-cells (to 0.43%; p<0.01) significantly increased. When compared with dual-dose mRNA regimen, IgG antibody response was lower (p<0.01), the neutralizing activity (p<0.01) and CD8 T-cell (p<0.01) response higher and no significant difference in CD4 T-cell response (p=0.54) between the two regimens. Cumulative training loss (3 days) did not significantly differ between vaccination regimens (p=0.46).

Conclusion: mRNA and vector vaccines against SARS-CoV-2 appear to induce different patterns of immune response in athletes. Lower immune induction after a single-shot vector vaccine was clearly optimized by a heterologous booster. Vaccine reactions were mild and short-lived.

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EIR 30 2024

INTRODUCTION

The world-wide coronavirus pandemic led to many medical, social, and health care system challenges. An infection with SARS-CoV-2 can cause severe COVID-19 with pathology including pulmonary inflammation, pulmonary fibrosis, or vascular thrombosis (1). Moreover, neurologic complications (2), olfactory and gustatory dysfunctions (3), and cardiac manifestations like myocarditis(4) may result. Important preventative/hygiene measures like frequent disinfection, wearing face masks and social distancing were recommended and used in the beginning of the pandemic(5) while different types of vaccines (vector-vaccine, mRNA vaccine, protein-based) were developed with some delay knowing that vaccinations are one of the most effective means to prevent the spread and severe courses of many infection diseases(6).

Due to vaccine shortage, it was initially necessary to prioritize older people, medical staff and other high-risk populations for vaccinations, mostly without individual choice of vaccine type. In May 2021, during the last preparation stages for the Olympic and Paralympic Games in Tokyo, aspirants for the German Olympic and Paralympic team were prioritized for vaccination based on a political decision of the German government, considering that vaccinating athletes against COVID-19 had been strongly advised (7). With different vaccine types available (and very little experience with mRNA vaccines in general), their immunogenicity and reactogenicity could be expected to differ and potentially differ in their impact on the training (e.g. time loss due to vaccine reactions) and the safety of athletes (e.g. protection from acquiring an infection) prior to and during the Olympic Games. The double-dose mRNA vaccines BNT162b2 (Comirnaty® by BioNTech) and mRNA-1273 (Spikevax® by Moderna) are based on non-replicating mRNA delivered via lipid-based nanoparticles. SARS-CoV-2 spike-encoding mRNA are translated by muscle cells or tissue resident antigen-presenting cells followed by its secretion and/or presentation on the cell surface. These viral spike proteins are recognised as foreign antigens and trigger cellular and humoral immune response(8). The mRNA vaccines were approved based on pivotal trials showing vaccination efficacy of 95%(9) and 94%(10), respectively. Overall, vaccine reactions were reported to be mild and short-lived (mean of 2-3 days) in these investigations (9, 10). The single-dose vector vaccine Ad26.COV.2 (Janssen® by Johnson&Johnson (renamed in 2022 as Jcovden®) is a recombinant, replication-incompetent human adenovirus type 26-based vector that encodes the SARS-CoV-2 spike protein, inducing expression and an immune response. It was officially approved with an effectiveness of 67% in the pivotal trial (11). At the time of the first athlete prioritization, only BNT162b2, mRNA-1273, and Ad26.COV.2 were available. It must be noted that at this time the double-dose ChAdOx1 nCoV-19 vector vaccine (by AstraZeneca) was no longer recommended for people under 60 years of age in Germany (12). Despite the considerably lower effectiveness of Ad26.COV.2 as demonstrated in the registration studies, Ad26.COV.2 was considered a practical choice for members of the German Olympic team in summer 2021 in Germany (7). A single-shot vaccination was considered promising by many athletes (and medical advisors) due to a potential induction of less vaccine side effects and possibly a faster build-up of SARS-CoV-2-specific immunity. The aspect of formally receiving a vaccinated state (meaning a certificate needed for traveling) more quickly added to the positive image of the vector vaccine particularly in the athletes.

Understanding that vaccinating athletes against SARS-CoV-2 is important, it also needs to be mentioned that sport may lead to changes in the immune system of athletes. Intensive training programs in the preparation phase for major competitions may result in an increased susceptibility to infections due to a reduction in the number of immune cells and an associated reduction in functionality(13). Therefore, it is important to understand the influence of COVID-19 vaccines on the immune system of athletes. In general data about vaccinating athletes is limited due to concerns in athletes about safety and efficacy of vaccinations - but it is important to understand more about the immune system of athletes (4, 14).

The aim of this study was to determine the immune response of elite athletes after COVID-19 vaccination as well as comparing the humoral und cellular immune response between double-dose mRNA vaccines and a single-dose vector vaccine in this population. We hypothesized a significant induction of the immune response after both vaccine types with a stronger induction of the immune response after double dose regimen compared to a single dose vector vaccine. We further hypothesized that vaccine related adverse events will overall be mild and short-lived but that training restrictions will be lower after a single dose compared to a double dose vaccine. Later changes in official vaccination policies putting more emphasis on booster vaccinations enabled us to carry out some comparison between homologous and heterologous booster vaccination in our elite athlete population.

METHODS

Participants

72 healthy elite athletes older than 16 years participated in this prospective study. Among individuals who were vaccinated with an mRNA vaccine (mean of 21 years ± 6 years (standard deviation)), 29 were females (28: BNT162b2, 1: mRNA-1273) and 27 were males (25: BNT162b2, 2: mRNA-1273). The mean age of the 5 female and 11 male athletes of the Ad26.COV.2 group was 28±5 years (standard deviation). In their respective sports discipline, the athletes performed on international or national level. Recruitment was supported by the Olympic Training Centre Saarbrücken, the University Hospital Charité Berlin and the Institute of Applied Training Science (IAT) in Leipzig mainly via personal communication with the athletes from May 2021 to September 2021. Exclusion criteria were hypersensitivity or allergy to one of the ingredients of the vaccines, a clinically relevant immunodeficiency, or an acute illness. Medication intake was not verified by means of blood profiling, but participants were explicitly asked about serious illnesses and possible treatments.

Ethics approval.

The study was carried out in accordance with the Helsinki

declaration and approved by the local ethics committee (133/21, Ärztekammer des Saarlandes, Saarbrücken, Germany). All participants were informed about the study procedures, prior to giving written informed consent. Parents signed informed consent for participants under the age of 18 years.

Study design

All participants received one out of three approved and vaccine regimens recommended at the time of the study. The regimen was chosen depending upon availability or personal preference, as a randomized controlled assignment of the vaccine was not intended and not possible under the circumstances in mid 2021. The available vaccines were mRNA-1273 (Spikevax® by Moderna, 3 athletes), BNT162b2 (Comirnaty® by BioNTech/Pfizer, 53 athletes) and Ad26.COV.2 (Jcovden® by Janssen, 16 athletes). mRNA-1273 and BNT162b2 are double-dose mRNA vaccines whereas Ad26.COV.2 was approved as a single-dose vector vaccine. Blood samples were taken before vaccination to determine baseline reactivity and exclude previous contact with SARS-CoV-2 antigens during asymptomatic infection. Moreover, short-term immunogenicity was analysed two weeks after the second dose in case of mRNA vaccines, and three weeks after the single dose vector vaccine (due to known differences in vaccine-induced peak immune responses after the first and the second vaccination(15)). Follow-up analyses were performed 6 months after the last vaccination. Further evidence for prior infection with SARS-CoV-2 was tested using an NCAP-ELISA that was performed at least once (primarily after second mRNA vaccination, or after the first Ad26.COV.2 vaccination to test for the presence of antibodies to the SARS-CoV-2 nucleocapsid protein). The study design is illustrated in figure 1. The athletes recorded all local and systemic adverse events such as pain, redness and swelling at the injection site as well as headache, fatigue, muscle pain, chills, and nausea during the first week after each vaccination by completing a standardized questionnaire. Each adverse event was rated by means of four different levels of severity. Experiencing no side effects was rated 0, whereas mild, moderate, or severe side effects were graded with 1, 2, and 3, respectively. Mild side effects were defined as adverse reactions that did not interfere with training and daily routine, moderate side effects impaired but still allowed training and daily routine, whereas severe side effects prevented training and daily routine for at least one day. Therefore, training restrictions in the context of this study were solely based on occurrence of moderate or severe side effects, whereas restrictions based on precaution were not considered. For regimens with two vaccination time points, all days with training restrictions were added to determine the total number of days lost.

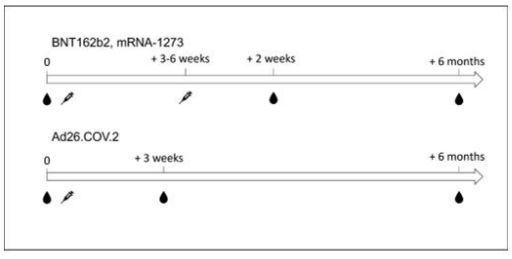

Figure 1. Overview of the study design with the vaccine regimens and their matching blood samples.

Necessary adjustments during the course of the study

After collecting the samples 2/3 weeks after vaccination and analysing the humoral and cellular immune response we found that the single-dose vector vaccine led to an insufficient humoral immune response in our athletes (e. g. median IgG antibodies after double-dose mRNA vaccination: 5702 BAU/ml, median IgG antibodies after single-dose vector vaccination: 61 BAU/ml). To provide adequate protection from COVID-19, recommendations for athletes were modified (and the study design had to be adjusted accordingly) by offering a heterologous boost vaccination to optimize the immune response in these athletes. This was carried out in 11 out of 16 athletes with the BNT162b2 vaccine after a median time of 119 days. An additional blood sample was taken two weeks after the heterologous boost to analyse the immune response. The adjusted study design can be seen in figure 2. The study adjustment was approved by the local ethics committee on September 6, 2021.

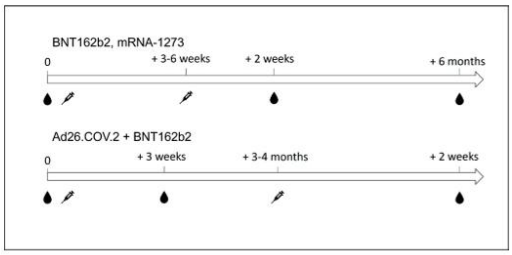

Figure 2. Overview of the adjusted study design with timelines for vaccination and blood sampling.

Procedures for immunological analyses

Lymphocyte subpopulations as well as vaccine-induced IgG antibody titers, neutralizing activity, and CD4 and CD8 T-cells were analysed from heparinized blood as previously described(16). Blood samples (9ml) were taken from an antecubital vein. The time of day was variable and deemed acceptable for our targeted parameters.

Vaccine-induced humoral immune responses were tested using ELISA assays as described by the manufacturer´s instruction (Euroimmun, Lübeck, Germany). An enzyme-linked immunosorbent assay (ELISA, SARS-CoV-2-QuantiVac) was used to quantify SARS-CoV-2 specific IgG antibodies against the receptor binding domain. Thresholds were set at <25.2 BAU/ml for being negative, ≥25.2 to <35.2 BAU/ml for being intermediate and ≥35.2 BAU/ml for being positive. An anti-SARS-CoV-2-NCP-ELISA was used to quantify SARS-CoV-2 specific IgG towards the nucleocapsid (N) protein. A surrogate neutralization assay that is based on antibody-mediated inhibition of soluble ACE2 binding to the plate bound S1 receptor binding domain (SARS-CoV-2-NeutraLISA) was used at a single serum dilution. Surrogate neutralizing capacity was calculated as percentage of inhibition (IH) by 1 minus the ratio of the extinction of the respective sample and the extinction of the blank value (16). The stimulus threshold was set according to manufacturer instructions with IH being negative under 20%, intermediate between 20 and 35% and positive over 35 %.

The protocol for quantification of SARS-CoV-2 spike-specific CD4 and CD8 T cells has been described before

(16). In brief, spike-19 specific CD4 and CD8 T-cells were quantified after a 6h stimulation with SARS-CoV-2 spike-derived overlapping peptides (each peptide 2 µg/ml, JPT, Berlin, Germany). Stimulation with 0.64% dimethyl sulfoxide (DMSO) and with 2.5 µg/ml of Staphylococcus aureus Enterotoxin B was used as a negative and positive control, respectively, to secure the specificity of the stimulation. Immunostaining was performed using anti-CD4 (clone SK3, 1:33.3), anti-CD8 (clone SK1, 1:12.5), anti-CD69 (clone L78, 1:33.3) and anti-IFNγ clone 4S.B3, 1:100, all antibodies from BD), and analyzed using flow-cytometry (BD FACS Canto II including BD FACSDiva software 6.1.3) (16). SARS-CoV-2-reactive CD4 or CD8 T-cells were identified as activated CD69-positive T-cells producing IFNγ. The percentage of specific T-cells was quantified by subtracting the percentage of T-cells after negative control stimulation from that after spike-specific stimulation. Detection limit was set at 0.03% as described before (16, 17).

Statistics

Statistical analysis was performed using R studio (version 4.0.5). Normal distribution of data was assessed using the Shapiro-Wilk test. No target parameter was distributed normally. Consequently, the nonparametric Wilcoxon test was used to analyse the quantitative parameters IgG antibody titre, neutralizing activity, CD4 and CD8 T-cells before and after vaccination. The Mann-Whitney-U-test was used to compare the immune response of the different vaccines and to analyse the vaccine side effects. The significance level was set at $p < 0.05$ for the α error. The effect size r for the Mann-Whitney-U and the Wilcoxon test was calculated with $|Z|/\sqrt{n}$ with Z being the standardised value and n the number of cases. Z was calculated with $x - \mu / \delta$. The effect size r is defined with r being small >0.10, medium >0.30 and large >0.50. No sample size analysis was performed because targeting a specific effect was not possible and intended; no comparable studies were available at that time.

RESULTS

Comparison of the immune response before and after vaccination

None of the athletes were tested NCAP-positive, which excluded a history of SARS-CoV-2 infection. The mRNA vaccines induced a significant immune response as indicated by an increase in IgG antibodies (z=-6.5, p<0.01, r=0.87), neutralizing antibodies (z=-6.5, p<0.01, r=0.87), as well as spike protein-specific CD4 (z=-6.5, p<0.01, r=0.87) and specific CD8 T-cells (z=-4.9, p<0.01, r=0.70). The aforementioned mRNA group comprises two vaccines, with mRNA-1273 being obtained from only three athletes. The IgG antibodies and neutralizing antibodies of the three athletes fall within the interquartile range of the mRNA group – which they belong to. Nevertheless, the values of the CD4 and CD8 T cells exhibit slight discrepancies, and thus, they are presented separately here (CD4 T cells: 0.05%, 0.48%, 0.67%; CD8 T cells: 0.01%, 0.05%, 0.11%). The Ad26.COV.2 vaccine also induced a significant increase in IgG antibodies (z=-4.2, p<0.01, r=0.88), neutralizing activity (z=-4.2, p<0.01, r=0.88), CD4 spike T-cells (z=-3.4, p<0.01, r=0.87) and CD8 spike T-cells (z=-4.2, p<0.01, r=0.88). Data are shown in table 1.

Comparison of the short-term immune response between the different vaccine regimens

When comparing immune-responses after vaccination, median IgG-levels were significantly higher after the mRNA vaccination (z=-6.1, p<0.01, r=0.71) than after the Ad26.COV.2 vaccination. This also held true for median neutralizing activity (z=-6.1, p<0.01, r=0.71), and CD4 T-cells (z=-4.4, p<0.01, r=0.52). In contrast, the Ad26.COV.2 vaccine induced a significantly higher CD8 T-cell response as compared to the mRNA vaccine (z=-4.1, p<0.01, r=0.48). Spike-specific IgG antibody levels and neutralizing activity as well as spike-specific CD4 and CD8 T-cell levels after vaccination are illustrated in figure 3.

Immune response after heterologous vaccination

A second heterologous mRNA vaccination with BNT162b2 was recommended for all individuals who had received a single dose of Ad26.COV.2. This led to a significant increase in both humoral and cellular immune responses (figure 3). IgG-levels increased from a

	mRNA			Ad26.COV.2		
Parameter	before	after	p-value	before	after	p-value
Spike specific IgG antibodies	4 BAU/ml (IQR 4 BAU/ml)	5702 BAU/ml (IQR 4343 BAU/ml)	<0.01	4 BAU/ml (IQR 2 BAU/ml)	61 BAU/ml (IQR 52 BAU/ml)	<0.01
Spike specific Neutralizing antibodies	0 % (IQR 0%)	99% (IQR 0.48%)	<0.01	0 % (IQR 0%)	11% (IQR 24%)′	<0.01
Spike specific CD4 T-cells	0 % (IQR 0.01%)	0.13 % (IQR 0.12%)	<0.01	0 % (IQR 0.01%)	0.05% (IQR 0.05%)	<0.01
Spike specific CD8 T-cells	0 % (IQR 0.005%)	0.02% (IQR 0.06%)	<0.01	0 % (IQR 0.003%)	0.15% (IQR 0.19%)	<0.01

Table 1 Blood parameters before and after vaccination with mRNA and Ad26.COV.2. Spike-specific IgG antibody levels [BAU/ml], neutralizing activity [%IC50], and the percentage of spike-specific CD4 and CD8 T-cells were quantified after two doses of a mRNA vaccine (n=56; BNT n=53, mRNA-1273 n=3) and after a single dose of Ad26.COV.2 (n=16), as well as before those vaccinations. Median values and interquartile range (IQR) are given.

77 Analysis of immune response and vaccine reactions

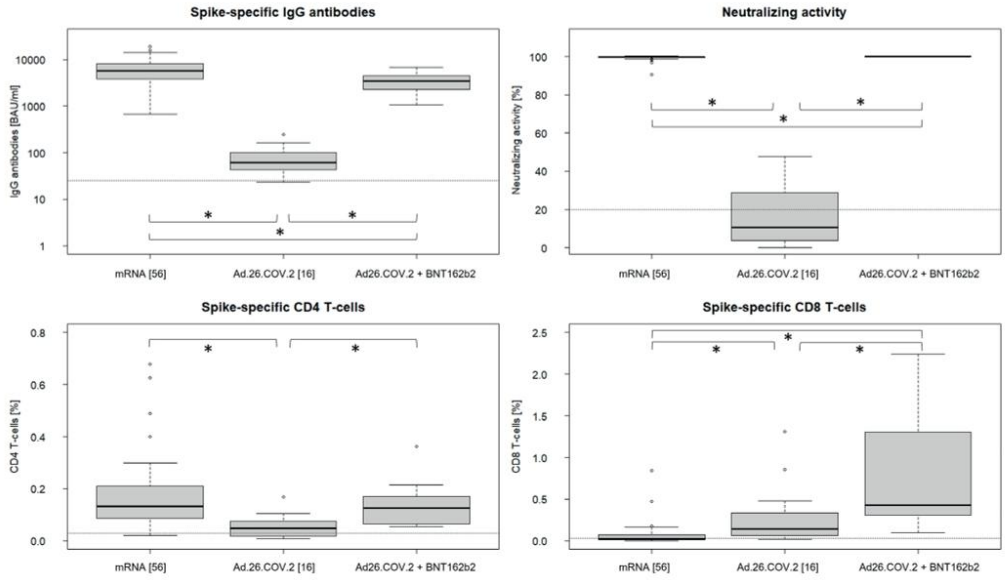

Figure 3. Vaccine-induced antibodies and T cells. Median spike-specific IgG antibody levels [BAU/ml], neutralizing activity [%IC50], and the percentage of spike-specific CD4 and CD8 T-cells were quantified after two doses of a mRNA vaccine (n=56; BNT n=53, mRNA-1273 n=3), a single dose of Ad26.COV.2 (n=16) or after heterologous combination of Ad26.COV.2 followed by BNT (n=11). Thresholds defining a negative response are indicated by a stippled line. Asterisks mark significance <0.05.

median of 61 BAU/ml (IQR 52 BAU/ml) to a median of 3456 BAU/ml (IQR 2209, z=-3.3, p<0.01, r=0.88) and the neutralizing activity from a median of 11% (IQR 24) to 100% (IQR 0.24, z=-3.3, p<0.01, r=0.88). Likewise, spike-specific CD4 T-cells increased from a median of 0.05% (IQR:0.05) to 0.13% (IQR 0.1, z=-2.6, p<0.01, r=0.75) and the CD8 T-cells from a median of 0.15% (IQR:0.19) to 0.43% (IQR 1, z=-2.6, p<0.01, r=0.75).

Comparison of the immune response after mRNA vaccine regimen and adjusted regimen

When compared to the homologous mRNA double dose vaccination regimen, IgG antibody levels after heterologous vaccination were moderately lower (z=-2.6, p<0.01, r=0.32), while the neutralizing activity (z=-3.6, p<0.01, r=0.45) and the CD8 T-cell response (z=-4.8, p<0.01, r=0.58) were significantly more pronounced. No difference was observed in CD4 T-cell levels (z=-0.6, p=0.54).

Long-term immune response after mRNA vaccine regimen

For the mRNA vaccines, all four chosen indicators significantly decreased after 6 months: IgG from a median of 5702 BAU/ml (IQR 4343 BAU/ml) to 1043 BAU/ml ((IQR 1112 BAU/ml), z=-7.7, p<0.01, r=0.87), neutralizing activity from a median of 99% (IQR 0.48) to 98% ((IQR 6), z=-4.8, p<0.01, r=0.70), CD4 T-cells from a median of 0.13 % (IQR 0.12) to 0.03% ((IQR 0.03),z=-5.9, p<0.01, r=0.86) and CD8 T-cells from a median of 0.02% (IQR:0.06) to 0.01% ((IQR 0.02),z=-3, p<0.01, r=0.45).

Due to necessary adaptions of the study design and limited numbers, a long-term follow-up after a single dose-vector vaccine (marginal reaction after 3 weeks) or heterologous regimen after Ad.26.COV.2 prime (too much delay) was not performed.

Adverse vaccine reactions

After the first dose of the mRNA vaccine, all athletes reported pain at the injection site lasting for a median time of 3 days (IQR 1). The most frequently reported systemic side effect was fatigue with 70% (median time: 2 days, IQR 3 days) and headache with 45% (median time: 0 days, IQR 1 day). The second mRNA dose caused pain at the injection site in 76% of cases for a median time of 2 days (IQR 2 days). Fatigue was reported by 71% (median time: 2 days, IQR 3 days) and headache by 59% (median time: 1 days, IQR 3 days) of the athletes. After the Ad26.COV.2 dose, all athletes reported pain at the injection site for a median time of 4 days (IQR 2 days). Fatigue was reported by 93% (median time: 3 days, IQR 2 days) and headache by 87% (median time: 3 days, IQR 1 day) of the athletes. The second heterologous mRNA dose led to local pain in 92% (median time: 2 days, IQR 1 day). Fatigue was reported by 84% (median time: 3 days, IQR 3 days) and headache by 75% (median time: 2 days, IQR 2.5 days) of the athletes. Occurrence of all collected local and systemic side effects is shown in figure 4.

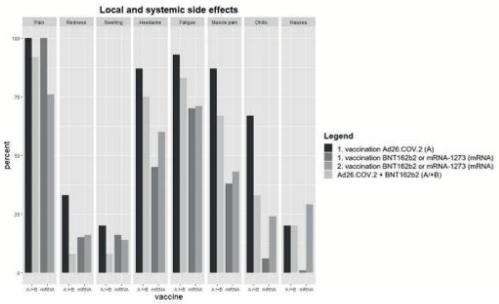

Figure 4. Local and systemic side effects. The different vaccine regimens are shown with their occurrence of local and systemic side effects.

Training Restrictions

Training restriction due to adverse events after the first and second mRNA vaccination lasted for a median of 2 days (IQR 1). The single dose Ad26.COV.2 vaccine led to a comparable training restriction of 2 (IQR1) days (z=-0.09, p=0.9, r=0.01).
The heterologous regimen after Ad26.COV.2 priming was followed by a cumulative restriction of training of 3 days (IQR 1), which was not significantly different from the two dose mRNA vaccines (z=-0.73, p=0.46, r=0.1).

DISCUSSION

The aim of our study was to evaluate the humoral and cellular response in elite athletes after vaccination with different regimes against COVID-19. The main findings were (i) the humoral and cellular immune response in athletes was induced after double-dose mRNA and single-dose vector vaccines, (ii) the mRNA and vector vaccines differed in their immunogenicity, with Ad26.COV.2 as single-dose being less potent for increasing IgG antibodies, neutralizing activity and CD4 T-cells, but more potent in inducing the CD8 T-cell response, (iii) a heterologous mRNA vaccination after Ad26.COV.2 priming was able to bring the humoral and cellular immune response close to double-dose mRNA vaccinations in all parameters, and (iv) there were no differences in training restrictions between the vaccine regimens. All side effects were minor and did not lead to substantial training loss.

Lo Sasso et al. (2021)(18) state that an effective immune response can be inferred from the increase in IgG antibodies and their related neutralizing activity, as well as from induction of CD4 and CD8 T-cells. Particularly, the neutralizing antibody titers are considered important for the protection against acquisition of SARS-CoV-2 infection due to their ability to inhibit spike protein attachment to the ACE-2 receptor, and consequently inhibit entry of the coronavirus (18). Initial studies on the immunogenicity of a single dose of the Ad26.COV.2 vaccine among non-athlete healthy individuals reported adequate induction of neutralizing antibody titers against the wild type and the Alpha variant, and some studies even showed durable and sufficient responses against new variants of the coronavirus (19–21). In contrast, the current study showed that the single dose of the Ad26.COV.2 vaccine only induced poor neutralizing antibody activity in elite athletes, which may indicate insufficient protection against infection and transmission. Similar findings have been reported for immunocompetent individuals in general by Self et al. (2021) (21) who claim that the single-dose vector vaccine is the least immunogenic one of the available vaccines. On the other hand, it induced a comparably strong CD8 T-cell response, which in concert with a low neutralizing antibody function may still protect from severe courses of COVID-19 disease once infected. Thus, the Ad26.COV.2 vaccine may protect athletes from serious outcomes of the infection, but it is potentially less effective in protecting against an acquisition of the infection and transmitting it to other athletes; it should therefore not be considered an effective choice for elite athletes participating in major sport events who want to avoid SARS-CoV-2 infections.

The double-dose mRNA vaccines showed a clearly stronger induction of neutralizing antibody titers and CD4 T-helper cells compared to the single-dose vector vaccine. This aligns with findings from Tada et al. (2021)(23) who showed significantly lower neutralizing antibody titers against all variants after Ad26.COV.2 compared to BNT162b2 and mRNA-1273. Collectively, findings support the notion of an inadequate humoral immune response after a single-dose vector vaccine, thereby explaining the increased rate of breakthrough infections (24), thus necessitating a second immunization following Ad26.COV.2 vaccine to increase protection from virus acquisition. Moreover, it is likely that transmission between athletes cannot be effectively prevented by the Ad26.COV.2 vaccine to control the virus spread in settings typical for sport and major sports events.

However, CD4 and CD8 T cells also contribute to the effectiveness of vaccinations. Grifoni et al. (2020)(25) showed that individuals who had contact with the virus develop CD4 T-cells in 100% and CD8 T-cells in 70% of cases and inferred that this mobilisation of the adaptive immune system may assist in the prevention of severe courses of COVID-19. In our study, the double-dose mRNA vaccinations led to a larger induction of CD4 T-cells than the single dose vector vaccine, whereas the latter induced a moderately higher CD8 T-cell response. Therefore, prevention of severe courses can be assumed for both vaccine regimen.

Under consideration of these findings, athletes vaccinated with Ad26.COV.2 were offered an additional vaccination to improve their immune response. A study by Atmar et al. (26) showed that the humoral immune response can be significantly improved with a heterologous boost after Ad26.COV.2 priming, leading to similar immune responses as homologous mRNA booster vaccination. Our data confirm these findings by showing a large improvement in all investigated immunological parameters. Moreover, a comparison of vaccine-induced immune responses after homologous mRNA vaccination with heterologous vector/mRNA vaccination in immunocompetent non-athlete individuals using exactly the same analysis methods also revealed significantly higher CD8 T-cell levels after heterologous vaccination, which is in line with our findings in elite athletes (16, 27). Accordingly, in October 2021, the Standing Committee on Vaccination (STIKO) at the Robert Koch Institute, the relevant council for vaccination policies in Germany, recommended a heterologous mRNA boost vaccination to all persons who have received the Ad26.COV.2 vaccine to optimize immunity against SARS-CoV-2 (24).
Typical vaccine related adverse events may lead to training restrictions and are therefore important aspects to consider when vaccinating athletes, particularly during their preparation for major sport events like Olympic Games. In the current study, there were no significant differences in (cumulative) training restrictions between the double-dose homologous mRNA, the single dose-vector vaccine, and the heterologous vector-mRNA regimens. Median training restriction was 2-3 days. In our study only training restrictions were considered that were caused by side effects with a score larger than 1, although it has to be taken into account that there may be additional reasons for athletes not to train than only side effects, e.g. general caution after vaccination. Comparable results have been found in British Olympic athletes where side effects after mRNA vaccination lasted for 1-2 days (28). Thus, adverse events in elite athletes appear to be generally

mild and short-lived with limited impact on training. However, individual athletes may be affected considerably longer (up to 9 days; (28)) so that - if possible - vaccinations should be planned well in advance of the next competition. Of note, training restrictions after vaccination are considerably lower and more predictable compared to an infection with SARS-CoV-2 (29).

Lastly, there was an expected large decline in the immune response 6 months after the double-dose mRNA vaccines. Accordingly, a third vaccine dose with BNT16b2 or mRNA-1273 can be considered to boost the immune response and increase the protective effect(30), which was generally recommended at a later stage of the pandemic.

Limitations

Due to the vaccine shortage and local differences in vaccine availability at the time of prioritizing Olympic Games aspirants for vaccination, it was not possible to control and randomize assignment of the vaccine regimens, which precluded a more rigorous study design. This is similar to many COVID-19 related studies, which arose from the circumstances at that time. Moreover, the time interval of the heterologous boost after the first dose of the Ad26.COV.2 vaccine was longer than between the first and second mRNA vaccinations, which may contribute to altered immune responses as compared to the dual dose mRNA regimen (31). However, at the time of planning the study, the less pronounced immune response after single-dose vector vaccine was unforeseeable. Altogether, some unpredictable changes in the national COVID-19 policy had a relevant influence on our study protocol without invalidating the measurements per se (but weakening the conclusions).

Perspective

This study helps to understand the induced immune response after COVID-19 vaccinations in athletes, and vaccine related training restrictions and side effects. In addition, it would be interesting to investigate the association of the analysed immune response with the number of athletes that experience SARS-CoV-2 infection, as well as the severity and duration of their symptoms. This could provide better insights in the actual risk of infections after vaccination and the protection that is assumed by the immune response. Another new question that can be explored in the future is more detailed analysis of the side effects. Detection of side effects and training limitations was performed in our study using paper-based questionnaires. It would also be interesting to investigate limitations using objective measurement devices including fitness watches or other biometric devices. These can detect parameters such as heart rate, heart rate variability, sleep phases and skin temperature that may be associated with the vaccination and documented side effects. This has been previously investigated using a wrist-worn biometric device, but not specifically in elite athletes(32).

Conclusion

In contrast to double-dose mRNA vaccination, a single-dose vector vaccination does not seem to protect athletes sufficiently against acquisition of COVID-19. Receiving a booster dose seems to induce a sufficient immune response in all cases. There were no indications for a compromised immune response to vaccination in elite athletes. Based on both the strong immunogenicity and limited side effets, this study does not provide any evidence against vaccinating elite athletes against COVID-19.

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Conflict of Interest and Source of Funding

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A.6 Curriculum vitae

Aus datenschutzrechtlichen Gründen wird der Lebenslauf in der elektronischen Fassung der Dissertation nicht veröffentlicht.