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ORIGINAL ARTICLE

Drug Allergy, Insect Sting Allergy, and Anaphylaxis

The value of the basophil activation test in the evaluation of patients reporting allergic reactions to the BNT162b2 mRNA COVID-19 vaccine

Marina Labella[1,2] | Jose Antonio Céspedes[1] | Inmaculada Doña[1,2] | Mohamed H. Shamji[3,4] | Ioana Agache[5] | Cristobalina Mayorga[1] | Maria José Torres[1,2,6]

[1]Allergy Research Group, Instituto de Investigación Biomédica de Málaga-IBIMA, Málaga, Spain

[2]Allergy Unit, Hospital Regional Universitario de Málaga, Málaga, Spain

[3]National Heart and Lung Institute, Imperial College London, London, UK

[4]NIHR Imperial Biomedical Research Centre, London, UK

[5]Faculty of Medicine, Transylvania University, Brasov, Romania

[6]Departamento de Medicina, Universidad de Málaga, Malaga, Spain

Correspondence
Cristobalina Mayorga, Instituto de Investigación Biomédica de Málaga-IBIMA, 29009 Málaga, Spain.
Email: mayorga@ibima.eu

Maria José Torres, Hospital Regional Universitario de Málaga, Plaza del Hospital Civil S/N, 29009 Málaga, Spain.
Email: mjtorresj@gmail.com

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Abstract

Background: mRNA-based COVID-19 vaccines have been reported to induce hypersensitivity reactions (HSR) in a small number of individuals. We aimed to evaluate the real-world incidence of the BNT162b2 mRNA COVID-19 vaccine HSR and to determine the value of the basophil activation test (BAT) in the allergological workup of patients reporting these reactions.

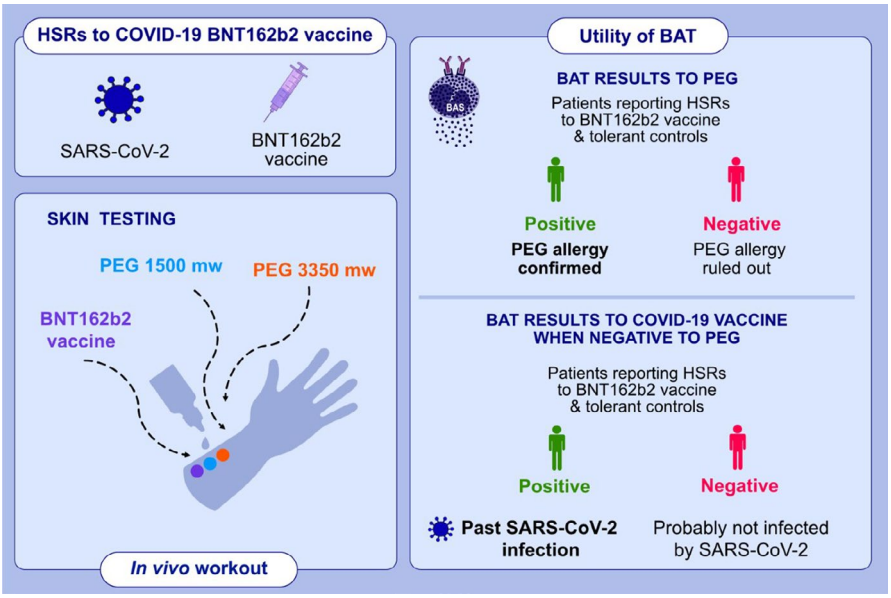
Methods: We prospectively enrolled patients with a clinical history indicative of HSR to the BNT162b2 mRNA COVID-19 vaccine. The allergological workup included skin testing (STs) and BAT with polyethylene glycol (PEG) and the vaccine. In those with negative allergy assessments, the administration of the second dose of the BNT162b2 mRNA COVID-19 vaccine was offered.

Results: Seventeen adults were included. Eleven cases (64.7%) tested negative in the allergological workup and tolerated the re-administration of the second dose of the vaccine and considered non-allergic. Six cases (35.3%) were considered allergic and classified into three groups: 2 subjects displayed positive STs and/or BAT to PEG (Group A), two individuals displayed positive BAT to the vaccine (Group B), and in 2 patients with moderate or severe reactions, the culprit was not identified, tested negative to STs and BAT to both PEG and vaccine (Group C). We further evaluated the value of BAT when the results were positive to the vaccine and negative to PEG by performing BAT in controls groups, finding positive BAT results in 50% of controls, all of them recovered from COVID-19 infection. In contrast, BAT was negative in patients who had not suffered from COVID-19 disease.

Conclusions: BAT can be used as a potential diagnostic tool for confirming allergy to PEG excipient but not to the vaccine as a positive result in BAT may indicate a past COVID-19 infection instead of an allergy.

KEYWORDS
allergic reactions, basophil activation test, BNT162b2, COVID-19, vaccines

Marina Labella, Jose Antonio Céspedes and Inmaculada Doña equal contributors.



GRAPHICAL ABSTRACT

The allergological workup of the patients reporting reactions after the vaccine administration is crucial to achieve a precise diagnosis. BAT can be used as a potential diagnostic tool for confirming allergy to PEG excipient. A positive result in BAT to COVID-19 BNT162b vaccine may indicate a past COVID-19 infection instead of an allergy.

1 | INTRODUCTION

The declaration of the pandemic induced by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in March 2020 by the World Health Organization (WHO) has led to an unprecedented challenge for healthcare systems.[1,2] The development of mRNA-based vaccines to prevent infection with SARS-CoV-2 is a landmark achievement of basic, translational, clinical and regulatory science.[3,4] Following regulatory approval, hypersensitivity reactions (HSRs) were reported in a small number of subjects receiving the vaccine, which resulted in public distress and a loss of confidence in vaccination safety.[5] Regulatory agencies introduced a summary of product characteristics, which included potential HSRs to mRNA-based vaccines and promptly issued recommendations on the avoidance of a second dose following a HSR to the first dose.

The European Academy of Allergy and Clinical Immunology (EAACI) issued recommendations[6,7] for the safe administration of COVID-19 vaccines, reviewed the allergic adverse reactions that can potentially occur after vaccination, and evaluated all vaccine components with allergenic potential.[8]

HSRs to customary anti-infectious vaccines have been estimated to account for 1–5 per million administered doses,[6] with IgE-mediated HSRs (anaphylaxis) to vaccines occurring in less than 1 case per million applications. For the BNT162b2 mRNA COVID-19 vaccine, 11.1 cases occurred per million administered doses.[9]

The underlying immunological mechanisms of the rare severe allergic reactions to the COVID-19 vaccines are poorly understood and need to be investigated. Immediate reactions to vaccines may be induced by excipients that act as preservatives, stabilisers or adjuvants in contrast to other types of drug allergy, where reactions are usually related to the active drug.[10-12]

Unless the patient has a history of an allergic reaction to any of the vaccine excipients or a severe allergic reaction to the first dose of COVID-19 vaccine, there is no contraindication to COVID-19 vaccines administration, nor HSR assessment is needed[8,12,13] The diagnostic workup in patients suspected of HSR includes *in vivo* skin tests (STs) and or *in vitro* approach, such as a basophil activation test (BAT). This evaluation is needed before excluding patients with suspected HSRs from receiving the vaccine and thus putting them at risk of severe COVID-19 infection.[6]

In this work, we evaluate the accuracy of an allergological workup, including BAT, to manage patients with HSRs to the first dose of the BNT162b2 mRNA COVID-19 vaccine for the safe administration of the second dose in order to achieve complete vaccination. Our results confirm that BAT is a potential tool for the diagnosis of HSRs to PEG excipient. However, BAT is not helpful to determine an allergy to the vaccine, as a positive result in BAT may indicate a past COVID-19 infection instead of an allergy.

2 | METHODS

2.1 | Cross-sectional evaluation of patients with a reaction to the first dose of the BNT162b2 mRNA COVID-19 vaccine

This study enrolled 17 adult patients referred to the Allergy Unit of the Regional University Hospital of Málaga starting with January 2021 to evaluate a possible HSR to the SARS-CoV-2 vaccine (BioNTech,

Pfizer, USA). The Allergy Unit is a tertiary public referral centre for all drug allergy evaluation in an area of 1,900,000 inhabitants.

Reactions were classified into immediate reactions (IRs) or non-immediate (NIRs), depending on whether the symptoms appeared within 1–6 h or later than 1 h, respectively, after administering the dose.[14] Reaction severity was classified as grade 1 (mild: skin and subcutaneous tissues); grade 2 (moderate: symptoms suggesting respiratory, cardiovascular, or gastrointestinal involvement); and grade 3 (severe: hypoxia, hypotension or neurologic compromise).[15]

The allergological workup started with a comprehensive clinical history performed according to the EAACI questionnaire,[16] followed by skin tests (STs) which included skin prick test (SPT) and if negative intradermal test (IDT) with polyethylene glycol (PEG) and SPT with the BNT162b2 vaccine.[17] No patients included in our study had dermatographism or any other disease that might interfere with assessing the response to STs. In all cases reporting IRs, BATs with PEG and the BNT162b2 vaccine were carried out.

According to the allergological workup, patients with STs and BAT negative were offered to receive the second dose of the BNT162b2 vaccine and if tolerated they were considered as non-allergic. Patients with STs and or BAT positive or grade 2 or 3 reactions were considered allergic (Figure 1). Further evaluation of the patients included serum tryptase, C3, C4, total IgE and SARS-CoV-2 IgG titres.

The study was approved by the Ethical Committee of Málaga and conducted according to the principles of the Declaration of Helsinki, and all the participants gave written informed consent.

2.2 | Skin tests

SPTs were carried out as previously described[18] using the vaccine Comirnaty (BioNTech, Pfizer, USA) 1:1, PEG 1500 (Roxall, Biscay, Spain) using 0.1%, 1% and 10% sequentially tested, and PEG 3350 (Movicol®) (Norgine, Madrid, Spain) at 55 mg/ml. If negative, IDTs were carried out[18] using PEG 1500 (Roxall, Biscay, Spain) at 0.01%, and PEG 3350 (Movicol®) (Norgine, Madrid, Spain) at 0.55 and 5.5 mg/ml as recommended.[12,19-21] Positivity criteria for SPTs were the development of a wheal larger than 3 mm surrounded by erythema, with a negative response to the control saline and for IDTs were an increase greater than 3 mm in the diameter of the initial wheal area surrounded by erythema.[22]

2.3 | Administration of the second dose of vaccine

In subjects with NIRs and negative STs to PEG and vaccine, the second dose of BNT162b2 was administered at the vaccination point. Subjects with IRs: If the patient had positive STs or BAT to PEG and or BNT162b2 vaccine, the administration of a vaccine other than the mRNA vaccine (Vaxzevria, AstraZeneca, Oxford, United Kingdom, and Vaccine Janssen, Janssen-Cilag International NV, Beerse, Belgium) was offered in a single dose under the supervision of an allergist[23]; If the patient had a suggestive clinical history of HSR but negative allergological workup, administration of the second dose of BNT162b2 under allergist supervision was offered.

2.4 | Basophil activation test

Patients from the cross-sectional study and those from the longitudinal study were evaluated with BAT. BAT was performed as previously described with some modifications.[24] One hundred μL of heparinized whole blood and 20 μl of stimulation buffer (NaCl at 0.78%, KCl at 0.037%, $CaCl_2$ at 0.078%, $MgCl_2$ at 0.033·, 78%, KCl at 0.037, HSA at 0.1%, HEPES at 1 M and IL-3 at 10 μg/ml) were added per test. After this step, 100 μl of PEG2000 (Sigma, St Louis, MO, USA) at 100, 10, 1 and 0.1 μg/ml and BNT162b2 vaccine at 10, 1, 0.1 and 0.01 μg/ml were added and incubated for 25 min at 37°C. As a negative control, 100 μl of washing solution was added, and as a positive control, 100 μl of anti-human IgE (BD Pharmingen, 0.5 mg/ml) was used. Cells were stained with monoclonal antibodies, anti-CCR3-APC, CD63-FITC and CD203c-PE (all from Caltag Laboratories, Burlingame, CA) and acquired in a FACSCalibur flow cytometer (Becton-Dickinson Bioscience, San Jose, CA) by obtaining at least 500–1000 basophils per sample selected as $CCR3^+CD203c^+$ cells. Results were analysed using FlowJo® software (FlowJo LLC, Becton Dickinson, Ashland, OR), and activation was expressed as stimulation index (SI) using CD63 as an activation marker.[25] SI was calculated as the ratio between the percentage of activated basophils ($CD63^+CCR3^+CD203c^+$ cells) in samples stimulated with either PEG or the BNT162b2 vaccine and in the unstimulated samples. The percentage of spontaneously activated basophils was required to be around 2.5% to calculate the SI. To confirm that positive basophil activation with PEG2000 or BNT162b2 vaccine was IgE-mediated, we used the wortmannin test at 1 μM.[26]

2.5 | BAT results in control patients

The control group for BAT included: 5 cases recovered from COVID-19-not vaccinated (C-NV), 5 cases recovered from COVID-19-vaccinated with BNT162b2 with no allergic reaction (C-V), 4 cases not infected by SARS-COV-2-not vaccinated (NC-NV), and 4 cases not infected by SARS-CoV-2-vaccinated with BNT162b2 with no allergic reaction (NC-V) (Figure 1). None of the controls revealed any history allergic clinical symptoms neither to any of the vaccine compounds like PEG or polysorbate 80 nor to the vaccine itself.

We also analysed BAT results in a longitudinal follow-up of subjects receiving the COVID-19 vaccine. Thirty adults were evaluated at four different time points: before the administration of the first dose of the BNT162b2 vaccine (T0), 21 days after the administration of the first dose of vaccine (before the administration of the second dose) (T1), and 20 days (T2) and 3 months (T3) after the administration of the second

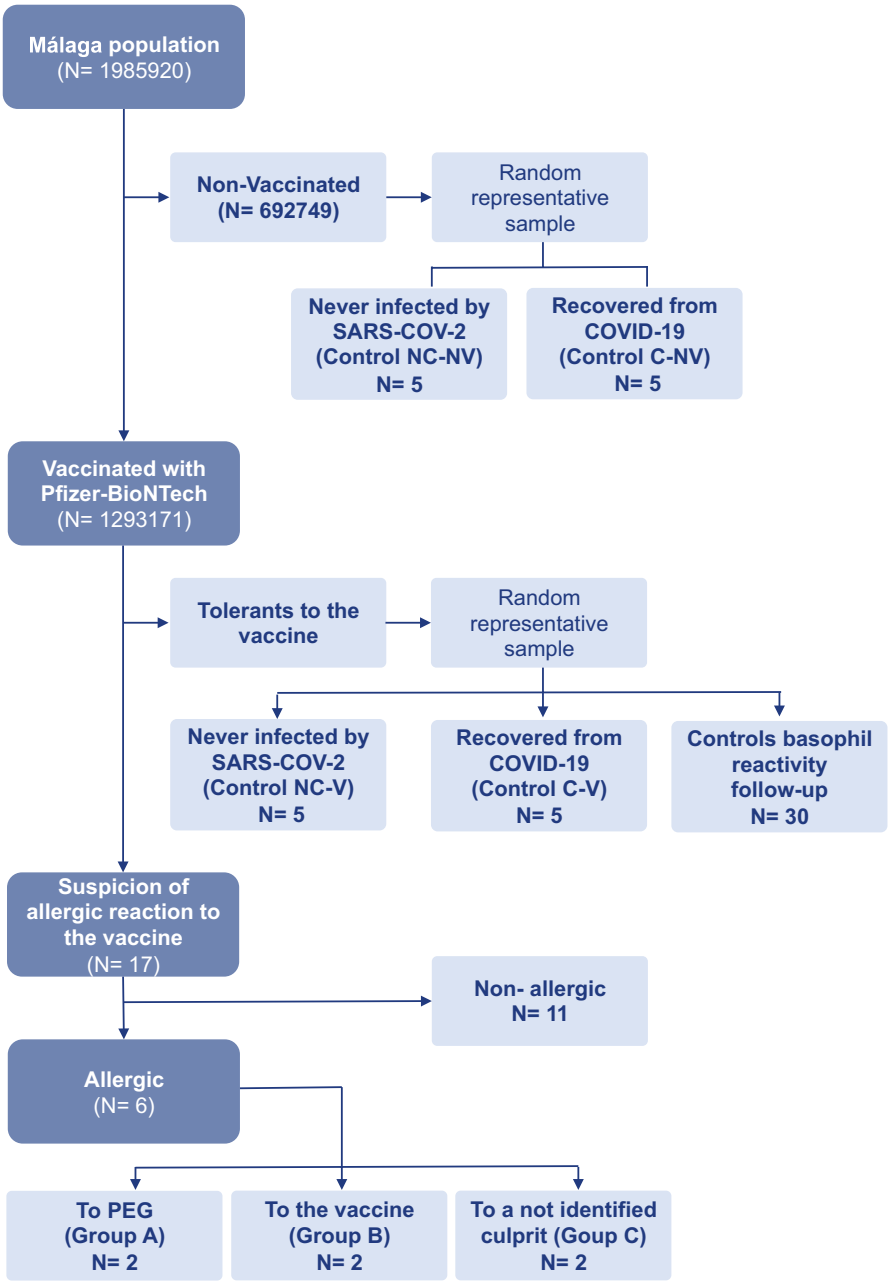


FIGURE 1 Flowchart for the distribution of study participants

dose of the BNT162b2 vaccine. In all cases, blood samples were obtained for performing BAT with both PEG and the BNT162b2 vaccine.

2.6 | Statistical analysis

Description of the quantitative variable was performed, including the median and interquartile range. Differences between qualitative variables were analysed by the chi-square test (non-related samples) and the McNemar test (related samples).

Comparisons between quantitative variables were performed by Mann-Whitney *U* test (non-related samples) and by Wilcoxon test (related samples). All statistical analyses were carried out using the software package GraphPad PRISM v7. A value of $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Cross-sectional evaluation of patients with a reaction to the first dose of BNT162b2 COVID-19 vaccine

Seventeen patients were sent for evaluation to our unit (Table 1 and Table S1). One case was confirmed by positive SPTs to PEG (Pt 1). Five cases with negative STs reported unequivocal allergic symptoms after the first dose vaccine administration. Despite the negative SPTs, they declined the administration of the second dose of BNT162b2, and one patient (Pt4) received Vaccine Janssen (Janssen-Cilag International NV, Beerse, Belgium) with good tolerance. The description of these patients is shown in (Table 2). Interestingly, 4 out of 6 were health workers who are subjects at

TABLE 1 Characteristics of patients referred for allergy evaluation following a reaction to the first dose of the BNT162b2 vaccine

N	17
Age (median, IR, years old)	56.5 (51–62)
Gender (*N*, % of females)	13 (76.47)
Co-morbidities	
Hypertension	7 (41.18)
Diabetes	5 (29.41)
Allergic rhinitis	2 (11.76)
Self-reported drug allergy	5 (29.41)
Food allergy	1 (5.88)
Symptoms recorded (*N*, %)	
Generalized urticarial	6 (35.29)
Localized urticaria (face)	2 (11.76)
Non-severe angioedema (face)	2 (11.76)
Lip/tongue angioedema	6 (35.29)
Dysphonia	1 (5.88)
Throat tightness	2 (11.76)
Oropharyngeal pruritus	4 (23.53)
Cough	1 (5.88)
Dyspnoea	2 (17.65)
Wheezing	1 (5.88)
Chest tightness	1 (5.88)
Dizziness	3 (17.65)
Malaise	3 (17.65)
Tachycardia	1 (5.88)
Severity	
Mild (Grade I Brown)	13 (76.47)
Moderate (Grade II Brown)	3 (17.65)
Severe (Grade III Brown)	1 (5.88)
Interval between the vaccine administration and the onset of the reaction (median, IR, min)	30 (10–1440)
Immediate	10 (8–25)
Non-Immediate	1440 (1110–6120)
Type of reaction (*N*, %)	
Immediate (≤6 h)	9 (52.94)
Non-Immediate (>1 h)	8 (47.06)
Management of the reaction—setting	
At hospital	7 (41.18)
At primary care	8 (47.06)
None (spontaneous recovery)	2 (11.76)
Management of the reaction—treatment	
Adrenaline IM	2 (11.11)
Corticosteroids	12 (70.59)
Intravenous route	4 (23.52)
Intramuscular route	7 (41.17)
Oral route	1 (5.88)
Antihistamines	11 (64.71)
Intravenous route	3 (17.65)
Intramuscular route	5 (29.41)
Oral route	3 (17.64)
Interval time between the reaction and the allergological workup (median, IR, days)	42 (30–51)
Blood tests before allergological workup Tryptase (median, IR, ng/ml)	8.35 (6.3–9.05)
C3 (median, IR, mg/dl)	121 (106.25–143)
C4 (median, IR, mg/dl)	26 (20–35)
Total IgE (median, IR, KU/L)	1304 (644.25–2045)
Post-vaccine IgG SARS-Cov-2 (median, IR, U/ml)	1.82 (1.1–9.18)

Abbreviations: IR, interquartile range; IM, intramuscular; IgE, immunoglobulin E; IgG, immunoglobulin G.

risk of infection and need a fast response for confirming their allergy before continuing their vaccination. Eleven out of 17 patients (64.7%) tolerated the second dose of BNT162b2 after a negative allergological workup (Figure 1).

3.1.1 | Comparison of patients with confirmed and excluded allergy to the BNT162b2 vaccine

Patients with confirmed allergic reaction to the BNT162b2 vaccine, either by STs or unequivocal clinical history, displayed a higher percentage of IRs (100% vs 27.27%, $p > .05$) and of vaccine-induced dizziness and malaise in the reported symptoms (50% vs 0%, $p = .02$, respectively) as compared to individuals in whom the diagnosis was not confirmed (Table 3).

3.2 | Basophil activation test for PEG and the BNT162b2 vaccine

For BAT studies, we first performed ROC curves for both PEG and vaccine. The area under curve (AUC) for PEG at 100 μg/ml was 0.7154 ($p = .2097$), for vaccine at 10 μg/ml was 0.6868 ($p = .1062$) and vaccine at 1 μg/ml was 0.6593 ($p = .1682$). Therefore, we selected as cut-off points 3 for PEG at 100μg/ml, and 2 and 2.5 for vaccine at 10 μg/ml and 1 μg/ml, respectively (Figure S1).

In the group of patients referred for suspected HSR to BNT162b2, we found 2 cases with BAT positive to PEG and vaccine BNT162b2 (Pt 1 and Pt 2) and 2 cases with BAT positive only to the vaccine (Pt 3 and Pt 4) (Table 2). In 2 cases with unequivocal symptoms of allergic reaction to vaccine BNT162b2, BAT was negative to both PEG and

TABLE 2 Results of the allergological workup in allergic cases

Group/ Patient	Age (years)/ Gender	Underlying diseases	Health workers	Time interval (min)	Reaction	Treatment/Time to resolution	ST			BAT	
							PEG 1500	PEG 3350	BNT162b2 Vaccine	PEG 2000	BNT162b2 Vaccine
A/Pt1	59M	AH, DM, dyslipidaemia, deep vein thrombosis	No	10	GM, dizziness and labial/lingual AE	CS, and AntiH/2h (AE 48 h)	SPT +	SPT +	–	+ (4.57)	+ (3.1)
A/Pt2	33F	None	Yes	5	Pharyngeal itching and difficulty in swallowing	CS and AntiH/1 h	–	–	–	+ (3.1)	+ (4.79)
B/Pt3	44F	AH, DM, allergy to latex and LTP, allergic rhinitis	Yes	25	Oropharyngeal, retroauricular and palmar pruritus	CS and AntiH/1 h	–	–	–	–	+ (3.19)
B/Pt4	36F	None	Yes	8	Dyspnoea, dysphonia, oropharyngeal itching, throat tightness, cough, GM, dizziness, tachycardia, hypotension	CS, AntiH, bronchodilator, fluids/3 h	–	–	–	–	+ (2.88)
C/Pt5	51F	None	Yes	25	Dizziness, generalized pruritus and urticaria	CS and AntiH/20 min	–	–	–	–	–
C/Pt6	55F	HIV, CHV, cryoglobulinemia	No	First/ 30 min	GM, facial urticaria, labial/lingual AE, throat tightness, difficulty in swallowing, breathing and speaking	CS and AntiH/2 h	–	–	–	–	–

Abbreviations: AE, angioedema; AH, arterial hypertension; AntiH, antihistamines; BAT, basophil activation test; CS, corticosteroids; DM, diabetes mellitus; F, female; GM, general malaise; H, hour; LTP, lipid transfer protein; M, male; Min, minutes; PEG, polyethylene glycol; Pt, patient; ST, skin testing; Time interval, Time between the vaccine administration and the appearance of the symptoms.

Patient classification: Group A: Patient allergic to PEG. Group B: Patient allergic to Pfizer-BioNTech vaccine. Group C: Patient with suggestive clinical history but negative STs and BAT.

TABLE 3 Characteristics of allergic and non-allergic patients

	Confirmed allergic $N = 6$	Allergy excluded $N = 11$	p value
Age (mean, IR, years old)	50 (39.25–53.25)	59 (55–75.5)	.026
Gender (*N*, % of females)	5 (83.3)	8 (72.7)	1
Underlying diseases			
Hypertension	2 (33.3)	5 (45.5)	1
Diabetes	2 (33.3)	3 (27.3)	1
Allergic rhinitis	1 (16.7)	1 (9.1)	1
Drug allergy	2 (33.3)	3 (27.3)	1
Food allergy	1 (16.7)	-	.352
Symptoms manifested in reaction (*N*, %)			
Generalized urticaria	1 (16.7)	5 (45.45)	.333
Localized urticaria (face)	1 (16.7)	1 (9.09)	1
Non-severe angioedema (face)	–	2 (18.18)	.514
Lips/tongue angioedema	2 (33.37)	4 (36.36)	1
Dysphonia	1 (16.7)	–	.352
Throat tightness	2 (33.33)	–	.110
Oropharyngeal pruritus	3 (50)	1 (9.09)	.098
Cough	1 (16.7)	–	.352
Dyspnoea	2 (33.3)	1 (9.09)	.514
Wheezing	1 (16.7)	–	.352
Chest tightness	1 (16.7)	–	.352
Dizziness	3 (50)	–	.029
Malaise	3 (50)	–	.029
Tachycardia	1 (16.7)	–	.352
Severity			
Mild (Grade I Brown)	3 (50)	10 (90.9)	.098
Moderate (Grade II Brown)	2 (33.3)	1 (9.1)	
Severe (Grade III Brown)	1 (16.7)	–	
Interval between the vaccine administration and the onset of the reaction (mean ± SD, min)	17.5 (8.5–25)	1440 (67.5–3600)	.043
Type of reaction (*N*, %)			
Immediate (≤1 h)	6 (100)	3 (27.27)	.009
Non-Immediate (>1 h)	-	8 (72.72)	
Management of the reaction setting			
At hospital	3 (50)	4 (36.4)	.81
At primary care	3 (50)	5 (45.5)	
None	–	2 (18.2)	
Management of the reaction – treatment			
Adrenaline IM	1 (16.7)	1 (9.09)	1
Corticosteroids	6 (100)	6 (54.54)	.102
Intravenous route	3 (50)	1 (9.09)	.098
Intramuscular route	2 (33.3)	5 (45.45)	1
Oral route	1 (16.7)	–	.375
Antihistamines	4 (66.6)	7 (63.63)	1
Intravenous route	2 (33.37)	1 (9.09)	.514
Intramuscular route	2 (33.3)	3 (27.27)	1
Oral route	-	3(27.27)	.514
Interval time reaction-allergological evaluation (mean, IR, days)	42 (32.25–93.75)	48 (31.5–50.5)	.801
Blood tests before allergological workup			
Tryptase (mean, IR, ng/ml)	8.1 (5.7–9.2)	8.9 (7.875–11.2)	1
C3 (mean, IR, mg/dl)	127.5 (108.25–143)	117 (107.75–129.25)	.830
C4 (mean, IR, mg/dl)	23.5 (19.25–30.75)	30.5 (23.75–36.75)	.334
Total IgE (mean, IR, KU/L)	1466.5 (1034.75–1898.25)	1304 (981–1627)	.914
Post-vaccine IgG SARS-Cov-2 (mean, IR, U/ml)	2.305 (1.22–11.73)	1.55 (1.1625–4.255)	–

Abbreviations: IR, interquartile range; IM, intramuscular; IgE, immunoglobulin E; IgG, immunoglobulin G.

TABLE 4 Demographic data and basophil activation test result in controls

GROUP	ID	Age (years)/ Gender	Underlying diseases	Health workers	BAT	
					PEG 2000	BNT162b2vaccine
C-NV	Ctrl1	46/Female	None	No	–	+ (11.43)
C-NV	Ctrl 2	47/Male	None	No	–	+ (7.18)
C-NV	Ctrl 3	23/Female	Allergic rhinitis, food allergy	No	–	–
C-NV	Ctrl 4	40/Male	Allergic rhinitis and asthma	No	–	–
C-NV	Ctrl 5	34/Male	Allergic rhinitis	No	–	+ (3.09)
C-V	Ctrl 6	41/Female	None	Yes	–	+ (8.04)
C-V	Ctrl 7	28/Female	None	Yes	–	+ (6.25)
C-V	Ctrl 8	40/Female	None	Yes	–	–
C-V	Ctrl 9	30/Female	Allergic rhinitis	Yes	–	–
C-V	Ctrl 10	36/Male	None	Yes	–	–
NC-NV	Ctrl 11	46/Female	Atopic dermatitis, allergic rhinitis and asthma	Yes	–	–
NC-NV	Ctrl 12	45/Female	None	Yes	–	–
NC-NV	Ctrl 13	55/Female	Hypothyroidism	Yes	–	–
NC-NV	Ctrl 14	40/Female	None	Yes	–	–
NC-V	Ctrl 15	32/Female	None	Yes	–	–
NC-V	Ctrl 16	28/Female	None	Yes	–	–
NC-V	Ctrl 17	37/Female	Hypothyroidism	Yes	–	–
NC-V	Ctrl 18	45/Male	Food allergy	Yes	–	–

Note: C-NV, Controls COVID-19 recovered, not vaccinated; C-V, Controls COVID-19 recovered, vaccinated with BNT162b2 without allergic reaction; NC-NV, Control not suffered from COVID-19, not vaccinated; NC-V, Control not suffered from COVID-19, vaccinated with BNT162b2 without allergic reaction.

vaccine (Pt 5 and Pt 6). Therefore, we were not able to confirm the causal agent (Table 2).

According to clinical history, STs and BAT results, patients were classified into three groups: (i) Group A: allergic to PEG (STs and or BAT positive to PEG); (ii) Group B: sensitized to the vaccine (STs and or BAT positive to the vaccine); and (iii) Group C: with suggestive clinical history and severe clinical symptoms occurring within first 30 min after vaccine administration, although negative STs and BAT to both PEG and vaccine (unidentifiable trigger). (Figure 1 and Table 2).

We further evaluated BAT value for the diagnosis of HSR to the BNT162b2 vaccine by conducting BAT (with PEG and with vaccine) in four different control groups (Table 4). Positive BAT results to the vaccine BNT162b2 were found in 5 out of 10 controls (50%); all of them recovered from COVID-19 infection (3 C-NV and 2 C-V). In controls who did not suffer COVID-19 (NC-V and NC-NV), BAT to BNT162b2 was negative in all cases (Figure 2). In all controls, BAT to PEG was negative. In all subjects, including patients and controls (C-NV and C-V), positive BAT with either PEG or Vaccine, wortmannin experiments confirmed that basophil activation was mediated by IgE. (Figures 3 and 4).

3.3 | Longitudinal follow-up of vaccinated patients

In the follow-up of the 30 subjects receiving the BNT162b2 vaccine, the BAT results indicated no differences in the SI to either PEG or BNT162b2 before and at different time points after the administration of the first and the second dose ($p > .05$). (Figure 5).

4 | DISCUSSION

This study reports on the value of the allergological workup for patients with reactions to the first COVID-19 vaccine dose, with a particular emphasis on BAT. It also evaluates the immunological profile and safety of the COVID-19 vaccine longitudinally up to 3 months after the second dose, using BAT.

The diagnosis of HSR to BNT162b2 due to PEG 2000 was confirmed by BAT in only 2 out of 17 (11.7%) patients reporting a reaction after vaccination. PEG is an excipient contained in the mRNA vaccines as well as in multiple drugs and cosmetic products. Although allergy to PEG is rare, it has been previously described as a compound that can induce severe allergic reactions[21,27]; therefore, the precise diagnosis is crucial. The correct diagnosis of PEG HSR is important due to the widespread use of this molecule which behaves as a 'hidden' allergen.[28] Positive STs (SPTs and IDTs) and BAT to PEG has been reported in small series and cases reports, raising the possibility of IgE-mediated type HSRs.[10,29] This study supports BAT to PEG as a valuable tool to exclude the diagnosis of PEG allergy.

To our knowledge, there are no cases reported with positive SPTs to the BNT162b2 vaccine.[30] Therefore, the last EAACI position

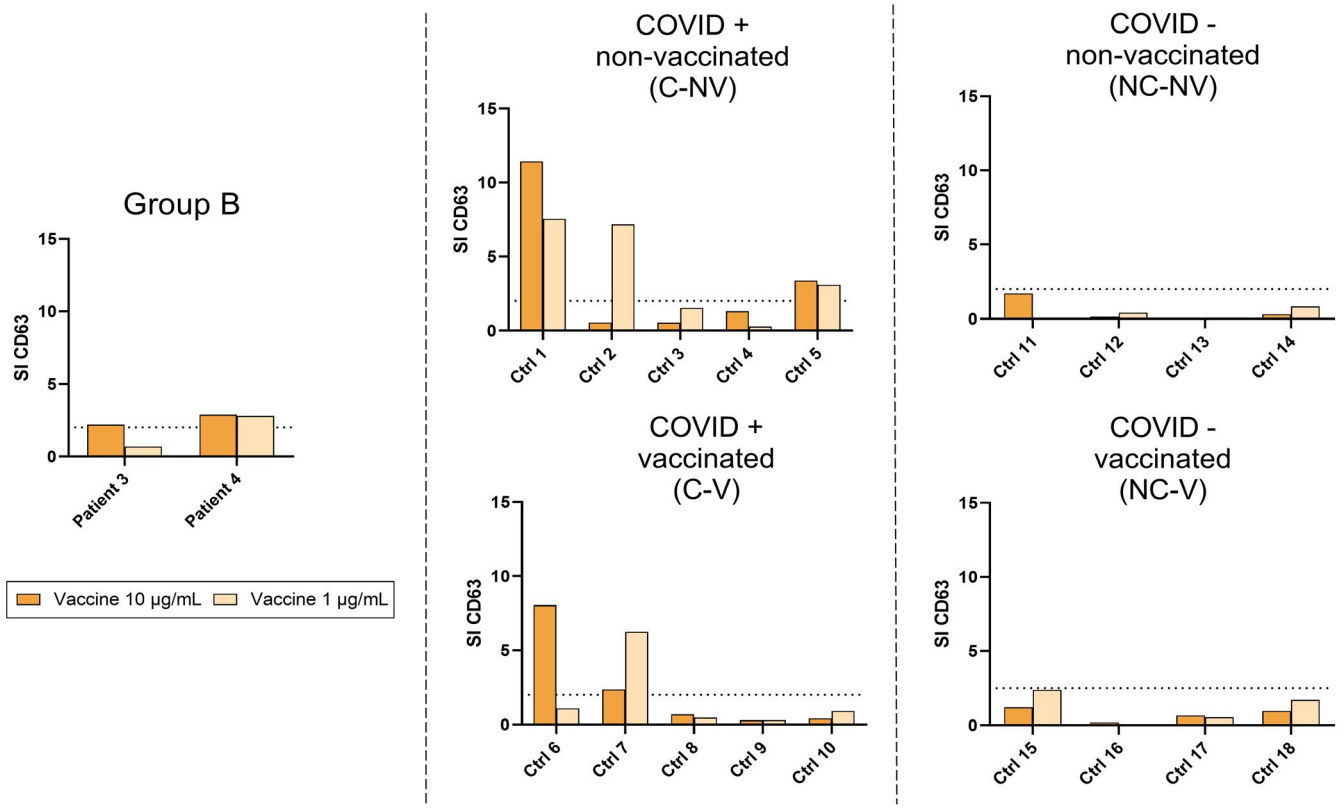


FIGURE 2 Effect of vaccine at 10 and 1 µg/ml on stimulation index (SI) for activation marker CD 63 from patients and different controls groups. Group B: STs and or BAT positive to the vaccine. Group C-NV: Controls COVID-19 recovered, not vaccinated. Group C-V: Controls COVID-19 recovered, vaccinated with BNT162b2 without allergic reactions. Group NC-NV: Control not suffered from COVID-19 and not vaccinated. Group NC-V: Control not suffered from COVID-19 and vaccinated with BNT162b2 without allergic reaction

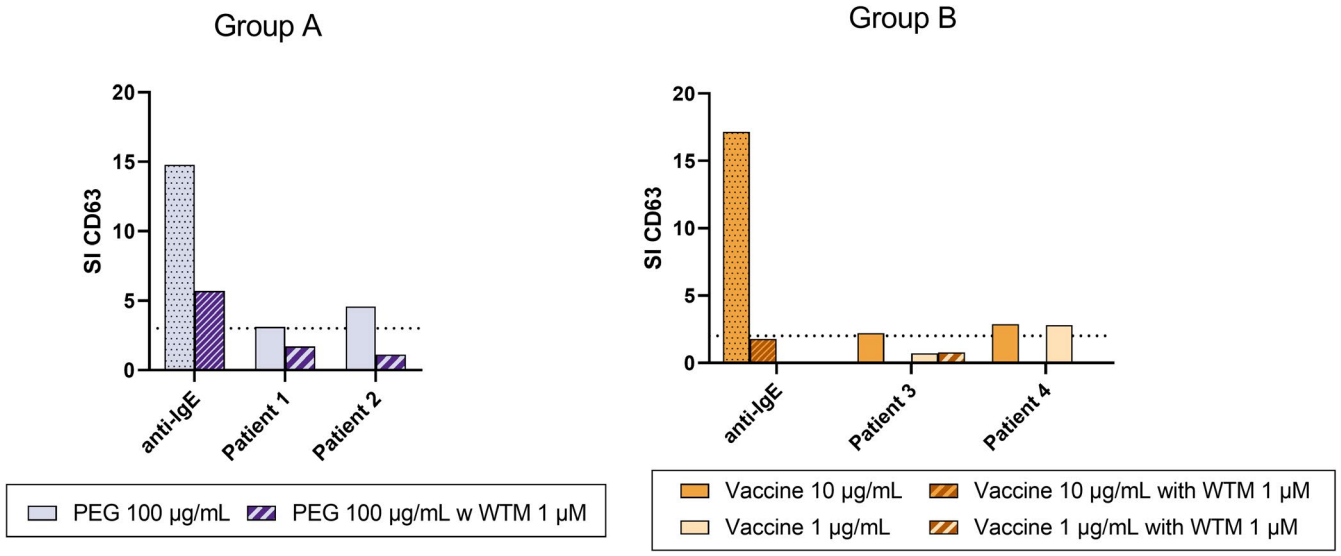


FIGURE 3 Effect of PEG at 100 µg/ml and vaccine at 10 and 1 µg/ml and effect of combination for each condition with wortmannin at 1 µM on stimulation index (SI) for activation marker CD 63 for group A and B of patients. Group A: STs and or BAT positive to PEG. Group B: BAT positive to the vaccine

paper[6] recommends as a matter of urgency the evaluation of the utility of the BAT for the management of suspected HSRs to the BNT162b2 vaccine. It has been reported that BAT may be useful for the assessment of COVID-19 vaccines.[31] However, in our study, BAT with the vaccine did not prove a useful test for differentiating patients with suspected allergic reactions to the vaccine from those who

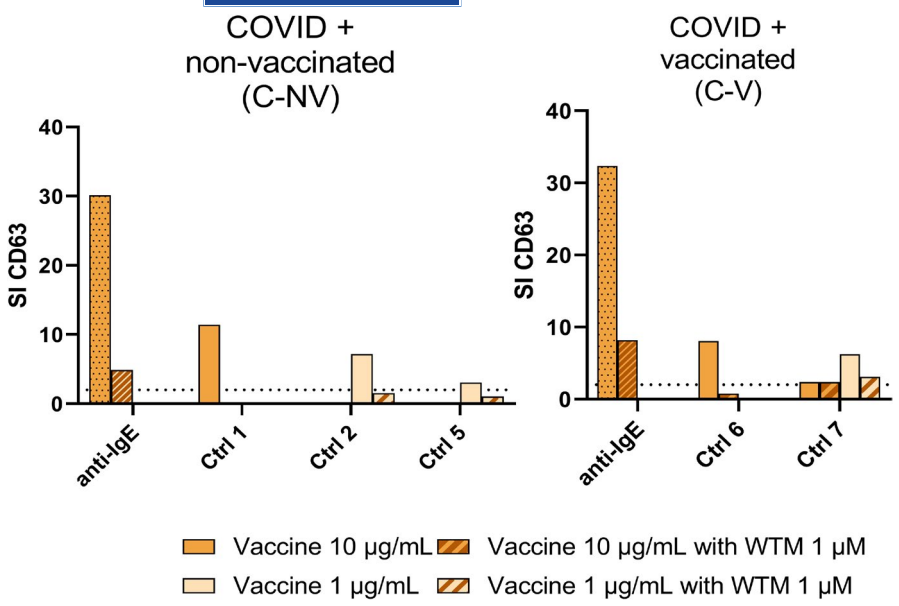


FIGURE 4 Effect of vaccine at 10 and 1 µg/ml and effect of combination for each condition with wortmannin at 1 µM on stimulation index (SI) for activation marker CD 63 for both controls group COVID-19 recovered. Group C-NV: Controls COVID-19 recovered, not vaccinated. Group C-V: Controls COVID-19 recovered, vaccinated with BNT162b2

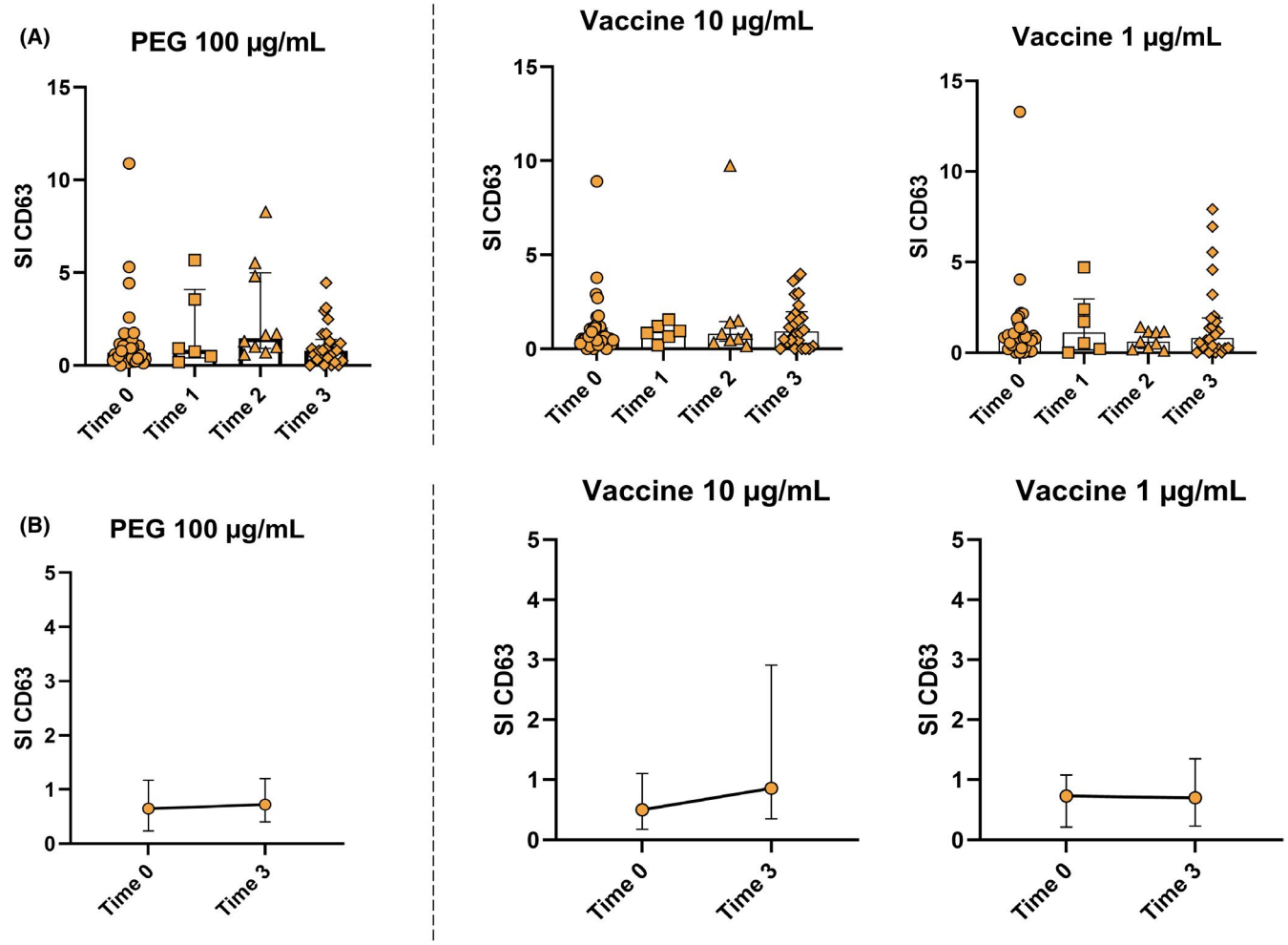


FIGURE 5 (A) Effect of PEG at 100 µg/ml and vaccine at 10 and 1 µg/ml at different times after the first and the second dose vaccine administration on stimulation index (SI) for activation marker CD 63. (B) Effect of PEG at 100 µg/ml and vaccine at 10 and 1 µg/ml on stimulation index (SI) for activation marker CD 63 from the same patients at two different times after the first and the second dose vaccine administration

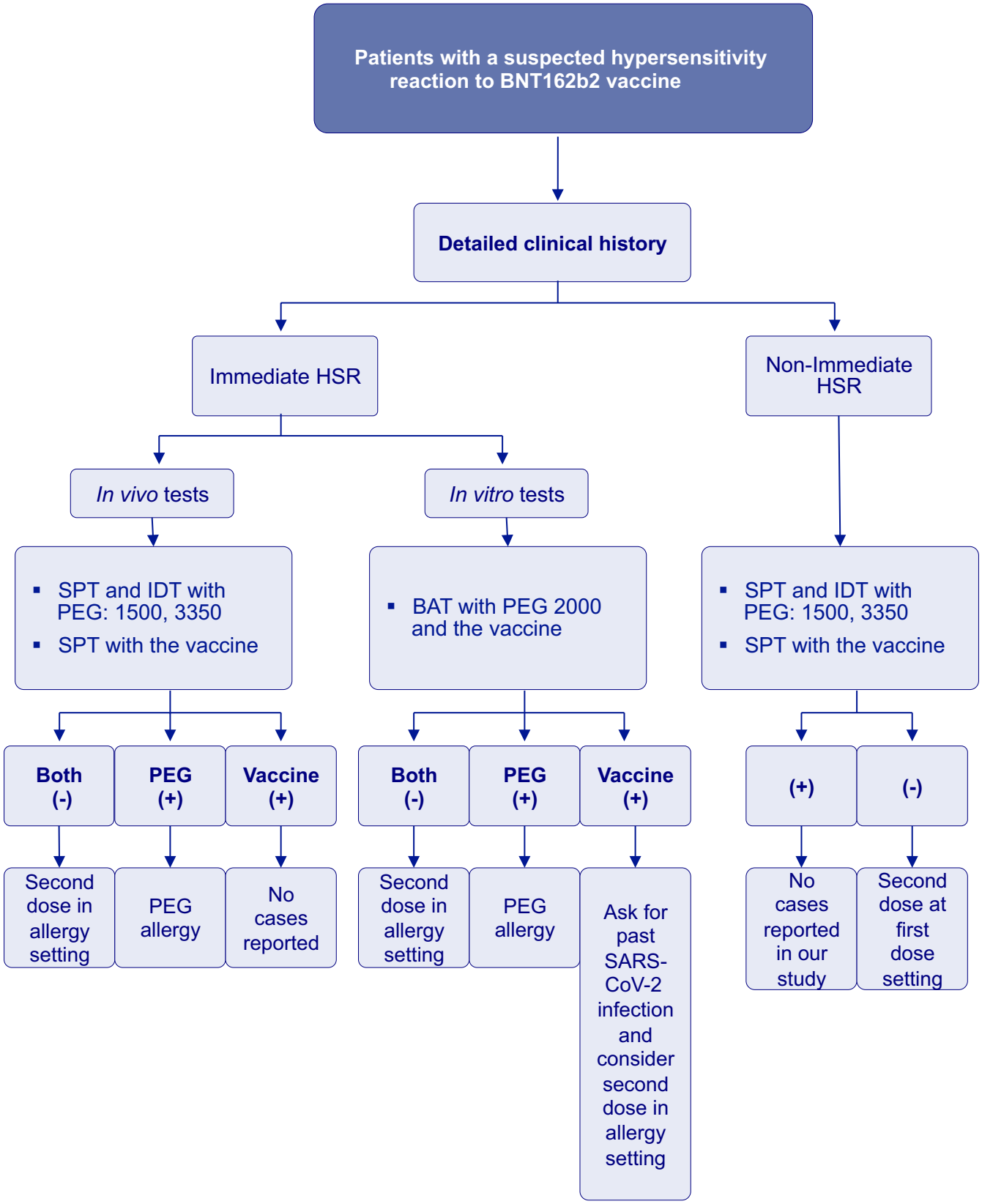


FIGURE 6 Proposed algorithm for the management of patients with reactions to the BNT162b2 vaccine

tolerate it. Furthermore, in order to assess whether the activation of basophils induced by the vaccine, which is a compound intended to produce an immunological response, is specific, we included different controls. In this regard, we observed that BAT with the vaccine was positive in 50% of cases recovered from COVID-19 and none of the non-infected cases. Therefore, BAT positivity to the vaccine is likely

indicative of SARS-COV-2 infection rather than to vaccine sensitization. Moreover, analysing the BAT results with the vaccine in controls performed during the follow-up period, we found that the vaccination status did not influence the basophil reactivity.[32]

In our study, we did not find any case with positive SPTs to vaccine. Therefore, in those patients reporting suspected HSR to the vaccine in which we have ruled out allergy to PEG by STs and BAT, the administration of the second dose of the vaccine under medical supervision and using is the only method to confirm or exclude the diagnosis of HSR.[33,34] Nevertheless, this procedure is not risk-free. In cases with moderate/severe and suggestive allergic reactions with a negative allergy assessment, the drug administration should be done only under allergist supervision and in fractionated doses in order to achieve a complete and efficient immunization. Although patients were receiving the BNT162b2 vaccine after the first dose of ChAdOx1-S (Vaxzevria, AstraZeneca, Oxford, UK) vaccine developed a robust immune response, with an acceptable and manageable reactogenicity profile,[35,36] it is currently unknown whether a first BNT162b2 dose followed by a different vaccine dose provides efficient protection from SARs-COV-2 infection.

Based on the data obtained from our study, we propose an algorithm for the diagnostic approach of suspected allergic reaction to the COVID-19 BNT162b2 vaccine, as described in (Figure 6).

In conclusion, HSRs due to BNT162b2 vaccines are very rare, and their over-diagnosis must be avoided to ensure a complete and efficient vaccination. Therefore, the allergological workup of the patients reporting reactions after the vaccine administration is crucial to achieve a precise diagnosis. BAT is a promising tool for confirming the diagnosis of HSRs to excipient PEG. However, BAT has shown not to be helpful to determine an allergy to the vaccine, as a positive result in BAT probably indicates a past SARS-COV-2 infection rather than vaccine sensitization. The administration of the second dose of BNT162b2 under strict clinical supervision and in incremental doses is recommended in patients with suspected HSRs when PEG allergy has been previously excluded.

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CONFLICT OF INTEREST

No author has any conflicts of interest to disclose.

ORCID

Marina Labella https://orcid.org/0000-0001-9618-4067
Inmaculada Doña https://orcid.org/0000-0002-5309-4878
Mohamed H. Shamji https://orcid.org/0000-0003-3425-3463
Ioana Agache https://orcid.org/0000-0001-7994-364X
Cristobalina Mayorga https://orcid.org/0000-0001-8852-8077
Maria José Torres https://orcid.org/0000-0001-5228-471X

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SUPPORTING INFORMATION

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