# PAVING THE WAY TOWARDS PRECISION VACCINOLOGY: THE PARADIGM OF MYOCARDITIS AFTER COVID-19 VACCINATION

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**Running title**

mRNA vaccine–associated myocarditis

**Abstract**

Systems vaccinology approaches have introduced novel tools for the evaluation of the safety profile of novel vaccine antigens by developing biomarkers of vaccine reactogenicity associated with potential adverse events. The use of such approaches may prove extremely advantageous in the context of a global pandemic where accelerated approval of new vaccine formulations for all ages is essential for the containment of the epidemic. The spread of SARS-COV-2 has had devastating effects on global health, but the emergency-authorization of mRNA vaccines significantly reduced SARS-COV-2-associated morbidity and mortality. Despite their favourable safety profile in adult populations, recent reports have raised concerns about an association of the mRNA-based vaccines with acute myocarditis predominantly among male adolescents and young adults following the second vaccine dose.

Here, we review data on myocarditis epidemiology following SARS-CoV-2 mRNA vaccination and describe potential mechanisms involved, that may explain the sex- and age-related differences, focusing on mRNA immune reactivity. The case of vaccine-associated myocarditis highlights the need to incorporate precision vaccinology approaches for the development of safe and effective vaccines for everyone.

**Keywords**

Myocarditis, mRNA COVID-19 vaccine, adverse events, adolescents

## Introduction

Despite the significant public health impact of vaccines, their full potential has yet to be reached. Vaccine development has been limited to traditional approaches against infectious agents targeting entire populations, without considering one's distinct immunological characteristics. Current cutting-edge progress in genetic engineering, adjuvant design and systems biology marked the beginning of a new era in vaccine research that takes into consideration demographic factors (e.g., age, sex, genetics, and epigenetics), paving the way towards precision vaccinology [1].

The pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has rapidly unfolded resulting in hundreds of thousands of deaths worldwide [2].Disease severity generally varies, but significantly increases with older age and comorbidities [3]. On the other hand, children have been largely spared from this pandemic [4-5]. This exception of young children from the severe morbidity and mortality associated with COVID-19 is an immunological 'paradox' implying that the distinct characteristics of immune responses in different ages are critical for the underlying mechanism.

The pathogenesis of COVID-19 is a compelling "interplay" between an infectious disease and an immune/autoinflammatory disorder where the immune system is of paramount importance in disease severity. Immune response observed in the elderly is associated with a massive release of cytokines and chemokines ("cytokine storm"), leading to devastating pulmonary and systemic tissue damage, clinically presented as severe COVID-19 including Acute Respiratory Distress Syndrome (ARDS) and multiorgan dysfunction (MODS). Importantly, cardiovascular complications have been described not only in the acute phase of COVID-19[6-8], but also well beyond the acute phase of COVID-19 and after the first 30 days post-infection, including cerebrovascular disorders, dysrhythmias, ischemic and non-ischemic heart disease, pericarditis, myocarditis, heart failure and thromboembolic disease regardless of age, sex, race and other predisposing conditions [9].

On the other hand, in paediatric populations, the immunological and inflammatory characteristics of immune responses provide protection against severe COVID-19 and may explain the different faces of COVID-19 [10-11].

The distinct profile of immunity and inflammation observed following natural infection has also been observed following vaccination with COVID-19 vaccines. A number of different vaccines against COVID-19 approved for emergency use in less than a year completely changed the course of the pandemic and significantly reduced COVID-19 related morbidity and mortality worldwide among individuals of different ages, highlighting the success of current vaccinology [12-16]. A novel mRNA platform used for the development of two of the most extensively used COVID-19 vaccines was highly successful in the induction of adequate immune responses, although with noticeable differences in immunogenicity and duration of protection among different populations, i.e. young adults, the elderly and individuals with immunocompromising conditions [17-19]. Similarly despite the favourable safety profile of mRNA vaccines confirmed through several clinical trials, post licensure surveillance showed that local and systemic reactions following vaccination were more frequent among younger compared to older individuals [20]. Most importantly, an association of the mRNA-based vaccines with acute myocarditis mainly among male adolescents and young adults has been recently confirmed, raising safety issues of the vaccination in such individuals and putting further hurdles in the implementation of COVID-

19 vaccination policies in this age group.

Here, we review the available epidemiological data on myocarditis following mRNA COVID-19 vaccination and discuss age and sex-related differences in the immune responses to mRNA platforms that could explain this significant disparity. This approach highlights that is imperative to consider multiple factors for the development of safe and effective vaccines for all ages.

**Epidemiology**

The first reports of post-vaccine myocarditis were recorded in Israel, where 148 myocarditis cases among 10.4 million vaccinated individuals were captured, about 4 months after the introduction of a massive vaccination program in the country. Most cases occurred following the second dose, mainly among males 16 to 30 years of age. The prevalence of myocarditis among the latter age-group was about 5/100 000 compared with 1/100 000 in the general population receiving the same vaccine. The accumulation of cases among adolescents and young adults soon after vaccination was up to 5 times higher than the pre-pandemic incidence of all-cause acute myocarditis in the general population [21-22]. Following these early findings, active surveillance programs were initiated in several countries to monitor post-vaccine myocarditis. Special focus was given to patients' clinical symptoms and imaging findings that should fulfil the CDC criteria for confirmed myocarditis including elevated cardiac enzymes (troponin I, troponin T or creatine kinase-MB), new onset or increased degree of severity of focal or diffuse depressed LV function by imaging, abnormal imaging findings indicating myocardial inflammation (CMR with gadolinium, gallium 67 scanning, anti-myosin antibody scanning). Later studies in Israel confirmed the early findings reporting an incidence of 2.13/100000, mainly among males aged 16-29 years, who had received at least one dose of Pfizer-BioNTech mRNA vaccine; in details, the incidence among males of all ages was 4.12/100000 while among males aged 16-29 years 10.69/100.000 [23].

In agreement with these studies, CDC reported an elevated risk for myocarditis in mRNA COVID-19 vaccine recipients, particularly among males aged 12–29 years, with an incidence range 3.9–4.7 per 100000 second mRNA COVID-19 vaccine doses [24]. Notably, the incidence based on CDC criteria, in adolescents 12-15 years is about 2.1 cases per 100000 second doses according to the VAERS (0.39/100.00 for girls and 3.9/100.00 for boys [25].

Similarly, most one-centre studies coming from different countries worldwide have shown even more increased incidence ranging from 1/500 to 1/10000 in these age groups [26]. In general, most studies agree that the vast majority of post-vaccine myocarditis occurs among males with a ratio of 2.5 males/1 female, while the same ratio appears to be even higher in adolescents (6-8 boys/1 girl), according to VAERS and EudraVigilance [27-28].

Most post-vaccine myocarditis cases (almost 60%) were recorded after the second dose of mRNA COVID-19 vaccine, while among boys aged 6-17 years the second dose caused more than 70% of the post-vaccine myocarditis [27]. According to CDC, myopericarditis adverse events are more likely to happen within six weeks after receiving a vaccine dose, and especially following booster doses [29]. Table 1 summarizes the studies showing the incidence of post-COVID-19 vaccine myocarditis in different age groups.

To date, the vast majority of documented post-COVID-19 vaccine myocarditis cases have been mild with rapid resolution of signs and symptoms and requiring at most a hospitalization of 3-6 days [31]. Bozkurt et al reported a mortality rate of post-COVID-19 vaccine myocarditis about 0,1-1 per 100.000 for persons aged 12-29 years [31]. However, higher morbidity and mortality rates of vaccine-related myocarditis have been recorded due to the limitations of the reporting systems used to extrapolate these data [29]. In detail, VAERS [36] serves as a post-marketing safety surveillance program, collecting information about adverse events (possible side effects) that occur after the administration of U.S. licensed vaccines. According to VAERS, 246 deaths were recorded out of more than 14.000 reported cases (approximate mortality rate 1.5%), over 6.000 cases of which required hospitalization [27]; EudraVigilance is the system for managing and analysing information on suspected adverse reactions to medicines which have been authorised or being studied in clinical trials in the European Union. according to EudraVigilance, 135 deaths and 259 resolving/recovering cases with sequelae were recorded out of more than 10.000 post-vaccine myocarditis cases in Europe, (approximate rate 1.2% and 2,5% respectively) [28]. It is apparent that caution is highly required when interpreting such data considering the limitations of the corresponding database systems and entries from both such database systems need further filtering by myocarditis CDC criteria on a single case basis in order to avoid mass misinterpretation of data and thus overestimation of vaccine-related myocarditis prevalence [36].

**Mechanisms involved in SARS-CoV-2 m RNA vaccine-associated myocarditis**

The underlying mechanisms responsible for myocarditis cases after immunization with SARS-CoV-2 mRNA vaccines are not yet clear. As the majority of cases are mild and self-limiting, cardiac biopsy has been rarely performed [31], hindering the elucidation of the immunological factors that trigger and contribute to the development of vaccine-associated myocarditis. In the few cases where cardiac biopsy was performed, no traditional lymphocytic or eosinophilic myocarditis or myonecrosis on cardiac histopathology were found, suggesting a novel mechanism of myocardial injury caused by immunization with SARS-CoV-2 mRNA vaccines.

mRNA immune reactivity has been proposed as the most prominent mechanism for systemic adverse reactions and myocariditis post-immunization with SARS-CoV-2 mRNA vaccines. It is known that exogenous mRNA is intrinsically immunostimulatory and it is recognized by a variety of cell surface, endosomal and cytosolic innate immune receptors [37]. This innate immune response to mRNA enhances vaccine immunogenicity, as it can provide adjuvant activity to drive dendritic cell (DC) maturation and thus elicit robust T and B cell immune responses. In this way, mRNA vaccine antigens can serve as both 'the antigen ' by encoding the viral protein and 'the adjuvant' due to its immunostimulatory properties.

In some cases however, the innate response to mRNA can cause an exacerbated immune response, known as 'mRNA immune reactivity', where dendritic cells and Toll-like receptor(TLR)-expressing cells express cytokines and activation markers that trigger a cascade of hyper-inflammation causing unfavorable systemic reactions with detrimental effects in different organs including the myocardium[38-39].

In order to minimize these phenomena, the current SARS-CoV-2 mRNA vaccines contain purified, in vitro-transcribed single-stranded mRNA with modified nucleotides that reduce binding to TLR and immune sensors, thus limiting excessive production of type I

interferon [40]. However, in selected individuals of all ages, the immune response to mRNA may not be turned down, activating immune pathways that may play a role in the development of vaccine-associated myocarditis. Since myocarditis presents predominantly in male adolescents and young adults, it is apparent that younger age and male sex contribute as independent factors to susceptibility to m RNA immune hyperactivity.

In support of mRNA immune reactivity as the causing mechanism of vaccine–associated myocarditis, most reported cases have presented shortly after the second dose of a SARS-CoV-2 vaccine, when the innate immune response to the vaccine is known to reach peak levels. A recent report on Systems vaccinology of the BNT162b2 m RNA vaccine showed that the second dose stimulated a significantly higher innate immune response compared to primary immunization, consisting of high frequency of CD14+CD16+ inflammatory monocytes and Interferon- gamma (IFNγ) and a prominent transcriptional signature of innate antiviral immunity [41]. It is therefore possible that within this timeframe of heightened innate responses, hyper inflammation may occur in certain individuals.

Younger age was associated with greater changes in monocyte, inflammatory response, and platelet-related gene expression shortly after the second dose of BNT162b2 vaccine, compared to older subjects who had increased response in B and T cell gene modules [41]. This higher innate response to the second dose of SARS-CoV-2 m RNA vaccines might be responsible for the increased susceptibility of younger individuals to vaccine-related adverse events [42].

Sex-related hormonal differences may be also be responsible for the male predominance in vaccine-associated myocarditis cases. Testosterone is thought to inhibit anti-inflammatory cells and promote commitment to a Th1-type immune response [43-44] . On the other hand, estrogen has inhibitory effects on pro-inflammatory T cells, resulting in a decrease in cell-mediated inflammatory response [45].

The quantity of mRNA antigen contained in each vaccine formulation may also be a factor to mRNA reactivity, as most cases of myocarditis have been associated with the formulations containing the higher loads of m RNA per dose [33,46-47].

Molecular mimicry between the spike protein of SARS-CoV-2 and cardiac self-antigens resulting in cross-reacting auto-antibodies directed to the myocardium post immunization has also been proposed as another possible mechanism of vaccine-associated myocarditis, regardless of age. However, molecular mimicry alone is less likely to suffice to cause myocadiac injury, as myocarditis is not seen with the Adenovirus vector, inactivated virus and protein/adjuvant vaccines and it does not explain the accumulation of cases within a certain age group. Moreover, it seems that the autoantibodies found in the peripheral blood of patients with symptomatic myocarditis might result from myocardial inflammation and injury and be the product and not the cause of myocarditis [48].

**Precision vaccinology: the way to move forward**

The devastating effects of COVID-19 on public health and economy globally called for emergency immunization practices that aimed to the rapid accomplishment of immunity on a population-wide basis. Under such immense pressure SARS-CoV-2 m RNA vaccines using a novel antigen delivery platform obtained license for emergency use based on convincing preclinical safety and effectiveness data obtained from large adult studies [15,49].

The extension of their emergency use in younger ages, however, was based on relatively small studies showing similar safety and immunogenicity profile to adult studies. These studies were not powered to reveal the relatively rare but serious events of vaccine-associated myocarditis among young males. Post vaccine myocarditis, although rare and with no serious sequelae in most cases caused disproportionate publicity and had an impact on vaccine uptake among adolescents, as the debate concerning the risks of acute myocarditis associated with SARS-CoV-2 infection and COVID-19 mRNA vaccination has gained intense social media attention. It should be highlighted that the incidence of COVID-19-associated cardiac complications or myocarditis is estimated to be 100 times higher (1,000–1,400 per 100,000 people with COVID-19) than that of COVID-19 mRNA-vaccine-related myocarditis [21]. Notably, among adolescents and early adults the incidence of COVID-19 myocarditis is estimated to be 133 per 100,000 COVID-19 infected people < 16 years of age and 98 per 100,000 among COVID-19 infected people aged 16-24 years, based on a CDC report [50]. Moreover, despite the overall mild clinical presentation and favourable outcome of post COVID-10 mRNA vaccine myocarditis, wild-type COVID-19 infection is linked with a major risk of cardiovascular complications [51] Thus, the risks of hospitalization and death associated with COVID-19 are consistently greater than the risks associated with the vaccine. Furthermore, clinical trials and real-world reports have shown that vaccines against COVID-19 are highly effective against symptomatic disease, reducing the risk of COVID-19-related hospital and intensive care admissions and deaths in both young individuals and the elderly [49]. COVID-19 vaccination reduces the risk of COVID-19-associated acute kidney injury, arrhythmia and thrombosis [49]., while decreasing the risk of heart lesion and myocarditis about 1,000-fold in the general population, with a minor 1–5-fold increased risk of mild myocarditis in young adults [21]. Several studies have demonstrated the favourable balance in the risk-benefit assessment of COVID-19 vaccination, regarding myocadiac injury (Table-2). Hence, given that post-mRNA-vaccine myocarditis is rare and usually resolves within a few days or weeks, the pros of COVID-19 vaccination definitely outweigh its cons, among different age groups. Notably, a recent study concludes that the best way to prevent even. Long COVID and its myriad complications, including the risk of serious cardiovascular sequelae in the long term, is to prevent SARS-CoV-2 infection in the first place through the wide implementation of the vaccine [9]

Nonetheless, when it comes to individualized protection, the risks of rare but serious adverse events associated with vaccines that could occur in certain at-risk populations, as seen with COVID19 associated myocarditis in adolescents and young adults, raise an important issue. Such adverse events are unlikely to be revealed in preclinical studies of relatively small number of participants especially under the emergency approval of novel vaccine platforms forced by the urgency of the pandemic. In contrast, precision vaccinology could identify inflammatory biomarkers that could predict hyperactivity to novel vaccines and could therefore offer an immense support in the urgent preclinical evaluation of COVID-19 vaccines in different population groups.

Previous studies have demonstrated the relevance of a systems biology approach to correlate the upregulation of genes associated with innate immunity, cytokine production, and responses to virus infection, particularly IFN-inducible genes, with adverse events seen

in human trials and paved the way towards better understanding of the underlying mechanisms of side effects [52-53].

The paradigm of mRNA vaccine-associated myocarditis demonstrates the need to include personalized vaccinology approaches to the global efforts for rapid evaluation of novel platforms to fight existing and novel global infectious threats.

Moreover, while it is obvious that ‘‘one-size-does not fit-all”, the promise of systems vaccinology is to identify specific immune response profiles, immune signatures, and biomarkers that predict vaccine safety and/or efficacy, which will lead to diversification of immunization practices in terms of kind and quantity of vaccine antigens, number and timing of doses.

As our understanding grows on how, and by what mechanisms, vaccines induce protective innate and adaptive immune responses, personalized vaccinology may be the way to go forward in order to optimize vaccine efficacy and safety for all.

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Table 1. Summary of studies showing the incidence of post-mRNA vaccination myocarditis (per 100.00 people) regarding the age and the received dose.

| Age group | Block et al USA 1st dose [30] | Block et al USA 2nd dose [30] | Bozkurt et al (USA) 2nd dose [31] | Chua et al (Hong Kong) 1st dose [32] | Chua et al (Hong Kong) 2nd dose [32] | Gellad et al (Denmark) 1st and 2nd dose [33] | Witberg et al (Israel) 1st dose [23] | Mevorach et al (Israel) 1st dose [34] | Mevorach et al (Israel) 2nd dose [34] | UK gov Moderna 1st dose [35] | UK gov Moderna 2md dose [35] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| <18 years | 3,6 | 3,8 | 1 | 3,4 | 21,22 | 1 | -` | 0,6 | 8 | - | - |
| 18-24 years | 3,8 | 4 | 0,5 | - | - | 1,8 | 5,5 | 1 | 5,9 | 5,5 | 7 |
| 25-29 years | 3,8 | 4 | 0,2 | - | - | 1,8 | 5,5 | 0,6 | 3,6 | 5,5 | 7 |
| 30-39 years | 3,9 | 4 | 0,4 | - | - | - | 1,1 | 0,2 | 2 | 5,5 | 5,5 |

Table 2. Studies that have highlighted the overall reduced relative risk of myocarditis after mRNA-vaccination compared to the risk associated with COVID-19.

| Study (Country) | Main findings |
|---|---|
| Heyman et al (Netherlands) [21] | COVID-19 vaccination decreases the risk of myocardial injury and myocarditis about 1,000-fold in the general population |
| Bozkurt et al (USA) [31] | Despite the rare cases of post-mRNA vaccination myocarditis, the benefit-risk assessment for COVID-19 vaccination shows a favourable balance for all age and sex groups, reducing the myocarditis associated with COVID-19 |
| Gellad et al (Denmark) [33] | The mRNA vaccines against COVID-19 are remarkably effective and provide tremendous benefit to recipients. The risks of myocarditis or myopericarditis are low and must be balanced against these many benefits. |
| Boehmer et al (USA) [50] | Patients with COVID-19 had nearly 16 times the risk for myocarditis compared with patients who did not have COVID-19. It is clear that vaccination reduces the public health impact of COVID-19 and its associated complications. |