



# To aspirate or not to aspirate? Considerations for the COVID-19 vaccines

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## Abstract

Syringe aspiration when vaccinating intramuscularly was not recommended before the pandemic due to the lack of conclusive evidence that it provides any benefit. However, *in vivo* evidence suggests that intravenous injection of mRNA vaccine can potentially lead to myocarditis, while introducing adenoviral vector to bloodstream can possibly result in thrombocytopenia and coagulopathy. These rare reactions were recorded in humans following the administration of the COVID-19 vaccines. Although the syringe aspiration may increase the level of pain at the injection site, it represents a simple technique to decrease the risk of vaccine introduction into the vascular system and potentially decrease the risk of severe reactions to mRNA and adenoviral vaccines. We are of the opinion that this cannot be disregarded if one considers that the COVID-19 vaccines will continue to be administered globally in the form of initial and booster doses. Therefore, the aspiration when giving mRNA and adenoviral vaccines appears to be fully in line with the precautionary principle.

**Keywords** Pandemic · Massive vaccination · SARS-CoV-2 · Myocarditis · Thrombosis · Acute side effects

## Introduction

The first coronavirus disease 2019 (COVID-19) vaccines received emergency authorizations within a year of the first documented COVID-19 outbreak was reported in Wuhan, China. The landscape of COVID-19 vaccine candidates was highly diverse in 2020 [1], with eventually over 20 approved in different parts of the world [2]. Although some of these vaccines are based on a more classical approach, *i.e.*, inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or recombination proteins, the major role in global vaccination campaigns is played by vaccines based on innovative solutions employing adenoviral vectors and

messenger RNA (mRNA) enveloped by lipid nanoparticle carriers [3]. As yet, all these vaccines are given as an intramuscular injection, although intranasal versions are under development in clinical trials [4].

Post-authorization monitoring has proven that adenoviral vector and mRNA-based vaccines are generally safe. However, various rare side effects were reported, including myocarditis, pericarditis [5, 6], appendicitis [7], liver [8], pancreatic [9] and kidney injuries [10], and thrombotic thrombocytopenia [11, 12]. The exact mechanisms behind these events require further investigations, although several potential explanations were offered [5, 13–15]. Notably, some of these events were documented in animals after an intravenous vaccine administration, *i.e.*, heart inflammation in case of mRNA vaccine [16] and acute thrombocytopenia and coagulopathy in the case of the adenoviral vector vaccine [13].

These observations fuel the discussion of whether the administration of the COVID-19 vaccines should be preceded with the syringe aspiration for 5–10 s after the needle is introduced intramuscularly [17, 18]. This technique was specifically developed in the past to ensure the medication is not inadvertently delivered into a blood vessel. Before the pandemic, the aspiration has generated numerous

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discussions and controversies, with no conclusive evidence to understand whether such a procedure is beneficial or unwarranted due to the absence of randomized clinical trials [19, 20]. At present, the World Health Organization, and various national authorities, including the U.S. Centers for Disease Control and Prevention [21], do not recommend aspirating prior COVID-19 vaccine administration. Contrary to this, Danish Serum Institute is still recommending this practice and provides a guideline on how to perform it correctly [22].

The aspiration is sometimes performed at various COVID-19 vaccination points depending on the approach of local consensuses or healthcare personnel habits. This paper briefly presents the pros and cons of these practices, explicitly addressing mRNA and adenoviral vaccines, to help in further considerations regarding the aspiration during the global COVID-19 vaccination campaigns.

### Arguments favoring aspiration during COVID-19 vaccination

Aspiration is a technique practiced to avoid accidental vaccine injection into a vessel during intramuscular administration. The appearance of blood in the syringe indicates that this is a case and shall result in another vaccination attempt. In this situation, the needle should be withdrawn, the syringe discarded, and another injection (prepared using new vaccine dose and equipment) should be given in a different location [17, 23]. The deltoid muscle is the preferred injection site for SARS-CoV-2 vaccines. Although the usual spot, 5–7 cm below the acromion, is relatively distant from big vessels, in some cases, the posterior circumflex humeral artery can be present in this area [24]. But even if major blood vessels in their typical locations are not in immediate proximity, anatomical variants and smaller branches can cause accidental intravessel administration of the vaccine or a part of it. While it may not represent a significant risk for various vaccines approved pre-pandemic and based on a more classical approach, it is not necessarily a case about mRNA and adenoviral vector vaccines, which administration was limited to clinical trials before the COVID-19 pandemic. In 2021, over 2.5 billion doses of mRNA vaccines and 2.5 billion doses of adenoviral vector vaccines were globally given to humans [3]. In turn, evidence from experiments *in vivo* highlights that introducing both mRNA and adenoviral vector vaccines into the blood instead of muscle can result in acute adverse events resembling those seen in post-authorization pharmacovigilance for humans given the same vaccines.

As shown *in vivo* in mice, intravenous injection of the BNT162b2 vaccine (BioNTech/Pfizer, Germany/USA) resulted in histopathological changes characteristic for myopericarditis. Two days after treatment, the animals revealed

calcific deposits on the visceral pericardium, interstitial edema, pericardial and myocardial infiltration of white blood cells, and transiently upregulated inflammatory cytokines and chemokines cardiomyocytes degeneration, apoptosis, and necrosis. The serum troponin levels were also markedly elevated. Moreover, the amount of mRNA encoding SARS-CoV-2 spike protein and its subsequent myocardial expression was significantly higher in heart tissue when compared to the animals receiving the intramuscular injection. Notably, the histological changes of myopericarditis persisted for 14 days and were aggravated considerably by intravenous injection of the second dose of the BNT162b2 vaccine.

These findings indicate that introducing the mRNA vaccine into the circulatory system can lead to acute cardiac inflammation. The mechanism behind this requires further elucidation. However, it is speculated that this may be due to the pro-inflammatory properties of lipid nanoparticles (LNPs) used as carriers for mRNA, as some of LNPs were shown to induce lung inflammation when introduced intranasally [16]. The other possible mechanism behind the observed myopericarditis (as observed in treated animals) is related to the expression of spike protein in transfected cardiomyocytes leading to excessively activated cytokine production and inflammatory cell infiltration [16]. Notably, the study did not find any differences in reaction to vaccines between female and male mice, while the effect of age was not studied. Therefore, the results are insufficient to explain the association between myocarditis following mRNA vaccination and male gender and younger age [5, 25, 26]. Nevertheless, they clearly show that invalid administration of mRNA vaccines into circulation can increase the risk of acute cardiomyopathies and should be avoided at all costs. This is essential if one considers that although post-vaccination acute inflammatory heart disease events are rare, their risk is increased for the first 30 days compared to unvaccinated individuals [27].

Although the mechanism behind rare events of thrombotic thrombocytopenia following the administration of the adenoviral vector vaccines also requires further elucidation, and it is not a specific subject to this article, certain observations are needed to be considered in relation to the potential benefit of aspiration practice. There is compelling evidence that selected adenoviruses, used as vectors, can directly interact with platelets. Some of them can bind using the coxsackie and adenovirus receptor (CAR), which represents an initial step for virus entry into thrombocytes [28]. This has also been shown for replication-deficient recombinant chimpanzee ChAdOx1 vector, which is the main component of the AZD1222 vaccine (Oxford/AstraZeneca, UK/Sweden) [14, 29]. In the case of human adenovirus type 26, the replication-deficient recombinant version is the main component of the Ad26.COV2.S vaccine (Janssen/Johnson&Johnson, Leiden, Netherlands/New Brunswick,

NJ, USA), and sialic acid is a primary cell receptor [30]. In human platelets, sialic acid has been implicated in their aggregation and adhesion [31]. Moreover, as recently evidenced, ChAdOx1 and human adenovirus type 26 can bind to the platelet factor 4 (PF4) [14]. Importantly, PF4 has been implicated in heparin-induced thrombocytopenia, an autoimmune complication after the administration of heparin manifested by the generation of pathogenic antibodies that bind the complex of PF4 and heparin. Eventually, this complex interacts with platelets' Fc $\gamma$ RIIA receptor (also known as CD32a), subsequently shifting thrombocytes to a hypercoagulable state, causing the release of additional PF4 and promoting both arterial and venous thrombosis [32]. The thrombotic events following adenoviral vector COVID-19 vaccines administration resemble heparin-induced thrombocytopenia. The affected subjects, including those with no history of heparin use, were tested positive for anti-PF4-heparin antibodies complex. They also turned out positive in the platelet-activation assay in the presence of PF4 independent of heparin [33–35].

The binding of adenoviral particles to circulating platelets can lead to the activation of the latter and subsequent aggregation [36–40]. Importantly, intravenous administration of adenovirus vectors has been directly shown to induce acute thrombocytopenia and coagulopathy within 24 h in rodents, rabbits, and non-human primates [37–40]. All in all, rare translocation of adenoviral vector outside the injection site may potentially result in its interaction with platelets and increase the risk of thrombosis. However, one should note that each intramuscularly injected dose of the COVID-19 adenoviral vaccines contains approximately  $5 \times 10^{10}$  viral particles [41, 42]. Thus, improper vaccine administration can potentially lead to their rapid appearance and high presence in the blood.

Considering that initial COVID-19 vaccinations and booster strategies with mRNA-based and adenoviral vector vaccines will continue to be pursued globally [43], aspiration appears as a simple solution to ensure that the risk of their introduction to the vein and distant translocation with the potential adverse outcome is minimized. It should be stressed that rare acute side effects following COVID-19 vaccine administration are most likely multifactorial and related to individual vulnerability, and their exact elucidation requires further studies. To this end, aspiration should be perceived as a practice framed within the precautionary principle.

### Arguments against aspiration during COVID-19 vaccination

There is evidence that performing an aspiration during intramuscular injection (including vaccination) can increase pain at the injection site in different age groups [44, 45]. Further,

most syringes are not explicitly designed for easy aspiration. Therefore, lack of precision may exaggerate discomfort in vaccinated individuals [46, 47]. Moreover, one study has shown that only 3% of the healthcare personnel performing an aspiration during intramuscular injections is doing so for the recommended 5–10 s [48]. Furthermore, exercising two-handed aspiration is less challenging when vaccinating with a conventional syringe but leads to lower control of the patient by a vaccinator, which may, in turn, lead to local injury [19]. On the other hand, training on accurately performing aspiration (including one-handed aspiration) is cost-effective and not time-consuming. Importantly, not all auto-disabled syringes, use of which is generally recommended, permit performing aspiration for 5–10 s. Therefore, it is essential for the vaccinators to thoroughly understand the anatomy and landmarks of the injection site to decrease the potential risk associated with the elimination of the aspiration technique [19].

Notably, the deltoid muscle, the recommended site for COVID-19 vaccines' administration, does not have the proximity of major blood vessels [20] except the posterior circumflex humeral artery. However, it must be kept in mind that several branches of the posterior circumflex humeral artery supply the middle and posterior portions of the deltoid. Together with the thoracoacromial artery (bifurcating into the deltoid artery and the acromial artery) and their smaller branches, they form a blood vessel network of this muscle. Hence, the introduction of the vaccine into the blood is relatively low but not zero [49]. One should also consider that such a low risk corresponds to a rare incidence of thrombotic thrombocytopenia after COVID-19 adenoviral vaccines or myocarditis after mRNA vaccinations. It can be argued, though, that lack of aspiration and subsequent accidental introduction of the vaccine into the bloodstream cannot be responsible for all acute cases of myocarditis/pericarditis after mRNA vaccines or thrombotic thrombocytopenia after vaccination with adenoviral vaccines. This is due to demographical differences in incidences of these events, e.g., heart inflammation significantly more frequently reported after vaccination in younger, male adults [6]. Young males have substantially higher muscle mass, greater muscle thickness with more blood vessels. The injection technique and sometimes needle size must be individually adjusted. Basic needle size for intramuscular injection (deltoid muscle) in children (5 years and more), adolescents and adults are usually 0.5–0.6 mm and 25–30 mm (22–25 gauge, 1–1½") [50, 51]. One should also note that skin bunching, often performed during vaccination, can create a skin-to-muscle distance of 20 mm or greater, leading to insufficient muscle penetration, particularly in case of individuals with higher body mass index and arm circumference [52].

One should also note that routine aspiration during COVID-19 vaccination would increase the risk of wasting

vaccine doses [23]. On the other hand, more than 20 different COVID-19 vaccines were already available by the end of 2021 in different part of the world [2], while a major concern was not the production capacity, but the inequality of vaccine distribution and accessibility due to insufficient involvement of developed regions in supporting the vaccination campaigns in the low-income countries [2, 43].

## Conclusions

There is no definitive evidence that improper intramuscular administration of COVID-19 vaccines, leading to the introduction of components into the bloodstream, is behind the reported rare cases of myocarditis and pericarditis (in case of mRNA vaccines) and thrombotic thrombocytopenia (in case of adenoviral vector vaccines). On the other hand, experimental in vivo data suggests that such events can be induced after the intravenous administration of these vaccines. Although COVID-19 vaccines are intended for intramuscular injection, the deltoid muscle, a preferred site, has enough vascularity to accidentally and rarely lead to the vaccine's introduction into the bloodstream and its translocation to distant tissues. Although the aspiration may increase the level of pain at the injection site, it represents a simple technique to decrease the risk of vaccine introduction into the vascular system. It can potentially reduce the risk of acute severe reactions to mRNA and adenoviral vaccines. We are of the opinion that this cannot be disregarded if one considers that the COVID-19 vaccines will continue to be administered globally in the form of initial and booster doses. Therefore, the aspiration when giving mRNA and adenoviral vaccines appears to be fully in line with the precautionary principle, particularly given that many countries are already vaccinating children against COVID-19.

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## Declarations

**Conflict of interest** The authors declare that there are no conflicts of interest.

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