



ORIGINAL ARTICLE



Atopic Dermatitis, Urticaria and Skin disease

Eicosanoid mediator profiles in different phenotypes of nonsteroidal anti-inflammatory drug-induced urticaria

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Abstract

Background: The role of arachidonic acid metabolites in NSAID-induced hypersensitivity has been studied in depth for NSAID-exacerbated respiratory disease (NERD) and NSAID-exacerbated cutaneous disease (NECD). However, no information is available for NSAID-induced urticarial/angioedema (NIUA), despite it being the most frequent clinical entity induced by NSAID hypersensitivity. We evaluated changes in leukotriene and prostaglandin metabolites for NIUA patients, using patients with NECD and single-NSAID-induced urticaria/angioedema or anaphylaxis (SNIUAA) for comparison.

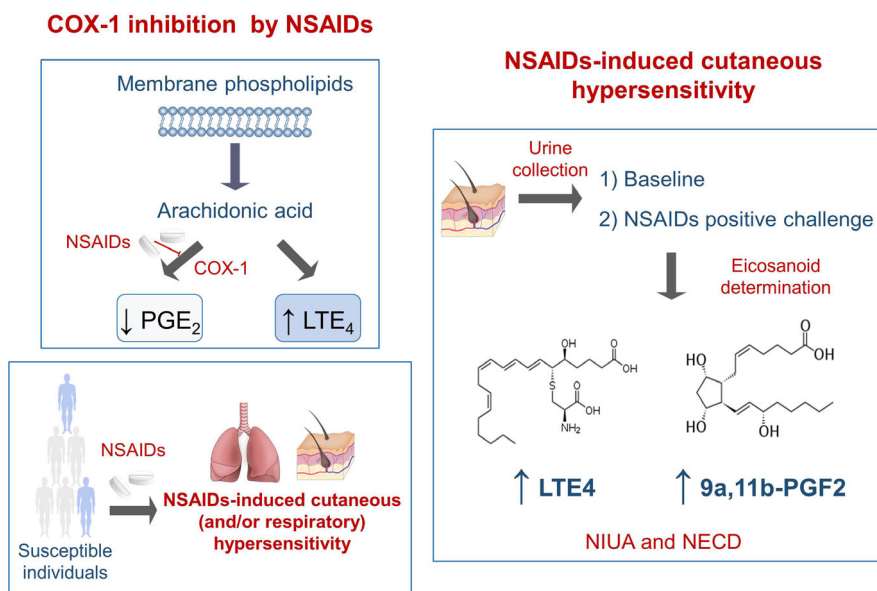
Methods: Urine samples were taken from patients with confirmed NSAID-induced urticaria and healthy controls, at baseline and at various time intervals after ASA administration. Eicosanoid measurement was performed using high-performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry.

Results: No differences were found between groups at baseline. Following ASA administration, LTE₄ and 9 α ,11 β -PGF₂ levels were increased in both NIUA and NECD patients compared to baseline, rising initially, before decreasing toward initial levels. In addition, the levels of these metabolites were higher in NIUA and NECD when compared with the SNIUAA and control groups after ASA administration. No changes were found with respect to baseline values for SNIUAA and control groups.

Conclusions: We present for the first time data regarding the role of COX-1 inhibition in NIUA. Patients with this entity show a similar pattern eicosanoid levels following ASA challenge to those with NECD. Further studies will help ascertain the cell populations involved and the underlying molecular mechanisms.

Abbreviations: ASA, acetyl salicylic acid; COX-1, cyclooxygenase 1; LTE₄, leukotriene E₄; NECD, NSAID-exacerbated cutaneous disease; NIUA, NSAID-induced urticaria/angioedema; NSAIDs, nonsteroidal anti-inflammatory drugs; PGE₂, prostaglandin E₂; 9 α ,11 β -PGF₂, 9 α ,11 β -prostaglandin F₂.

Inmaculada Doña and Raquel Jurado-Escobar contributed equally to this work.



GRAPHICAL ABSTRACT

The inhibition of COX-1 by NSAIDs shunts the metabolism of arachidonic acid from PGE₂ toward LTE₄ synthesis. After NSAIDs intake, some individuals develop cutaneous and/or respiratory hypersensitivity reactions. Although NIUA and NECD showed distinct urinary eicosanoid excretion profile curves, ASA challenge results in an increase of LTE₄ and 9α,11β-PGF₂ in both.

COX-1: Cyclooxygenase 1; LTE₄: Leukotriene E₄; NECD: NSAIDs-exacerbated cutaneous disease; NIUA: NSAIDs-induced urticaria/angioedema; NSAIDs: Nonsteroidal anti-inflammatory drugs; PGE₂: Prostaglandin E₂; 9α,11β-PGF₂: 9α,11β-prostaglandin F₂

1 | INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most highly consumed medicines worldwide,^{1,2} but they are also the main triggers of drug hypersensitivity reactions, with the skin being the most commonly affected organ.³⁻⁶ The latest classification of NSAID hypersensitivity from the European Academy of Allergy and Clinical Immunology is based on timing, clinical entities induced, and the presence or absence of cross-reactivity to other NSAIDs. Cross-reactive hypersensitivity can also be accompanied by underlying chronic cutaneous or respiratory disease, while selective (single-NSAID-induced) hypersensitivity cannot.⁷ Cross-reactive hypersensitivity is the most common pattern,⁸ with patients reacting to NSAIDs belonging to different chemical groups in the absence of specific immunological recognition. On the other hand, selective hypersensitivity occurs when patients develop a reaction against a single NSAID or to NSAIDs belonging to the same group, which in most cases is thought to occur through an IgE-mediated mechanism, with the most frequent clinical entity being single-NSAID-induced urticaria/angioedema or anaphylaxis (SNIUAA).^{7,9,10}

Cross-reactive hypersensitivity to NSAIDs includes at least three main phenotypes: NSAID-exacerbated respiratory disease (NERD, formerly known as aspirin-exacerbated respiratory disease or aspirin-intolerant asthma) in patients with rhinitis and/or asthma with or without nasal polyposis; NSAID-exacerbated cutaneous disease (NECD, also known as aspirin-exacerbated cutaneous disease) in patients with underlying chronic spontaneous urticaria; and NSAID-induced

urticaria/angioedema (NIUA, also known as aspirin-induced urticaria) in otherwise healthy individuals.⁷ According to the cyclooxygenase (COX)-1 hypothesis, the inhibition of this enzyme by NSAIDs shunts the metabolism of arachidonic acid from the production of prostaglandins toward the 5-lipoxygenase pathway, with the subsequent biosynthesis of cysteinyl (Cys)-LTs (LTC₄, LTD₄, and LTE₄) responsible for eliciting the clinical response in susceptible individuals.¹¹ CysLTs play a role in eosinophil chemotaxis,¹² vascular leakage,^{13,14} and airway remodeling,¹⁵ and are thought to be key proinflammatory mediators in cross-hypersensitivity to NSAIDs.¹⁶ The COX-1 hypothesis was first proposed to explain the etiology of NERD,¹¹ supported by studies that found increased concentrations of LTE₄ in urine,^{17,18} and nasal¹⁹ and bronchoalveolar lavage fluids²⁰ after oral aspirin (ASA) challenge in patients with this pathology. Other studies have found increased baseline levels of LTE₄.²¹⁻²⁴ In addition, the severity of the respiratory reaction during ASA oral challenge was associated with the degree of elevation of baseline urinary LTE₄.²⁵

Although less information is available for NECD regarding mediator release, increased basal urinary levels of LTE₄ have been described²⁶ as well as for 9α,11β-prostaglandin F₂ (9α,11β-PGF₂), the main metabolite of PGD₂, after ASA challenge.²⁷ Concerning NIUA, some genetic association studies suggest a role for arachidonic acid pathway abnormalities and COX-1 inhibition in the underlying mechanism.²⁸⁻³¹ However, there is a lack of studies concerning the participation of eicosanoid release in patients with NIUA in spite of it being the most frequent clinical entity induced by drug hypersensitivity.^{3,4,8}

In this study, we analyze the potential role of arachidonic acid metabolites in patients suffering from NIUA. We also included groups of patients with the other two phenotypes of NSAID-induced urticaria, NECD and SNIUAA. To the best of our knowledge, this is the first study that simultaneously analyzes metabolite levels in all these three groups.

2 | MATERIALS AND METHODS

2.1 | Subjects

We included patients aged 14–60 years with a confirmed diagnosis of urticaria induced by NSAID hypersensitivity who attended the Allergy Unit of the Malaga Regional University Hospital (Malaga, Spain) between 2014 and 2016. Cross-hypersensitive patients reported at least three episodes of urticaria after the intake of at least three NSAIDs belonging to different chemical groups. Patients who presented chronic spontaneous urticaria (reoccurrence of urticaria for more than 6 weeks with no external trigger)³² were classified as NECD, and those without a history of chronic spontaneous urticaria were classified as NIUA. Cross-reactive hypersensitivity was confirmed in both NIUA and NECD by a positive drug provocation test (DPT) to ASA. Those patients presenting episodes of urticaria to only one NSAID or to NSAIDs belonging to the same chemical group were diagnosed with SNIUAA if they gave a positive DPT to the culprit drug and a negative DPT to ASA. No patients had any history of anaphylaxis, rhinitis, asthma, or nasal polyposis (Figure S1).

We also included a control group containing individuals who did not take ASA before urine collection (Non-ASA Controls) and another control group of subjects that took ASA before urine collection (ASA Controls). All these individuals reported regularly taking NSAIDs without developing a clinical reaction and had no history of chronic spontaneous urticaria, drug hypersensitivity, rhinitis, and/or asthma or nasal polyposis.

All participants gave informed consent. The study was approved by the Ethics Committee of Malaga Regional University Hospital and conducted according to the principles of the Declaration of Helsinki.

2.2 | Dpt

DPT was performed in a single-blind manner as reported previously,⁸ by giving placebo capsules at different times on the 1st day. Drugs and placebo were given in opaque capsules prepared by the hospital pharmacy service. Other medications were withheld before testing, according to international guidelines.³³

For DPT to ASA, two doses were administered orally with an interval of 180 minutes (50 and 100 mg) on the 2nd day. If negative, two larger doses of ASA (250 and 500 mg) were administered on the 3rd day, with a 180-min interval. If cutaneous and/or respiratory symptoms or changes in vital signs (cardiac rhythm alterations, decrease in peak expiratory flow or hypotension)

appeared, the procedure was stopped and symptoms were evaluated and treated.³⁴ If no symptoms appeared during these periods, the therapeutic dose of ASA was achieved; this was followed by a 2-day/8-hours course of the therapeutic dose (500 mg) after a gap of 24 hours.³⁴

In addition to ASA DPT, SNIUAA patients were challenged with the culprit drug, at least one week after the ASA DPT. Drugs were given in increasing doses every 90 minutes, and if no symptoms appeared, this was followed by a 1-day/8-hours course or by a 2-day/8-hours course at the therapeutic dose after a gap of 24 hours. The doses administered were as follow: for ibuprofen 5, 50, 100, 200, and 250 mg on the 2nd day; for dipyrone 5, 10, and 50 mg during the 2nd day, and 50, 150, and 375 mg on the 3rd day; and for diclofenac 5, 15 and 30 mg on the 2nd day.⁸

Subjects included in the ASA Controls group were given a dose of 500 mg of ASA.

2.3 | Sample collection and determination of urinary eicosanoids

Urine samples from patients were collected at basal state, that is, before ASA DPT, and at three different time intervals after ASA DPT: T1, within the first three hours and after the appearance of symptoms; T2, within the third and sixth hour; and T3, within the sixth and ninth hour.

Concerning controls, urine samples from the ASA Controls group were also obtained before ASA administration (basal state) and at similar time intervals to patients after ASA administration (T1, T2, and T3). Samples were also obtained from the Non-ASA Controls group at these times, although ASA challenge was not performed. After collection, all urine samples were immediately stored at -80°C until assayed.

Urinary eicosanoid measurements were performed at the Division of Molecular Biology and Clinical Genetics, Jagiellonian University Medical School in Krakow (Poland) using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS²) for leukotriene E4 (LTE4) and for the following prostaglandin (PG) metabolites: 13,14-dihydro-15-keto-PGE2 (keto-PGE2), 13,14-dihydro-15-keto-tetranor-PGE2 (tetranor-PGE2), 13,14-dihydro-15-keto-PGD2 (keto-PGD2), 13,14-dihydro-15-keto-tetranor-PGD2 (tetranor-PGD2), tetranor-PGE-M, and tetranor-PGD-M. Gas chromatography-mass spectrometry (GC-MS) following chemical derivatization was used for measuring 9 α ,11 β -PGF2. All results were recalculated for urinary creatinine and expressed in pg/mg of creatinine in order to account for hydration status. Full analytical details have been published previously, and measurement was done on blinded samples.^{35,36}

2.4 | Statistical analysis

Descriptive statistics (frequency, mean and SD) are used to summarize data. Comparisons of eicosanoid levels between groups were performed using the Kruskal–Wallis test followed by Mann–Whitney when necessary. Basal and post-ASA DPT eicosanoids levels from

TABLE 1 Demographic and clinical data for patients

	Clinical entity	Gender	Age (y)	No. episodes	Drugs involved	Time interval drug intake reaction (min)
Pat. No. 1	NIUA	F	55	4	ASA, ibuprofen (2), dipyrone	30
Pat. No. 2	NIUA	M	53	4	ASA, diclofenac, dipyrone, ibuprofen	60
Pat. No. 3	NIUA	M	14	4	Dexketoprofen, ibuprofen, dipyrone, diclofenac	120
Pat. No. 4	NIUA	M	19	5	ASA, ibuprofen (3), diclofenac	15
Pat. No. 5	NIUA	F	34	3	Dipyrone, ibuprofen, piroxicam	20
Pat. No. 6	NIUA	M	15	3	ASA, ibuprofen, diclofenac	60
Pat. No. 7	NIUA	F	49	3	Dipyrone, ibuprofen, diclofenac	15
Pat. No. 8	NIUA	F	22	3	ASA, ibuprofen, dipyrone	20
Pat. No. 9	NIUA	F	15	3	ASA, diclofenac, dexketoprofen	15
Pat. No. 10	NIUA	F	27	3	ASA, dipyrone, ibuprofen	10
Pat. No. 11	NIUA	M	18	3	ASA, ibuprofen, dipyrone	60
Pat. No. 12	NIUA	M	48	3	Diclofenac, dipyrone, naproxen	15
Pat. No. 13	NIUA	F	55	5	ASA, ibuprofen (3), indomethacin	30
Pat. No. 14	NIUA	M	53	4	ASA, diclofenac, dipyrone, ibuprofen	60
Pat. No. 15	NIUA	M	14	4	ASA, ibuprofen, dexketoprofen, dipyrone	120
Pat. No. 16	NIUA	M	19	5	ASA, ibuprofen (3), indomethacin	15
Pat. No. 17	NIUA	F	34	3	Dipyrone, ibuprofen, diclofenac	20
Pat. No. 18	NIUA	M	15	4	ASA, ibuprofen (2), diclofenac	60
Pat. No. 19	NIUA	F	49	3	ASA, dipyrone, naproxen	15
Pat. No. 20	NIUA	F	22	3	ASA, ibuprofen, diclofenac	20
Pat. No. 21	NIUA	F	15	3	ASA, ibuprofen, dipyrone	15
Pat. No. 22	NIUA	F	27	3	Dipyrone, ibuprofen, lornoxicam	10
Pat. No. 23	NIUA	M	18	3	ASA, ibuprofen, aceclofenac	60
Pat. No. 24	NIUA	M	48	3	Diclofenac, dipyrone, naproxen	30
Pat. No. 25	NECD	F	15	3	ASA, ibuprofen, propifenazone	45
Pat. No. 26	NECD	M	52	3	ASA, dipyrone, ibuprofen	30
Pat. No. 27	NECD	F	15	3	Ibuprofen, dipyrone, diclofenac	30
Pat. No. 28	NECD	M	52	4	Ibuprofen (2), ASA, dipyrone	60
Pat. No. 29	NECD	F	15	3	Ibuprofen, dipyrone, diclofenac	120
Pat. No. 30	NECD	F	52	4	ASA, ibuprofen (2), dipyrone	60
Pat. No. 31	SNIUAA	F	42	2	Ibuprofen	25
Pat. No. 32	SNIUAA	F	37	3	Dipyrone	15
Pat. No. 33	SNIUAA	F	54	2	Diclofenac	30
Pat. No. 34	SNIUAA	M	42	2	Dipyrone	15
Pat. No. 35	SNIUAA	F	32	4	Dipyrone	30
Pat. No. 36	SNIUAA	M	24	2	Ibuprofen	15
Pat. No. 37	SNIUAA	M	48	2	Dipyrone	15

ASA, aspirin (acetylsalicylic acid); NECD, NSAID-exacerbated cutaneous disease; NIUA, NSAID-induced urticaria/angioedema; SNIUAA, single-NSAID-induced urticaria/angioedema or anaphylaxis.

the different groups were compared using one-way repeated-measures ANOVA followed by a paired 2-tailed t test if the ANOVA *p* value was <0.05. Correlation between variables was estimated with the Spearman correlation coefficient. All analyses were performed with GraphPad Prism version 7.04 for Windows (GraphPad Software, La Jolla, Calif; www.graphpad.com). All *P*-values ≤0.05 were considered statistically significant.

3 | RESULTS

3.1 | Clinical data

A total of 37 NSAID-hypersensitive patients were included: 24 NIUA (12 female), 6 NECD (4 female), and 7 SNIUAA (4 female). In addition, 42 healthy controls were included: 25 Non-ASA Controls (16

TABLE 2 Eicosanoid levels at basal state

	NIUA	NECD	SNIUAA	Non-ASA Controls	ASA controls	P-value
LTE4	52.3 ± 33.1	52.1 ± 26.3	44 ± 18.7	52.9 ± 15.9	51.4 ± 21.6	0.763
Keto-PGE2	261.9 ± 137.9	277.4 ± 67.4	280.5 ± 107.9	257.1 ± 107.9	262.1 ± 123.1	0.943
Tetranor-PGE2	401.7 ± 662.6	427.3 ± 231.8	381.5 ± 66.7	355.9 ± 294.6	411.6 ± 299.7	0.307
PGE-M	11593.6 ± 6259.8	11748.2 ± 5631.6	8955.1 ± 6542.9	9883.3 ± 6303.2	11389.5 ± 6392.1	0.735
Keto-PGD2	146.7 ± 74.5	159.4 ± 64.7	144.7 ± 19.1	128 ± 77.6	159.3 ± 120.1	0.774
Tetranor-PGD2	490.5 ± 160.5	480.6 ± 175.2	495.9 ± 174.6	481.2 ± 224.6	518.7 ± 203.1	0.979
PGD-M	3040.9 ± 1087.8	3491.6 ± 668.4	3321.4 ± 825.7	3180.3 ± 3040.2	3288.2 ± 1677.8	0.684
9α,11β-PGF2	439.5 ± 129.1	405.3 ± 259	408.4 ± 71.9	429 ± 104.4	420.4 ± 115.5	0.934

Results are expressed as pg/mg creatinine.

ASA, aspirin (acetylsalicylic acid); keto-PGE2, 13,14-dihydro-15-keto-PGE2; keto-PGD2, 13,14-dihydro-15-keto-PGD2; LTE4, leukotriene E4; NECD, NSAID-exacerbated cutaneous disease; NIUA, NSAID-induced urticaria/angioedema; SNIUAA, single-NSAID-induced urticaria/angioedema or anaphylaxis; PGE-M, tetranor-PGE-M; 9α,11β-PGF2, 9α,11β-prostaglandin F2; PGD-M, tetranor-PGD-M; tetranor-PGE2, 13,14-dihydro-15-keto-tetranor-PGE2; tetranor-PGD2, 13,14-dihydro-15-keto-tetranor-PGD2.

female) and 17 ASA Controls (10 female). No gender differences were found between groups ($P = 0.882$). No statistically significant differences were found between groups in terms of age (30.8 ± 15.9 years for NIUA; 33.5 ± 20.3 years for NECD; 39.9 ± 9.9 years for SNIUAA; 32.7 ± 6.0 years for Non-ASA Controls; and 34.3 ± 6.1 for ASA Controls; $P = 0.360$; Table S1). We did not find significant differences in the mean number of episodes reported in the clinical history between the three groups of patients (3.5 ± 0.7 for NIUA, 3.3 ± 0.5 for NECD, and 2.4 ± 0.8 for SNIUAA; $P = 0.767$; Table S1). Although the time interval between drug administration and the appearance of clinical symptoms following DPT was lower in SNIUAA (20.0 ± 7.3 minutes) when compared with NIUA and NECD (37.3 ± 31.7 and 57.5 ± 33.4 minutes, respectively), these differences were not statistically significant ($P = 0.158$; Table S1). More detailed information is included in Table 1.

3.2 | Eicosanoid levels at basal state

We did not find any significant statistical differences for LTE4 at baseline between the different groups ($P = 0.763$; Table 2). In addition, no differences were found between groups for either PGE2 metabolites ($P = 0.943$ for keto-PGE2; $P = 0.307$ for tetranor-PGE2; and $P = 0.735$ for PGE-M) or PGD2 metabolites ($P = 0.774$ for keto-PGD2; $P = 0.979$ for tetranor-PGD2; $P = 0.684$ for PGD-M; and $P = 0.934$ for 9α,11β-PGF2; Table 3).

3.3 | Eicosanoids levels between groups after ASA DPT

We did not find any differences between Non-ASA Controls and ASA Controls at any time interval for any of the eicosanoids measured (Table S2); therefore, we only used the ASA Controls group for subsequent comparisons with patient groups. We did not find differences between SNIUAA and ASA Controls at any time interval for any of the mediators included in this study, as shown in Figures 1 and Figure S2.

Among all the metabolites tested, we found statistically significant differences after DPT with ASA for LTE4 and 9α,11β-PGF2 when considering all four groups. However, no differences were found for these metabolites between NIUA and NECD at any time interval (Table 3). Although tetranor-PGE-M showed a tendency to decrease after ASA challenge, these differences were not statistically significant (Figure S2).

Concerning LTE4 levels, there was an increase at T1 for both NIUA and NECD when compared to the ASA Controls group ($P = 0.004$ and $P = 0.011$, respectively) and the SNIUAA group ($P = 0.006$ and $P = 0.012$; Table 3). These levels were also higher at T2 for NIUA and NECD ($P = 0.011$ and $P = 0.017$, compared to ASA Controls; and $P = 0.045$ and $P = 0.020$, compared to SNIUAA; Table 3). In addition, LTE4 levels were still higher in NECD patients at T3 when compared to the ASA Controls and SNIUAA groups ($P = 0.009$ and $P = 0.050$, respectively; Table 3).

At T1, the levels of 9α,11β-PGF2 were increased in NIUA and NECD patients when compared to ASA Controls ($P = 0.005$ and 0.003 , respectively) and to SNIUAA ($P = 0.021$ and $P = 0.05$, respectively). These levels were still significantly increased at T2 in NIUA and NECD compared to ASA Controls ($P = 0.014$ and $P = 0.05$) and to SNIUAA ($P = 0.012$ and $P = 0.023$; Table 3).

3.4 | Time course of eicosanoid levels after ASA DPT

Concerning LTE4 levels, there was a statistically significant increase for NIUA patients from the basal state to T1 (52.3 ± 33.1 to 131.9 ± 128.8 pg/mg creatinine; $P = 0.007$), which remained significantly increased at T2 (96.1 ± 86.8 pg/mg creatinine; $P = 0.028$), with no differences at T3 (72.1 ± 47.5 pg/mg creatinine; Tables 2 and 3; and Figure 1, P -values not shown in figure). In addition, differences were also significantly lower at T2 compared to T1, and at T3 compared to T2 ($P = 0.047$ and $P = 0.017$; Table 3 and Figure 1, P -values not shown in figure). Similarly, for NECD we also found that LTE4 levels were higher at T1 and T2 with respect to basal state

TABLE 3 LTE4 and 9 α ,11 β -PGF2 levels after ASA administration

	Time	NIUA	NECD	SNIUAA	ASA Controls	NIUA vs ASA Controls P-value	NECD vs ASA Controls	SNIUAA vs ASA Controls	NIUA vs NECD	NIUA vs SNIUAA	NECD vs SNIUAA
LTE4 (pg/mg creatinine)	T1	131.9 $\pm$ 128.8	158.9 $\pm$ 71.1	49.7 $\pm$ 22.2	46.8 $\pm$ 20.2	0.004	0.011	0.755	0.629	0.006	0.012
	T2	96.1 $\pm$ 86.8	161.9 $\pm$ 80.9	53.1 $\pm$ 27.3	46.1 $\pm$ 19.7	0.011	0.017	0.487	0.104	0.045	0.020
	T3	72.1 $\pm$ 47.5	91.4 $\pm$ 42.2	47.1 $\pm$ 22.1	47.5 $\pm$ 28.1	0.062	0.009	0.972	0.372	0.190	0.050
9 α ,11 β -PGF2 (pg/mg creatinine)	T1	625.1 $\pm$ 329.9	739.5 $\pm$ 303.9	388.8 $\pm$ 173.8	373.3 $\pm$ 133.9	0.005	0.003	0.815	0.469	0.021	0.050
	T2	607.6 $\pm$ 328.5	810.6 $\pm$ 402.1	394.1 $\pm$ 114.6	410.6 $\pm$ 143.4	0.014	0.059	0.790	0.205	0.012	0.023
	T3	452.1 $\pm$ 257.8	463.4 $\pm$ 292.1	402.4 $\pm$ 159.1	397.3 $\pm$ 151.6	0.438	0.483	0.942	0.926	0.635	0.642

Bold value indicates statistically significant differences.

Results are expressed as pg/mg creatinine.

ASA, aspirin (acetylsalicylic acid); LTE4, leukotriene E4; NECD, NSAID-exacerbated cutaneous disease; NIUA, NSAID-induced urticaria/angioedema; SNIUAA, single-NSAID-induced urticaria/angioedema or anaphylaxis; 9 α ,11 β -PGF2, 9 α ,11 β -prostaglandin F2.

(52.1 $\pm$ 26.3 pg/mg creatinine at baseline, 158.9 $\pm$ 71.1 pg/mg creatinine at T1, and 161.9 $\pm$ 80.9 pg/mg creatinine at T2; $P = 0.016$ and $P = 0.024$, respectively). In contrast to NIUA patients, LTE4 levels were still increased at T3 with respect to basal state (91.4 $\pm$ 42.2 pg/mg creatinine; $P = 0.043$; Tables 2 and 3; and Figure 1, P -values not shown in figure). Although these levels were higher in NECD patients at T1 and T2 with respect to T3, differences were not statistically significant ($P = 0.051$ and $P = 0.071$, respectively; Figure 1, P -values not shown).

Levels of 9 α ,11 β -PGF2 were increased in NIUA patients when compared to basal state at T1 and T2 (439.5 $\pm$ 129.1 pg/mg creatinine at baseline, 625.1 $\pm$ 329.9 pg/mg creatinine at T1, and 607.6 $\pm$ 328.5 pg/mg creatinine at T2; $P = 0.005$ and $P = 0.007$, respectively; Tables 2 and 3; and Figure 1, P -values not shown in figure). In addition, these levels were significantly higher at T1 and T2 with respect to T3 (452.1 $\pm$ 257.8 pg/mg creatinine at T3; $P = 0.001$ and $P = 0.018$, respectively; Table 2 and Figure 1, P -values not shown in figure). For NECD, 9 α ,11 β -PGF2 levels were also increased at T1 and T2 (405.3 $\pm$ 259 pg/mg creatinine at baseline, 739.5 $\pm$ 309.9 pg/mg creatinine at T1, and 810.6 $\pm$ 402.1 pg/mg creatinine at T2; $P = 0.05$ and $P = 0.017$, respectively; Tables 2 and 3; and Figure 1, P -values not shown in figure). These levels were also higher in both T1 and T2 with respect to T3 (463.4 $\pm$ 292.1 pg/mg creatinine at T3; $P = 0.02$ and $P = 0.019$, respectively; Table 2 and Figure 1, P -values not shown).

3.5 | Correlation analysis between variables

We found a positive correlation between LTE4 and 9 α ,11 β -PGF2 at T1 for NIUA patients (Spearman $r = 0.439$, $P = 0.032$). No correlations were found between any of the other eicosanoid metabolites.

4 | DISCUSSION

The inhibition of COX-1 and the subsequent generation of inflammatory mediators from the arachidonic acid pathway have long been recognized as holding a central role in the etiopathogenesis of NERD and are also thought to participate in NECD.^{16,37,38} This mechanism has also been hypothesized to extend to NIUA, even in the absence of experimental data.⁷

Although different biological fluids are suitable for eicosanoid quantification in NSAID hypersensitivity,^{39–43} urinary LTE4 determination is the best validated model.^{44–47} In recent years, the utility of 9 α ,11 β -PGF2, the stable urinary metabolite of PGD2, has received great interest as a potential biomarker of NSAID hypersensitivity.^{48,49}

In this study, we provide for the first time information concerning urinary eicosanoids levels, including both LTE4 and 9 α ,11 β -PGF2, in patients with NIUA. We simultaneously analyzed such levels in patients suffering from NECD, the other type of NSAID-induced urticaria by cross-reactive hypersensitivity.⁷ In addition, we also included a group of patients with SNIUAA, the third type of NSAID-induced urticaria, even though this entity is thought to be due to an

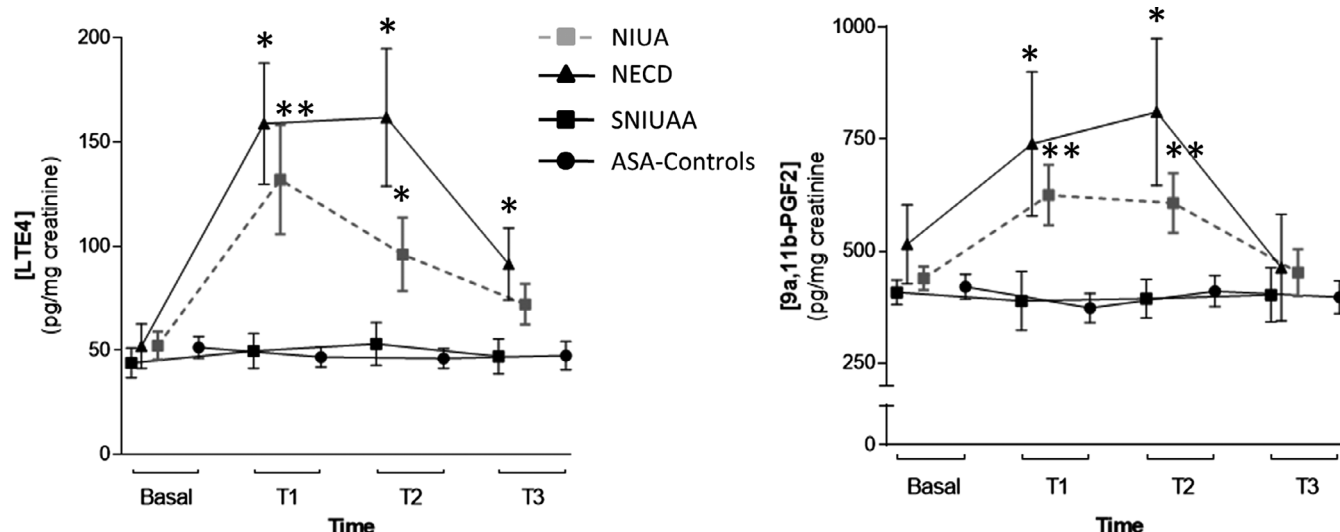


FIGURE 1 Leukotriene E4 and $9\alpha,11\beta$ -prostaglandin F2 in patients with NSAID-induced urticaria and ASA Controls (* $P \leq 0.05$, ** $P < 0.01$). ASA, aspirin (acetylsalicylic acid); LTE4, leukotriene E4; NECD, NSAID-exacerbated cutaneous disease; NIUA, NSAID-induced urticaria/angioedema; SNIUAA, single-NSAID-induced urticaria/angioedema or anaphylaxis; $9\alpha,11\beta$ -PGF2, $9\alpha,11\beta$ -prostaglandin F2

immunological IgE-mediated reaction.⁵⁰ Our results highlight similarities and differences in the role of eicosanoids in the three recognized types of NSAID-induced immediate urticaria.⁵⁰

Of all metabolites analyzed in this study, we only found significant differences for LTE4 and $9\alpha,11\beta$ -PGF2. These were found for the NIUA and NECD groups with respect to both the ASA Controls and the SNIUAA groups. Concerning SNIUAA, we did not find any statistically significant differences with respect to the ASA Controls group. Although this phenotype is widely accepted to be induced by an immunological mechanism rather than being related to COX-1 inhibition, nevertheless we felt it important to explore the potential participation of eicosanoids in this group, as occurs in related pathologies.⁵¹ In fact, increased urinary LTE4 and $9\alpha,11\beta$ -PGF2 levels have been reported in a patient suffering from anaphylaxis to salicylamide characterized by generalized urticaria, angioedema, lacrimation, abdominal pain, dyspnea, and throat tightness.⁵² However, our SNIUAA patients only developed isolated urticaria after NSAIDs intake without other organ system involvement and eicosanoid release differs depending on the severity of the reaction.^{25,53}

We have not found statistically significant differences between any of the four groups considered at the basal state. These results highlight an important difference between NIUA and NERD, for which high baseline LTE4 levels have been repeatedly reported^{17,22,44,45,54,55} which may be a reflection of the underlying respiratory disease.⁵⁶ This is the first time such information has been made available for NIUA; however, contrasting results exist for NECD. Di Lorenzo et al⁵⁷ did not report baseline differences for LTE4 in patients with chronic urticaria and hypersensitivity to ASA or food additives, whereas Mastalerz et al⁴⁹ reported that baseline LTE4 levels were increased in NECD patients with a positive ASA challenge with respect to those with a negative ASA challenge; however, they found no changes for $9\alpha,11\beta$ -PGF2. No changes at basal state were reported for these two mediators in another study.²⁷

We have previously described time-course variations in nasal lavages in NERD that were not found in NIUA.⁴² However, in this study ASA was not administered orally but by nasal provocation test with Lys-ASA, with most NIUA patients giving a negative result, and those cases with a positive test not developing an overt clinical reaction.⁴² In the present study, we demonstrate that patients with NIUA and NECD show a different urinary eicosanoids profile after oral ASA challenge compared with SNIUAA and ASA Controls. LTE4 levels were found to be higher in NIUA and NECD patients at T1 with respect to both groups. Whereas NECD showed similar LTE4 levels at T1 and T2, only starting to decrease after T2, in NIUA there was a sharp decline at T2, which continued until T3. For both NIUA and NECD, urinary LTE4 appeared to be returning to basal state levels at T3. Concerning $9\alpha,11\beta$ -PGF2 levels, they were increased with respect to their basal state values at T1 and T2 in both NIUA and NECD and also with respect to the SNIUAA and ASA Control values at these time intervals, decreasing markedly after T2.

Our results for NIUA and NECD share some similarities with those reported by other authors for NERD and NECD. A significant increase in urinary LTE4 at similar time intervals to T1 and T2 over basal levels has been described for NERD after ASA challenge together with a higher level of $9\alpha,11\beta$ -PGF2 at T1, which correlated with that of LTE4.⁵⁵ Urinary levels of LTE4 and $9\alpha,11\beta$ -PGF2 were also increased with respect to baseline three hours after ASA challenge in NERD in another study.⁴⁵ For NERD, an increase in urinary LTE4 above baseline has been reported following administration of intravenous Lys-ASA during the first three hours, and $9\alpha,11\beta$ -PGF2 also increased at similar time intervals to T1 and T2.²² The $9\alpha,11\beta$ -PGF2 metabolite has been reported to increase at around three hours following administration in NECD patients,²⁷ whereas another study showed a sustained rise of urinary LTE4 until 6 hours after ASA challenge as well as an early increase in plasma $9\alpha,11\beta$ -PGF2, but with no changes in urine.⁵⁸

Interestingly, although we found that for NIUA and NECD both LTE4 and 9a,11b-PGF2 increased to similar levels at T1, after this time interval they showed different behaviors: for NIUA, levels decreased at T2 and T3, while for NECD, they increased slightly at T2 followed by a decrease at T3. Thus, even if we did not find statistically significant differences for these mediators when comparing NIUA and NECD at any time, their excretion profile curves were different in magnitude, with a longer interval of mediator release in NECD. Levels of 9a,11b-PGF2 have also been shown to increase from basal state and to be sustained at similar time intervals to T1 and T2 after ASA challenge in AIA,⁴⁶ as well as another PGD2 metabolite.^{46,59} However, the reasons for these different profiles of mediator release are currently unknown. The depletion of protective PGE2 by NSAIDs may promote the unrestrained synthesis of CysLTs and the release of such mediators as PGD2 metabolites from mast cells, a process that may be enhanced and long-lasting in NECD by additional elements which could include sensitization to autoantibodies or the existence of histamine-releasing factors.^{60,61}

Another interesting control group could include patients with CSU that take NSAIDs without an aggravation of their underlying disease. However, LTE4 urinary levels have been shown not to change in CSU patients without ASA or food additive hypersensitivity after specific challenge, whereas they increased in CSU individuals with ASA or food additive hypersensitivity.⁵⁷ Another study by the same group also showed similar levels of LTE4 in CSU patients without NSAID hypersensitivity before and after ASA challenge, with no differences compared to a control group. However, LTE4 was significantly increased in NECD patients after ASA intake compared to basal levels and to controls.⁶² Nevertheless, CSU and NECD may share some characteristics. For example, CSU patients showed positivity to the autologous serum skin test,^{63–66} which has also been associated with NECD.^{67,68} It has been proposed that NECD should be considered as a clinically distinct phenotype to CSU, as the former has been shown to exhibit a significantly longer duration of the disease, and a higher prevalence of angioedema and atopy, which could suggest a different pathogenic mechanism and/or a more severe disease.⁶⁹

In conclusion, our study provides, for the first time, strong evidence for the participation of COX-1 inhibition and the generation of proinflammatory eicosanoid mediators in NIUA, with all previous studies having been carried out with NERD and NECD patients. In addition, cross-reactive hypersensitivity to NSAIDs may involve additional sources of inflammatory mediators, such as eosinophils and platelets,⁷⁰ which may potentially play different roles in NIUA and NECD. Further studies are required to shed light on the cells populations involved and the underlying mechanisms at the molecular level.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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SUPPORTING INFORMATION

TITLE: EICOSANOID MEDIATOR PROFILES IN DIFFERENT PHENOTYPES OF NONSTEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED URTICARIA.

AUTHORS: Inmaculada Doña, MD PhD^{1,2*}; Raquel Jurado-Escobar, BSc^{3*}; James R. Perkins, PhD³; Pedro Ayuso, PhD³; María Carmen Plaza-Serón, PhD³; Natalia Pérez-Sánchez, MD¹; Paloma Campo, MD PhD^{1,2}; Gador Bogas-Herrera, MD PhD¹; Joan Bartra, MD PhD^{4,5,6}; María José Torres^{1,2}; Marek Sanak, MD PhD⁷; José Antonio Cornejo-García, PhD^{2,3}.

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Supplementary Table S1. Clinical and demographic data for patients and controls.

	NIUA (n=24)	NECD (n=6)	SNIUAA (n=7)	Non ASA- Controls (n=25)	ASA- Controls (n=17)	p- value
Gender (female), n (%)	12 (50)	4 (66.7)	4 (57.1)	16 (64)	10 (58.8)	0.882
Age (years), mean $\pm$ SD	30.8 $\pm$ 15.9	33.5 $\pm$ 20.3	39.9 $\pm$ 9.9	32.7 $\pm$ 6	34.3 $\pm$ 6.1	0.360
No. of episodes, mean $\pm$ SD	3.5 $\pm$ 1.1	3.3 $\pm$ 0.5	2.4 $\pm$ 0.8	NA	NA	0.767
Culprit NSAIDs, No. of episodes, n (%)						
<i>Propionic acid derivatives</i>	34 (40.5)	8 (40)	4 (23.5)	NA	NA	0.647
<i>Acetylsalicylic acid</i>	17 (20.2)	4 (20)	-	NA	NA	0.187
<i>Pyrazolones</i>	16 (19)	6 (30)	11 (64.7)	NA	NA	0.028
<i>Acetic acid derivatives</i>	13 (15.5)	2 (10)	2 (11.8)	NA	NA	0.829
<i>Others</i>	4 (4.8)	-	-			0.419
Time interval DPT-symptoms (min), mean $\pm$ SD	37.3 $\pm$ 31.7	57.5 $\pm$ 33.4	20.7 $\pm$ 7.3	NA	NA	0.158

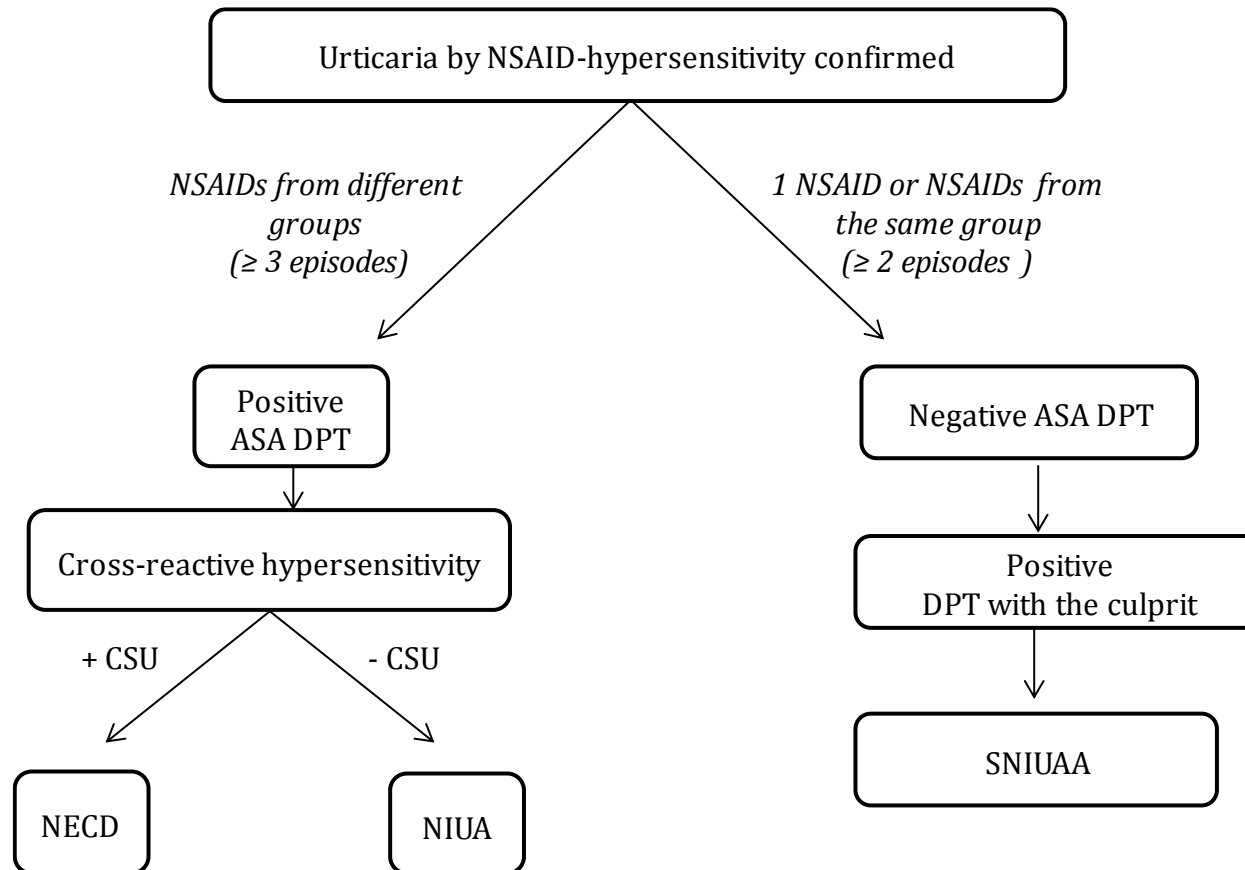
Abbreviations: **ASA**, aspirin (acetylsalicylic acid); **DPT**, drug provocation test; **NECD**, NSAIDs-exacerbated cutaneous disease; **NIUA**, NSAIDs-induced urticaria/angioedema; **SNIUAA**, single-NSAID-induced urticaria/angioedema or anaphylaxis.

Supplementary Table S2. Eicosanoid levels in Non ASA-Controls and ASA-Controls. Results are expressed as pg/mg creatinine.

Mediators (pg/mg creatinine)	Time	Non ASA- Controls	ASA-Controls	p-value
LTE4	T1	51.9±16.7	46.8±20.2	0.373
	T2	54.6±23.1	46.1±19.7	0.222
	T3	51.7±25.3	47.5±28.1	0.612
Keto-PGE2	T1	246.4±98.3	252.4±82.2	0.838
	T2	255.6±75.1	241.3±86.1	0.573
	T3	244.3±48.6	240.5±151.6	0.908
Tetranor- PGE2	T1	338.4±261.1	384.7±271.3	0.582
	T2	343.5±195.3	400.9±248.9	0.408
	T3	335.5±266.4	360.1±299.7	0.782
PGE-M	T1	9938.6±5396.1	10091.4±6401.9	0.934
	T2	11031.2±9823.2	9832.5±5859.3	0.655
	T3	9844.8±6882.1	10682.9±6127.3	0.688
Keto-PGD2	T1	137.4±44.1	147.1±41.5	0.475
	T2	137.7±42.9	152.7±41.2	0.263
	T3	137.5±73.1	152.3±76.9	0.533
Tetranor- PGD2	T1	512.3±430.1	539.9±371.4	0.830
	T2	524.4±264.7	516.1±245.2	0.918
	T3	464.4±267.6	515.9±174.3	0.489
PGD-M	T1	3306.4±1781.3	3396.3±1872.4	0.876
	T2	3244.5±2174.9	3318.1±1753.4	0.908
	T3	2891.1±2169.8	3168.9±1209.5	0.635
9 α ,11 β -PGF2	T1	417.1±146.1	373.3±133.9	0.330
	T2	441.8±132.3	410.6±143.4	0.473
	T3	443.9±142.6	397.3±151.6	0.317

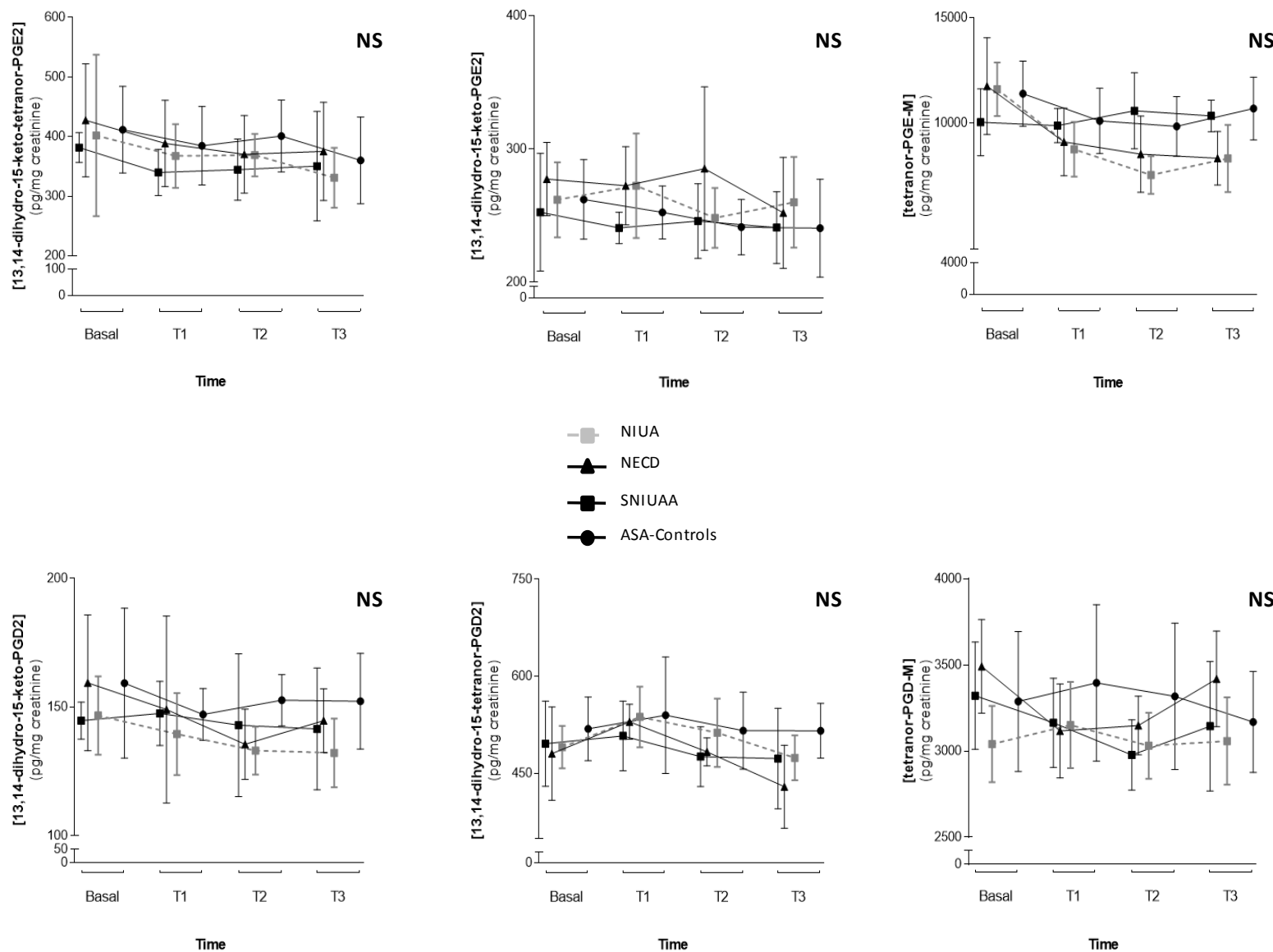
Abbreviations: ASA, aspirin (acetylsalicylic acid); **keto-PGE2**, 13,14-dihydro-15-keto-PGE2; **keto-PGD2**, 13,14-dihydro-15-keto-PGD2; **LTE4**, leukotriene E4; **NECD**, NSAIDs-exacerbated cutaneous disease; **NIUA**, NSAIDs-induced urticaria/angioedema; **SNIUAA**, single-NSAID-induced urticaria/angioedema or anaphylaxis; **PGE-M**, tetranor-PGE-M; **9 α ,11 β -PGF2**, 9 α ,11 β -prostaglandin F2; **PGD-M**, tetranor-PGD-M; **tetranor-PGE2**, 13,14-dihydro-15-keto-tetranor-PGE2; **tetranor-PGD2**, 13,14-dihydro-15-keto-tetranor-PGD2.

SUPPLEMENTARY FIGURE S1. Patients' selection.



Abbreviations: **ASA**, aspirin (acetylsalicylic acid); **CSU**, chronic spontaneous urticaria; **DPT**, drug provocation test; **NIUA**, NSAIDs-induced urticaria/angioedema; **SNIUAA**, single-NSAID-induced urticaria/angioedema or anaphylaxis.

SUPPLEMENTARY FIGURE S2. Prostaglandin E2 and D2 metabolites in patients with NSAIDs-induced urticaria and ASA-Controls.



Abbreviations: **ASA**, aspirin (acetylsalicylic acid); **keto-PGE2**, 13,14-dihydro-15-keto-PGE2; **keto-PGD2**, 13,14-dihydro-15-keto-PGD2; **NECD**, NSAIDs-exacerbated cutaneous disease; **NIUA**, NSAIDs-induced urticaria/angioedema; **NS**, not significant; **SNIUAA**, single-NSAID-induced urticaria/angioedema or anaphylaxis; **PGE-M**, tetranor-PGE-M; **PGD-M**, tetranor-PGD-M; **tetranor-PGE2**, 13,14-dihydro-15-keto-tetranor-PGE2; **tetranor-PGD2**, 13,14-dihydro-15-keto-tetranor-PGD2.