Small non-coding RNA profile in anaphylaxis

To the Editor,

Anaphylaxis is the most severe manifestation of allergic disorders. This consists of a severe systemic hypersensitivity reaction that evolves rapidly and can provoke the patient's death. When it happens, a series of signs and symptoms can affect the cutaneous, cardiovascular, respiratory, digestive and nervous systems. Currently, the treatment of choice is the administration of adrenaline [1]. The pathophysiological mechanisms by which the anaphylactic reaction occurs are mainly due to the release of mediators by mast cells and basophils. This degranulation is mostly induced in an IgE-dependent manner by antigens of different nature (food, drugs, stings ...). However, it is necessary to increase the knowledge of the underlying molecular bases considering other endotypes of anaphylaxis have been described [2]. Since the diagnosis of this condition is based on clinical symptoms which can be common to other pathologies, diagnosis should be supported with the identification of *in vitro* biomarkers. Currently, in clinical practice, the only one used is serum tryptase, but it does not correlate with a large part of the cases and in food anaphylaxis practically does not increase. Therefore, it is necessary to find more reliable and stable markers which could even be related to the severity of the reaction [3].

Non-coding RNA (ncRNA) were considered for many years as 'junk' DNA, although it is now known to be functional. This ncRNA can be classified by length: small-ncRNA (sncRNA), when they have 18-200 nucleotides, and long-ncRNA (lncRNA) when they have >200. This sncRNA correspond to microRNA, piRNA, transference RNA derived fragments (tRF), YRNA derived fragments (yRF), small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA) [4]. Among these, the most studied are microRNAs. However, knowledge of the rest of sncRNA, although rare, has increased in recent years and has been related to epigenetic regulation, DNA stability, transcription and translation control [5]. Furthermore, those molecules participate in cell-cell communication modulating several physiological and pathological processes [6]. SncRNA are very stable, resistant to degradation in body fluids and highly correlated with the pathological situation. Besides, they are measurable, have lower complexity and there are not known post-processing modifications. Thus, they have been postulated as potential diagnostic/prognostic non-invasive biomarkers of several pathologies [6,7]. Moreover, diverse clinical trials are investigating their therapeutic potential [7]. Recently, their role

in various pathological processes such as cancer [4] and cardiovascular diseases has been studied [7]. In the allergy field, most of the research done is focused on microRNA and lncRNA. However, although their role in anaphylaxis has never been studied, other sncRNA have also been associated with asthma [8]. Specifically, Isidoro-García *et al*. observed that allergic patients had a differential YRNA profile [9]. Besides, a greater knowledge of ncRNAs could increase the understanding of the etiopathogenesis of allergic diseases and their possible use as diagnostic biomarkers [8].

Therefore, in order to carry out both biological functional assignment and clinical translation strategies, our purpose is to analyze the expression profile of sncRNA in anaphylactic patients. To perform this study, serum was obtained from 5 children (<18 years) and 5 adults (> 18) collected from controlled challenge tests and the emergency department. These samples were extracted from each patient in two different situations: during anaphylaxis (acute) and its baseline (14 days after the reaction) (basal). Subjects were included after providing informed consent, and the study was approved by the IIS-FJD ethics committee. Anaphylaxis were triggered by food or drugs and graded as moderate/severe according to their clinical features confirmed by allergists [10]. Demographic characteristics and details of the reactions are shown in **Table1**. Sera were sent to the Qiagen Genomics Service where RNA was extracted, retro-transcribed and quantified by Next Generation Sequencing (NGS). In addition, libraries and technique quality controls were carried out from all the samples. NGS is a powerful transcriptomic tool that allows to characterize the ncRNA expression profile and their capacity as biomarkers [6].

In total, 2037 sncRNA were identified which 42 were piRNA, 364 snoRNA, 504 snRNA, 539 tRF and 588 yRF. The distribution is shown in **Fig.1A**. Through a statistical analysis, those sncRNA that did not present significant differences ($p < 0.05$) between acute and basal phases, were discarded. The normalization of the obtained data was carried out using two different approaches: by trimmed mean of M-values as independent values (TMM) and pairing sample-data from both phases in each patient (Paired). Among those filtered as significant, the distribution observed in children is similar applying both normalization methods, being 88 (TMM) and 80 (Paired) sncRNA detected including many tRF and any piRNA (**Fig.1B**). However, adults' samples showed a reduced total number of sncRNA, indicating less similarities by applying both methods, TMM (22) and

Paired (33) (**Fig.1C**). A detailed list indicating the statistically different sncRNA is shown in **Table2.**

Our data reveal differences in the sncRNA expression pattern profiles in anaphylaxis serum samples. tRF were the group with the greatest variations detected in children. Those participate in gene suppression, apoptosis, epigenetic inheritance and most importantly, they have been associated with inflammatory processes [5,6]. yRF are the secondary group detected with the largest variations presenting a relevant expression in anaphylaxis adult samples. They are related to apoptotic processes and pathologies such as cancer and cardiovascular diseases [5,9]. Absolute numbers of snRNA and snoRNA levels were homogeneous between both groups. Finally, piRNA are almost absent compared to the rest of the sncRNA and essentially none of them are statistically different. The fact that piRNA mainly regulate the germ line would explain the very low levels found in serum samples, although they have been detected in several differentiated cells [8].

TMM normalization would favor the identification of diagnostic biomarkers for anaphylaxis, considering brute values of each situation (acute and basal). However, paired normalization would contribute to a better interpretation of the molecular basis carried out in these reactions. Therefore, divergences in patterns observed in our study would be attributed to different reasons. The detection of statistically differential sncRNA is higher in the children group, suggesting that those molecules may play a larger role in the regulation of the anaphylactic molecular bases in the childish frame-time. In addition, adults are more likely to suffer severe reactions than children [2] and the scarce number of significant sncRNA found in adults may suggest the loss of serum sncRNA throughout patients' lifetime. In fact, the small number of sncRNA found in this group shows different patterns when normalizing by TMM or paired. On the other hand, the studied population of children reactions were elicited by food while in adults were drugs. Most importantly, drugs could favor non IgE underlying mechanisms while food are IgE related. This fact indicates that sncRNA pattern differences observed could be attributed to variations associated with triggers and/or their underlying molecular mechanisms pointing to tRF as candidates playing a role under food anaphylaxis.

In conclusion, this study shows the first identification of the sncRNA profile in sera from anaphylactic patients. The results obtained suggest that sncRNA could participate in the regulation of the underlying molecular bases of anaphylaxis. However, knowledge of the

function of these sncRNA is still poor. Moreover, they are very stable, easy and fast molecules to obtain and they reflect the pathological state of the individual making them promising prognosis and diagnostic biomarkers. However, it would be necessary to extend the data obtained in an independent larger cohort of patients with a greater number of samples.

Conflict of interest statement:

The authors declare no competing financial interests except PRR that received reports honoraria for lectures from ALK-Abello, FAES, LETI, Merck, Allergy Therapeutics and MEDA Pharma.

Funding information:

This research was funded by the Spanish Government (Ramón y Cajal Program 12880-2013) and grants from the Instituto de Salud Carlos III (PI16/00888 and PI18/00348) co-supported by FEDER Thematic Networks and Cooperative Research Centers: ARADYAL (RD16/0006/0013, RD16/0006/0033, RD16/0006/0026). E. N-B is granted by funding from the Community of Madrid included in the project FOOD-AL (FOODAL-CM_P2018/BAAA-4574).

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Figure and Table legends

Figure 1. Graphical distribution of total sncRNA and with statistical differences between acute and basal phases. (**A**) Percentage distribution of total sncRNA identified by NGS in 10 anaphylactic serum samples. piRNA: 42, snoRNA: 364, snRNA: 504, tRF: 539, yRF: 588. (**B**) Percentage distribution of significant sncRNA in anaphylactic children serum samples. TMM: 0 piRNA, 5 snoRNA, 6 yRF, 7 snRNA, 70 tRF. Paired: 0 piRNA, 4 snoRNA, 6 snRNA, 16 yRF, 54 tRF. (**C**) Percentage distribution of significant sncRNA in anaphylactic adults' serum samples. TMM: 0 piRNA, 2 tRF, 6 snRNA, 7 snoRNA, 7 yRF. Paired: 1 piRNA, 1 snRNA, 4 snoRNA, 7 tRF, 20 yRF. TMM = Normalization by trimmed mean of M-values. Paired = Normalization by each patient**.**

Table 1. Clinical features of anaphylactic reactions included in this study. Gender: M (Male), F (Female). Clinical symptoms: Skin: E (Erythema), U (Urticaria), Pr (Prurito), Sw (Sweating); Mucous: Cj (Conjunctivitis), Ag (Angioedema); Respiratory: Dp (Dyspnoea), W (Wheezing), R (Rhinitis), Cg (Cough); Digestive: N (Nausea), V (Vomit), Dr (Diarrhoea), Ab (Abdominal pain); Vascular: Hy (Hypotension), Sy (Syncope), Pl (Paleness), T (Tachycardia); Neurological: Dz (Dizziness), He (Headache). Clinical signs: Fc (Heart rate), spO2 (Oxygen saturation).

Table 2. List of statistically significant differential sncRNA detected in serum from anaphylactic patients. FC = Logarithm of fold change. Pval = Level of significance of the p-value. TMM = Normalization by trimmed mean of M-values. Paired = Normalization by each patient**.**