Optimizing investigation of suspected allergy to polyethylene glycols

Maria Anna Bruusgaard-Mouritsen, MD,[a,b] Bettina Margrethe Jensen, MSc, PhD,[b] Lars K. Poulsen, PhD, DMSc,[b] Jeanne Duus Johansen, MD, DMSc,[a,b] and Lene Heise Garvey, MD, PhD[b,c] *Copenhagen, Denmark*

Background: Polyethylene glycols (PEGs) are polymers of varying molecular weight (MW) used widely as excipients in drugs and other products, including the mRNA vaccines against coronavirus disease 2019. Allergy to PEGs is rare. Skin testing and graded challenge carries a high risk of inducing systemic reactions.
Objective: We evaluated skin prick test (SPT) results and *in vitro* reactivity over time to different MW PEGs and assessed cross-sensitization patterns in PEG allergy.
Methods: Ten patients with previously diagnosed PEG allergy underwent SPT twice with PEGs 26 months apart. Lower MW (PEG 300, 3000, 6000) were tested, followed by PEG 20,000, in stepwise, increasing concentrations. Cross-sensitization to polysorbate 80 and poloxamer 407 was assessed. SPT was performed in 16 healthy controls. *In vitro* basophil histamine release (HR) test and passive sensitization HR test were performed in patients and controls.
Results: Patients previously testing positive on SPT to PEG 3000 and/or 6000 also tested positive to PEG 20,000. Patients with a longer interval since diagnosis tested negative to lower MW PEGs and positive mainly to higher concentrations of PEG 20,000. Three patients developed systemic urticaria during SPT. Eight patients showed cross-sensitization to poloxamer 407 and 3 to polysorbate 80. All controls tested negative. *In vitro* tests showed limited usefulness.
Conclusions: Skin test reactivity to PEG can decrease over time, but titrated SPT with increasing concentrations of PEG 20,000 can be diagnostic when lower MW PEGs test negative. To avoid systemic reactions, stepwise SPT is mandatory. (J Allergy Clin Immunol 2022;149:168-75.)

Key words: *Drug allergy, anaphylaxis, polyethylene glycol, PEG, macrogol, skin prick test, basophil histamine release, COVID-19 vaccine*

Abbreviations used
COVID-19: Coronavirus disease 2019
HR: Histamine release
MW: Molecular weight
PEG: Polyethylene glycol
PMA: Phorbol 12-myristate 13-acetate
PS: Passive sensitization
SPT: Skin prick test

Polyethylene glycols (PEGs) or macrogols are hydrophilic polymers of varying molecular weight (MW) used as excipients in many different products, including drugs and cosmetics.[1] PEGs have recently gained renewed interest because PEG 2000 is an excipient in the BioNTech/Pfizer and Moderna mRNA vaccines against coronavirus disease 2019 (COVID-19). Two cases of anaphylaxis in the first days of vaccination in the United Kingdom directed the suspicion against PEGs.[2] Allergy to PEGs is rare, but an increasing number of patients have been diagnosed over the past 2 decades.[1] A review by Wenande and Garvey[1] identified 37 case reports of PEG allergy between 1977 and 2016. In the United States, there are approximately 4 PEG-associated cases of anaphylaxis caused by laxatives per year, and the US Food and Drug Administration has registered 133 reports associating PEG with anaphylaxis since 1989.[3] The true prevalence of PEG allergy is unknown but is suspected to be significantly underreported, and a rise in the incidence of PEG allergy is expected as a result of the continued extensive use and increased focus on this hidden allergen.[1,3,4]

PEGs are synthesized by polymerization of ethylene oxide and addition of water, and they vary in MW and chain length. Low MW PEGs are viscous, clear liquids, while high MW PEGs are waxy, white solids.[1,5] There is potential for cross-sensitization to structurally related derivatives sharing the same chemical groups as PEG.[3,5-8] Other excipients, such as polysorbates derived from pegylated sorbitan or poloxamers comprising a trimer consisting of 1 moiety of polypropylene glycol surrounded by 2 moieties of PEGs, have been reported to show cross-sensitization (Fig 1).[1,9] Cross-sensitization is likely underestimated and rarely investigated; its clinical significance is not clear.

Diagnosing patients with PEG allergy is challenging. They often present with repeated, severe allergic reactions/anaphylaxis to structurally different drugs/products, and PEGs are rarely suspected. When suspected, performing a skin prick test (SPT) with a panel of different MW PEGs is the recommended investigation, although systemic reactions have been reported on SPT.[1,10] Intradermal testing and graded challenge with PEG-containing products should only be performed with caution as a result of the relatively

From [a]the National Allergy Research Centre and [b]Allergy Clinic, Copenhagen University Hospital at Gentofte, and [c]the Department of Clinical Medicine, University of Copenhagen.

This work was supported by grants from the Danish Environmental Protection Agency to the National Allergy Research Centre and Kongelig Hofbundtmager Aage Bangs Fond.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication January 4, 2021; revised May 7, 2021; accepted for publication May 19, 2021.

Available online May 28, 2021.

Corresponding author: Lene Heise Garvey, Allergy Clinic, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen, Gentofte Hospitalsvej 8, 1st floor, 2900 Hellerup, Denmark. E-mail: lene.heise.garvey@regionh.dk.

0091-6749

https://doi.org/10.1016/j.jaci.2021.05.020

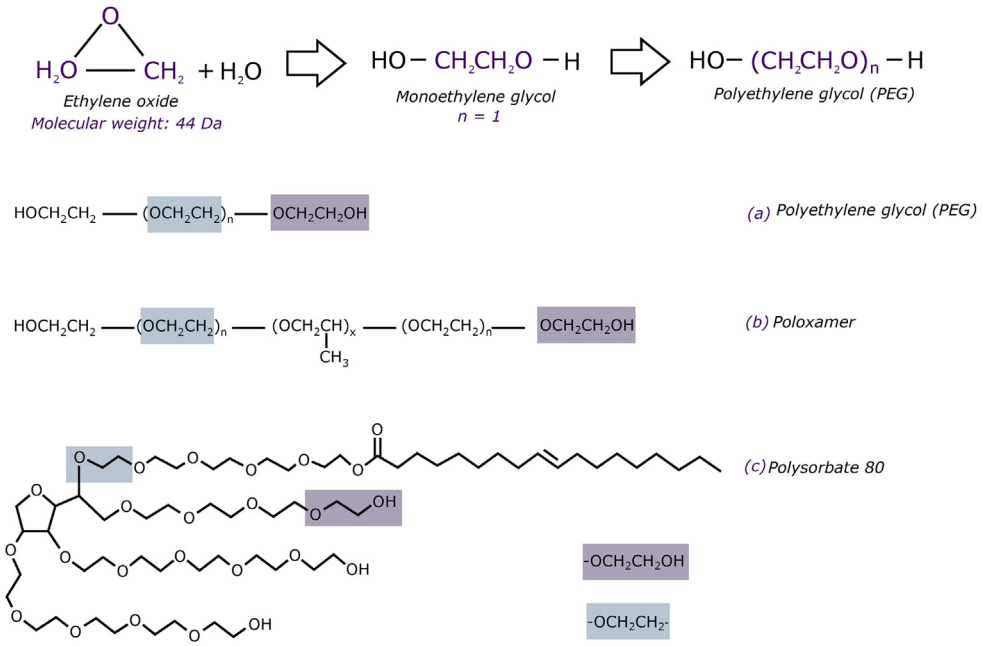

FIG 1. Molecular structure and polymerization of PEG and the derivatives poloxamer and polysorbate 80, which share 2 chemical moieties, $–(OCH_2CH_2)–$ and $–OCH_2CH_2O$. Image reproduced with permission from *Clinical & Experimental Allergy* from Wenande and Garvey.[1]

high risk of inducing anaphylaxis.[1,3,7,10-15] Skin test reactivity may decrease over time, showing negative results on titrated SPT with recommended low MW (PEG 300, 3000, 6000) despite a strong clinical suspicion, putting patients at risk of inadvertent reexposure if the diagnosis is not confirmed.

To date, there is limited knowledge about skin test reactivity over time, cross-sensitization to structurally related polymers, and supplemental diagnostic tests. In this study, therefore, we evaluated skin test reactivity over time and cross-sensitization patterns in 10 patients with confirmed allergy to PEG. We investigated whether titrated SPT with increasing concentrations of a PEG 20,000 MW can increase diagnostic sensitivity of SPT in PEG allergy. Further, because a reliable *in vitro* test would minimize the risk to patients, we assessed the basophil histamine release test with and without passive sensitization (PS). We present an investigation algorithm that is based on the study findings.

METHODS

Study design

The study included SPT results and histamine release test results from the time of diagnosis and initial allergy assessment. In addition, prospective testing was performed with a PEG SPT series developed for the study, as well as blood samples at 2 different time points 26 months apart. Blood samples were analyzed with histamine release test with and without PS.

Written and oral informed consent was obtained from all patients as well as participants in the control group. The study was approved by the regional ethical committee (file H-17021145).

Patients

Twelve patients were diagnosed with PEG allergy at the Allergy Clinic at Gentofte Hospital from September 2010 to August 2019. The diagnosis was made at the initial assessment by a history of 1 or more allergic reactions to PEG-containing products combined with a positive SPT to 1 or more low MW PEGs.

In the current study, we included 10 patients diagnosed with PEG allergy aged ≥18 years at the time of inclusion in the study. One patient had died, and another declined participation.

We included 8 patients diagnosed until 2017, who consented to participate twice, with a second visit 2 years later in 2019. One patient later declined the second visit. We consecutively invited patients newly diagnosed with PEG allergy between 2017 and 2019. Two patients were included after 2017, and these only participated once in the study.

Control group

The control group comprised 16 healthy, nonallergic individuals matched for age and sex who had a blood sample drawn and were tested once with the study PEG SPT series.

Skin prick testing

PEGs and derivatives were prepared in sterile water at the Laboratory of Medical Allergology, Gentofte Hospital, Hellerup, Denmark (see Table E1 in this article's Online Repository at www.jacionline.org). SPT was performed stepwise one concentration at a time with 20 minutes' observation between each step. The PEG SPT series developed for the study comprised the following: lower MW PEGs: PEG 300 (100%), PEG 3000 (50% wt/vol), PEG 6000 (50% wt/vol), polysorbate 80 (20% wt/vol), and poloxamer 407 (10% wt/vol). These were tested first in stepwise fashion. If only local reactions occurred on testing, SPT was performed stepwise with PEG 20,000 in concentrations of 0.01%, 0.1%, 1%, 10%, and 20% (wt/vol) until a positive response was reached. Three patients with very strong local responses or systemic urticaria to lower MW PEG were not tested with PEG 20,000 for ethical and safety reasons. SPT was performed on the forearm with a positive control with histamine 10 mg/mL and a negative control with saline. Duplicate testing was performed if the test was negative. The control subjects were tested with all components in duplicate. A positive reaction was defined as a wheal diameter of ≥3 mm.

Blood sampling and histamine release tests

Prospective blood samples were drawn before SPT at both visits. Histamine release test were performed on the day of blood sampling on 10 PEG-allergic patients and 16 healthy controls using the method previously described by Larsen et al.[16] PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, polysorbate 80 (Sigma-Aldrich, St Louis, Mo), anti-IgE (KPL, Gaithersburg, Md), and phorbol 12-myristate 13-acetate (PMA) + ionomycin (both from Sigma-Aldrich) were all tested in 6 concentrations. The percent HR (%HR) equals released histamine of stimuli divided by maximum histamine release induced by PMA + ionomycin stimulation. Histamine release >10% was considered positive if found in 2 consecutive concentrations. If anti-IgE response was <10%, the test was considered inconclusive (see this article's Methods section in the Online Repository at www.jacionline.org).

RESULTS

Six men and 4 women participated in the study. The median age was 35 years (range, 18-64 years). Three patients had a history of allergic rhinoconjunctivitis; none had a history of reactions to food, PEG-free drugs, venoms, or vaccinations. None of the patients had received a vaccination containing polysorbate 20, polysorbate 80, or PEG since diagnosis.

For all patients, median time from first reaction to PEG until diagnosis was 20 months (range, 2-120 months). Median time from diagnosis to first study visit was 30 months (range, 1-86 months).

The most common PEG exposures were oral medications, such as analgesic tablets, antacids, antibiotic tablets and laxatives, and depot steroid injections. Table I lists the culprit agents. The mean number of reactions before diagnosis was 3 (range, 2-6). Eight patients had at least 1 reaction fulfilling the criteria for anaphylaxis and requiring epinephrine. Clinical patient data are reported in detail elsewhere.[17]

Skin prick tests

In 9 patients, the diagnosis was made at initial allergy assessment by a positive SPT to PEG 3000 and/or PEG 6000 (Table I). Patient 4, who experienced cardiac arrest after insertion of poloxamer 407–containing bone cement during hand surgery, only tested positive on SPT with the PEG derivatives poloxamer 407 and polysorbate 80 at initial allergy assessment 1 month after his reaction. He later tested positive to PEGs of varying MW at the first study visit three and a half years later.

All patients previously testing positive to PEG 3000 and PEG 6000, and who lost reactivity to these concentrations over time, still tested positive on PEG 20,000. Patients with a longer interval since diagnosis (patients 1, 2, 5, and 8) tested negative to lower MW and positive only to the higher concentrations of PEG 20,000 (Table I). Three patients (patients 6, 7, and 10) were not tested with PEG 20,000 in the study because they developed systemic urticaria during SPT with lower MW PEG. Patient 6 had tested positive for PEG 20,000 0.01% at the time of diagnosis. Symptoms were treated successfully with PEG-free oral antihistamines in all 3 patients.

All 16 participants in the control group tested negative in all SPT concentrations in duplicate.

Changes in SPT reactivity to PEG over time. In 7 patients, reactivity decreased over time, with loss of reactivity to the lower MWs. Decreased reactivity median time was 41 months (range, 26-82 months). In patients 3 and 9, reactivity increased over time after 26 and 16 months, respectively. In patient 10, who was newly diagnosed, reactivity did not change over 9 months.

Cross-sensitization. Eight patients showed cross-sensitization to PEG derivatives (all 8 to poloxamer 407 and 3 to polysorbate 80) at some point between diagnosis and last study visit. Patients 1 and 2, who had the longest delays since diagnosis (7 and 4 years, respectively), tested negative to both derivatives during the study. Neither had been tested with poloxamer 407 and polysorbate 80 before, as we had not been aware of the potential for cross-sensitization at the time of their diagnosis.

Histamine release test

Blood samples from patients or healthy controls were investigated for basophil reactivity when stimulated with PEGs. Four patients had a HR test performed at the time of diagnosis, and 2 patients (patients 1 and 2) tested positive to relevant MW PEGs, while 2 tested negative. Patients 1 and 2, who had tested positive in HR test at diagnosis but who tested negative at the first study visit, remained positive on SPT.

At the first study visit, 2 of 10 patients tested positive. Patient 7 tested positive for a relevant MW PEG, while patient 4 only showed partial concordance between SPT and HR test (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org). Four patients (patients 3, 5, 6, and 10) had inconclusive tests, most likely due to nonreleasing basophils, a phenomenon found in 10% to 20% of the general population where basophils are found to be unresponsive (ie, histamine is not released) when using IgE-dependent stimuli.[18] Three of these patients (patients 3, 6, and 10) had systemic urticarial reactions during SPT in this study. Four patients had a negative HR test to PEG and PEG derivatives despite having a positive skin test. HR test was not performed at the second study visit.

PS histamine release test

To circumvent the problem of nonreleasing basophils, the technique of PS was used, where blood from blood donors with releasing basophils was passively sensitized with IgE from patients or healthy control serum. PS HR was performed at both study visits. Only 1 patient (patient 1) showed a positive response of doubtful clinical relevance. Six patients had negative results and 3 patients had inconclusive results on samples from the first study visit. Seven patients who had a blood sample analyzed at the second visit all tested negative (Table E2).

HR and PS HR were negative in all tests in all 16 controls.

DISCUSSION

In this study of 10 patients with PEG allergy, which is to our knowledge the largest cohort of PEG-allergic patients reported to date, we found that SPT reactivity to PEGs may decrease over time, but that the diagnosis can still be made by SPT with higher MW PEGs. All patients who had lost skin test reactivity over time to low MW PEG (PEG 3000 and/or PEG 6000) tested positive to PEG 20,000 in varying concentrations. In 7 patients, reactivity decreased over time with loss of reactivity to a lower MW PEG, while reactivity increased over time in 2 patients and remained stable in 1 patient. The 2 patients (patients 1 and 2) with the

TABLE I. SPT results over time in 10 patients with confirmed PEG allergy

Patient no., year of diagnosis	Age, sex	Culprit agents	Latest reaction to diagnosis (months)	SPT at diagnosis (SPT 0)	SPT 0 to SPT A (months)	SPT at first study visit (SPT A) 2017*	SPT at second study visit (SPT B) 2019
1, 2010	28, F	Intramuscular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Balancid Novum (magnesium hydroxide) reflux tablet (PEG 6000) Effexor (venlafaxine) antidepressant tablet (PEG 400)	7	PEG 300 **PEG 3350 (+)** **PEG 6000 (+)** Poloxamer and polysorbate not tested	82	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	Declined
2, 2014	63, F	Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350)	2	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer and polysorbate not tested	46	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **10% (+)** Poloxamer 407 Polysorbate 80
3, 2014	37, M	Vepicombin (phenoxymethylpenicillin) antibiotic tablet (PEG 6000) Vepicombin (phenoxymethylpenicillin) antibiotic tablet during drug provocation (PEG 6000) Burana (ibuprofen) tablet (PEG 6000) Mucoangin (ambroxol) throat lozenge (PEG 6000) Xerodent (sodium fluoride) oral tablet (PEG 6000) Balancid Novum (magnesium hydroxide) reflux tablet (PEG 6000) Sensodyne Dental floss (PEG 6000)	4	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** **0.01% (+)** Poloxamer 407 **(+)** Polysorbate 80	45	PEG 300 PEG 3000 **PEG 6000** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** Polysorbate 80
4, 2014	33, M	Accell Connexus DBM putty (poloxamer 407) during hand surgery	1	PEG 300 PEG 3000 **PEG 6000** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	41	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 Polysorbate 80
5, 2014	53, M	Unidentified perioperative exposure during coronary stent insertion	5	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 **(+)** Polysorbate 80	39	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **1% (+)** Poloxamer 407 Polysorbate 80
6, 2016	30, M	Migea (telfenamic acid) tablet (PEG 6000) Burana (ibuprofen) tablet (PEG 6000) Panodil (paracetamol) Zapp tablet (PEG 6000)	5	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** **0.01% (+)** Poloxamer 407 **(+)** Polysorbate 80	20	PEG 300 **PEG 3000 (+)** Polysorbate 80 *Systemic urticaria*	PEG 300 PEG 3000 **PEG 6000 (+)** Polysorbate 80 *Systemic urticaria*

(Continued)

TABLE I. *(Continued)*

Patient no., year of diagnosis	Age, sex	Culprit agents	Latest reaction to diagnosis (months)	SPT at diagnosis (SPT 0)	SPT 0 to SPT A (months)	SPT at first study visit (SPT A) 2017*	SPT at second study visit (SPT B) 2019
7, 2017	37, M	Vepicombin (phenoxymethylpenicillin) tablet (PEG 6000) Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350)	3	**PEG 300 (+)** **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	1	**PEG 300 (+)** **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)** *Systemic urticaria*
8, 2017	22, F	Vepicombin Novum (phenoxymethylpenicillin) tablet (PEG 6000) Balancid Novum (magnesium hydroxide) tablet (PEG 6000) Movicol (macrogol) laxative (PEG 3350) Panodil (paracetamol) tablet (PEG and polysorbate 80)	1	PEG 300 PEG 3000 **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	1	PEG 300 PEG 3000 **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **1% (+)** Poloxamer 407 Polysorbate 80
9, 2018	16, M	Movicol (macrogol) laxative (PEG 3350) Dulcosoft (macrogol) laxative (PEG 4000) Diprospan (betamethasone) intra-articular injection (PEG 3350)	27	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	16	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	Newly diagnosed, second study visit not possible
10, 2019	33, F	Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Sanex body lotion (PEG 4400)	5	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	8	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)** *Systemic urticaria*	Newly diagnosed, second study visit not possible

Positive (+) SPT results are indicated in boldface.
*Patient 9 in 2018 and patient 10 in 2019.

longest time interval (7 and 4 years, respectively) since diagnosis lost reactivity to the lower MW PEGs and tested positive only to the higher concentrations (10-20%) of PEG 20,000. Two patients (patients 5 and 8) with shorter time intervals (2 to 3 years) since diagnosis had also lost reactivity to lower MW PEGs but tested positive to the lower concentrations (0.1-1%) of PEG 20,000. This indicates that SPT with increasing concentrations of a PEG 20,000 can be used to increase diagnostic sensitivity of SPT even if there is a long delay between clinical reaction and allergy assessment. Despite careful stepwise SPT with increasing concentrations, 3 patients developed systemic urticaria during testing, even with lower MW PEG (PEG 3000), thus confirming that SPT with PEGs can be hazardous to patients with a history of severe allergic reactions if not performed with stepwise increasing concentrations.[1,10]

Severe systemic reactions on intradermal and provocation testing with PEGs have been repeatedly reported, so avoiding these test modalities would be a safer option.[1,3,7,10-14] On the basis of the results of our study, we suggest an investigation algorithm that is based on a titrated stepwise approach of SPT only (Fig 2). If results of SPT with lower MW PEGs are negative and clinical suspicion of PEG allergy is strong, we suggest to test PEG 20,000 in increasing concentrations using a stepwise approach. We believe that this approach will minimize the need for more hazardous test modalities such as intradermal test and graded challenge, which we only recommend if clinical suspicion is strong and the full algorithm has shown negative results. In patients with a low pretest probability of PEG allergy, such as patients with a history of reactions to several drugs not consistently containing PEGs, we only perform SPT with low MW PEGs, poloxamer 407, and polysorbate 80 without the stepwise approach. In our study, 5 patients tested positive only to PEG 20,000 at the last study visit, although they had previously tested positive to lower MW PEGs. If these patients had been referred with a long delay since their initial reaction, SPT with lower MW PEGs could have turned out falsely negative, and intradermal test or graded challenge might have been performed, putting the patients at risk of experiencing systemic reactions on testing.

Because many health care professionals are unfamiliar with the clinical presentation of PEG allergy, we provide in Table II a list of clinical scenarios where allergy to PEGs should be suspected. Because the mRNA COVID-19 vaccines contain PEG 2000, it is important that patients with suspected allergy to PEGs are investigated before vaccination, and we have included PEG 2000 in the new algorithm for this reason.

Another important finding of this study is that if lower MW SPT tested positive, PEG 20,000 would also test positive, suggesting that allergenicity increases with increasing MW[10]

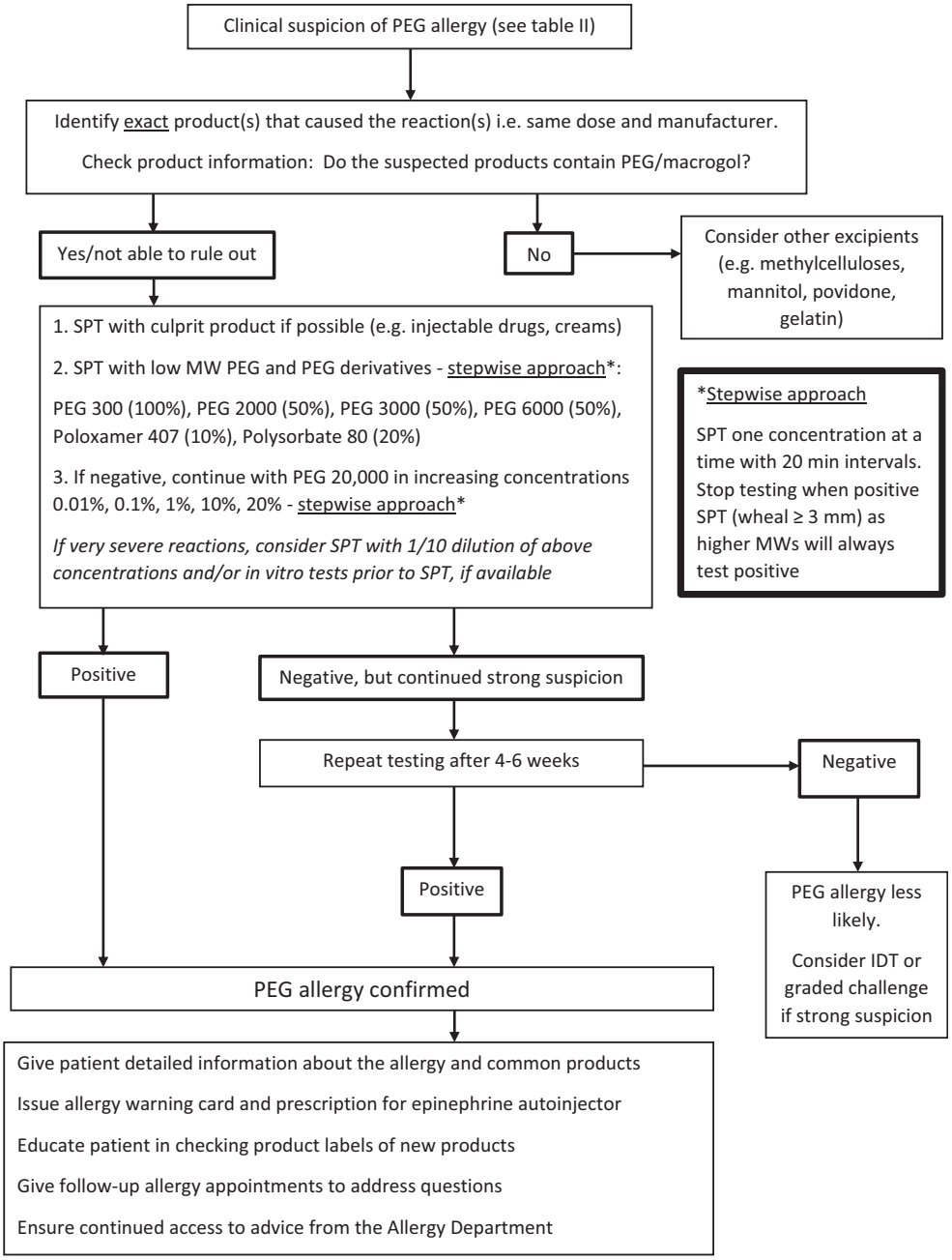

FIG 2. Investigation algorithm for patients with suspected PEG allergy. A stepwise approach should always be used in patients with severe reactions and strong suspicion of PEG allergy. In patients with milder reactions and weak suspicion of PEG allergy, several tests can be performed simultaneously after individual risk evaluation.

TABLE II. Clinical history where allergy to PEG should be considered[17]

- Repeated, severe allergic reactions/anaphylaxis to ≥2 structurally different drugs/products (eg, tablets, depot injections, antacids, PEG-based laxatives).
- Severe allergic reactions to only some formulations, or doses, of same generic drug.
- Severe allergic reaction to drugs, where allergy to the active ingredient has been excluded on testing (eg, antibiotics, analgesics).
- Severe allergic reaction to drugs containing PEG or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reaction to vaccines containing PEG (mRNA vaccines) or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reaction to PEGylated drugs, where allergy to the active drug is excluded.
- Severe unexplained allergic reactions in connection with surgery or invasive procedures.

and that there is no upper threshold for positivity.[1] This means that if SPT is positive to PEG 3000, further testing with higher MW PEGs is not necessary and may put the patient at risk of a systemic reaction. We have included this important information in the algorithm. Whether the threshold on SPT translates into a threshold for clinical reactivity has not been confirmed. Other groups have suggested that a lower threshold can be identified and that patients can use products with PEGs of MWs testing negative on SPT or challenge.[3,10] In our center, however, we adopt the more cautious approach of warning against PEGs of all MWs even if lower MWs test negative. This is supported by the results of the current study, where 2 patients showed an increase in reactivity by testing positive on lower MW at the second study visit. This may be explained by unknown accidental re-exposure to PEG. There is a high risk of accidental re-exposure, as PEGs are widely used in daily life and in the health care setting. It is possible that minor asymptomatic exposure—for example, from soaps, creams, cosmetics, or tablet coatings containing PEG—can be enough to maintain or increase allergenic reactivity. Finally, a lack of standardized labeling and the possibility of admixture with other MW PEGs means that the MW stated on drugs and other products cannot always be trusted.[1,19]

Recommendations for investigation of patients with suspected hypersensitivity to PEG are generally based on experiences from very few patients, making it difficult to assess specificity and sensitivity of individual tests. However, on the basis of negative SPT results for all MWs and concentrations in 16 healthy controls in this study, as well as negative SPT results with PEG 300, PEG 3000, PEG 6000, polysorbate 80, and poloxamer 407 in 314 non–PEG-allergic patients investigated as part of routine allergy assessment in our clinic during 2012-19, the specificity of our SPT series with PEG is likely to be high.

Although SPT is the recommended investigation when diagnosing patients with PEG allergy, even this procedure, generally considered very safe for most other allergens, may lead to systemic allergic reactions if performed with too high concentrations in highly reactive patients, such as those with severe reactions or when testing takes place soon after the allergic reaction. In our study, 3 patients developed systemic urticaria during SPT but responded quickly to treatment with oral antihistamines not containing PEG. This emphasizes the need for a stepwise approach. Indeed, using this protocol, we have never induced anaphylaxis on SPT. We suggest that a 1/10 dilution of our recommended concentrations may be used initially in patients with a strong suspicion of PEG allergy and/or severe or recent reactions. Because of the risk of systemic reactions, testing should always be performed in a specialized setting with equipment and expertise in treating immediate-type allergic reactions. It should be ensured that antihistamine tablets without PEG are available for treating early symptoms.[10] On the Danish market, presently only a single oral antihistamine is PEG-free.

Cross-sensitization between PEGs and structurally related polymers have only been rarely investigated.[1,3] Eight patients showed cross-sensitization to PEG derivatives in our study, all 8 to poloxamer 407 and 3 to polysorbate 80. Patient 4 is the only patient in this study with a history of a clinical reaction to poloxamer 407. Another patient from our clinic diagnosed with PEG allergy, who had died before this study, did have a clinical reaction to polysorbate 80.[7]

Some patients showed a decrease in skin test reactivity to these other polymers over time, while others maintained their reactivity. The clinical relevance of cross-sensitization is unknown and urgently needs elucidating because polysorbate 80 is used in many drugs[3] and vaccines, including some of the upcoming vaccines against COVID-19.

To our knowledge, the skin test reactivity over time in patients with PEG allergy has not been previously investigated. It is not known whether allergenic reactivity remains dormant until reactivated by re-exposure or if it can disappear permanently. In this study, some patients (patients 2, 4, and 5) only had a single reaction, and they seemed to be less reactive on SPT.[17] It may be that they can tolerate limited exposure to PEG. Others have had repeated severe reactions with years in between and may never lose their reactivity. Although there may be individuals who truly lose sensitization to PEG, the risk of severe reactions on re-exposure means that until more affirmative information is available to prove otherwise, in our clinic, we tell patients that PEG allergy covers all MW PEGs and is for life.

If a reliable *in vitro* test for allergy to PEG were available, the risk of inducing systemic reactions on SPT or other test modalities would be eliminated. However, such a test is presently not available. For other allergens, *in vitro* test reactivity can decrease or even be lost over time with lack of exposure; this has been shown for IgE to chlorhexidine, ethylene oxide, and penicillin.[20-23] It has been suggested that PEG allergy is caused primarily by an IgE-mediated mechanism.[4,24] An assay for detecting anti-PEG IgE has been reported to show promising results in a small cohort of patients.[25]

In this study, we investigated the direct HR test with and without PS. HR testing is used in some centers in Denmark and shows good results for allergy to things like chlorhexidine, peanut, and pollen.[16,26,27] Previously our group published promising results on HR and PS for PEG in a single patient (patient 1) on blood sampled close to the clinical reactions.[4] On testing in this study 82 months later, this patient had lost reactivity. In the current study, direct HR was only positive in 2 patients, and another Danish group showed similar results.[15] One of these patients had been diagnosed just 1 month previously and with proper titration of the PEG substances; direct HR test may have a place in patients where investigations take place within a few months of exposure. Four patients showed inconclusive results, likely due to nonreleasing basophils, a well-known limitation of this test.[18] In the present study setting, HR test with PS was negative in all patients and was not considered helpful in the diagnosis of allergy to PEG. Improved *in vitro* diagnostic tests for patients with allergy to PEG (and structurally related derivatives) therefore remains to be developed. In addition the potential for improving the sensitivity of biologic tests, a serologic assay identifying specific IgE to PEGs would be useful.[4,25] However, at the moment, no commercially validated specific IgE assay for PEGs or structurally related polymers is available.

In general, optimization of safe diagnostic tests is of great importance to PEG-allergic patients and health care personnel. However, there are still many gaps in the current knowledge. The rarity of the allergy and its unknown true prevalence make it difficult to describe the epidemiology and future prognosis. In addition, there is a potential lack of generalizability across health care systems and countries. The pathway to sensitization is unknown, and basic immunologic mechanisms remain to be identified. There is only limited experience with allergy investigation, primarily based on SPT, but data on positive and negative predictive value are sparse. Skin test reagents are not standardized, and the role of intradermal and *in vitro* testing remain to be

defined. Total avoidance of PEG causes considerable stress to patients in terms of the large number of products they need to avoid. Developing a safe method for determining a lower threshold for reactivity, thereby potentially allowing exposure to small amounts of PEG, would be helpful. Not much information is available on cross-reactivity patterns with polysorbate 80 and other polymers; this should be addressed because the BioNTech/Pfizer and Moderna mRNA COVID-19 vaccines contains PEG 2000, while most of the other available non-mRNA vaccines contain varying amounts of polysorbate 80. There is therefore an urgent need to identify a COVID-19 vaccine that can be used safely in PEG-allergic patients.

In conclusion, we have presented novel results of skin test reactivity to PEGs over time and cross-sensitization patterns in 10 patients with allergy to PEGs. On the basis of our experience as well as the results of this study, we suggest an optimized investigation algorithm for patients with suspected allergy to PEGs that is based on titrated stepwise SPT with PEGs of increasing MW, utilizing the fact that higher MW PEGs are likely to test positive even after many years. We therefore minimize the need for other tests that carry a high risk of inducing anaphylaxis. Cross-sensitization between PEGs and poloxamer 407 and polysorbate 80 is common, but the clinical implications remain unknown. Although *in vitro* tests would be the safest option for patients, we confirm the findings of others that *in vitro* testing so far has limited use in the investigation of allergy to PEGs.

Clinical implications: An algorithm using a stepwise approach of skin prick testing to polyethylene glycols (PEGs) of increasing molecular weights and concentrations can be used to diagnose allergy to PEGs.

REFERENCES

1. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. Clin Exp Allergy 2016;46:907-22.
2. Garvey LH, Nasser S. Anaphylaxis to the first COVID-19 vaccine: is polyethylene glycol (PEG) the culprit? Br J Anaesth 2021;126:e106-8.
3. Stone CA, Liu Y, Relling MV, Krantz MS, Pratt AL, Abreo A, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. J Allergy Clin Immunol Pract 2019;7:1533-40.e8.
4. Wenande EC, Skov PS, Mosbech H, Poulsen LK, Garvey LH. Inhibition of polyethylene glycol–nduced histamine release by monomeric ethylene and diethylene glycol: a case of probable polyethylene glycol allergy. J Allergy Clin Immunol 2013;131:1425-7.
5. Calogiuri G, Foti C, Bilo MB, Garvey LH. Macrogols: a misleading cause of drug hypersensitivity diagnosis. Clin Immunol Endocr Metab Drugs 2016;4(1):9-13.
6. Yamasuji Y, Higashi Y, Sakanoue M, Katsue H, Kawai K, Arai N, et al. A case of anaphylaxis caused by polyethylene glycol analogues. Contact Dermatitis 2013;69:183-5.
7. Wenande E, Kroigaard M, Mosbech H, Garvey LH. Polyethylene glycols (PEG) and related structures: overlooked allergens in the perioperative setting. A A Case Rep 2015;4:61-4.
8. Badiu I, Guida G, Heffler E, Rolla G. Multiple drug allergy due to hypersensitivity to polyethylene glycols of various molecular weights. J Investig Allergol Clin Immunol 2015;25:368-9.
9. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Vol 23, Pharmaceutical research. Amsterdam: Springer; 2006. p. 2709-28.
10. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol–induced systemic allergic reactions (anaphylaxis). J Allergy Clin Immunol Pract 2021;9:670-5.
11. Borderé A, Stockman A, Boone B, Franki AS, Coppens MJ, Lapeere H, et al. A case of anaphylaxis caused by macrogol 3350 after injection of a corticosteroid. Contact Dermatitis 2012;67:376-8.
12. Oyarzabal NA, Longo Areso N, Bernedo Belar N, Popolizio IG, Arregui AV, Balza de Vallejo OV. Anaphylactic shock due to allergy to macrogol 4000 contained in SonoVue®. Case Rep Clin Med 2017;06:143-7.
13. Antón Gironés M, Roan Roan J, de la Hoz B, Sánchez Cano M. Immediate allergic reactions by polyethylene glycol 4000: two cases. Allergol Immunopathol (Madr) 2008;36:110-2.
14. Dewachter P, Mouton-Faivre C. Anaphylaxis to macrogol 4000 after a parenteral corticoid injection. Allergy 2005;60:705-6.
15. Brandt N, Garvey LH, Bindslev-Jensen U, Kjaer HF, Bindslev-Jensen C, Mortz CG. Three cases of anaphylaxis following injection of a depot corticosteroid with evidence of IgE sensitization to macrogols rather than the active steroid. Clin Transl Allergy 2017;7:2.
16. Larsen LF, Juel-Berg N, Hansen KS, Clare Mills EN, van Ree R, Poulsen LK, et al. A comparative study on basophil activation test, histamine release assay, and passive sensitization histamine release assay in the diagnosis of peanut allergy. Allergy Eur J Allergy Clin Immunol 2018;73:137-44.
17. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. Clin Exp Allergy 2021;51:463-70.
18. Nguyen KL, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. J Allergy Clin Immunol 1990;85:1020-9.
19. Povoski SP, Davis PD, Colcher D, Martin EW Jr. Single molecular weight discrete PEG compounds: emerging roles in molecular diagnostics, imaging and therapeutics. Expert Rev Mol Diagn 2013;13:315-9.
20. Opstrup MS, Poulsen LK, Malling HJ, Jensen BM, Garvey LH. Dynamics of plasma levels of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure. Clin Exp Allergy 2016;46:1090-8.
21. Opstrup MS, Malling HJ, Krøigaard M, Mosbech H, Skov PS, Poulsen LK, et al. Standardized testing with chlorhexidine in perioperative allergy—a large single-centre evaluation. Allergy Eur J Allergy Clin Immunol 2014;69:1390-6.
22. Opstrup MS, Mosbech H, Garvey LH. Allergic sensitization to ethylene oxide in patients with suspected allergic reactions during surgery and anesthesia. J Investig Allergol Clin Immunol 2010;20:269-70.
23. Blance M, Garcia J, Vega JM, Miranda A, Carmona MJ, Mayorga C, et al. Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. Clin Exp Allergy 1996;26:335-40.
24. Bommarito L, Mietta S, Nebiolo F, Geuna M, Rolla G. Macrogol hypersensitivity in multiple drug allergy. Ann Allergy Asthma Immunol 2011;107:542-3.
25. Zhou ZH, Stone CA, Jakubovic B, Phillips EJ, Sussman G, Park J, et al. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. J Allergy Clin Immunol Pract 2021;9:1731-3.e3.
26. Garvey LH, Krøigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, et al. IgE-mediated allergy to chlorhexidine. J Allergy Clin Immunol 2007;120:409-15.
27. RefLab. Standard/non-standard materials. Available at: https://reflab.dk/allergy-testing/histamine-release-test/standard-materials/. Accessed June 26, 2020.

METHODS

Histamine release (HR) tests were performed on the day of blood sampling on 10 polyethylene glycol (PEG)-allergic patients and 16 healthy controls. Blood was drawn before skin prick test (SPT) on the study day. On the day of blood sampling, blood was centrifuged, and plasma replaced with piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) buffer (RefLab, Copenhagen, Denmark). Glass fiber–coated microtiter plates (RefLab) were added 50 μL diluted blood and 50 μL stimulant (polyclonal goat anti-human IgE [VWR International, West Chester, Pa], PMA, and ionomycin [both from Sigma-Aldrich, St Louis, Mo], or PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, or polysorbate 80 [Sigma-Aldrich]) in 6 concentrations. The plates were incubated for 60 minutes at 37°C, and released histamine was determined by making o-phthaldialdehyde–histamine fluorescent complexes, which were quantified on a Histareader (RefLab).

To perform the passive sensitization (PS) HR test, fresh buffy coat blood obtained from the local blood bank (Rigshospitalet, Copenhagen, Denmark) was added 10 pg/mL recombinant human IL-3 (Trichem, Skanderborg, Denmark) and stored overnight at 8°C. The buffy coat blood was washed with PIPES buffer followed by ice-cold stripping buffer (RefLab) to remove IgE from donor basophils. IgE-stripped cells were then incubated with serum for 1 hour at 37°C, and the cell suspension (25 μL) and stimulants (25 μL) were added to glass fiber–coated microtiter plates, with released histamine quantified as described above.

Percent HR (%HR) was calculated as the released histamine of stimuli divided by maximum HR induced by PMA + ionomycin stimulation. Participants who had a %HR of <10% to anti-IgE stimulation were designated as nonreleasing. Those with nonreleasing basophils did respond to PMA + ionomycin (Fig E1). Participants not responding to 2 consecutive PEG doses with ≥10% basophil reactivity were considered nonreacting. A test was regarded conclusive if the basophils reacted to anti-IgE or PEG stimulation, and inconclusive if the participant was both nonreleasing and nonreacting.

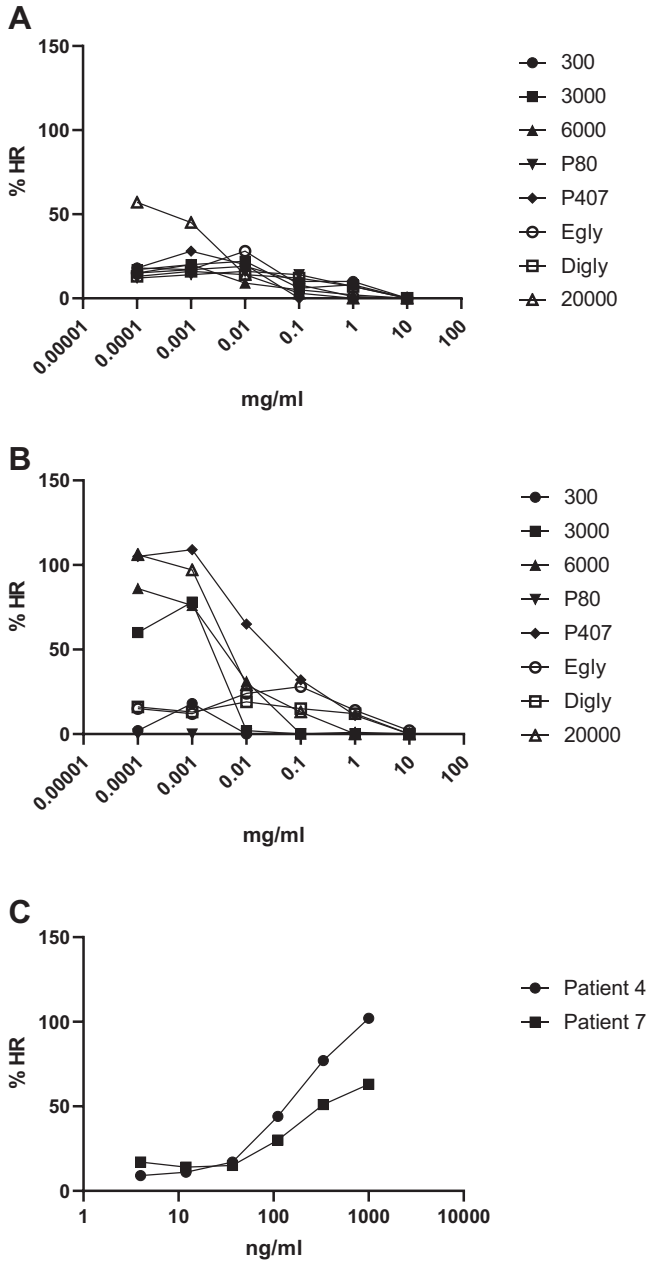

FIG E1. Direct basophil HR tests in response to PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407 or polysorbate 80, ethylene glycol, and diethylene glycol for **(A)** patient 4 (positive for PEG 20,000), **(B)** patient 7 (positive for PEG 3000, PEG 6000, PEG 20,000, and poloxamer 407), and **(C)** anti-IgE for patients 4 and 7 at first study visit in 2017. Unfortunately, the 10-fold dilution range of PEGs from 10 mg/mL to 0.1 ug/mL was not sufficiently long; in some cases, it only allowed demonstration of a positive response at the lowest concentrations (ie, at right part of the bell-shaped dose–response curve normally seen for HR). For logistical reasons, it was not possible to repeat the experiments using higher dilutions.

TABLE E1. Detailed procedure for preparing solutions for SPT for PEG, poloxamer 407, and polysorbate 80 at the Laboratory for Medical Allergology, Allergy Clinic, Gentofte Hospital, Denmark

Compound	Manufacturer product no.*	Dilution	Amount for 10 mL solution	Production method†
PEG 300	81162	No dilution	10 mL	PEG 300 is used undiluted; just form 10 mL aliquots of the solution.
PEG 3000	03997	50% (wt/vol)	5 g	For PEG 3000, PEG 6000, and poloxamer 407, weigh the appropriate amount and transfer to a 15 mL tube containing 5 mL sterile water. Poloxamer is difficult to dissolve, so always add sterile water before the compound. Tighten the lid and seal with parafilm. Place the tube on a tube rotator at 37°C for 2 hours. Ensure that the compound is dissolved. If not, leave the tube on the rotator at 37°C until dissolved. Centrifuge the tubes (500 × *g*, 5 minutes, 20°C), adjust the volume to 10 mL with sterile water, and vortex the suspension to ensure correct mixing.
PEG 6000	03394	50% (wt/vol)	5 g	
Poloxamer 407	16758	10% (wt/vol)	1 g	
PEG 20,000 (average molecular weight)	81300	0.01-20% (wt/vol)	2x2 g	For PEG 20,000, twice the amount is made up because more is needed for serial dilutions. Weigh 4 g and transfer to a 50 mL tube containing 14 mL sterile water. Tighten the lid and seal with parafilm. Place the tube on a tube rotator at 37°C for 2 hours. Ensure that the compound is dissolved. If not, leave the tube on the rotator at 37°C until dissolved. Centrifuge the tubes (500 × *g*, 5 minutes, 20°C), adjust the volume to 20 mL with sterile water, and vortex the suspension to ensure correct mixing. *Prepare 4 new tubes for serial dilution:* ● 10% PEG 20,000: Mix 8 mL of 20% PEG 20,000 with 8 mL sterile water. ● 1% PEG 20,000: Mix 2 mL of 10% PEG 20,000 with 18 mL sterile water. ● 0.1% PEG 20,000: Mix 2 mL of 1% PEG 20,000 with 18 mL sterile water. ● 0.01% PEG 20,000: Mix 2 mL of 0.1% PEG 20,000 with 18 mL sterile water. Vortex each dilution to ensure correct mixing before preparing the next dilution step. You will end up having more than 10 mL in the final solution.
Polysorbate 80	P1754	20% (v/v)	2 mL	Pipette 2 mL into a 15 mL tube containing 8 mL sterile water. Vortex the suspension to ensure correct mixing.

Once solutions are made following this procedure, they will stay in solution at room temperature. Solutions are transferred to a sterile vial for multiple use. Solutions are used for 6 months, but no studies have been done on stability or sterility. Solutions should be presumed to be nonsterile and should only be used for SPTs. The compounds used are classified as laboratory chemicals. Use of these substances for SPT may be subject to local legislation and is at the responsibility of the doctor ordering the test.

*Sigma-Aldrich (Sigmaaldrich.com).

†Description of method for production of 10 mL solution (transfer into sterile vials for multiple use and store at room temperature).

TABLE E2. Direct HR test and PS test results in 10 patients with confirmed PEG allergy

Patient no.	HR 0	HR A	PS 0	PS A	PS B
1	PEG 3350 PEG 6000	−	PEG 3350 PEG 6000	Poloxamer 407	NT
2	PEG 3000 PEG 6000	−	NT	−	−
3	−	(−)	NT	−	−
4	−	PEG 20,000	NT	−	−
5	NT	(−)	NT	−	−
6	NT	(−)	NT	−	−
7	NT	PEG 3000 PEG 6000 PEG 20,000 Poloxamer 407	NT	−	−
8	NT	−	NT	(−)	−
9	−	−	NT	(−)	NT
10	NT	(−)	NT	(−)	NT

Only positive results are provided in full. For HR, results are shown from initial reaction (0) and study visit A. For PS, results are shown for study visit A and study visit B. All patients were tested with PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, or polysorbate 80 in 6 concentrations at study visit A and B. 0 indicates time of diagnosis; A, first study visit; and B, second study visit; and − indicates negative; (−), inconclusive; and NT, not tested.