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Basal Tryptase High Levels Associated with a History of Arterial Hypertension and Hypercholesterolemia Represent Risk Factors for Severe Anaphylaxis in Hymenoptera Venom-Allergic Subjects over 50 Years Old

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Keywords
Hymenoptera venom · Tryptase · Age · Arterial hypertension · Hypercholesterolemia

Abstract

Introduction: Allergy to Hymenoptera venom (HV) may lead to life-threatening anaphylaxis. Some of the factors influencing the symptom's severity are still undetermined. The aim of this study was to identify the clinical aspects associated with the most severe reactions in a population with HV allergy, by comparing clinical and immunochemical biomarkers between patients with previous local large reactions (LLRs) and systemic reactions (SRs). ***Methods:*** We selected adult patients with a history of HV allergy, with positive diagnostic tests and a correlation with one single Hymenoptera species. Age, gender, atopy, serum basal tryptase (sBT) value, total IgE, venom-specific IgE, history of hypertension, cardiovascular diseases, and hypercholesterolemia were compared between patients with previous LLRs and SRs. ***Results:*** 460 adult patients (381 SRs, 79 LLRs) were included. Age ($p = 0.0097$), male gender ($p < 0.0001$), arterial hypertension ($p = 0.046$), hypercholesterolemia ($p = 0.009$), and higher sBT levels ($p = 0.0004$) were significantly associated with severe reactions as independent variables. Moreover, considering the previous variables as risk factors, there was a significant and progressive increase in the odds of being Mueller III + IV as the number of positive variables increased. Patients with sBT ≥6.4 ng/mL adjusted for any of the positive variables had increased the risk of Mueller grade IV reaction ($p < 0.0001$). ***Conclusion:*** According to our results, older age, male gender, arterial hypertension, hypercholesterolemia, and increased levels of sBT ≥6.4 ng/mL are risk factors for severe anaphylaxis to HV in adults. Atopy and allergic asthma do not increase the risk of HV-induced SRs.

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Introduction

Anaphylaxis has been identified as "the latest allergy epidemic" [1] being drugs, insect stings, and foods the most common causes. Many studies on anaphylaxis [2–6] demonstrate that Hymenoptera venom (HV) anaphylaxis is an increasing condition worldwide, and that more efforts should be made to identify the risk factors for life-threatening reactions. As concerned risk factors, in the UK, it was observed that the fatality for insect-sting induced anaphylaxis was higher in the group aged 60 years and older, also observed in Australia and USA, confirming the role of higher age in conditioning the severity of the reactions. Other risk factors were male gender and mastocytosis [7]. An increased value of tryptase, even in the absence of mastocytosis, has been demonstrated to be a factor generally predisposing to anaphylaxis [8]. In a recent study, we found that a tryptase value of approximately 7 ng/mL was associated with a more frequent occurrence of anaphylactic reactions in venom-allergic subjects. A similar value was also a predictor of major cardiac events in a population of patients with a history of acute coronary syndrome (ACS), showing that the mast cells in the cardiac muscle may be implied in the pathophysiology of necrosis and that tryptase can be an expression of cardiovascular diseases [9]. Other causes, however, could determine an increased basal tryptase value and could in their turn predispose to anaphylaxis. We thus planned to look for these factors by comparing clinical and immunological parameters in subjects with previous large local reactions (LLRs) and in those with previous systemic reactions (SRs) to HV. LLRs usually do not evolve in SRs regardless of the specific IgE levels. Recently, Pucci et al. [10] found only a 4.2% of SRs to insect stings in patients with a previous history of only one LLR, but none of the patients with at least 2 previous LLRs developed a SR to a subsequent sting. However, a prospective multi-centric study by Bilò et al. [11] showed that patients with one previous LLR, if re-stung, reported a risk of SRs significantly higher (24%) with severe SRs in 11% of patients. Therefore, we conducted a retrospective study in our population of venom-allergic patients in order to relate basal tryptase levels to the severity of the reactions and the presence of pathological conditions (especially of the cardiovascular and respiratory compartment related to a mast cells [MC] increase) by comparing this occurrence in patients with a history of at least 2 LLRs and in those with SRs.

Materials and Methods

Patients

For this retrospective study, we collected data from adult outpatients (>15 years old) consecutively referred to the Allergy and Immunology Unit of the Niguarda Ca' Granda Hospital (Milan, Italy) for a documented history of allergic reactions to HV. Diagnosis of Hymenoptera venom allergy was based on the European Academy of Allergy and Clinical Immunology guidelines [12]. Skin tests were performed with standard venom extracts of *Apis mellifera*, *Vespula* spp., and *Polistes dominula* (Stallergènes®, Antony, Hauts-de-Seine, France; Anallergo®, Florence, Italy). The study population included patients with a history of at least 2 SRs to Hymenoptera stings. Large local reaction was characterized by oedema, erythema, or itch with an area of oedema >10 cm of diameter and a peak between 24 and 48 h [13]. Systemic reactions were graded into 4 classes according to Mueller's classification (grades of severity I–IV) [14]. For each patient, the following data were collected: age, gender, atopy (presence of positive IgE against one or more inhalant allergens and a history of asthma and/or rhinitis and/or atopic dermatitis), history of mastocytosis (bone marrow biopsy and clinical documentation), bronchial asthma (following GINA: https://ginasthma.org) or other respiratory diseases, arterial hypertension (history of p_{max} > 140 mm Hg values, p_{min} > 90 mm Hg values), previous ischemic heart disease or other cardiovascular diseases (clinical documentation), hypercholesterolemia (if present before specific therapy), the medications at the time of the sting, in particular antihypertensive drugs such as, angiotensin-converting enzyme inhibitors, selective or nonselective β-blockers, angiotensin receptor blockers, *total* serum IgE (value sensitization >100 kU/L), *venom-specific IgE* (positive value >0.10 kUA/L), and basal serum tryptase (sBT) (normal range <11.4 ng/mL). The primary end point of the study was the comparison of all these data in patients with SRs and in those with LLRs in order to identify the clinical aspects and biochemical parameters increasing the risk of developing a severe systemic reaction. The study protocol was approved by the Ethics Committee of ASST Grande Ospedale Metropolitano Niguarda (Milan, Italy) on October 26, 2018, protocol ID 538-102018.

In vitro Tests

Serum total IgE and specific IgE levels against *A. mellifera*, *V.* spp., and *P. dominula* were determined by using the ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden). A specific IgE value >0.10 kUA/L was considered positive. The sBT level was determined by using UniCAP Tryptase (Thermo Fisher Scientific, Uppsala, Sweden), with a normal value <11.4 ng/mL [15]. These tests were performed at least 3 weeks after venom exposure and resolution of the reactions.

Statistical Analysis

All the variables were analyzed using the suitable descriptive methods: more precisely, continuous variables were given as mean and standard deviation or as median and range, on the basis of their distribution (which was verified by a visual check of the histogram and also – if needed – by the Shapiro–Wilk test), and categorical and nominal variables were described by frequency tables. Cross-tabulations of categorical variables were analyzed using the Fisher's exact test. The differences between continuous variables were checked by 2-sided/2-tailored Student's *t* test (in the case,

Table 1. Demographic data

	Total	SRs	LLRs	*p* value
Patients	460	381 (82.8%)	79 (17.2%)	
Gender, M/F	284/176	265/126	29/50	<0.0001
Mean age (min–max), years	49 (15–84)	49 (15–84)	45 (15–83)	0.0097

LLRs, local large reactions; SRs, systemic reactions.

with the Welch's correction for different variances) or by Mann–Whitney U test with exact algorithm.

The continuous variables of interest, as well as the possible confounders such as age and gender, were then fitted as independent regressors in a set of ordinary least squares and logistic univariate and multivariate models, using the Wald's test to verify the significance of each independent variable. Statistical significance was assumed for $p < 0.05$; all calculations were carried out using the Stata/SE 15.0 statistical package.

Table 2. Mueller's classification of patients with SRs

Mueller's classification	
Grade I; %	50/381; 13.1%
Grade II; %	105/381; 27.5%
Grade III; %	110/381; 28.9%
Grade IV; %	116/381; 30.4%

Results

Patients

A total of 460 patients (284 males and 176 females; mean age 49 years, range 15–84 years) with a diagnosis of HVA were included in this study. The study population included 79 patients (17.2%; 29 males and 50 females, mean age 45 years, and range 15–83 years) selected for having presented at least 2 LLRs and 381 subjects (82.8%; 265 males and 126 females, mean age 49 years, and range 15–84 years) with SRs. Sixteen of 381 patients (4.1%; 16 males; mean age 55 years, range 31–74 years) with a diagnosis of mastocytosis based on bone marrow biopsy were excluded from the statistical analysis to avoid any mast cell disorder interference. A summary of patient's demographic and clinical data are shown in Tables 1 and 2 that consider only the adult population.

Comparison of the Various Clinical Parameters between Patients with LLRs and SRs

Age and Gender in Relation to Reaction Severity and Venom-Specific IgE Values

Patients with a history of SRs Mueller grade III + IV were significantly older (mean age 49 years) than patients with LLRs (mean age 45 years) (Mann-Whitney U test: $p = 0.0097$), in particular each arbitrary unitary increase in age determines a 2.4% increase in the odds of having a Mueller grade III + IV reaction versus LLR (CI 95%: 0.8–4.1% $p < 0.0001$). A significant association was observed between gender and severity of the reaction: males reported more frequently systemic reactions than females (66.9 vs. 33.1%; $p < 0.0001$).

Atopy and Asthma in Relation to Severity of the Reaction

Patients with LLRs (43/79) were more frequently atopic than those with SRs (106/381) (54.4 vs. 27.8%; $p < 0.0001$) and more frequently asthmatic (12.7 vs. 6.0%; $p = 0.050$) than those with SRs in whom asthma does not appear to be a risk factor for severe anaphylaxis. In general, in our population, asthma was significantly more frequent in atopic patients than in not atopic patients (19.3 vs. 1.5%; $p < 0.0001$).

Arterial Hypertension and Antihypertensive Drugs in Relation to Reaction Severity

Patients with severe SRs (Mueller grades III + IV) had a more frequent history of hypertension than patients with LLRs (21.8 vs. 15.2%; $p = 0.046$ Fisher's exact test). Moreover, we observed that the use of angiotensin receptor blockers (ARBs) was more frequent in patients with Mueller grades III + IV rather than in patients with Mueller grades I + II ($p = 0.015$). Instead, the intake of β-blockers or angiotensin-converting enzyme (ACE) inhibitors or other antihypertensive drugs showed no statistically significant differences in patients with Mueller grades III + IV compared to patients with Mueller grades I + II ($p = 0.591$; $p = 0.333$; and $p = 0.184$, respectively).

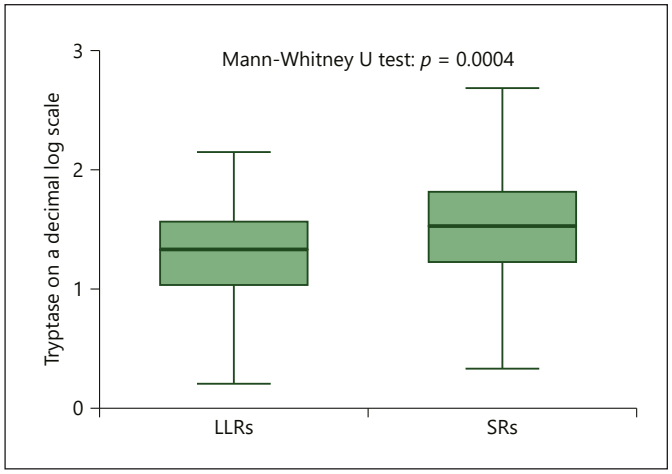

Fig. 1. Tryptase levels in LLRs patients and in SRs patients. LLRs, local large reactions; SRs, systemic reactions.

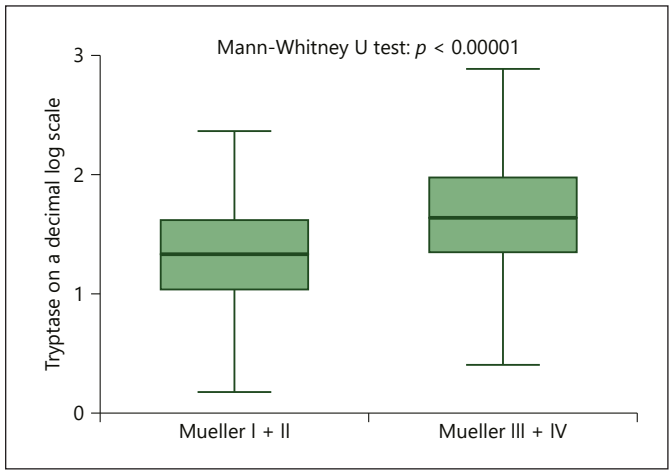

Fig. 2. Tryptase levels in Mueller I + II patients and in Mueller III + IV patients.

Previous Major Cardiac Event

A total of 9 patients had a previous history of ACS with myocardial infarction and subsequent complications; all of these patients were aged >60 years old and presented Mueller grade III + IV reactions and mean sBT value of 6.5 ng/mL.

Hypercholesterolemia

Hypercholesterolemic patients experienced severe reactions more frequently (Mueller grades III + IV) than LLRs (27.4 vs. 12.7%; $p = 0.009$ Fisher's exact test).

Tryptase

Multivariate logistic regression analysis showed that sBT values were significantly higher in patients with SRs (median sBT values 5.9 ng/mL, range 1–137 ng/mL) than in LLRs patients (median sBT values 4.29 ng/mL, range 0.1–24.1 ng/mL) ($p = 0.0004$, Mann-Whitney Exact test) (shown in Fig. 1). Furthermore, sBT levels in SRs were significantly higher in patients with Mueller grade III + IV than in patients with Mueller grade I + II ($p < 0.00001$) (shown in Fig. 2). A bivariate logistic regression analysis demonstrates an independent significant impact of age and sBT on the risk of having a severe reaction. Each single unit increase in sBT levels, adjusted for age, increased the odds of having an SR Mueller III + IV by 34.8% (CI95%: 20.4–50.8%; $p < 0.0001$), and each single unit increase in age, adjusted for sBT levels, increased the odds of having a SR Mueller III + IV by 3.0% (CI 95%: 1.5–4.5%; $p < 0.0001$). Moreover, a bivariate logistic regression model showed that tryptase and arterial hypertension were independent risk factors for a more severe reaction and that both were significantly important in determining the Mueller grades III + IV. In fact, each arbitrary unitary increase in the tryptase value, after adjusting for hypertension, determines a 28% increase in the odds of having a Mueller grade III + IV reaction (CI 95%: 12–47%; $p < 0.0001$, Wald test); the presence of hypertension, adjusted for sBT, caused a 123% increase in the odds to have a Mueller grade III + IV reaction (CI 95%: 6–70%; $p = 0.035$). The association between tryptase and symptoms severity (Mueller grades III + IV) was still persistent even if we considered 3 bivariate models including tryptase and specific IgE to *P. dominula*, *V.* spp. or *A. mellifera*. In all 3 models, tryptase increases the odds of having a severe reactions (Mueller grades III + IV). In particular, each single unit increase in sBT levels, adjusted for anti-*P. dominula* IgE levels, increased the odds of having a SRs Mueller grade III + IV of 28.2% (CI 95%: 11.7–47%; $p < 0.0001$). Moreover, when adjusted for anti-*V.* spp. IgE levels, it increased the odds of having a SRs Mueller grade III + IV of 26.7% (CI 95%: 10.6–45.1%; $p = 0.001$), and when adjusted for anti-*A. mellifera* IgE levels, the odds of having a SRs Mueller grade III + IV increased by 26.3% (CI 95%: 10.5–44.4%; $p = 0.001$).

Serum Basal Tryptase (sBT) Cutoff

We analyzed sBT values according to the Youden method, assigning appropriate cutoff values (shown in Fig. 3). The risk of grade IV reactions increased together with sBT values. In particular, we found that 6.4 ng/mL

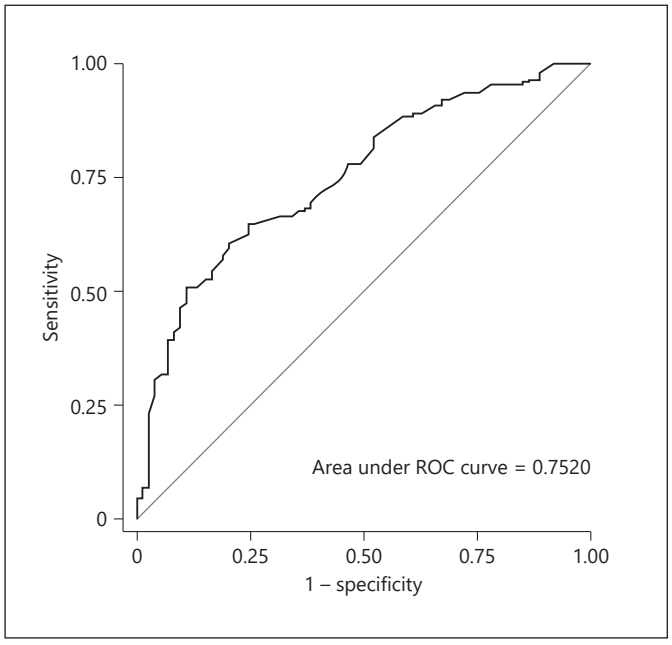

Fig. 3. ROC curve regarding tryptase levels. ROC, receiver operating characteristic.

was the optimal cutoff value with a sensitivity of 44.7% (CI 95%: 35.4–54.3%) and a specificity of 93.5% (CI 95%: 88.5–96.9%); a PPV of 83.6% (CI 95%: 71.9–91.8%) and an NPV of 69.7% (CI 95%: 63–75.9%). Switching from a tryptase <6.4 ng/mL to a tryptase ≥6.4 ng/mL, adjusting for hypertension, the odds of SRs Mueller IV increased by 1,077% (CI 95%: 460–2,374%, $p < 0.0001$).

Multivariate Model of Risk Factors

We used a multivariate model including tryptase and one or more of these variables: arterial hypertension, previous ischemic heart disease, and hypercholesterolemia. In this model, switching from a tryptase <6.4 ng/mL to a tryptase ≥6.4 ng/mL, adjusting for one of the 3 variables, the odds of grades IV reactions increased by 9,078% ($p < 0.0001$, Wald test). In the same model, patients who had one of the 3 clinical conditions associated to a tryptase ≥6.4 ng/mL were at risk of presenting SRs Mueller IV with an odds increase of 297% (IC95%: 118–624%; $p < 0.0001$); when patients who had two of the 3 clinical conditions were at higher risk of presenting SRs Mueller IV with an odds increase of 361% (IC95%: 123–854%; $p < 0.0001$), and patients who had all the 3 clinical conditions were at further increased risk of presenting SRs Mueller IV with an odds increase of 657% (IC95%: 91–2,904%; $p = 0.004$).

Serum Total IgE and Specific Venom IgE

Regarding serum total IgE levels, we did not find any difference in total IgE serum levels between LLRs and SRs (Mann-Whitney exact test: $p = 0.4448$). Concerning specific IgE to the different venoms, we found that anti-*P. dominula* IgE levels, anti-*V. spp.* IgE levels, and anti-*A. mellifera* IgE levels were significantly higher in patients with SRs than in patients with LLRs ($p = 0.0001$; $p < 0.0001$ and $p = 0.361$, respectively; Mann-Whitney exact test).

Discussion

Systemic reactions to Hymenoptera stings can lead to fatal anaphylaxis [16]. Therefore, it is important to identify the clinical conditions characterizing patients with a history of very severe reactions. In our study, we compared an array of biochemical and clinical parameters such as, age, gender, atopy, asthma, arterial hypertension, previous ischemic heart diseases, hypercholesterolemia, antihypertensive drugs, total IgE, venom-specific IgE, and basal serum tryptase level in subjects with at least 2 previous LLRs and in subjects with SRs, in order to identify the possible risk factors for developing severe anaphylaxis. We observed that an older age and male gender significantly predisposes to systemic reactions as already reported in literature [17, 18]. Moreover, we found that atopy was significantly more frequent in the LLRs cohort than in SRs patients ($p < 0.0001$), hence appearing unrelated to the risk of anaphylaxis [19]. In agreement with this observation, we found that a history of asthma was more common in atopic patients ($p < 0.0001$) and was not associated with severe systemic reactions. As expected and as known in literature [20, 21], sBT levels were significantly higher in SRs patients (median value 5.9 ng/mL) than in LLRs ones (median value 4.29 ng/mL) ($p = 0.0004$). Higher sBT levels were detected in patients with more severe reactions, as the level of 6.4 ng/mL significantly correlated with grade IV reactions. Furthermore, each single unit increase in tryptase value, when adjusted for age, enhanced the risk of a SRs Mueller III + IV by 34.8% ($p < 0.0001$). It is not clear what causes the increase in basal tryptase values in the subjects with the most severe HV reactions and not suffering from mastocytosis (HV-allergic subjects with mastocytosis were excluded from the statistical analysis) or other clinical conditions suggesting a mast cell activation syndrome, such as recurrent episodes of urticaria, flushing, pruritus, and angioedema, as recently described [22]. Therefore, looking for the possible mecha-

nisms at the basis of the tryptase increase, we investigated the clinical history of diseases other than allergic ones usually associated with a MC increase. However, the patients in our study population did not present renal failure or other kidney diseases, autoimmune disorders, or other inflammatory conditions. We have recently found that serum tryptase levels in patients with ACS were significantly related to either cardiovascular complexity during acute event [23] or the development of major adverse cardiovascular events in the 2 years following ACS [24]. In the present study, only 9 out of 460 patients had a history of ACS, although all of them presented a HV-induced Mueller grade III + IV SRs and a mean sBT value of 6.5 ng/mL, significantly higher than median sBT value (5.9 ng/mL) in SRs. However, this was not true for each single patient and the number of patients reporting a previous severe cardiovascular condition was too low to justify such a significant sBT general increase. The second objective was to study the history of arterial hypertension, given its frequent association with atherosclerosis and inflammatory alterations of vascular walls. We did not observe any significant association between hypertension and sBT increased values. However, we found that a history of hypertension was more frequent in patients with Mueller grade III + IV reactions than in patients with LLRs (p = 0.046). Hypertension and tryptase resulted to be independent, highly significant risk factors for more severe reactions with a significant additive effect, while not being interrelated among each other. Moreover, we found that a history of hypercholesterolemia (p = 0.009) was significantly more frequent in patients with the most severe reactions (Mueller III + IV). A possible explanation for this significant report could be cited for the recently demonstrated role of statins in making PAF more available, given their role in decreasing LDL cholesterol, which, as is known, is the PAF-acetylhydrolase carrier, an enzyme whose deficiency has been related to the severity of HV anaphylaxis [25, 26]. Furthermore, several studies suggested that MC may play an important role in the pathogenesis of atherosclerotic plaque; therefore, a significant relationship between hypercholesterolemia and the most severe reactions may further confirm their interdependence [27]. However, we did not have the pretreatment cholesterol basal values; hence, further prospective studies will be necessary to ascertain the real role of hypercholesterolemia in conditioning an increase in sBT value. Given the significant association of hypertension, hypercholesterolemia, and sBT values with severe reactions, we looked to clarify the relationship among these factors. By using a multivariate model including sBT level and one or more variables (arterial hypertension, previous ACS, or hypercholesterolemia), we found that progressively adding each of them, when documented in the history, in the presence of an sBT value ≥6.4 ng/mL, there was a progressive significant increase in the odds of presenting a Mueller IV SRs. In fact, we observed a 297% odds increase with one risk factor, passing to 361% increase with two risk factors, until to 657% increase with all of them. Thus, we believe that our data may be an important support in the clinical practice to improve accuracy in estimating each patient's potential risk profile. In conclusion, our study allowed the identification of at least some of the risk factors predisposing HV-allergic patients to severe SRs. In particular, patients over 50 years old with increased sBT levels and a history of hypertension or hypercholesterolemia or previous ACS present higher risks of severe SRs, and this risk increases as the number of such diseases increases. This has to be bear in mind when we evaluate a patient with mild SR, who following European Academy of Allergy and Clinical Immunology guidelines, has not necessarily to be admitted to VIT. If we detect the over-described characteristics, we suggest this patient has to undergo VIT as he is at high risk of presenting a more severe reaction at the following sting and has to be supplied with self-injectable epinephrine.

Statement of Ethics

The study protocol was approved by the Ethics Committee of ASST Grande Ospedale Metropolitano Niguarda (Milan, Italy) on October 26, 2018, protocol ID 538-102018.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All the authors have contributed equally in the design, data collection, realization, and writing of this manuscript.

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