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Predictive value of skin testing with excipients for COVID-19 vaccines

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Abstract

Coronavirus disease 2019 (COVID-19) was declared a global pandemic by the World Health Organization (WHO) in March 2020. Despite the availability of therapies and the adoption of security measures, the most effective method to fight COVID-19 remains the induction of immunity through vaccines. Scientific communities have developed several types of COVID-19 vaccines since the beginning of the pandemic, including those with innovative messenger RNA (mRNA) technology. Patients with a history of allergic reactions may have an increased risk of hypersensitivity reactions to COVID-19 vaccines. Therefore, it is important that these patients are evaluated by an allergist to help monitor immediate-type adverse reactions and identify what vaccine component may elicit an allergic reaction. Various strategies have been suggested to prevent hypersensitivity reactions, including performing skin tests or *in vitro* tests before vaccination in high-risk patients, administering a different vaccine for the second dose in subjects reporting adverse reactions to the first dose, fractional dosing, or pretreating with anti-immunoglobulin E (IgE) monoclonal antibody. The scope of this review is to evaluate, through current evidence available in the literature, the accuracy of skin testing to the excipients of COVID-19 vaccines, especially polyethylene glycol (PEG) and polysorbate, in predicting allergic reactions to vaccination, despite the existing discordance of data and approaches to the question from the various clinical experiences, as to permit the safe administration of COVID-19 vaccines to populations around the globe.

Keywords

Coronavirus disease 2019 vaccines, anaphylaxis, vaccine allergy, excipient allergy, polyethylene glycol allergy, polysorbate allergy, ethylenediaminetetraacetic acid allergy, trometamol allergy

Introduction

Coronavirus disease 2019 (COVID-19), an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, was declared a global pandemic by the World Health Organization (WHO) in March 2020. The clinical spectrum of the disease ranges from mild illness to severe pneumonia associated with acute respiratory distress syndrome (ARDS). Most patients are either asymptomatic carriers or have a mild influenza-like illness. Moderate and severe cases may require hospitalization as well as intensive therapy which includes antipyretics, antivirals, antibiotics, corticosteroids, and non-invasive or invasive ventilation. Immunomodulatory drugs or plasma exchange therapy may be required in complicated cases [1]. Despite the availability of therapies and the adoption of security measures such as masks and social distancing, the induction of immunity through vaccines has been shown to be the most effective method to fight COVID-19. Scientific communities throughout the world have been striving to create several COVID-19 vaccines, including nucleic acid vaccines, viral vector vaccines, subunit vaccines, attenuated vaccines, and inactivated vaccines, since the outbreak of the pandemic [2–4].

Nucleic acid vaccines are currently messenger RNA (mRNA)-based and consist of a modified mRNA encoding the full-length spike (S) protein and encapsulated in lipid nanoparticles containing polyethylene glycol (PEG, PEG 2000). The lipid nanoparticles serve as stabilizers preventing the premature degradation of the vaccine mRNA by ribonucleases. The viral mRNA is released into the cytosol, where the host ribosome machinery translates it to make the SARS-CoV-2 viral S protein in the prefusion conformation, allowing the production of neutralizing antibodies and memory cells [2, 3, 5]. Even though PEG is used as an excipient in many pharmaceutical products in oral, topical, and parenteral dosage forms, this is the first time it has been used in a vaccine [6]. This platform is used in the production of the Pfizer-BioNtech (BNT162b2-Comirnaty) and Moderna (mRNA-1273) vaccines [2, 5, 7, 8].

Viral vector vaccines use a non-replicating viral vector to deliver genes to the host cells to produce specific antigens, thus allowing them to mount an immune response. In this case, the gene encoding for the SARS-CoV-2 S protein is integrated into the genome of a different virus, such as adenovirus (Ad), which has been engineered in a modified harmless version [2–4]. A non-replicating chimpanzee Ad is used in Vaxzevria (AZD1222) manufactured by AstraZeneca. The Janssen Johnson & Johnson COVID-19 vaccine (JNJ-78436725) uses an Ad26 vector and the Gam-COVID-Vac vaccine (Sputnik V, not approved for use in Europe) is based on two recombinant replication-deficient human (Ad26 and Ad5) [2, 3, 5].

A subunit vaccine is a vaccine that contains purified parts of the pathogen that are antigenic. Nuvaxovid (NVX-CoV2373) is a subunit vaccine against the SARS-CoV-2 virus, produced by Novavax, containing recombinant S protein and matrix-M1 adjuvant protein [2, 3, 5].

Both viral vector and subunit vaccines contain polysorbate 80 (PS80) as the most noteworthy excipient [5].

A live-attenuated vaccine is prepared by introducing the virus into a “non-host” species in which it does not replicate well, hence reducing the virulence of the virus [2, 3]. No live-attenuated vaccines have yet been approved for SARS-CoV-2. Inactivated vaccines are not live, they cannot replicate and cannot cause disease. SinoVac Biotech generated an inactivated vaccine for SARS-CoV-2 (CoronaVac) which does not contain PEG or PS [9].

Anaphylaxis is a potential risk associated with all vaccines and drugs. Allergic reactions to SARS-CoV-2 vaccines are thought to be very rarely directed to the active component of the vaccine, but are usually directed towards the inactive excipients that stabilize the vaccine, such as PEG and PS, with both immunoglobulin E (IgE)-mediated and non-IgE-mediated mechanisms hypothesized [4]. Apart from PEG

and PS, other excipients of allergologic interest include tromethamine as a buffering agent in the Moderna vaccine, aluminum hydroxide in the CoronaVac vaccine, disodium ethylenediaminetetraacetic acid (EDTA) in the AstraZeneca and Sputnik V vaccines [5, 10], and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) as part of the lipid nanoparticles in the Pfizer-BioNtech and Moderna vaccines [7, 8, 10]. DSPC has been recently considered a potential culprit as it contains a quaternary ammonium (QA) group which the patient could have been sensitized to by previous exposures to QA-containing compounds in cosmetics, disinfectants, or drugs like codeine and its derivatives [8].

A higher risk of hypersensitivity reactions to COVID-19 vaccines may exist in patients who have a history of allergic reactions to medications, foods, or other vaccines. It is crucial that an allergist evaluates these patients to guide the patients appropriately on upcoming vaccines [10]. The scope of this article is to evaluate the accuracy of skin testing to the excipients of vaccines, especially PEG and PS, in predicting allergic reactions to vaccination, as to permit the safe administration of COVID-19 vaccines to populations around the globe.

Excipients of COVID-19 vaccines

PEG

PEGs are a family of hydrophilic polymers, formed through polymerization of ethylene oxide, frequently used in culinary, medicinal, cosmetic, and industrial products [11]. PEGs have different chain lengths and molecular weights (MWs). They can also have their methylether caps removed [methoxy PEGs (mPEGs) or PEG membrane metalloendopeptidase (MME)] [12]. In addition, there are also many structurally related polymers including PEG laureths, ceteths, ceteareths, oleths, PEG castor oils, PEG sorbitans (PSs), and PEG-propylene glycol copolymers (poloxamers) [11].

In the cosmetic industry, the number associated with the word "PEG" is related to the average number of ethylene oxide units in the cosmetic industry whereas, in the pharmaceutical industry, it indicates the average MW. The available MWs vary from 200 g/mol to 35,000 g/mol. Depending on the MW, PEGs may present as clear viscous liquids (MW < 400 g/mol) or as opaque solids or powders (MW > 1,000 g/mol).

PEGs are frequently used as excipients in a wide range of pharmaceutical products including tablet surface coatings, parental liquid preparations, suppositories, and ultrasound gel. Due to their water-binding properties, they are also applied in the manufacturing of hydrogels, bone tissue engineering, and neurosurgical dural sealants. PEG can be covalently attached to a drug, in a process known as PEGylation, increasing its MW. Increasing the drug's MW can increase its half-life and can decrease its immunogenicity by preventing opsonization [13]. When administered orally, the toxicity of PEG decreases with increasing MW, due to a slower absorption [14]. Moreover, it appears that PEGs can engage in P-glycoprotein (P-gp)-based drug interactions, which could have important implications for potential toxicity [15].

In the cosmetic and fragrance industry, PEGs can be found in creams, lotions, shampoos, hair gels, lipsticks, and oral hygiene products [16, 17].

PEG has been pointed out as a possible culprit of anaphylactic reactions to mRNA COVID-19 vaccines [18–21]. PEG allergy is a frequently underdiagnosed hypersensitivity, although some cues during clinical history-taking should be investigated further. The history of previous reactions to laxatives or bowel cleaning preparations before a colonoscopy, reactions towards many drugs with unrelated chemical structures, previous hypersensitivity to PEGylated drugs, or a seemingly innocent intolerance to some cosmetics applied topically, should alert the clinician to be in front of a patient with an occult sensitization to PEG [22].

PSs

PS80 (or Tween 80 and labeled E433 according to the European directive on food additives) is a non-ionic surfactant polymer produced by ethoxylation of sorbitan, esterified with oleic acid [22]. Its solubilizing properties render it ideal for use in creams, ointments, lotions, as an additive in tablets, and other medical preparations (such as vaccinations) [23, 24].

PS80 is an excipient of the three COVID-19 viral vector vaccines AZD1222 (AstraZeneca), JNJ-78436725 (Johnson & Johnson), Gam-COVID-Vac (Sputnik), and of the subunit vaccine NVX-CoV2373 (Novovax).

As PEG and PS80 are structurally similar, there is a potential risk of reactions to both vaccination components. While clinical cross-reactivity between PEG and PS80 has been documented in the literature, this is very rare [25].

Generalized reactions have been reported after the administration of parenteral drugs containing PS including human papillomavirus vaccine adalimumab and ustekinumab [26, 27], erythropoietin and darbepoetin [28], and omalizumab (PS20) [29].

EDTA

EDTA is a metal chelator first synthesized from ethylenediamine ($C_2H_8N_2$) in 1935 [30]. EDTA is available in several salts including disodium EDTA, sodium calcium edetate, and tetrasodium EDTA. Disodium EDTA is used to treat hypercalcemia due to its chelating capacity towards calcium. EDTA is used in food, cosmetic, and industrial productions, and in the AstraZeneca vaccine, for its conservation and stabilization capabilities. EDTA can inactivate metal cations, preventing them from binding with other ingredients of the cosmetic formulation and undermining the stability of the product itself and it can also enhance the antimicrobial action of some preservatives, especially against gram-negative bacteria [31].

Tromethamine (trometamol)

Tromethamine (or trometamol) is an organic amine proton acceptor. At room temperature, it appears as an odorless white solid. Tromethamine is used as a biological buffer in topical products, cosmetics, medications (fosfomycin, ketorolac), and some vaccines such as the Moderna vaccine [32]. It is an excipient in various contrast agents, including gadolinium and iodate agents [33].

The main COVID-19 vaccines available and their excipients are listed in Table 1.

Table 1. COVID-19 vaccines and their excipients

Vaccine	Type of vaccine	Excipients
Pfizer-BioNtech BNT162b2 (Comirnaty)	mRNA	PEG 2000, DSPC
AstraZeneca AZD1222 (Vaxzevria)	Non-replicating chimpanzee Ad	PS80, EDTA
Moderna mRNA-1273	mRNA	PEG 2000, DSPC
Johnson & Johnson COVID-19 vaccine (JNJ-78436725)	Recombinant, replication-incompetent, Ad-based vaccine	PS80
Gam-COVID-Vac (Sputnik V)	Recombinant, replication-incompetent, Ad-based vaccine	PS80, EDTA
Novavax NVX-CoV2373 (Nuvaxovid)	Protein based vaccine	PS80
SinoVac Biotech COVID-19 vaccine (CoronaVac)	Inactivated vaccine	-

-: No excipients

Note. Adapted with permission from "Allergies and COVID-19 vaccines: an ENDA/EAACI position paper," by Barbaud A, Garvey LH, Arcolaci A, Brockow K, Mori F, Mayorga C, et al. Allergy. 2022;77:2292–312 (https://onlinelibrary.wiley.com/doi/10.1111/all.15241). © 2022 EAACI and John Wiley and Sons A/S.

Proposed immune mechanisms for vaccine-induced immediate hypersensitivity reactions

According to the international consensus on allergic reactions to vaccines, immediate allergic reactions occur less than 4 h post-vaccination and delayed reactions appear more than 4 h after administration of the vaccine. With the COVID-19 vaccines, reactions occurring within 2 h of administration are considered immediate [5, 34].

There are different proposed mechanisms for COVID-19 vaccine-induced immediate hypersensitivity reactions. The most prevalent and known mechanism is the IgE-dependent pathway. In a previously sensitized individual, an allergen binds to its specific IgE bound to FCεRI on mast cells, leading to cross-linking and activation of mast cells which release their immune mediators. This frequently results in reactions within minutes. A late phase release of cytokines can cause another reaction typically 2–6 h after the first signs and symptoms and peaks in activity after 6–9 h [35]. IgE-mediated allergy to PEG has been well-documented in the literature [21]. Although less common, IgE-mediated reactions to PS80 can occur. A Spanish study reported two patients who developed immediate hypersensitivity reactions after being administered an intramuscular corticosteroid containing PS80 as an additive [36]. They both had positive skin tests for PS80 and corticosteroids containing the excipient. In both patients, allergic symptoms peaked hours after drug administration, but immediate positive skin test results suggest an IgE-mediated mechanism. Immediate positive skin test results suggested an IgE-mediated mechanism. The slow absorption of PS80 could explain the delayed symptoms [36].

It has been suggested that the PEG-micellar carrier system in the mRNA vaccines can lead to activation of the complement system in a second proposed mechanism of acute hypersensitivity reactions called complement activation-related pseudoallergy (CARPA). CARPA involves the production of anaphylatoxins (such as C3a, C4a, and C5a) by the complement system when vaccine allergens bind to IgG or IgM. These complement peptides can bind to mast cell complement receptors, thus causing the release of immune mediators during mast cell degranulation [34, 35]. According to Radice et al. [7], it is not a single PEG but the presence of highly repetitive domains in lipid nanoparticles resembling a pathogen surface that can induce the so-called CARPA. Binding of preexisting anti-PEG IgM to liposomes with subsequent complement activation has been proposed [37, 38]. PS80 and polyoxyethylated castor oil have also been associated with complement activation. They promoted the generation of biologically active complement products, C3a, C5a, and C5b-9 in human *in vitro* studies [39]. It was also demonstrated that the anti-cancer drugs paclitaxel and docetaxel, drugs formulated in polyoxyethylene castor oil and PS80 respectively, were capable of activating the complement system in a similar way [39]. Both drugs are associated with frequent hypersensitivity reactions [40]. The mechanism behind these hypersensitivity reactions is unlikely to be IgE-mediated, because of the rapid and overwhelming onset of most reactions during the first or second doses [41].

Another proposed non-IgE-mediated mechanism is the binding of the vaccine components and excipients to Mas-related G protein-coupled receptor X2 (MRGPRX2) protein, a class of G protein-coupled receptors expressed on mast cells, which may directly trigger mast cell activation. It has been demonstrated that many molecules, such as neuromuscular blocking agents and fluoroquinolones can activate MRGPRX2 [35].

Role of excipient testing: PEG and PS80

Skin tests

According to the guidelines issued jointly by the Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri (AAIITO) and the Italian Society of Allergy, Asthma and Clinical Immunology (SIAAIC) [42], skin tests for PEG should be carried out as follows (Table 2):

(1) PEG 3350 in its pure form: skin prick test (SPT) with initial dilutions from 1:100 up to 1:1, and a 30 min observation period after every dose step.

(2) PEG 3350 as an excipient of a corticosteroid drug (methylprednisolone acetate): SPT with undiluted form (40 mg/mL), followed by intradermal testing (IDT) at 1:1,000, 1:100, and 1:10, and a 30 min observation period after every dose step.

(3) Negative control with methylprednisolone sodium succinate-SPT with undiluted form (40 mg/mL), followed by IDT from 1:1,000, 1:100, and 1:10, and a 30 min observation period after every dose step.

Table 2. Non-irritating concentrations recommended for skin testing by Europen network on Drug Allergy (ENDA)/European Academy of Allergy and Clinical Immunology (EAACI) [5] guidelines and AAIITO/SIAAIC [42] guidelines

Excipients	Excipients-containing substances	Substance name	Concentration	ENDA/EAACI guidelines		AAIITO/SIAAIC guidelines	
				SPT	I.D.	SPT	I.D.
PEG	PEG-containing vaccines	Comirnaty (PEG 2000)	0.03 mg/0.03 mL	1:1	Starting at very low concentration	-	-
		Moderna (PEG 2000)	0.1 mg/0.5 mL	1:1	Starting at very low concentration	-	-
	PEG-pure substances	PEG 300	-	1:1	-	-	-
		PEG 400		1:1			
		PEG 500		50% in water			
		PEG 2000		1%, 10%, and 50% in water			
		PEG 3000		10% in water			
		PEG 4000		50% in water			
		PEG 6000		50% in water			
	PEG-containing drugs	Depo-Medrol (methylprednisolone acetate, PEG 3350)	40 mg/mL	1:1	1:100 and 1:10	1:1	1:1,000, 1:100, and 1:10
		Macrogol powder for oral solution (PEG 3350)	170 mg/mL	1:100, 1:10, and 1:1	-	1:100, 1:10, and 1:1	-
	PEG-derivatives	Polaxamer 407 (PEG 4000)	-	10% in water	-	-	-
PS80	PS80-containing vaccines	AstraZeneca	2.5×10^8 Inf.U/0.5 mL	1:1	-	-	-
		Johnson & Johnson	≥ 8.92 $\log_{10}$ Inf.U/0.5 mL	1:1	-	-	-
	PS80-containing drugs	Kenacort (triamcinolone acetonide)	-	-	-	1:1	1:100, 1:10, and 1:1
		Optive plus eye drops (carboxymethylcellulose)	-	-	-	1:1	1:100 and 1:10
	Tween 80	-	-	1%, 10%, and 20% in water	0.1%, 1%, and 10% in saline	-	-

Inf.U: infectious units; I.D.: intradermal. -: No data available

Note. Adapted with permission from "Allergies and COVID-19 vaccines: an ENDA/EAACI position paper," by Barbaud A, Garvey LH, Arcolaci A, Brockow K, Mori F, Mayorga C, et al. Allergy. 2022;77:2292–312 (https://onlinelibrary.wiley.com/doi/10.1111/all.15241). © 2022 EAACI and John Wiley and Sons A/S.

The ENDA/EAACI position paper [5] recommends a prick-to-prick test with the suspected vaccine in addition to SPTs with the excipients (Table 2). IDT with the mRNA vaccine starting at very low concentrations should be carried out in the case of positive prick tests to PEG but negative prick-to-prick tests to mRNA vaccines. It has been observed that IDT with mRNA vaccines can cause unspecific delayed reactions in patients and controls, but an immediate IDT reading is more specific [5].

IDT with PEG carries a risk of systemic reactions. Restivo et al. [43] recommend that only a PEG 0.01% dilution should be used for IDT in SPT-negative patients. Moreover, they advise avoiding IDT in high-risk patients because of the possibility of systemic reactions even with a negative skin test [43]. IDT with PS carries a risk of irritant effects. Wagner and Podda [44] tested 30 patients intracutaneously: each received 0.05 mL of PS-containing solvent, 0.02 mL of PS-containing solvent, and 0.02 mL of PS-free solvent. The results show that false positive skin test results in 77% of patients when a volume of 0.05 mL is applied

during IDT. The authors' take-home message is to use small volumes when carrying out IDT for PS to prevent false positive results.

The positive and negative predictive values of skin testing for PEG and PS are still to be defined. For this reason, the allergist is strongly encouraged to carefully select patients to undergo skin testing based on a detailed history [42, 45]. AAIITO/SIAAIC guidelines recommend testing three main patient groups: those with a history of hypersensitivity reactions to laxatives or bowel preparation containing high MW PEG, those with a history of hypersensitivity reactions to echocardiography contrast dye, and patients with a history of hypersensitivity reactions to intravenous drugs containing PEG, especially PEGylated drugs, or PSs [42]. A risk stratification score for initial administration of COVID-19 vaccine by Mass General Brigham and Vanderbilt allergy expert consensus is based on a history of anaphylaxis to an injectable drug/previous vaccine/another allergen such as food, venom, or latex, and a history of an immediate or severe allergic reaction to PEG-, PS-, or polyoxy 35 castor oil-containing injectable drug or vaccine [45]. Expanded skin testing and clinical phenotyping by an allergist is recommended for high-risk individuals [45].

Eight healthcare workers who were referred for PEG testing with anaphylactic reactions after the first dose of the BNT162b2 (Pfizer-BioNtech) mRNA vaccine, had PEG allergy ruled out by skin testing, challenge, and tolerance test history. All went on to receive a second dose of the same vaccine after premedication with antihistamines, without symptoms or significantly milder symptoms than experienced with the first dose. Moreover, in 5 out of the 8 patients, serum tryptase levels checked 30–90 min after the first dose were negative. These findings suggest a non-IgE-mediated mechanism behind the reactions [46].

Wolfson et al. [47] performed skin testing on a cohort of 80 patients with a reported allergic reaction after the first dose of mRNA COVID-19 vaccine and 25 controls. Adopting a protocol devised by Banerji et al. [45], PEG testing was performed using MiraLAX and methylprednisolone acetate, while triamcinolone acetonide and refresh tears were used for PS80 testing. Four patients reacted to PEG testing. Two patients tested positive for IDT methylprednisolone acetate and were rechallenged successfully with a second dose of mRNA vaccines. The third patient refused a second vaccine dose and the other opted for a non-mRNA vaccine. Positive skin tests for PS80 were only seen with refresh drops but an irritant effect of refresh tears was seen in several non-allergic controls, suggesting that it is not ideal to test for PS80. The authors concluded that skin testing is of limited utility and should be only considered in patients with previous anaphylaxis to the first dose of COVID-19 mRNA vaccine or oral PEG-containing drugs.

ALMuhizi et al. [48] investigated possible PEG or PS80 allergies among 44 patients reporting prior reactions to mRNA COVID-19 vaccines (40 patients) or with previous allergic reactions to PEG-related compounds (4 patients). Two patients with a previous reaction to PEG-containing compounds had PEG allergy confirmed by skin testing, with both receiving and tolerating two doses of the AstraZeneca vaccine. Two patients who had reported generalized cutaneous symptoms after the first dose of mRNA vaccine had delayed positive skin tests to PEG but both were administered a second dose of mRNA COVID-19 vaccine. A fractionated dosing regimen was used and both patients only reported mild symptoms, with one requiring an oral antihistamine due to the onset of pruritus and a subjective throat globus sensation, while the other patient only reported a small itchy spot after vaccination. One of the patients who had reported allergic symptoms after the second dose of mRNA vaccine subsequently had a delayed positive test on skin testing to PS80. Among patients who had a negative SPT, the majority tolerated the second dose except for two patients who had mild reactions. The findings of this study suggest that the prevalence of PEG allergy is low and there is no clear evidence of cross-sensitization between PEG and PS80. In most of the patients, a reaction to the first dose of the mRNA vaccine did not preclude revaccination.

Otani et al. [49] studied a cohort of 44 patients who developed allergic symptoms following the first dose of mRNA COVID-19 vaccination, 57% of them had a previous history of at least one drug allergy, but none had ever reported a reaction to PEG or PS. All received a second mRNA vaccine dose but only 14 patients underwent skin testing before proceeding to the second dose. They adopted the Banerji et al. [45] testing protocol, excluding triamcinolone IDT 40 mg/mL due to the authors' previous clinical experience with false positives at that dilution. Testing for PS80 with refresh drops was not done, but instead, prevnar

13 was used. Skin testing was negative except for 3 doubtful positive results (methylprednisolone succinate, *n* = 1, and prevnar 13, *n* = 2). One patient experienced a dubious irritating reaction to MiraLAX (SPT 17 mg/mL), but subsequent testing revealed negative results for both methylprednisolone acetate and MiraLAX at a higher dilution (SPT 170 mg/mL), demonstrating the limitations of PEG 3350 testing. After receiving the second COVID-19 mRNA vaccine dose, only 4 out of 44 patients manifested symptoms that were visible or required treatment (Pfizer 3, Moderna 1). All four reactions occurred after negative excipient skin testing. In this cohort of patients, skin testing failed to identify the culprit allergen in patients who experienced symptoms following the first dose of the mRNA COVID-19 vaccine and failed to predict those who were able to safely receive a second dose.

Otani et al. [49] also included two other cohorts of patients in their study. The first cohort consisted of patients with previous reactions to PEG and PS documented in electronic medical records (*n* = 202); all subsequently received mRNA COVID-19 vaccination safely. The second cohort consisted of patients referred to allergy and immunology with a history of PEG, PS, vaccine, or paclitaxel reaction (*n* = 50). Skin testing was done in 7 of these patients, with negative results. All patients in the second cohort were cleared for COVID-19 vaccination and 37 patients went on to receive COVID-19 vaccination. All tolerated the vaccine except for 5 patients who developed mild symptoms: mild delayed rash (*n* = 2), mild immediate rash around the injection site (*n* = 1), and hypertension (*n* = 2).

A practical approach to using PEG and PS testing to guide the safe vaccination of patients with a history of allergic reactions to these excipients is provided by Mortz et al. [50]. They re-evaluated 25 patients with a previous diagnosis of PEG and/or PS allergy (clinical history + positive SPT and/or basophil histamine release test (BHRT) and/or challenge test) months to years after initial diagnosis. The allergy status of the patients was re-classified as certain, possible, or unlikely, based on history and testing on primary valuation and re-evaluation. Based on this information, after the re-evaluation, the patients were classified into one of 5 groups and it was decided whether to offer patients vaccination and if yes, which vaccine to offer them.

The groups were the following:

(1) A certain PEG and PS allergy, with sensitization to PEG or PS80, or both; in 4 patients—no COVID-19 vaccine was offered.

(2) A certain history of PEG allergy and sensitization, with no clinical history of PS80 allergy but SPT sensitization to PS; in 2 patients—no COVID-19 vaccine was offered.

(3) A possible PS80 allergy and unlikely PEG allergy, with sensitized/challenge positive to PS; in 1 patient—PEG vaccine was offered, and accepted by the patient.

(4) A certain/possible history of allergy to PEG and not to PS with sensitized/challenge positive to PEG but not PS; in 11 patients—PS vaccine was offered, and accepted by 3 patients.

(5) Unlikely PEG and PS allergy, there were negative SPT and IgE and transient positive BHRT to a single PEG or PS; in 2 patients—PEG vaccine was offered, and accepted by both.

In this cohort, half of the patients had lost their skin test reactivity at re-evaluation highlighting that timing of allergy testing is important to get reliable results as also seen with other drug allergens. For SPT to PS, a concentration of 100% was used instead of the usually used 20%, which may result in false positive reactions. In this cohort, however, there were only a few positive SPT reactions to PS80 in 100% and no severe reactions to skin testing. Moreover, BHRT seemed to be a poor marker of PS allergy. Overall, this approach led to safe vaccination in most of the patients in the study. It should be noted that this was a Danish study and PS-containing vaccines by Johnson & Johnson and AstraZeneca are not recommended by Danish authorities due to the risk of thrombotic side effects.

Vidal Oribe et al. [51] evaluated the sensitization to COVID-19 vaccines in 5 patients with a prior diagnosis of PS80 allergy. Three had a history of anaphylaxis, and 2 reported acute urticaria, after the administration of Inzitan (cyanocobalamin, dexamethasone, lidocaine, thiamine, and PS80). Skin tests carried out at the time of diagnosis (between 3 years and 7 years before the study) yielded positive results

with PS80 and corticosteroids containing that excipient. SPT and IDT were performed with COVID-19 vaccines Pfizer-BioNtech, Moderna, AstraZeneca, and PS80.

Test results were negative in four out of 5 patients, but since a remission of the sensitization to PS80, as seen with other drugs like penicillin, cannot be ruled out, AstraZeneca vaccination was avoided and mRNA COVID-19 vaccination was opted for instead, with no ensuing adverse reactions. The remaining patient had a positive IDT with PS80 and with all the vaccines tested, so she was advised to avoid vaccination entirely.

In a study by Bruusgaard-Mouritsen et al. [52], ten patients with previously diagnosed PEG allergy underwent SPT with PEGs. SPT of low MW PEGs [PEG 300 (100%), PEG 3000 (50% weight/volume), and PEG 6000 (50% weight/volume)] were carried out, and if negative, were followed by SPT of PEG 20000 (0.01% dilution, tenfold increases in concentrations until a positive response is reached, up to 20% weight/volume). All healthy controls tested negative on SPT. Patients with a longer interval since diagnosis tested negative for lower MW and positive only for the higher concentrations of PEG 20000. All patients who had previously tested positive for PEG 3000 and PEG 6000, over time became less reactive to those doses but only tested positive for PEG 20000.

Klimek et al. [53] recommend a more cautious approach to PEG SPT with stepwise dilutions starting from 0.001% going up to a maximum of 10%. Moreover, they suggest that testing should be performed with PEG excipients of 2,000 g/mol MW that are used in both COVID-19 vaccines. However, there is a risk of missing a diagnosis of PEG allergy when using a single concentration of low MW PEG for testing. In fact, a patient who developed hypersensitivity after receiving the Pfizer-BioNTech COVID-19 vaccine had negative SPT results with PEG 2000 at 0.1% dilution and with vaccine vial residue. However, when SPT was carried out again with PEG 4000 at a higher concentration (1%), it led to anaphylaxis [54]. SPT utilizing just PEG 2000 at 0.1% dilution would not have identified the patient's risk of hypersensitivity before the vaccination.

Patch testing has historically been of limited value in examining the delayed effects of other vaccinations. For COVID-19 vaccine reactions occurring after 4 h, patch testing can be carried out with commercially available patch test materials for PEG 400 1:1 and PS80 at 5% in petrolatum [5].

In vitro tests

After a thorough history, the determination of basal tryptase is necessary. If elevated, a *KIT* mutation analysis in peripheral blood or bone marrow should be done to exclude mastocytosis [5, 21].

Some authors think that *in vitro* tests specific to vaccine excipients are not relevant [35, 42]. Klimek et al. [53] suggest that such testing may have a role in high-risk individuals with suspected allergy to excipients of the vaccine.

According to clinical history, specific IgE against PEG could be tested. The enzyme-linked immunosorbent assay (ELISA) is the most widely used test to detect antibodies to PEG but has not yet been standardized [55]. Mouri et al. [55] analyzed PEG-IgE and -IgG antibodies in patients presenting with allergic symptoms after COVID-19 mRNA vaccination. Raised levels of these antibodies were found to be higher in both immediate allergy and delayed allergy groups compared to controls. PS-IgE was higher in the immediate allergy group and significantly higher in the delayed allergy group compared to controls. To better understand the function of PEG-specific IgE in delayed allergy, additional research involving more patients is required. Skin tests were conducted in the same trial, and results indicated a positive connection between positive skin tests and positive IgE to PEG and/or PS. These findings imply that PEG- and PS-IgE may both be helpful in the detection of immediate-type allergy to mRNA vaccinations. There is no commercially available, specifically validated IgE assay for PEGs or other structurally similar polymers [52].

If IgE, IgG, and/or IgM antibodies against PEG 2000 are not available, a basophil activation test (BAT) can be considered, but no certified and validated test systems are currently available [5]. BAT requires incubating whole blood or enriched blood leukocytes with allergens. Cellular-bound specific IgE is indirectly measured by the flow cytometric identification of surface activation markers (e.g., CD63, CD203c) produced following allergen stimulation or the mediators released by basophils. However, it should be noted that stimuli that are IgE-independent also cause basophil activation. Two to six concentrations

should be utilized with PEG compounds, vaccinations, and other medications [52, 56, 57]. In a patient with an anaphylactic reaction to the Pfizer-BioNTech vaccine PEG sensitization was later verified by BAT, with a positive signal upon stimulation of whole blood *in vitro* with PEG 4000 at 0.2 mg/mL [58]. BAT testing in three known PEG allergy sufferers showed dose-dependent activation of all patients' basophils *ex vivo* with the BNT162b2 (Pfizer-BioNtech) vaccine, but not PEG alone. PEGylated liposomal doxorubicin was able to elicit a similar basophil activation, suggesting that PEGylated lipids within nanoparticles, but not PEG in its original state, are effectively able to promote degranulation. PEG conformation on nanoparticle surfaces increases the avidity, enhancing IgE-cross-linking on basophil surfaces [57–60].

Bruusgaard-Mouritsen et al. [52] used PEG-derivatives at different MW (PEG 300, 3000, 6000, and 20000), poloxamer 407, and PS80 in six concentrations and performed histamine release tests on 10 PEG-allergic patients and 16 healthy controls. They observed that *in vitro* reactivity over time to different MW PEG can decrease or even be lost over time with lack of exposure, so direct histamine release tests may be limited to those patients who are investigated within a few months of exposure. Moreover, BHRT is also considered a poor marker of PS allergy [50].

If there is a convincing history of an immediate-type reaction to a COVID-19 vaccine or PEGs, cellular assays, preferably BAT, can be successfully incorporated into the allergy testing methodology. mRNA vaccines or modified compounds containing PEG are preferred over unmodified PEGs, which less often lead to positive histamine release tests [59]. Positive results (threshold in the BAT to be established, perhaps > 10–15% activated basophils) seem to point to a PEG allergy [57, 59]. A negative BAT (< 5% activated basophils) or histamine release test to a vaccine should encourage vaccination with the tested vaccine. The tolerance of a COVID-19 vaccine following negative BAT and BHRT tests seems to reflect a good negative predictive value of these cellular tests [57]. According to Hung et al. [35], the BAT has a specificity of 80–96% and a sensitivity of 55–80% for acute hypersensitivity reactions caused by drugs and vaccines.

Role of excipient testing: EDTA and trometamol

EDTA

Two patients [13] with a known allergy to EDTA diagnosed after anaphylaxis to radiocontrast media (RCM) were investigated with skin tests and BAT to Vaxveria vaccine, Comirnaty vaccine, and EDTA. Both patients had IDT positive for EDTA at concentrations of 0.3 mg/mL and 3 mg/mL respectively but had negative *in vitro* and/or *in vivo* testing for the Vaxzevria vaccine which contains EDTA. They both subsequently received and tolerated the vaccine [13].

Trometamol

Delayed local reactions to the Moderna vaccination could be due to sensitization to trometamol. The typical reaction, known as the "COVID arm", is characterized by erythema, pruritus, and induration at the injection site. Generally, it occurs 8 days after the first dose of the Moderna vaccination, but it can also be seen 2 days after the second dose. Seven patients who presented with non-immediate local reactions occurring over 6 h after the injection of the Moderna vaccine, tested positive with IDT to both Moderna vaccine and trometamol [61].

A case of immediate hypersensitivity reaction (generalized urticaria) 1 h after the inoculation of the Moderna vaccine was reported [62]. Five weeks after vaccination, SPT and IDT were performed with non-irritant concentrations of the excipients or excipient-containing drugs, including PEG 3350, PEG 1500, gadobutrol (Gadovist, which contains tromethamine), and gadoteric acid. The only positivity resulting from IDT was that of gadobutrol (60.5 mg/mL) which confirmed the culpability of tromethamine [62].

Discussion

Since the initial reports of anaphylaxis after COVID-19 vaccination, many groups have looked with keen interest towards PEG and derivatives or other vaccine excipients, including skin tests, IgE assays, and BAT to elaborate safe algorithms for the correct management of vaccine hypersensitivity. A recent systematic

review and meta-analysis found skin testing to have high specificity but very poor sensitivity for predicting immediate allergic reactions in patients who are revaccinated with an mRNA COVID-19 vaccine after reporting an allergic reaction to the first dose [62]. Skin testing with PEG and PS would detect only 3 out of 100 people who will develop immediate revaccination reactions [63]. Studies have shown it is possible to safely administer the second dose of an mRNA vaccine with antihistamine premedication [45] or without premedication [36]. In high-risk patients, switching the patient to a PEG-free alternative vaccine could be an option. Isolated PS allergy is rare. Korean authors [64] performed an oral desensitization protocol to PEG using the PEG formulation for bowel preparation before administering the vaccine. Their 13-step desensitization schedule takes two days to be performed [64]. Alternatively, a desensitization protocol to the vaccine itself has been elaborated by Canadian and European researchers, allowing it to reach the full dose in about 2 h [8].

Despite the poor sensitivity of *in vivo* and *in vitro* testing, a study evaluating the effectiveness of allergy testing in increasing COVID-19 vaccination rate found that testing significantly decreased anxiety related to fear of allergic reactions and vaccine hesitancy in both subjects who were not vaccinated before testing and subjects who reported allergic reactions after the first dose [65]. In both groups, there was a strong female predominance, a gender difference identified in all studies.

Identifying at-risk patients has far-reaching consequences beyond COVID-19 vaccination. Individuals who are allergic to parenteral formulations with PEG may have tolerance to food or tablets containing PEG but could react to laxatives containing PEG because the amount of oral intake of PEG through laxatives will be higher [66]. It is also important to de-label patients "misdiagnosed" with a PEG or mRNA vaccine allergy, as mRNA technology has important prospects for vaccinology [66] and cancer treatment [26].

Conclusions

The SARS-CoV-2 pandemic has caused a global emergency that primarily involved the intensive care unit departments, and later other departments like neurology, cardiology, and lung care rehabilitation for the treatment of long COVID or post COVID patients. Allergists and immunologists were "recruited" to manage an innovative class of vaccines based on mRNA technology, specifically to evaluate potential hypersensitivity reactions to these vaccines and their excipients, to identify which vaccine component may elicit an allergic reaction and which patients could repeat the dose of vaccine safely, despite a previous allergic response. It caused an overwhelming influx of allergy consults while an international debate unfolded regarding the true usefulness of skin testing of vaccines and their excipients, with different research groups often reaching contradictory conclusions. The complexity of skin testing of COVID-19 vaccines or their components is compounded by the fact that no diagnostic kit for drug excipients skin testing is commercially available. Clinicians must generally rely on other drugs containing PEG such as macrogol from bowel cleansing preparations used in colonoscopies or specific parenteral steroid formulations, with the necessity of obtaining serial dilutions, leading to a time-consuming preparation.

Probably, from an economic point of view, the same principle behind de-labeling patients with penicillin allergy have been applied to investigate COVID-19 vaccine hypersensitivity: in the long term, it is more expensive to maintain this "label" than to remove.

Abbreviations

AAIITO: Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri

Ad: adenovirus

BAT: basophil activation test

BHRT: basophil histamine release test

CARPA: complement activation-related pseudoallergy

COVID-19: coronavirus disease 2019

DSPC: 1,2-distearoyl-*sn*-glycero-3-phosphocholine

EAACI: European Academy of Allergy and Clinical Immunology

EDTA: ethylenediaminetetraacetic acid

ENDA: Europen network on Drug Allergy

IDT: intradermal testing

IgE: immunoglobulin E

mRNA: messenger RNA

MWs: molecular weights

PEG: polyethylene glycol

PS80: polysorbate 80

S: spike

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

SIAAIC: Italian Society of Allergy, Asthma and Clinical Immunology

SPT: skin prick test

Declarations

Author contributions

FV and DGS: Investigation, Writing—original draft, Writing—review & editing. GC: Investigation, Writing—original draft, Conceptualization, Writing—review & editing. DP, FP, IZ, GL, MAL, GP, CMC, and VN: Investigation, Writing—original draft. DDB: Investigation, Writing—original draft, Validation, Supervision. EN: Conceptualization, Investigation, Writing—original draft, Validation, Supervision. All authors read and approved the submitted version.

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The authors declare that they have no conflicts of interest.

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