NSAIDs-hypersensitivity often induces a blended reaction pattern involving multiple organs

Inmaculada Doña1,2, Esther Barrionuevo1,2, María Salas1,2, José Julio Laguna3,4, José Agúndez5,6, Elena García-Martín5,6, Gádor Bogas1, James Richard Perkins7, José Antonio Cornejo-García2,7 & María José Torres1,2

Non-steroidal anti-inflammatory drugs (NSAIDs)-induced hypersensitivity reactions are classified by the European Network on Drug Allergy (ENDA) as either cross-reactive or selective. The former is the most frequent type and includes patients with exclusively respiratory symptoms (NSAIDs-exacerbated respiratory disease, NERD) or exclusively cutaneous symptoms: NSAIDs-induced urticaria/angioedema (NIUA); and NSAIDs-exacerbated cutaneous disease (NECD). However, although not reflected in the current classification scheme (ENDA), in clinical practice a combination of both skin and respiratory symptoms or even other organs such as gastrointestinal tract symptoms (mixed or blended reactions) is frequently observed. This entity has not been sufficiently characterised. Our aim was to clinically characterize blended reactions to NSAIDs, comparing their clinical features with NERD and NIUA. We evaluated patients with symptoms suggestive of hypersensitivity to NSAIDs who attended the Allergy Unit of the Regional University Hospital of Malaga (Malaga, Spain) between 2008 and 2015. We included 880 patients confirmed as cross-reactive based on clinical history, positive nasal provocation test with lysine acetylsalicylate (NPT-LASA), and/or positive drug provocation test (DPT) with acetylsalicylic acid (ASA), who were classified as blended (261; 29.6%), NERD (108; 12.3%) or NIUA (511; 58.1%). We compared symptoms, drugs, underlying diseases and diagnostic methods within and between groups. Among blended patients the most common sub-group comprised those developing urticaria/angioedema plus rhinitis/asthma (n = 138), who had a higher percentage of underlying rhinitis (p < 0.0001) and asthma (p < 0.0001) than NIUA patients, showing similarities to NERD. These differences were not found in the sub-group of blended patients who developed such respiratory symptoms as glottis oedema; these were more similar to NIUA. The percentage of positive NPT-LASA was similar for blended (77%) and NERD groups (78.7%). We conclude that blended reactions are hypersensitivity reactions to NSAIDs affecting at least two organs. In addition to classical skin and respiratory involvement, in our population a number of patients also develop gastrointestinal symptoms. Given the high rate of positive responses to NPT-LASA in NERD as well as blended reactions, we suggest that all patients reporting respiratory symptoms, regardless of whether they have other associated symptoms, should be initially evaluated using NPT-LASA, which poses less risk than DPT.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequent triggers of drug hypersensitivity reactions (DHRs)1–4. Several classifications have been proposed5–8, including a recently published and heavily cited publication from the European Network on Drug Allergy (ENDA) group from the European Academy of Allergy and Clinical Immunology (EAACI)9. They classify hypersensitivity reactions to NSAIDs according to the clinical symptoms induced, the number of NSAIDs involved, and the presence or absence of underlying reactions. One of the classification schemes is based on the number of NSAIDs involved: NSAIDs-exacerbated respiratory disease (other than NSAID-exacerbated chronic obstructive pulmonary disease) (NERD); NSAIDs-induced urticaria/angioedema (NIUA); and NSAIDs-exacerbated cutaneous disease (NECD). However, although not reflected in the current classification scheme (ENDA), in clinical practice a combination of both skin and respiratory symptoms or even other organs such as gastrointestinal tract symptoms (mixed or blended reactions) is frequently observed. This entity has not been sufficiently characterised. Our aim was to clinically characterize blended reactions to NSAIDs, comparing their clinical features with NERD and NIUA. We evaluated patients with symptoms suggestive of hypersensitivity to NSAIDs who attended the Allergy Unit of the Regional University Hospital of Malaga (Malaga, Spain) between 2008 and 2015. We included 880 patients confirmed as cross-reactive based on clinical history, positive nasal provocation test with lysine acetylsalicylate (NPT-LASA), and/or positive drug provocation test (DPT) with acetylsalicylic acid (ASA), who were classified as blended (261; 29.6%), NERD (108; 12.3%) or NIUA (511; 58.1%). We compared symptoms, drugs, underlying diseases and diagnostic methods within and between groups. Among blended patients the most common sub-group comprised those developing urticaria/angioedema plus rhinitis/asthma (n = 138), who had a higher percentage of underlying rhinitis (p < 0.0001) and asthma (p < 0.0001) than NIUA patients, showing similarities to NERD. These differences were not found in the sub-group of blended patients who developed such respiratory symptoms as glottis oedema; these were more similar to NIUA. The percentage of positive NPT-LASA was similar for blended (77%) and NERD groups (78.7%). We conclude that blended reactions are hypersensitivity reactions to NSAIDs affecting at least two organs. In addition to classical skin and respiratory involvement, in our population a number of patients also develop gastrointestinal symptoms. Given the high rate of positive responses to NPT-LASA in NERD as well as blended reactions, we suggest that all patients reporting respiratory symptoms, regardless of whether they have other associated symptoms, should be initially evaluated using NPT-LASA, which poses less risk than DPT.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequent triggers of drug hypersensitivity reactions (DHRs)1–4. Several classifications have been proposed5–8, including a recently published and heavily cited publication from the European Network on Drug Allergy (ENDA) group from the European Academy of Allergy and Clinical Immunology (EAACI)9. They classify hypersensitivity reactions to NSAIDs according to the clinical symptoms induced, the number of NSAIDs involved, and the presence or absence of underlying

---

1 Allergy Unit, IBIMA-Regional University Hospital of Malaga-UMA, Malaga, Spain. 2 ARADyAL network RD16/0006/0003, New York, USA. 3 Allergy Unit and Allergy-Anaesthesia Unit, Hospital Central Cruz Roja, Madrid, Spain. 4 ARADyAL network RD16/0006/0033, New York, USA. 5 Department of Pharmacology, University of Extremadura, Caceres, Spain. 6 ARADyAL network RD16/0006/0004, Caceres, Spain. 7 Research Laboratory, IBIMA-Regional University Hospital of Malaga-UMA, Malaga, Spain. Inmaculada Doña, Esther Barrionuevo, José Antonio Cornejo-García and María José Torres contributed equally. Correspondence and requests for materials should be addressed to I.D. (email: inmadd@hotmail.com)
Several NSAIDs from the same chemical group but tolerance to other non-chemically related NSAIDs. SNIUAA and SNIDHR are characterized by immediate or non-immediate reactions to a given NSAID or an NSAIDs and indomethacin; (ii) having experienced less than 3 episodes of respiratory symptoms, with or without other NSAIDs from different chemical groups including a strong COX-1 inhibitor (acetylsalicylic acid (ASA) and/or a strong COX-1 inhibitor as: (i) NERD if they had respiratory symptoms (rhinitis and/or asthma); (ii) NIUA if they had skin symptoms (urticaria and/or angioedema); and (iii) blended if they had a combination of skin, respiratory and/or gastrointestinal symptoms induced by less than 3 different NSAIDs and giving a positive NPT-LASA; (iii) having had less than 3 episodes of cutaneous symptoms, with or without respiratory and/or gastrointestinal symptoms induced by less than 3 different NSAIDs and giving a positive NPT-LASA or drug provocation test (DPT) to ASA (Fig. 1).

Exclusion criteria included the following: patients with delayed DHRs such as fixed drug eruption, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis complex or acute generalised exanthematic pustulosis; pregnant or breastfeeding patients; patients taking beta-blockers or ACE inhibitors or with contraindications for epinephrine administration; patients with acute infections and/or underlying cardiac, hepatic or renal diseases that contraindicated DPT; patients with CSU that was exacerbated by NSAIDs (NECD); patients with psychosomatic disorders; patients reporting throat tightness not associated with dysphonia, difficulty breathing and swallowing and glottis oedema was observed not by fibroscope; and patients reporting gastrointestinal symptoms such as epigastric burning and hemorrhage related to alterations in the gastroduodenal mucosa secondary to the pharmacology action of NSAIDs.

The study was conducted according to the principles of the Declaration of Helsinki and approved by the Provincial Investigational Ethics Committee of Malaga. All participants were informed orally about the study and signed the corresponding informed consent. In the case of participants under the age of 18 years, informed consent was obtained from a parent and/or legal guardian.

Patients. We evaluated patients aged 14–80 years old with symptoms suggestive of DHRs to one or more NSAIDs who attended the Allergy Unit of the Regional University Hospital of Malaga (Malaga, Spain) between 2008 and 2015. Of these, we included patients with a confirmed diagnosis of cross-reactive hypersensitivity to NSAIDs, defined by meeting at least one of the following criteria: (i) having experienced 3 or more episodes of cutaneous (urticaria and/or angioedema), respiratory (rhinitis, asthma and/or glottis oedema) and/or gastrointestinal symptoms (periumbilical colic pain, vomiting and/or diarrhoea) after the intake of at least 3 distinct NSAIDs from different chemical groups including a strong COX-1 inhibitor (acetylsalicylic acid (ASA) and/or indomethacin); (ii) having experienced less than 3 episodes of respiratory symptoms, with or without other organs involved after the intake of 2 different or only one NSAID, and giving a positive nasal provocation test with lysine acetyl salicylate (NPT-LASA); (iii) having had less than 3 episodes of cutaneous symptoms, with or without respiratory and/or gastrointestinal symptoms induced by less than 3 different NSAIDs and giving a positive NPT-LASA or drug provocation test (DPT) to ASA (Fig. 1).

Patient classification. Patients were classified according to the symptoms experienced after ASA or other strong COX-1 inhibitor as: (i) NERD if they had respiratory symptoms (rhinitis and/or asthma); (ii) NIUA if they had skin symptoms (urticaria and/or angioedema); and (iii) blended if they had a combination of skin, respiratory and/or gastrointestinal symptoms (urticaria; angioedema; rhinitis; asthma; throat tightness associated with dysphonia, difficulty breathing and swallowing; periumbilical colic pain; vomiting and/or diarrhoea). Glottis oedema was observed using a fibroscope in patients reporting throat tightness associated with dysphonia, difficulty breathing and swallowing.
Clinical history. Patients were questioned about the symptoms that appeared after NSAIDs intake, the time interval between drug intake and reaction onset, the number of episodes, the number of NSAIDs involved in the episodes, underlying nasal and bronchial symptoms (sneezing, itching, watery nose, nasal blockage, difficulty breathing, cough and wheezing), food allergy (urticaria, angioedema, oral allergy syndrome, anaphylaxis and shock), and CSU.

Atopic status. Atopic status was assessed by skin prick test (SPT) using a panel of 20 common inhalant allergens, including pollens, house dust mites, moulds and animal dander, and 27 common food allergens including animal, fruit and vegetable allergens (ALK, Madrid, Spain). Histamine hydrochloride (10 mg/ml) and phenolated glycerol saline were used as positive and negative controls, respectively. Patients were requested to stop taking any antihistamine medication at least 8 days before undertaking SPT. A positive SPT response was defined as a wheal diameter of 3 mm or larger to at least one of these allergens and any patient producing such a wheal was considered atopic.

Nasal and oral drug provocation testing. NPT-LASA was carried out as described in patients reporting respiratory symptoms regardless of the other organs involved. Results were considered positive if an increase ≥ 30% in the total nasal symptoms and a decrease ≥ 30% in the total volume of both nasal cavities from 2 to 6 cm (vol 2–6 cm), measured by acoustic rhinometry, was observed.

Oral drug provocation testing (DPT) was performed in NIUA patients, and in blended and NERD patients who gave negative NPT-LASA results. DPT was performed in a single-blind manner as reported. Briefly, placebo capsules were given at different times on the 1st day; three doses of ASA were administered orally at intervals of 90 min (10, 50 and 50 mg) on the 2nd day; and, if negative, three doses of ASA (125, 125 and 250 mg) were administered on the 3rd day. If cutaneous and/or respiratory symptoms or changes in vital signs (rhythm alterations, decrease in FEV1 or hypotension) appeared, the procedure was stopped and symptoms were evaluated and treated. If no symptoms appeared during drug administration, the therapeutic dose of ASA was achieved and this was followed by a 2 day/8 h course at maximum dose, after a gap of 24 h. ASA and placebo were given in opaque capsules prepared by the hospital pharmacy service. Other medications were withheld before testing, according to international guidelines.

Statistical analysis. Descriptive statistics (frequency, mean, median and range) were used to present results, as indicated. Chi-square analysis was used to test differences for nominal variables, and the Fisher test was used when criteria for using the chi-square test was not met. For quantitative variables, non-parametric Mann-Whitney and Kruskal-Wallis tests were used. All reported p-values represented two-tailed tests, with values < 0.05 considered significant.

Results
A total of 2848 patients with symptoms suggestive of NSAIDs hypersensitivity were evaluated in the Allergy Unit of the Regional University Hospital of Malaga between 2008 and 2015. Of these, diagnosis could not be achieved for 1662 patients (1230 could not undergo DPT to ASA due to age or comorbidities, 376 refused to perform the study, and 56 were excluded due to pregnancy), and 267 individuals were diagnosed with selective reactions. This...
Table 1. Demographic and clinical data of study participants. IQR: interquartile range; NS: Not significant.

|                          | Blended n = 261 | NERD n = 108 | NIUA n = 511 | p-value Blended vs NERD vs NIUA | p-value Blended vs NERD | p-value Blended vs NIUA | p-value NERD vs NIUA |
|--------------------------|------------------|--------------|--------------|---------------------------------|------------------------|------------------------|----------------------|
| Age median (IQR)         | 39 (30–48.5)     | 38 (28–50.7) | NS           | NS                              | NS                     | NS                     | NS                   |
| Gender (female), n (%)   | 176 (67.4)       | 73 (67.6)    | 296 (58)     | 0.03                            | NS                     | 0.01                   | 0.01                 |
| Underlying rhinitis, n (%)| 158 (60.5)      | 77 (71.3)    | 185 (36.2)   | <0.0001                         | NS                     | 0.0002                 | <0.0001              |
| Underlying asthma, n (%)| 123 (47.1)       | 70 (64.8)    | 98 (19.2)    | <0.0001                         | <0.0001                | <0.0001                | <0.0001              |
| Nasosinusal polyposis, n (%)| 24 (9.2)      | 42 (38.9)    | 0            | <0.0001                         | <0.0001                | <0.0001                | <0.0001              |
| Food allergy, n (%)      | 0                | 0            | 15 (3)       | 0.004                           | NS                     | 0.005                  | NS                   |
| Atopy, n (%)             | 187 (71.6)       | 59 (54.6)    | 335 (65.5)   | NS                              | 0.002                  | NS                     | NS                   |
| Positive to at least one inhalant allergen, n (%)| 181 (69.3) | 59 (54.6)    | 328 (64.2)   | NS                              | NS                     | NS                     | NS                   |
| Loliun;                  | 91 (34.9)        | 19 (17.6)    | 82 (16)      | 0.001                           | 0.03                   | 0.003                  | 0.002                |
| Cupressus;               | 28 (10.7)        | 1 (0.9)      | 48 (9.4)     | 0.014                           | NS                     | 0.002                  | 0.005                |
| Olea;                    | 98 (37.5)        | 19 (17.6)    | 159 (31.1)   | 0.004                           | NS                     | NS                     | NS                   |
| Parietaria;              | 19 (7.3)         | 9 (8.3)      | 30 (5.9)     | NS                              | NS                     | NS                     | NS                   |
| Salsola;                 | 22 (8.4)         | 2 (1.8)      | 40 (7.8)     | NS                              | NS                     | NS                     | NS                   |
| D. pteronyssinus;        | 108 (41.4)       | 32 (29.6)    | 219 (42.8)   | NS                              | NS                     | NS                     | NS                   |
| Alternaria;              | 46 (17.6)        | 6 (5.5)      | 37 (7.2)     | 0.016                           | 0.02                   | 0.008                  | NS                   |
| Dog dander;              | 76 (29.1)        | 12 (11.1)    | 73 (14.3)    | 0.003                           | 0.005                  | 0.002                  | NS                   |
| Cat dander;              | 67 (25.7)        | 13 (12)      | 88 (17.2)    | NS                              | 0.03                   | NS                     | NS                   |
| Positive to at least one food allergen, n (%)| 81 (31)    | 32 (29.6)    | 83 (16.2)    | NS                              | NS                     | NS                     | 0.02                 |
| Pru p 3                  | 28 (10.7)        | 0            | 26 (5.1)     | 0.0001                          | 0.0003                 | NS                     | 0.01                 |
| Apple                    | 24 (9.2)         | 0            | 10 (3.8)     | <0.0001                         | 0.001                  | NS                     | NS                   |
| Peanut                   | 49 (18.7)        | 15 (13.9)    | 14 (2.7)     | 0.03                            | NS                     | 0.04                   | NS                   |
| Walzetnut                | 49 (18.7)        | 15 (13.9)    | 15 (2.9)     | 0.03                            | NS                     | 0.04                   | NS                   |
| Melon                    | 23 (8.8)         | 0            | 22 (4.3)     | 0.0009                          | 0.001                  | NS                     | 0.02                 |
| Shrimp                   | 29 (11.1)        | 10 (9.2)     | 14 (2.7)     | 0.01                            | NS                     | 0.04                   | 0.04                 |

left a total of 919 patients (32.3%) diagnosed as having cross-reactive hypersensitivity. Of these, only 4.2% were classified as NECD, and were therefore not further considered.

Of the 880 patients finally included, 511 (55.1%) were diagnosed with NIUA, 261 (28.4%) classified as suffering blended reactions and 108 (11.7%) diagnosed with NERD. A total of 560 patients (63.6%) were female and the median age at diagnosis was 38 years (IQR: 28–50). Five hundred and eighty-one (66%) patients were atopic, the most common allergens being Dermatophagoides pteronyssinus (359; 40.8%), Olea europaea (276; 31.4%), and Lolium perenne (192; 21.8%). A total of 420 (47.7%) had underlying rhinitis; 291 (33.1%) had asthma; 66 (7.5%) had nasosinusal polyposis and 15 (1.7%) had food allergy (4 to nuts, 9 to shellfish and 4 to melon).

A comparison of the demographic and clinical data between the different groups is shown in Table 1. We found that the proportions of patients having underlying rhinitis and asthma were higher in the blended group compared to NIUA (p = 0.0002 and p < 0.0001, respectively), but lower than NERD (p > 0.05 and p < 0.0001, respectively). In addition, polyposis was also less frequent in blended than NERD but more frequent than NIUA (p < 0.0001 for both comparisons). The proportion of atopic patients was similar when comparing blended with NIUA, but higher when comparing blended with NERD (p = 0.002) (Table 1 and Supplementary Table). No NIUA patient had nasosinusal polyposis and no patient diagnosed with NERD or as having blended reactions had food allergy.

Analyses of patient reactions according to clinical records are shown in Tables 2 and 3. Patients reported a median of 3 episodes after NSAIDs intake, and a median of 2 different NSAIDs were involved, with no differences between groups. The median onset time interval after NSAIDs intake was 45 minutes when considering patients from all groups (IQR: 20–120). When comparing between groups, blended reactions and NERD showed similar onset times (median: 30 minutes; IQR: 20–90, and median: 30; IQR: 15–120, respectively). However, this interval was shorter in patients with blended reactions compared to NIUA (median: 60; IQR: 30–120) (p = 0.0003) (Table 2).

Considering all groups together, 545 patients (61.9%) reported reactions to ibuprofen; 343 to dipyrone (39%); 333 to ASA (37.8%); 185 to diclofenac (21%); 208 to paracetamol (23.6%); 70 to dexketoprofen (7.9%); 60 to naproxen (6.8%); 28 to piroxicam (3.2%); 18 to lysine clonixinate (2%); 10 to indomethacine (1.1%), 9 to a selective COX-2 inhibitor (1%) and 5 to meloxicam (0.6%).

The percentage of reactions induced by dipyrone was higher in blended reactions compared to NERD (41.4% and 27.8%, p = 0.004). It was also higher for NIUA then NERD (40% and 27.8%, p = 0.03), as was paracetamol (27.5% vs 13.9%, p = 0.02). No significant differences found for other drugs (Table 2).

Analysis of the clinical symptoms in the blended reactions group shows several general patterns (Table 3). Patients can be classified into 4 sub-groups: patients developing skin symptoms (urticaria/angioedema) and rhinitis/asthma (Sub-group I); patients developing skin symptoms (urticaria/angioedema) and glottis oedema (Sub-group II); patients developing skin symptoms (urticaria/angioedema), rhinitis/asthma and glottis oedema (Sub-group III); and patients experiencing a combination of gastrointestinal symptoms (abdominal pain,
diarrhoea, nausea, vomiting) with skin symptoms (urticaria/angioedema) and/or rhinitis/asthma (Sub-group IV) (Table 3). Sub-group I was the most frequent \( n = 138; 52.9\% \), followed by Sub-group II \( n = 100; 38.3\% \), Sub-group III \( n = 15; 5.7\% \) and Sub-group IV \( n = 8; 3.1\% \). The proportion of underlying rhinitis, asthma, nasosinusal polyposis and atopy in the 4 Sub-groups of patients developing blended reactions is compared to NERD and NIUA in Fig. 2. Sub-group I contained a similar proportion of patients with underlying rhinitis (98; 71.01%) and asthma (85; 61.59%) to NERD patients (rhinitis: 77; 71.3%; asthma: 70; 64.8%), but different to NIUA (rhinitis: 185; 36.2%; asthma: 98; 19.2%) \( p < 0.0001 \) for both comparisons. In Sub-groups II, III and IV, the proportions of patients with underlying rhinitis (Sub-group II: 50; 50%; Sub-group III: 7; 46.66%; and

| Group | Symptoms | \( n (\%) \) |
|-------|----------|-------------|
| Skin + Rhinitis/Asthma (Sub-group I) \( n = 138 \) | AE + Asthma | 48 (18.4) |
| | Urticaria + Asthma | 48 (18.4) |
| | AE + Rhinitis | 17 (6.5) |
| | Urticaria + AE + Asthma | 8 (3.1) |
| | AE + Rhinitis + Asthma | 7 (2.7) |
| | Urticaria + AE + Rhinitis + Asthma | 5 (1.9) |
| | Urticaria + Rhinitis + Asthma | 3 (1.1) |
| | Urticaria + AE + Rhinitis | 2 (0.8) |
| Blended \( n = 261 \) | Skin + GE (Sub-group II) \( n = 100 \) | AE + GE | 59 (22.6) |
| | Urticaria + AE + GE | 24 (9.2) |
| | Urticaria + GE | 17 (6.5) |
| | Urticaria + Asthma + GE | 5 (1.9) |
| | AE + Rhinitis + GE | 4 (1.5) |
| | AE + Asthma + GE | 4 (1.5) |
| | Urticaria + Rhinitis + Asthma + GE | 2 (0.8) |
| | GI + Skin/Rhinitis/Asthma/GE (Sub-group IV) \( n = 8 \) | GI + Rhinitis + Asthma | 4 (1.5) |
| | GI + AE + Asthma | 4 (1.5) |
| NERD \( n = 108 \) | Rhinitis | 20 (18.5) |
| | Asthma | 64 (59.2) |
| | Rhinitis + Asthma | 24 (22.2) |
| NIUA \( n = 511 \) | Urticaria | 89 (17.4) |
| | AE | 124 (24.3) |
| | Urticaria + Angioedema | 298 (58.3) |

Table 2. Clinical characteristics of the reactions based on data reported by patients. IQR: interquartile range; NS: Non significant.

| Time interval drug-reaction, median (IQR) (min) | Blended \( n = 261 \) | NERD \( n = 108 \) | NIUA \( n = 511 \) | p-value Blended vs NERD vs NIUA |
|-----------------------------------------------|-----------------|-----------------|-----------------|------------------------------|
| 30 (20–90) | 30 (15–120) | 60 (30–120) | 0.001 | NS | 0.0003 | NS |
| ASA | 105 (39.9) | 34 (31.5) | 194 (38) | NS | NS | NS |
| Indomethacin | 3 (1.1) | — | 7 (1.4) | NS | NS | NS |
| Diclofenac | 59 (22.6) | 20 (18.5) | 106 (20.7) | NS | NS | NS |
| Ibuprofen | 142 (54.4) | 66 (61.1) | 337 (65.7) | NS | NS | NS |
| Naproxen | 18 (6.9) | 6 (5.5) | 36 (34.8) | NS | NS | NS |
| Dipyrone | 108 (41.4) | 30 (27.8) | 205 (40) | 0.004 | 0.004 | NS |
| Piroxicam | 9 (3.4) | 3 (2.8) | 16 (3.1) | NS | NS | NS |
| Paracetamol | 52 (19.9) | 15 (13.9) | 141 (27.5) | 0.003 | NS | NS |
| Meloxicam | 2 (0.8) | 1 (0.9) | 2 (0.4) | NS | NS | NS |
| Lysine clonixinate | 7 (2.7) | 2 (1.8) | 9 (1.8) | NS | NS | NS |
| Selective COX-2 | 3 (1.1) | — | 6 (1.2) | NS | NS | NS |
| Number of episodes, median (IQR) | 3 (2–4) | 3 (2–4) | 3 (3–4) | NS | NS | NS |
| Number of drugs involved, median (IQR) | 3 (1.25–3) | 2 (1–3) | 2 (2–3) | NS | NS | NS |
| Time interval between the last reactions and diagnosis, median (IQR) | 7 (3–24) | 3 (2–4) | 7 (5.5–8.5) | NS | NS | NS |

Table 3. Symptoms induced by NSAIDs according to patient report. AE: Angioedema; GE: Glottis oedema; GI: Gastrointestinal symptoms.
Sub-group IV: 3; 37.5%), asthma (Sub-group II: 31; 31%; Sub-group III: 4; 26.66%; and Sub-group IV: 3; 37.5%) and nasosinusal polyposis (zero for Sub-groups II, III and IV) were lower than in NERD, whereas underlying rhinitis and asthma showed similar proportions to NIUA. However, these differences were only significant for rhinitis and asthma between Sub-group II and NERD (\( p = 0.03 \) and \( p = 0.005 \), respectively). The percentage of atopy was similar for all subgroups of patients developing blended reactions, being similar to that of NIUA and higher than NERD, although significant differences were only found for Sub-group I, when compared to both NERD and NIUA (\( p = 0.001 \) and \( p = 0.02 \), respectively).

When considering the chronological appearance of clinical symptoms after NSAIDs intake by blended reaction patients, we found that 197 (75.5%) developed cutaneous symptoms first, followed by respiratory manifestations; 59 (22.6%) developed respiratory symptoms followed by skin and/or gastrointestinal symptoms, and 5 (1.9%) developed gastrointestinal symptoms followed by respiratory and/or skin symptoms.

The mean time interval between last reaction and diagnosis was 6 months (IQR: 2.5–24), with no statistically significant differences between groups.

The proportion of patients that could be diagnosed using only clinical history varied between groups (\( p < 0.0001 \)). For NIUA it could be used for the diagnosis of 340 patients (66.5% of the total NIUA patients), for NERD it could be used for 22 (20.4% of the total NERD patients) and for blended it was 41 (15.7% of the total blended patients). In 286 patients (32.5%) the diagnosis was confirmed by NPT-LASA: 85 from the total of NERD patients (78.7%) and 201 from the total of blended patients (77%) (Table 4). Considering the 4 sub-groups of patients with blended reactions mentioned above, no statistically significant differences were found in terms of the percentage of patients giving positive NPT-LASA: 84.8% for Sub-group I, 66% for Sub-group II, 80% for Sub-group III, and 75% for Sub-group IV. None of the patients who underwent NPT-LASA suffered bronchial symptoms or a significant fall in FEV1 upon administration.

The proportion of patients that were diagnosed using DPT also varied between groups (\( p < 0.0001 \)). For NIUA it could be used for the diagnosis of 172 patients (33.6% of the total NIUA patients), for NERD it could be used for 3 (2.7% of the total NERD patients), and for blended it was 19 (7.3% of the total blended patients). Patients reacted to a median cumulative ASA dose of 250 mg (IQR: 100–500) with a median time interval of 75 minutes (IQR: 30–120) after the last administered dose. Although no statistical differences were found, NERD patients tended to react to a lower ASA dose (median: 75 mg; IQR: 46.25–200) compared to NIUA (median: 300 mg; IQR: 100–500) and blended reaction patients (median: 250 mg; IQR: 113.75–500) (Table 5). Patients with positive DPT responses to ASA experienced similar symptoms to those recorded in their clinical history, however they were generally milder, disappearing within 1–2h after taking corticosteroid and antihistamine drugs and, if bronchial symptoms occurred, inhaled salbutamol. None of the patients required adrenaline to resolve their reaction.

**Discussion**

Not all entities induced by hypersensitivity to NSAIDs fit neatly within the ENDA classification\(^9\). For example, patients often develop cutaneous and respiratory symptoms simultaneously\(^5\)–\(^8\),\(^10\),\(^16\). By evaluating a large series of patients with cross-reactive hypersensitivity to NSAIDs we found such blended reactions to be the second most frequent reaction type after NIUA in our population, representing more than 28% of cases in this study, in agreement with previous findings\(^10\).
Furthermore, we used NIUA patients to represent patients with exclusively cutaneous reactions, in our patients the gastrointestinal symptomatology is acute and includes periumbilical colic pain, vomiting and diarrhea, being related to the hypersensitivity induced by NSAIDs. In this sense, patients may be considered ana-phylactic. Therefore, we suggest guidelines for NSAIDs hypersensitivity diagnoses and classification should take into account that cross-hypersensitivity reactions may have an anaphylactic component. Despite having a rather different clinical profile to NERD patients, these patients also gave a high percentage of positive response to NPT-LASA.

The aim of this study was to characterize patients developing blended reactions and to compare them to those developing exclusively respiratory or cutaneous symptoms. In our population, no NEDC patients experienced respiratory symptoms in combination with cutaneous symptoms after NSAIDs intake and no blended patients had underlying chronic urticaria. Therefore, we used NIUA patients to represent patients with exclusively cutaneous manifestations for comparisons purposes and NEDC patients were not further considered. Although it is thought that both NERD and NIUA may involve COX-1 inhibition, they are also thought to represent two distinctive phenotypes, as can be seen in the response to NPT-LASA and the release of inflammatory mediators. In blended patients there is an overlap of clinical entities as they share the same NSAIDs-induced respiratory and cutaneous with NERD and NIUA, respectively. Moreover, blended patients have a similar proportion of underlying rhinitis than NERD and atopy than NIUA. However, although the proportion of underlying asthma and nasosinusal polyposis is higher than NERD, is lower than NERD. It is unclear whether blended reactions represent a further phenotype or are part of a NERD-NIUA spectrum. This is especially important for those patients that develop throat tightness. Such tightness is considered as glottis oedema, a form of angioedema. As such, it should be decided whether these patients belong to the NIUA category or to NERD, as they have difficulty breathing due to upper airway involvement. Strikingly, we find that patients experiencing rhinitis and/or asthma accompanied with urticaria and/or angioedema have a similar clinical profile to NERD with respect to underlying diseases, and this is different from the profile of patients experiencing glottis oedema plus urticaria and/or angioedema, as well as NIUA patients. In addition, the percentage of positive responses to NPT-LASA in blended reactions is similar to NERD, with no significant differences between patients experiencing urticaria and/or angioedema accompanied by rhinitis and/or asthma and/or glottis oedema. Therefore, although patients with glottis oedema had a different clinical profile to NERD, appearing to be more similar to NIUA, the positive response to NPT-LASA allows it to be differentiated from NIUA. Previously, it has been reported that 12% of NIUA patients gave positive NPT-LASA. However, these patients would be better classified as blended, as they reported both palpebral angioedema and glottis oedema after NSAIDs intake. Importantly, the NIUA patients in this study that gave negative NPT-LASA did not report glottis oedema or other respiratory symptoms in combination with skin symptoms. This has important implications for diagnosis, which we will discuss below.

We have also detected patients showing gastrointestinal plus respiratory involvement and/or skin symptoms. NSAIDs are known to induce alterations in the gastroduodenal tract due to direct action on the mucosa as well as prostaglandin synthesis. This can lead to a wide range of tissue damage from mucosal erosions to ulcer and perforation, inducing generally symptoms as epigastric burning and hemorrhage. The symptoms referred by our patients experiencing blended reactions are not related to such alterations in the gastroduodenal mucosa secondary to the action of NSAIDs, in our patients the gastrointestinal symptomatology is acute and includes periumbilical colic pain, vomiting and diarrhea, being related to the hypersensitivity induced by NSAIDs. In this sense, patients may be considered ana-phylactic. Therefore, we suggest guidelines for NSAIDs hypersensitivity diagnoses and classification should take into account that cross-hypersensitivity reactions may have an anaphylactic component. Despite having a rather different clinical profile to NERD patients, these patients also gave a high percentage of positive response to NPT-LASA. The high percentage of positive response to NPT-LASA in patients with blended reactions, especially in those that previously reported severe symptoms, is an important finding, with potentially important implications for diagnosis as this test is known to be safer than oral DPT. Therefore, we suggest that NPT-LASA should be the first diagnostic approach in blended patients independently of the organ involved and we propose the inclusion of this test in the diagnostic algorithm for NSAIDs hypersensitivity reactions (Fig. 1).

Blended reactions represent a systemic clinical entity involving at least 2 organs, that can be severe and may be confused with SNIUAA. However, DPT with ASA can be used to differentiate between selective and cross-reactive hypersensitivity reactions. This has important clinical implications, as patients with selective reactions can tolerate other NSAIDs, whereas in cross-reactive hypersensitivity a safe alternative NSAID must be found.

| Group      | Diagnosis method n (%) | Clinical history | NPT-LASA | Positive DPT to ASA |
|------------|------------------------|------------------|----------|---------------------|
| Blended n = 261  | 41 (15.7)               | 201 (77)         | 19 (7.3) |
| NERD n = 108      | 20 (18.54)              | 85 (78.7)        | 3 (2.8)  |
| NIUA n = 511      | 340 (66.5)              | —                | 172 (33.6)|
| p                 | <0.0001                 | NS               | <0.0001 |

Table 4. Methods used to achieve diagnosis for each clinical entity.

| Group      | Cumulative dose ASA in positive DPT, median (IQR) | Interval (minutes) ASA dose-positive response in DPT, median (IQR) |
|------------|--------------------------------------------------|---------------------------------------------------------------|
| Blended n = 261  | 250 (113.7–500)                                  | 60 (30–112.5)                                              |
| NERD n = 108      | 75 (46.2–200)                                    | 60 (51.2–75)                                               |
| NIUA n = 511      | 300 (100–500)                                    | 90 (30–165)                                                |
| p                 | 0.4002                                            | 0.6519                                                      |

Table 5. Cumulative dose ASA and time interval between ASA administration and reaction in positive DPT. IQR: interquartile range.
Symptoms induced by ASA or other strong COX-1 inhibitors are crucial to define the phenotype, as the potency with which NSAID inhibit COX-1 can influence the symptoms induced by the drug. For example, meloxicam or paracetamol can induce cutaneous symptoms exclusively, whilst ASA induces skin and respiratory symptoms in the same patient. In our population, patients tended not to vary in terms of reported symptoms for repeated episodes, and for most of them a strong COX-1 inhibitor was involved. Patients with positive DPT responses to ASA experienced similar symptoms to those recorded in their clinical history, although generally milder, as they were challenged in a controlled manner and responded to lower doses. Only in 14 (5.36%) patients there was a difference in the phenotype established by clinical history (they reported only one episode which manifested as skin symptoms after paracetamol, meloxicam or etoricoxib intake and no strong COX-1 inhibitor was taken between the reaction and the study) compared to that established by DPT (skin and respiratory symptoms in DTP to ASA). Therefore, the phenotypes in our population were very reproducible.

Reports have shown that dipyrone and paracetamol are relatively safe drugs as therapeutic alternatives in NERD patients23,24 whereas up to 30% of subjects with NIUA may also be intolerant to these drugs5,10,25,26. Here, we found the number of NSAIDs involved and the percentage of patients having reactions induced by dipyrone and paracetamol to be higher in patients with blended reactions than for NERD. It would appear that patients developing blended reactions to NSAIDs show varying degrees of overlap with NERD and NIUA. Nevertheless, we do not know if NERD patients can develop skin symptoms over time, whilst long term studies of NIUA patients did not show the development of respiratory symptoms in the natural course of their disease37,38. Further longitudinal studies alongside investigation of the underlying mechanisms may help clarify the nature of the relationship between the different entities. Whilst the inhibition of COX-1 is thought to participate in these reactions39,40, and this has been studied in depth in NERD subjects23, additional studies are required to shed light on the mechanisms of NIUA and blended reactions.

Summarizing, the results of this study indicate that blended reactions are a frequently occurring, complex and potentially severe condition for which fewer alternative drugs may exist than for NERD, due to increased sensitivity to dipyrone and paracetamol. Given the frequency of these reactions, we would suggest further investigation with the aim of extending the ENDA guidelines in terms of including patients with blended reactions, with potential subdivisions within this category. We have also shown that NPT-LASA represents a useful diagnostic approach for these reactions and should be considered when dealing with a patient with blended reaction symptoms. Future studies should aim to clarify the pathomechanisms involved and the natural evolution of these reactions.

References
1. Gomes, E. et al. Self-reported drug allergy in a general adult Portuguese population. Clin. Exp. Allergy. 34, 1597–1601 (2004).
2. Messaad, D. et al. Drug provocation tests in patients with a history suggesting an immediate drug hypersensitivity reaction. Ann. Intern. Med. 140, 1001–1006 (2004).
3. Chalabanlloo, F. et al. Clinical characteristics of patients with drug hypersensitivity in Norway: a single-centre study. Pharmacoepidemiology. Drug Saf. 20, 506–513 (2011).
4. Doña, I. et al. Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. J. Investig. Allergol. Clin. Immunology. 22, 363–371 (2012).
5. Quiralte, J., Blanco, C., Castillo, R., Delgado, J. & Carrillo, T. Intolerance to nonsteroidal antiinflammatory drugs: results of controlled drug challenges in 98 patients. J. Allergy Clin. Immunol. 98, 678–685 (1996).
6. Stevenson, D. D., Sanchez-Borges, M. & Szczeklik, A. Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. Allergy. Asthma Immunol. Res. 87, 177–180 (2001).
7. Sanchez-Borges, M., Capriles-Behrens, E. & Caballero-Fonseca, F. Hypersensitivity to non-steroidal anti-inflammatory drugs in childhood. Pediatr Allergy Immunol. 15, 376–380 (2004).
8. Caimmi, S., Caimmi, D., Bousquet, P. J. & Demoly, P. How can we better classify NSAID hypersensitivity reactions? validation from a large database. Int. Arch. Allergy Immunol. 159, 306–312 (2012).
9. Kowalski, M. L. et al. Classification and practical approach to the diagnosis and management of hypersensitivity to nonsteroidal anti-inflammatory drugs. Allergy. 68, 1219–1232 (2013).
10. Doña, I. et al. Characteristics of subjects experiencing hypersensitivity to non-steroidal anti-inflammatory drugs: patterns of response. Clin. Exp. Allergy. 41, 86–95 (2011).
11. Zambonino, M. A. et al. Drug provocation tests in the diagnosis of hypersensitivity reactions to non-steroidal anti-inflammatory drugs in children. Pediatr Allergy Immunol. 24, 151–159 (2013).
12. Szczeklik, A., Gryglewski, R. J., Czerwiawska-Mysliw, G. & Zmuda, A. Aspirin-induced asthma. Hypersensitivity to fenoprofen and ibuprofen in relation to their inhibitory action on prostaglandin generation by different microsomal enzymic preparations. J. Allergy Clin. Immunol. 58, 10–18 (1976).
13. Samter, M. & Beers, R. F. Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. Ann. Intern. Medicine. 68, 975–983 (1968).
14. Canto, M. G., Andreu, I., Fernandez, J. & Blanca, M. Selective immediate hypersensitivity reactions to NSAIDs. Curr. Opin. Allergy Clin. Immunol. 9, 293–297 (2009).
15. Pichler, W. J. Delayed drug hypersensitivity reactions. Ann. Intern. Medicine. 139, 683–693 (2003).
16. Cousin, M., Chiriaca, A., Molinari, N., Demoly, P. & Caimmi, D. Phenotypical characterization of children with hypersensitivity reactions to NSAIDs. Pediatr Allergy Immunol. 27, 743–748 (2016).
17. Kidon, M. I. et al. Early presentation with angioedema and urticaria in cross-reactive hypersensitivity to nonsteroidal antiinflammatory drugs among young, Asian, atopic children. Pediatrics. 116, 675–680 (2005).
18. Blanca-Lopez, N. et al. Value of the clinical history in the diagnosis of urticaria/angioedema induced by NSAIDs with cross-intolerance. Clin. Exp. Allergy. 43, 85–91 (2013).
19. Campo, P. et al. Mediator release after nasal aspirin provocation supports different phenotypes in subjects with NSAID hypersensitivity reactions to ASA. Allergy. 68, 1001–1007 (2013).
20. Nizankowska-Mogilnicka, E. et al. EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. Allergy. 62, 1111–1118 (2007).
21. Blanca-Lopez, N. et al. ASA must be given to classify multiple NSAID-hypersensitivity patients as selective or cross-intolerant. Allergy. 71, 576–578 (2016).
22. Asero, R. Oral aspirin challenges in patients with a history of intolerance to single non-steroidal anti-inflammatory drugs. Clin. Exp. Allergy. 35, 713–716 (2005).
23. Stevenson, D. D. & Szczeklik, A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. J. Allergy Clin. Immunol. 118, 773–786 (2006).
24. Szczeklik, A. & Stevenson, D. D. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. J. Allergy Clin. Immunol. 111, 913–21 (2003).
25. Doña, I. et al. Response to a selective COX-2 inhibitor in patients with urticaria/angioedema induced by nonsteroidal anti-inflammatory drugs. Allergy 66, 1428–1433 (2011).
26. Asero, R. Risk factors for acetaminophen and nimesulide intolerance in patients with NSAID-induced skin disorders. Ann. Allergy Asthma Immunol. 82, 554–558 (1999).
27. Doña, I. et al. Natural evolution in patients with nonsteroidal anti-inflammatory drug-induced urticaria/angioedema. Allergy. 72, 1346–1355 (2017).
28. Doña, I. et al. NSAID-induced urticaria/angioedema does not evolve into chronic urticaria: a 12-year follow-up study. Allergy. 69, 438–444 (2014).
29. Sanchez-Borges, M. NSAID hypersensitivity (respiratory, cutaneous, and generalized anaphylactic symptoms). Med. Clin. North Am. 94, 853–864 (2010).

Acknowledgements

JR Perkins also contributed to the English version. We would like to thank the nurses from our Allergy Unit for performing SPT, NPT-LASA and DPT. The present study has been supported by the Andalusian Government (PI-0463-2013), Institute of Health Carlos III of the Ministry of Economy and Competitiveness (grants cofounded by European Regional Development Fund (ERDF): RETIC ARADyAL RD16/0006/0001 and PI17/1253) and the Spanish Society of Allergology (Fondo de Investigación de la Fundación de la SEAIC 2016). I Doña holds a Juan Rodes research contract (JR15/00036), G Bogas holds a Rio Hortega contract (CM16/0067), JA Cornejo-García received funding from the Miguel Servet Program (Ref CP14/00034), and JR Perkins from the Sara Borrell Program (Ref CD14/00242), all of them from the Carlos III National Health Institute, Spanish Ministry of Economy and Competitiveness (grants cofounded by European Social Fund, ESF).

Author Contributions

I. Doña and M.J. Torres contributed to study design. I. Doña, E. Barrionuevo, M. Salas, G. Bogas and M.J. Torres recruited patients and performed the clinical evaluations. I. Doña, J.J. Laguna, Agúndez J., García-Martín E, J.A. Cornejo-García, J.R. Perkins and M.J. Torres wrote the manuscript. I. Doña and J.R. Perkins prepared illustrations.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-34668-1.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018