Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vaccine 39 (2021) 4925–4927

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Commentary

The Novel Platform of mRNA COVID-19 Vaccines and Myocarditis: Clues into the Potential Underlying Mechanism

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Acute myocarditis is the second most common inflammatory heart disease after pericarditis, with an estimated annual incidence of 22 cases per 100,000 subjects [1]. The absence of specific pathognomonic features in conjunction with the wide spectrum of clinical manifestations that range from subclinical cases to sudden cardiac death, render myocarditis particularly challenging. Myocarditis is most often the result of viral infections. The currently pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as an additional causal agent of myocarditis.

Several highly effective vaccines that avert COVID-19 hospitalizations and deaths were available just over a year after the emergence of SARS-CoV-2. This record in the history of vaccinology has allowed for the re-opening of societies and the return to a new normalcy after strict lockdowns. Two of the leading vaccines that were granted emergency use authorization, Pfizer-BioNTech's Tozinameran (Comirnaty, BNT162b2) and Moderna's CX-024414 (mRNA-1273), are based on the novel platform of messenger RNA (mRNA). As mass vaccination efforts intensify, the meticulous safety monitoring of COVID-19 vaccines continues. Recent reports on the potential link between mRNA vaccination and myocarditis raised concerns among healthcare workers, the public and social media.

In more detail, Israel, that is leading the vaccination race, reported in late April that it is examining cases of myocarditis that occurred days after receipt of the Pfizer-BioNTech vaccine. A total of 62 cases (updated on June 2 to 275 cases between December 2020 and May 2021) were recorded (56 after the second- and 6 after the first dose) out of 5 million vaccinated, or 1 in 100,000 who received a second dose (0.001%); notably, however, this percentage was five times higher in the 16–30 years age group (1/20,000 or 0.005%). Of the 62 cases, 60 were hospitalized but recovered and were discharged, while two young, previously healthy people died (a 22-year-old female and a 35-year-old male). Abu Mouch *et al.* just presented in detail six cases of myocarditis, with a mild clinical course, in young males shortly after BNT162b2 vaccination [2]. Additional myocarditis cases post mRNA COVID-19 vaccination were recorded in European countries (e.g., in France), but with no further details disclosed. In the United States, myocarditis after vaccination was reported in military personnel [7]. The 23 recorded cases (16 Moderna/ 7 Pfizer-BioNTech recipients) in 2.8 million vaccinated (0.000821%) confirmed the rarity of event. Of the 23 cases, 20 occurred after the second dose and three after the first vaccine dose.

Side effects officially filed for COVID-19 vaccines were released by the European Medicines Agency (EMA) on May 29, 2021. Available data on acute myocarditis cases post mRNA vaccination are summarized in Table 1. Such a link was not observed after receipt of vector-based vaccines (Johnson & Johnson and AstraZeneca). Reported associations are indeed rare, predominantly found among males, and corresponding to 1.60 cases/million doses for Pfizer-BioNTech and 3.04 cases/million doses for Moderna. Most cases were in the working age population (18–64 years), some among older individuals (65–85 years) who were prioritized for vaccination, and a few were among adolescents 12–17 years whose vaccination has just been approved (as of May 10). Although rare, the identified association can be serious as shown by the finding that a large proportion of cases did not recover and by the (albeit few) fatalities. The association of myocarditis with male sex and younger age could be attributed to sex hormones which may account for a more intense inflammatory response. As suggested by experimental studies on myocarditis in mice, testosterone may be implicated in the inhibition of anti-inflammatory cells and the stimulation of immune responses mediated by Th1-lymphocytes [3]. But what could be triggering the inflammatory response that perhaps for some prone individuals is localized in the cardiac muscle tissue in the first place?

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https://doi.org/10.1016/j.vaccine.2021.07.016

Table 1
Description of acute myocarditis cases post mRNA COVID-19 vaccination by vaccine type.

Vaccine	**Moderna (CX-024414)**				**Pfizer-BioNTech (Tozinameran)**			
Sex	**Female**	**Male**	**Not specified**	**Total**	**Female**	**Male**	**Not specified**	**Total**
Age group								
Not specified	0	0	0	**0**	4	7	1	**12**
0–1 month	0	0	0	**0**	0	0	0	**0**
2 months – 2 years	0	0	0	**0**	0	0	0	**0**
3–11 years	0	0	0	**0**	0	0	0	**0**
12–17 years	0	1	0	**1**	0	16	0	**16**
18–64 years	13	48	0	**61**	57	166	3	**226**
65–85 years	2	3	0	**5**	18	12	0	**30**
> 85 years	0	0	0	**0**	0	0	0	**0**
Total	**15**	**52**	**0**	**67**	**79**	**201**	**4**	**284**
	Number of individual cases							
Reporter group								
Healthcare professional	62				211			
Non-healthcare professional	5				73			
Outcome								
Fatal	0				8			
Not recovered/Not resolved	19				70			
Recovered/Resolved	16				29			
Recovered/Resolved with sequelae	0				7			
Recovering/Resolving	12				58			
Unknown	20				112			

* Data (up to May 29, 2021) summarized from: European Medicines Agency. EudraVigilance-European database of suspected adverse drug reaction reports. Available at: https://www.adrreports.eu.
** The total number of COVID-19 vaccine doses administered in Europe was 352.61 million, as of May 29, 2021. The distribution of mRNA vaccine doses by manufacturer was as follows for Europe: Moderna 22,072,265 and Pfizer-BioNTech 177,656,584 doses, respectively (European Centre for Disease Prevention and Control, COVID-19 Vaccine Tracker. Available at: https://vaccinetracker.ecdc.europa.eu/). In the US, 293.49 million vaccine doses were administered as of May 29, 2021, with the following distribution by manufacturer: Pfizer-BioNTech 158.71 million, Moderna 124.07 million, Johnson & Johnson 10.71 million. Available at: https://ourworldindata.org/coronavirus.

The answer probably lies in the heart of mRNA vaccine technology. Two of the main hurdles that had to be overcome during the development of the platform, which had been known to work in principle since the 1990s (the injection of foreign mRNA into mouse muscle led to the expression of the encoded protein and the induction of cellular immunity), were the RNA's unwanted reactogenicity and fragility. Inflammatory reactions were mediated by innate immune sensors for both single-stranded (ss) and double-stranded (ds) RNA, e.g., by endosomal Toll-like receptor 3 (TLR3), TLR7 and TLR8 and cytoplasmic protein kinase R (PKR), RIG-I, MDA5, IFIT1, and 2′-5′-oligoadenylate synthetase, respectively. To minimize such undesired immune responses, the RNA is modified to contain N^1-methylpseudouridine instead of uridine [4]. Additional modifications of the mRNA and its encapsulation in lipid nanoparticles (LNPs) help overcome, but not eliminate, the inherent instability of the mRNA molecule under physiological conditions. This intrinsic property of the mRNA and bio- and nano-similars-related issues that may arise during the production of vaccine components (that takes places at multiple sites across different states or countries) are important variables that could be of concern. Minor differences in biophysical characteristics, such as particle size, homogeneity, shape and liposome lamellarity, may affect the physicochemical stability, bioactive moiety uptake, distribution or circulation times and immunogenicity [5].

Accordingly, some early commercial batches of the Pfizer-BioNTech's vaccine contained lower than expected levels of intact mRNA as shown by leaked EMA documents [6]. In an email dated November 23, an EMA official outlined a raft of issues on "truncated and modified mRNA species present in the finished product," with unknown consequences on vaccine safety and efficacy. Two "major objections" were filed with Pfizer by EMA regulators who were tasked with ensuring good manufacturing production (GMP) practices. EMA authorized Pfizer-BioNTech's vaccine on December 21, noting that "the quality of this medicinal product, submitted in the emergency context of the current (COVID-19) pandemic, is considered to be sufficiently consistent and acceptable." Approval of Moderna's vaccine, which is based on the same modality and subject to similar potential problems, followed.

Thankfully, both mRNA vaccines proved to be very safe and effective against the severe consequences of COVID-19, and, hopefully, this platform will be used to tackle other diseases in the near future. Minimal biosafety risks are entailed, as the mRNA offers transient gene expression and does not integrate into the host genome. Nevertheless, as the span of vaccinations is increasing beyond adults to include children aged 6 months to 12 years and given the fact that the rare potential association with myocarditis is more frequent among male adolescents, we propose the following two-tiered strategy: 1) Medical care should be sought in the presence of symptoms compatible with myocarditis, such as chest pain, dyspnea and palpitations, after COVID-19 vaccination, according to CDC's preliminary instructions; and 2) the purification of the mRNA from *in vitro* transcription contaminants and the mRNA-LNP product stability need to be improved and carefully monitored for each vaccine batch. An acceptable threshold, yielding the lowest stimulation of immune endpoints, should be established for the RNA impurities that are present in the final product [8]. Further studies are warranted to understand additional aspects of the modality, including the biodistribution of LNPs after the intramuscular injection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. A. Klein: research grant, scientific advisory board Kiniksa Pharmaceuticals, Ltd; scientific advisory board Swedish Orphan Biovitrum AB; scientific advisory board Pfizer, Inc.

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