

Platelet Activation in Bronchial Asthma according to asthma endotype and clinical severity

Short Title: Platelet Activation in Bronchial Asthma

Rossella Liani

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Isotta Altomare

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Paola Giustina Simeone

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Margherita Alfonsetti

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Matteo Mucci

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University

Stefano Lattanzio

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Alessia Ricci

University "G. d'Annunzio" of Chieti-Pescara

Anna Maria Draicchio

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Antonello Sanctis

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Paolo Mattia

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Francesco Papa

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Francesca Spadano

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Enrico Cavallucci

Allergy and Immunology Section, G. d'Annunzio University, School of Medicine

Francesco Cipollone

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Antonio Recchiuti

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University

Francesca Santilli

francesca.santilli@unich.it

Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara

Article

Keywords: bronchial asthma, endotypes, asthma severity, platelet, D-series resolvins, Immunothrombo-inflammation

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9528447/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Title: Platelet Activation in Bronchial Asthma according to asthma endotype and clinical severity

Short Title: Platelet Activation in Bronchial Asthma

Rossella Liani¹, Isotta Altomare², Paola Giustina Simeone¹, Margherita Alfonsetti¹, Matteo Mucci³, Stefano Lattanzio¹, Alessia Ricci⁴, Anna Maria Draicchio², Antonello De Sanctis², Paolo Di Mattia², Francesco Papa², Francesca Spadano², Enrico Cavallucci², Francesco Cipollone¹, Antonio Recchiuti³, Francesca Santilli¹

¹Department of Medicine and Aging, and Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

²Allergy and Immunology Section, Department of Internal Medicine, G. d'Annunzio University, School of Medicine, Chieti, Italy.

³Department of Medical, Oral and Biotechnological Sciences, and Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University, Chieti-Pescara, Italy

⁴Department of Pharmacy, University "G. d'Annunzio" of Chieti Pescara, Chieti, Italy

Corresponding author: Francesca Santilli MD, PhD, Full Professor, Department of Medicine and Aging, Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University. Address: Luigi Polacchi, 66013 Chieti, Italy. E-mail: francesca.santilli@unich.it Tel: +39-0871-541322. Fax: +39-0871-541261.

ABSTRACT

Bronchial asthma is a chronic disease characterized by distinct inflammatory endotypes and variable clinical severity. Beyond their role in hemostasis, platelets are increasingly implicated in asthma pathophysiology. Defective resolution of inflammation, mediated by D-series resolvins, may contribute to disease severity. This study investigated the association between platelet activation, asthma severity, inflammatory endotypes, and pro-resolving mediators.

Thirty-nine patients with bronchial asthma and twelve controls were enrolled. Asthma severity was classified as moderate (steps 3–4) or severe (step 5) according to GINA 2024 guidelines. Patients were stratified into type-2-allergic (GT2A), type-2-non-allergic (GT2NA), and non-type-2 (GNT2) endotypes. Platelet activation (P-selectin and CD40) and D-series resolvins were measured. Asthma was evaluated using the Asthma Control Test.

Asthma patients exhibited increased platelet activation, with higher P-selectin and CD40, in severe versus moderate disease. Platelet CD40 expression inversely correlated with

ACT scores, indicating poorer disease control. Type-2 asthma endotypes, especially GT2A, showed enhanced platelet activation compared with GNT2 asthma. Severe asthma showed reduced levels of RvD1 and 17(R)-RvD1, while RvD2 was reduced in GT2A.

These findings suggest that an imbalance between enhanced platelet activation and impaired resolution of inflammation may contribute to persistent airway inflammation and poor disease control, particularly in severe allergic asthma.

Keywords: bronchial asthma | endotypes | asthma severity | platelet | D-series resolvins, Immunothrombo-inflammation

Introduction

Bronchial asthma is a chronic inflammatory disease characterized by airway hyperreactivity and recurrent episodes of wheezing, dyspnea, chest tightness, and cough. Based on the features of underlying inflammation, asthma can be classified in two major endotypes: **type 2 (T2)**, allergic and/or eosinophilic, which is the most common form, and **non-type 2 (NT2)**, neutrophilic or paucigranulocytic, associated with late onset and comorbid factors such as cigarette smoking, pollution, and obesity [1]. According to the GINA 2024 guidelines, patients can be retrospectively stratified by disease severity into mild (steps 1–2), moderate (**steps 3–4**), and severe asthma (**step 5**), based on the intensity of treatment required to achieve adequate disease control.

Recent evidence has highlighted the active role of platelets in immune and inflammatory processes beyond their classical functions in hemostasis and thrombosis [2,3].

In bronchial asthma, several mechanisms of platelet activation have been described [1]. The first is inflammation-induced endothelial dysfunction, which triggers the production of strong platelet activators such as thrombin, von Willebrand factor, and thromboxane A₂ [4,5]. Exposure of subendothelial collagen leads to GPVI-dependent platelet activation [2]. IgE-dependent mechanisms during allergic sensitization represent an additional pathway of platelet activation [6]. In response to inflammatory stimuli, activated platelets express surface glycoproteins, such as CD40L, P-selectin, and thrombospondin-1, which are released into the circulation and contribute to asthma pathogenesis [1]. Activated platelets thus become capable of interacting with other immune system cells, including dendritic cells, eosinophils, and neutrophils, participating in allergic sensitization through the interaction of platelet CD40L with the CD40 receptor expressed on the surface of dendritic cells [1]. Following platelet activation, P-selectin contained in α -granules translocates to the platelet surface acquiring a high affinity for its ligand PSGL-1 [7,8] and platelets activate eosinophils and neutrophils. Such interactions (P-selectin/PSGL-1, α IIb β 3/MAC-1) allow platelets to form cellular aggregates with eosinophils, amplifying leukotriene production [9].

Impaired resolution of airway inflammation contributes to persistent and worsening clinical symptoms. Resolution is an active process involving chemical mediators to regulate immune cell reactivity [9]. Resolvins (Rv), natural lipid mediators derived from omega-3 fatty acids, play

a role in the resolution of inflammation in bronchial asthma and represent potential therapeutic targets [10]. A recent study shows that, among D-series Rvs, RvD1 and its epimer AT-RvD1 have strong anti-inflammatory and pro-resolving effects in a mouse model of allergic airway disease [10]. When administered before allergen exposure, RvD1 reduces eosinophilia, Th2 cytokines, mucus metaplasia, and NF- κ B activation, whereas post-challenge treatment with RvD1 and AT-RvD1 accelerates inflammation resolution, with AT-RvD1 more effectively shortening eosinophil resolution time and reducing IL-17, IL-23, and other inflammatory mediators. [11]. Asthma involves chronic T2 inflammation that current treatments only partially resolve. The pro-resolving mediator RvD2 reduces inflammation and airway hyperresponsiveness in mice via the DRV2 receptor, which is also elevated in asthma patients. Blocking DRV2 prevents RvD2's effects, suggesting RvD2–DRV2 signaling as a potential therapeutic target to resolve T2 inflammation in asthma [12]. However, the clinical association of RvDs with asthma severity and endotypes remains unknown.

This study aims to explore the relationship between the clinical severity of bronchial asthma [moderate (**steps 3–4**) and severe (**step 5**)] , the underlying inflammatory endotypes — allergic type 2 (**GT2A**), non-allergic type 2 (**GT2NA**), and non-type 2 (**GNT2**) — and key biological markers including platelet activation, oxidative stress, and pro-resolving mediators such as Rvs. The goal is to better understand the interplay between these factors and to identify potential targets for personalized therapeutic strategies in asthma.

Results

Clinical characteristics

Comparison according to disease severity

Table 1 and **2** presents a detailed description of the clinical characteristics. Compared with asthma patients, control subjects (CG) showed significantly lower waist circumference ($p=0.021$), diastolic blood pressure ($p=0.006$), and red blood cell distribution width, as well as reduced neutrophil ($p=0.041$), monocyte ($p=0.031$), and basophil counts ($p=0.013$). CG also exhibited a lower prevalence of chronic rhinosinusitis without nasal polyps (CRSsNP; $p<0.001$) and gastroesophageal reflux disease (GERD; $p=0.007$), and, as expected, did not receive asthma-related therapies, including ICS/LABA, leukotriene receptor antagonists, or long-acting muscarinic antagonists.

Among asthma patients, those in steps 3–4 had a higher prevalence of CRSsNP ($p=0.001$) and a lower prevalence of GERD compared with step-5 patients, in addition to the expected differences in treatment intensity driven by disease severity, **Table 2**.

Comparison according to patient endotype

Supplementary Table 1 and **2** summarizes the clinical and laboratory characteristics stratified by inflammatory endotype. Compared with all asthma endotypes (GT2A, GT2NA, GNT2), control subjects (CG) showed significantly lower waist circumference ($p=0.029$), diastolic blood pressure ($p=0.007$), total white blood cell count ($p=0.043$), neutrophils ($p=0.025$), monocytes ($p=0.010$), eosinophils ($p=0.005$), and basophils ($p=0.021$). In contrast, mean

corpuscular volume was higher in CG and GNT2 patients ($p=0.025$). As expected, CG exhibited a lower prevalence of chronic rhinosinusitis with (CRSwNP; $p=0.006$) and without nasal polyps (CRSsNP; $p=0.045$) and were less frequently treated with antihistamines ($p=0.000$) and intranasal corticosteroids ($p=0.041$), **Supplementary Table 1**.

Among asthma patients, no major clinical differences were observed between endotypes, except for higher eosinophil counts in T2 asthma ($p=0.021$) and a more frequent use of antihistamines in the T2 allergic group (GT2A; $p=0.003$), **Supplementary Table 2**.

Increased Platelet Activation in Patients with Severe Persistent Asthma

CG vs. asthma patients. CG patients showed a lower platelet activation profile compared with both moderate (step 3-4) and severe asthma (step 5). Specifically, CG displayed reduced platelet P-selectin expression (CD61+CD62p+, % $p=0.009$, MFI $p=0.512$) and lower platelet CD40 levels (CD61+CD40+, % $p=0.105$, MFI $p=0.009$) compared with patients with moderate asthma (step 3-4). **Figure 1A–D**. A similar pattern was observed when comparing CG with severe persistent asthma (step 5), with significantly lower platelet P-selectin (CD61+CD62p+, % $p<0.001$, MFI $p=0.323$) and CD40 expression (CD61+CD40+: %, $p=0.005$; MFI <0.001), **Figure 1A–D**.

Step 5 vs. step 3-4. Despite the more extensive use of anti-inflammatory treatments in step 5 asthma, flow cytometric analysis of whole blood revealed increased expression of platelet activation markers. In particular, P-selectin (CD61+CD62p+: %, $p=0.050$; MFI, $p=0.139$) and CD40 (CD61+CD40+: %, $p=0.023$; MFI, $p=0.019$) were significantly higher in patients with severe persistent asthma (step 5) as compared to steps 3-4, as illustrated in **Figure 1A–D**. Patients with worse disease control, as assessed by the ACT, which reflects how well asthma has been controlled over the last four weeks of therapy [13], showed higher platelet CD40 expression, **Figure 1E–F**. This was supported by an inverse correlation between ACT scores and platelet CD40 (CD61+CD40+) expression, both in terms of percentage ($\rho = -0.285$, $p=0.097$) and mean fluorescence intensity (MFI; $\rho = -0.412$, $p=0.014$), as shown in **Figure 1E–F**.

Type 2 Allergic Endotype Exhibits Enhanced Platelet Activation

CG vs. patients of different endotypes. The percentage of platelet expressing the activation markers P-selectin and CD40 was significantly increased in the GT2A group ($p<0.001$ and $p=0.024$, respectively) and in the GT2NA group ($p=0.062$ and $p=0.020$, respectively) compared with CG. No significant differences were observed between CG and GNT2, **Figures 2A and 2C**. CD40 mean fluorescence intensity (MFI) on platelets was significantly higher in GT2A and GT2NA compared with CG (both $p < 0.001$, **Figure 2D**). A significant increase was also observed in GNT2 compared with CG ($p = 0.021$, **Figure 2D**). p-Selectin MFI did not differ significantly among groups (**Figure 2B**).

Comparison among endotypes. Overall, GT2A and GT2NA exhibited a more pronounced platelet activation phenotype, primarily characterized by increased CD40 expression, whereas GNT2 showed a comparatively modest effect. When directly comparing allergic endotypes, GT2A showed a significantly higher level of platelet activation compared to the GNT2, as indicated by increased platelet P-selectin positive expression (%CD61+CD62p+: $p=0.045$,

Figure 2E). No differences were observed in P-selectin MFI (**Figure 2F**) and in CD40 (CD61+CD40+) expression between the groups (**Figure 2G-H**).

Differential Platelet Activation Within Type 2 Asthma Endotypes With Increasing Treatment Steps: Variations in P-Selectin and CD40 Expression. This trend for higher platelet activation in severe persistent asthma was further observed within the type 2 asthma endotypes, both allergic (GT2A) and non-allergic (GT2NA). For P-selectin, only the GT2NA subgroup showed a statistically significant increase in step 5 patients (CD61+CD62p+: %, $p=0.020$; MFI, $p=0.002$), while GT2A and non-T2 (GNT2) groups did not show significant differences (CD61+CD62p+: GT2A: $p=0.427$ and $p=0.657$; GNT2: $p=0.667$ and $p=0.333$), **Figure 3A-F**. Regarding CD40 expression, a significantly higher MFI was observed only in the GT2A group (CD61+CD40+: $p=0.006$), while no significant changes were detected in GT2NA (CD61+CD40+: $p=0.639$ and $p=0.943$) or GNT2 (CD61+CD40+: $p=0.333$ and $p=0.667$) subgroups, **Figure 3G-L**.

High-dose ICS/LABA therapy and platelet activation. In our cohort, patients receiving high-dose of inhaled corticosteroid/long-acting β_2 agonist (ICS/LABA) therapy, likely reflecting less controlled disease, showed significantly higher platelet activation markers, including CD40 expression (CD61+CD40+: %: $p=0.022$; MFI: $p=0.002$) and p-Selectin expression (CD61+CD62p+: %: $p=0.005$; MFI: $p=0.046$), as compared with patients not on high-dose ICS/LABA, as illustrated in **Figure 4A-D**.

Lower RvD1 and 17(R)-RvD1 levels in patients with severe persistent asthma. RvDs are important regulators of inflammatory responses and represent a promising therapeutic avenue, as their biosynthesis is often impaired in asthma. Since the previous results indicate a resolution gap in patients with asthma, we assessed serum concentration of RvDs in a subgroup of patients and CG. RvD1 and its epimer 17(R)-RvD1 were lower in CG compared to moderate asthma severity ($p=0.074$, $p=0.022$, respectively; **Figure 5A-B**), consistent with the notion that RvDs are biosynthesized in response to harmful stimuli. In addition, compared to moderate asthma, patients with severe asthma had significantly lower serum concentrations of RvD1 ($p=0.018$) and 17(R)-RvD1 ($p=0.045$) **Figure 5A-B**, suggesting that the resolution gap is more pronounced in these latter. In contrast, serum levels of RvD2, RvD3, and RvD4 did not differ significantly across clinical severity steps, with no differences observed between controls, moderate (steps 3–4), and severe asthma (step 5) (**Figure 5C**).

Lower RvD2 levels in type 2 allergic endotype. Upon stratification based on the allergic endotype, RvD2 levels were significantly higher in GT2NA ($p=0.008$) and GNT2 ($p=0.018$), but not GT2A, subjects compared to CG, **Figure 6A**.

Moreover, GT2A patients exhibited significantly lower RvD2 levels compared to GT2NA, ($p=0.040$, **Figure 6B**). A similar trend was observed in GT2NA patients, who also showed reduced RvD2 levels compared to the type 2 non-allergic group, although the difference was at

the limit of statistical significance ($p=0.057$, **Figure 6B**). No significant differences were observed for RvD1, 17(R)-RvD1, RvD3 and RvD4, **Figure 6C**.

Overall, these results demonstrate a selective impairment in RvD1 and D2 biosynthesis that is severity- and endotype-related.

Discussion

Asthma is a complex inflammatory disease involving both immune and structural airway cells, in which eosinophils, lymphocytes, and also platelets contribute to airway inflammation beyond their hemostatic role [14]. As immune effectors, platelets promote leukocyte recruitment and inflammatory amplification. [14]. Experimental and clinical studies show that platelet activation occurs early after allergen exposure and is enhanced in severe or uncontrolled asthma, although its relevance across disease severities and inflammatory endotypes remains unclear [14]. In addition to excessive inflammation, asthma features defective resolution pathways, whereby altered D-series resolvins fail to terminate inflammation and restore airway homeostasis, contributing to severe disease and treatment resistance. [15]. The interaction between platelet activation and endogenous resolution mechanisms in asthma is still poorly characterized. In this study, we analyzed the clinical characteristics, platelet activation and pro-resolving mediator profile in asthma patients stratified by severity and inflammatory endotype. Our findings indicated that severe asthma is associated with a marked state of platelet activation and impairment in pro-resolving pathways, suggesting a potential contribution of immuno-thrombotic mechanisms to poor disease control. Treatment typically involves inhaled glucocorticoids and bronchodilators, tailored to disease severity and guided by a stepwise management approach according to the latest GINA guidelines [16]. In recent years, platelets have been recognized as playing a significant role in asthma and other inflammatory diseases [17]. Platelets act as a bridge between innate and adaptive immunity and contribute to asthma by forming platelet-leukocyte aggregates, releasing pro-inflammatory mediators, and in lung tissue, they can directly promote tissue damage. [18]. Animal models support the idea that platelets are essential for the development of airway inflammation and altered lung function [19]. Upon activation, platelets express surface glycoproteins such as CD40L and P-selectin enabling interactions with various immune cells, including eosinophils, dendritic cells, and neutrophils [20]. In allergic asthma, IgE-mediated platelet activation promotes type 2 inflammation and leukocyte recruitment, consistent with increased P-selectin and CD40 expression and the immunomodulatory role of platelets beyond hemostasis. [21]. Our data showed higher P-selectin and CD40 in severe asthma compared with moderate disease, supporting the concept that platelet activation increases proportionally with disease severity [22]. This association is further reinforced by the inverse correlation between platelet activation (CD40) and asthma control (ACT score), in line with studies linking platelet activation, reduced lung function, and airway hyperresponsiveness [23]. Platelet profiling across inflammatory endotypes revealed higher platelet activation in the GT2A compared with GNT2, consistent with the established role of platelets in eosinophilic and IgE-driven inflammation through IL-33

release, eosinophil recruitment, and CD40L-mediated T-cell modulation. [24–26]. This result confirms that platelets are indeed more activated in individuals with asthma, as in many other inflammatory diseases. Platelet activation and the presence of platelet factor 4 in the circulation increase with the severity of asthma. After activation, P-selectin expressed on the platelet surface mediates the binding of platelets to eosinophils [24]. Platelet CD40 expression inversely correlated with disease control, emphasizing the potential role of platelets in asthma worsening [25]. The differential distribution of P-selectin and CD40 among T2 subgroups in our study suggests heterogeneity in the immune and thrombo-inflammatory mechanisms across endotypes. This aligns with evidence indicating that, even in phenotypically similar forms of asthma, molecular pathways of platelet activation may diverge depending on the predominant inflammatory environment [27]. High-dose ICS/LABA therapy, reflecting poorer disease control, was associated with increased platelet activation, consistent with evidence that platelet pathways may be partially resistant to conventional anti-inflammatory treatment and contribute to severe asthma refractoriness.[28].

Our study also showed a clear gradation of platelet activation that reflects disease severity. This graded pattern - observed across clinical steps, treatment intensity, and inflammatory endotypes - suggests that platelet activation does not merely accompany asthma but may scale with the underlying inflammatory burden, thereby offering additional insight into platelet involvement across the spectrum of disease severity. In a 2009 meta-analysis, Jiang et al. found a positive association between abdominal obesity and asthma (OR=1.47, 95% CI 1.35-1.59) [29]. Indeed, we observed that our patients with asthma had a higher waist circumference compared to the control group. Adipose tissue, especially visceral adipose tissue, is closely associated with the release of pro-inflammatory mediators, including leptin, adiponectin, interleukin-6, and tumor necrosis factor α . These mediators may directly influence the airways or induce alterations in immune responses and platelet activation [30].

Epidemiological studies have also reported an increased incidence of cardiovascular diseases in patients with asthma [31]. Beyond shared inflammatory pathways, platelet activation may represent a mechanistic link between asthma-related systemic inflammation and cardiovascular comorbidity. Severe asthma, aeroallergen sensitization, female sex, and older age are associated with higher cardiovascular risk [32], with hypertension and heart failure being the most frequently reported conditions [31,33,34]. Experimental and clinical data suggested increased myocardial vulnerability [35], impaired vascular function [36], and accelerated atherosclerosis [37] in allergic and asthmatic conditions. In this context, cytokine dysregulation, leukotriene overproduction, and the activation of inflammatory cells may contribute to pathogenic mechanisms, although the precise biological pathways remain to be clarified [31,37]. While cardiovascular outcomes were not directly assessed in this study, our findings provide a biological rationale for the increased cardiovascular risk reported in patients with severe asthma.

Specialized pro-resolving mediators, including RvDs, are produced during asthma and their synthetic stable analogs proved more potent than cysteinyl leukotrienes antagonists in reducing leukocyte recruitment and bronchial hyper-responsiveness [38]; however, their biosynthesis appears reduced in severe and uncontrolled disease [9].

Our study provides the first evidence that serum concentrations of select SPMs in patients with asthma are not only altered but follow a distinct pattern related to disease severity and endotype. Specifically, we observed that RvD1 and 17R-RvD1 concentrations were significantly higher in patients with moderate asthma compared to those with severe, therapy-resistant disease (**Fig. 6**). The physiological and therapeutic relevance of these findings is underscored by preclinical studies demonstrating that treatment with RvD1 or its epimer AT-RvD1 significantly reduced airway eosinophilia and mucus metaplasia in mouse allergic airway inflammation by lowering IL-5 levels and I κ B α degradation. Notably, AT-RvD1 exhibits superior efficacy in accelerating the resolution of lung eosinophilia and improving airway hyperreactivity. This enhanced potency is largely attributed to its metabolic stability; unlike RvD1, AT-RvD1 resists rapid enzymatic inactivation, allowing it to more effectively enhance allergen clearance and the phagocytosis of apoptotic cells by macrophages without triggering further release of pro-inflammatory factors. [11].

An intriguing observation in our study was that RvD1 and 17R-RvD1 levels were slightly lower in healthy controls than in patients with moderate asthma, while no significant differences were found between controls and patients with severe disease (Fig. 5). Although this pattern may initially seem counterintuitive - since Rvs are broadly considered impaired in chronic inflammatory disorders - it aligns with the dynamic and stimulus-dependent SPM biosynthesis. SPMs, including D-series resolvins, are actively biosynthesized in response to inflammatory stimuli as part of an endogenous program aimed at terminating inflammation and restoring homeostasis [39]. Indeed, Rv production typically increases during the early and intermediate phases of inflammation to counterbalance incoming pro-inflammatory signals and promote resolution. In moderate asthma, the inflammatory challenge likely triggers a compensatory upregulation of pro-resolving pathways, resulting in circulating levels that exceed the basal activity found in healthy individuals. However, this adaptive response can collapse in severe asthma. Hence, the significant reduction of RvDs in patients with higher clinical severity and poor therapy responsiveness suggests a failure of resolution, where the immune system does not produce sufficient counter-regulatory signals to balance persistent inflammatory amplification [39].

RvD2 is known to regulate type 2 inflammatory responses, promoting resolution in murine models through RvD2–DRV2 receptor interaction [12]. Our findings highlight that patients with severe persistent asthma (Step 5) exhibit a more pronounced pro-inflammatory profile, characterized by increased platelet activation (CD62p and CD40) and reduced production of pro-resolving mediators such as RvD1, 17(R)-RvD1, and RvD2. Notably, the type 2 allergic endotype is associated with greater platelet activation and lower RvD2 levels, suggesting an impairment in the resolution pathways of inflammation.

The finding of reduced RvD2 in the T2 allergic subgroup is particularly noteworthy, as RvD2 plays immunoregulatory roles in neutrophils and eosinophils—key components of T2 inflammation [40,41]. This may point to intrinsic biological differences within T2 endotypes.

Limitations of our study include its observational and largely cross-sectional design, which precludes causal inference; the sample size in stratified subgroups, which may limit power to detect smaller effects. Causal relationships cannot be inferred, and longitudinal

studies are needed to determine whether modulation of platelet activation or enhancement of resolution pathways translates into clinical benefit.

These findings support the hypothesis that specialized pro-resolving mediators and platelet activity modulation may represent novel therapeutic targets for patients with severe, uncontrolled asthma. However, the role of platelets in bronchial asthma is not yet fully elucidated, and the use of antiplatelet drugs remains controversial [42–44]. Importantly, long-term and large-scale clinical studies are lacking to confirm the efficacy of antiplatelet therapy in asthma, particularly given the increased bleeding risk associated with such treatments [45]. Therefore, retrospective analyses and prospective studies evaluating the efficacy and safety of antiplatelet agents in patients with asthma and thrombotic comorbidities will be crucial. Future therapeutic strategies should focus not so much on inhibiting platelet aggregation *per se*, but rather on disrupting the interactions between platelets and inflammatory cells — with promising targets such as P-selectin. In addition, there is a need for new therapies that, instead of acting as generic anti-aggregants, specifically block platelet–inflammatory cell interactions (e.g. via P-selectin) to minimize inflammation while preserving hemostasis. In conclusion, this study identifies platelets as active immunoinflammatory co-players in bronchial asthma, whose activation reflects disease severity, inflammatory endotype, and impaired resolution mechanisms. The concomitant increase in platelet activation markers and reduction in pro-resolving mediators supports a model of persistent inflammation driven by both inflammatory amplification and defective resolution. These findings highlight platelet activation markers and specialized pro-resolving mediators as promising biomarkers and potential therapeutic targets in asthma, opening new avenues for precision medicine approaches aimed at restoring immune balance rather than merely suppressing inflammation.

Methods

Study design. In a prospective observational study 51 patients, with (n=39) and without (n=12) bronchial asthma (Control Group, CG), were enrolled at the Unit of Allergology and Clinical Immunology of the S.S. Annunziata Hospital in Chieti.

Patients with the T2 inflammatory endotype were defined by the presence of at least one biomarker in their past clinical history (blood eosinophilia $\geq 150/\mu\text{L}$; FeNo ≥ 20 ppb; sensitization to aeroallergens: prick test and/or specific IgE). Based on the presence of allergic sensitization, T2 patients were divided into 2 different subgroups in allergic (GT2A, n=20) and non-allergic group (GT2NA, n=13).

Patients with asthma with a non-T2 inflammatory endotype (GNT2, n=6) were those patients in whom T2 inflammatory biomarkers were not identifiable.

Patients with asthma (GT2A, GT2NA, GNT2) were stratified according to GINA guidelines 2024 into moderate (step 3-4) and severe (step 5), based on the medications taken for asthma control.

At enrolment, patients in steps 3-4 (n=23) were receiving low (n=13) or medium-dose (n=10) ICS/LABA maintenance therapy, respectively. Patients in step 5 (n=16) were treated with medium (n=2) or high-dose (n=14) ICS/LABA, with optional add-on LAMA (n=5) and were in pharmacological washout from oral corticosteroids for at least two weeks. We enrolled only patients who had not yet initiated any biologic therapy, which represents an additional therapeutic option in severe asthma. Furthermore, some patients (n=5) were also receiving leukotriene receptor antagonists, which, according to the GINA 2024 guidelines, may be added at any treatment step to improve symptom control [46]. Each patient also had access to as-needed therapy, consisting of either an ICS–formoterol combination (according to the so-called Maintenance And Reliever Therapy regimen) or a Short-Acting β -Agonists such as salbutamol [46].

We also enrolled, as control group, non-asthmatic and non-allergic patients (CG, n=12) who were referred to our Clinic for an allergy consultation.

The protocol was approved by the Territorial Ethics Committee of the Abruzzo Region (Protocol number 3728). Written informed consent was provided for participants.

All methods were performed in accordance with the Declaration of Helsinki and the relevant institutional and national guidelines and regulations.

Each patient underwent a clinimetric test to evaluate asthma control over the previous four weeks (Asthma Control Test - ACT), baseline spirometry with a possible bronchodilator reversibility test in case of bronchial obstruction, blood sampling and urine collection.

Platelet receptors: Flow Cytometry Analysis

Evaluation of proteins expressed on the surface of activated platelets (P-selectin and CD40), in a citrate tube analyzed by flow cytometry (FACS Canto, BD). Whole blood (5uL) was stained with a mix of antibodies (CD61 347408 BD, CD40 11040973 eBiosciences, CD62P 550888 BD) and incubated for 15 minutes (RT, in the dark). Finally, samples were adequately diluted with PBS-/-, acquired (1×10^6 events/samples) by flow cytometry (FACS Canto, BD Biosciences) and analyzed the percentage (%) and the mean flow intensity (MFI) using FACS Diva (BD Biosciences) software.

LC-MS/MS lipidomics

Lipid mediators were extracted from serum samples in a subgroup of patients (n=20), and their profiles were analyzed following the methodology previously described by Serhan and colleagues (14) with adaptations to our analytical platform. Briefly, ice-cold methanol containing 10 pg of each deuterated internal standard—RvD1-d₅, RvD2-d₅, 17(R)-RvD1-d₅, and RvD3-d₅—was added to each sample. These internal standards were selected to represent distinct retention time regions and structural classes of specialized pro-resolving mediators (SPMs). Samples were incubated at -20 °C for 30 minutes to precipitate proteins, followed by centrifugation at 3,000 rpm for 10 minutes at 4 °C. Supernatants were collected and subjected to solid-phase extraction using Waters Sep-Pak C18 cartridges (3 cc, 200 mg). Columns were preconditioned with methanol and water; immediately prior to loading, samples were acidified

and diluted 1:3 with acidified water (pH=3.5). After washing with water and hexane, lipid mediators were eluted with methyl formate, evaporated under nitrogen at 37 °C, and reconstituted in 200 µL of 50:50 methanol:water for analysis.

Lipid mediators were identified and quantified using an Agilent 1260 Infinity II LC system coupled to an Ultivo Triple Quadrupole mass spectrometer equipped with a Jet Stream electrospray ionization (ESI) source (Agilent Technologies, USA). Chromatographic separation was performed using a Kinetex 2.6 µm Polar C18 100 Å, LC Column (100 × 3.0 mm, Phenomenex) maintained at 40 °C. The mobile phase consisted of water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B), with a gradient optimized for SPM and eicosanoid separation.

Multiple reaction monitoring (MRM) was performed in negative ionization mode using compound-specific Q1 (precursor ion) and Q3 (product ion) transitions. Two transitions were acquired per compound: a quantifier transition, used for concentration determination, and a qualifier transition, used for structural confirmation. Transitions used included RvD1 (375.5→215.2; 375.5→141.1), RvD2 (375.5→175.1; 375.5→141.1), RvD3 (375.2→147.0; 375.2→114.8), and RvD4 (375.2→357.1; 375.2→101.0). Signals were acquired and processed using Agilent MassHunter Quantitative Analysis Software version 12.1. Peaks were automatically integrated and manually inspected. Where needed, manual integration was performed to correct for errors in automatic peak selection.

Analyte identity was confirmed by retention time matching to authentic standards and qualifier/quantifier ion ratio consistency (±20%). A signal-to-noise ratio ≥5 was required for identification. Quantification was performed using external calibration curves generated from authentic standards and analyzed in parallel with the samples. Deuterated internal standards were used to correct for extraction efficiency and matrix effects. Calibration was performed using weighted linear regression ($1/x^2$) of internal standard-normalized peak areas versus nominal concentrations. Where authentic calibrants were unavailable (e.g., for RvD4), quantification was performed using calibration curves and internal standards from structurally related analogs (e.g., RvD3/RvD3-d₅). All analytes were monitored; however, only those detected above the instrumental limit of detection (LOD) were reported. Compounds detected below the validated lower limit of quantification (LLOQ) were considered present but were not quantified.

Statistical Analysis

We estimated that at least 10 patients in each group would be needed to detect an average difference in any of the investigated parameters equal to or greater than one standard deviation. The power and the probability of Type I error (alpha) associated with this null hypothesis test that the population means of the two groups are equal are 90% and 0.01, respectively. The alpha value was chosen to address the issue of multiple parameters being tested.

The univariable comparisons between groups were performed using the chi-square test or the Mann-Whitney U test. The Spearman rank correlation test was used to evaluate the relationships between continuous variables. $p < 0.05$ were considered significant.

References

1. Yue, M., Hu, M., Fu, F., Ruan, H. & Wu, C. Emerging Roles of Platelets in Allergic Asthma. *Front. Immunol.* **13**, 846055 (2022).
2. Scherlinger, M., Richez, C., Tsokos, G. C., Boilard, E. & Blanco, P. The role of platelets in immune-mediated inflammatory diseases. *Nat Rev Immunol* **23**, 495–510 (2023).
3. Koupenova, M., Clancy, L., Corkrey, H. A. & Freedman, J. E. Circulating Platelets as Mediators of Immunity, Inflammation, and Thrombosis. *Circulation Research* **122**, 337–351 (2018).
4. Glorieux, G., Cohen, G., Jankowski, J. & Vanholder, R. Platelet/Leukocyte Activation, Inflammation, and Uremia. *Seminars in Dialysis* **22**, (2009).
5. Margetic, S. Inflammation and hemostasis. *Biochem Med* 49–62 (2012)
doi:10.11613/BM.2012.006.
6. Capron, M. & Joseph, M. The low affinity receptor for IgE on eosinophils and platelets. *Monogr Allergy* **29**, 63–75 (1991).
7. Hernandez, J. M. & Janssen, L. J. Revisiting the usefulness of thromboxane-A₂ modulation in the treatment of bronchoconstriction in asthma. *Can. J. Physiol. Pharmacol.* **93**, 111–117 (2015).
8. Yun, S.-H., Sim, E.-H., Goh, R.-Y., Park, J.-I. & Han, J.-Y. Platelet Activation: The Mechanisms and Potential Biomarkers. *BioMed Research International* **2016**, 1–5 (2016).
9. Levy, B. D. Resolvin D1 and Resolvin E1 Promote the Resolution of Allergic Airway Inflammation via Shared and Distinct Molecular Counter-Regulatory Pathways. *Front. Immun.* **3**, (2012).

10. Peh, H. Y., Bruggemann, T. R., Wong, W. S. F. & Levy, B. D. Resolvin D2 Promotes the Resolution of Lung Inflammation in Allergen-Induced Asthma. *The Journal of Immunology* **206**, 94.13-94.13 (2021).
11. Rogerio, A. P. *et al.* Resolvin D1 and Aspirin-Triggered Resolvin D1 Promote Resolution of Allergic Airways Responses. *The Journal of Immunology* **189**, 1983–1991 (2012).
12. Peh, H. Y. *et al.* Resolvin D2 regulates type 2 inflammatory responses and promotes resolution of mouse allergic inflammation. *Allergy* **79**, 739–743 (2024).
13. Schatz, M. *et al.* Asthma Control Test: Reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *Journal of Allergy and Clinical Immunology* **117**, 549–556 (2006).
14. Yue, M., Hu, M., Fu, F., Ruan, H. & Wu, C. Emerging Roles of Platelets in Allergic Asthma. *Front Immunol* **13**, 846055 (2022).
15. Barnig, C., Frossard, N. & Levy, B. D. Towards targeting resolution pathways of airway inflammation in asthma. *Pharmacol Ther* **186**, 98–113 (2018).
16. Dubin, S., Patak, P. & Jung, D. Update on Asthma Management Guidelines. *Mo Med* **121**, 364–367 (2024).
17. Takeda, T., Morita, H., Saito, H., Matsumoto, K. & Matsuda, A. Recent advances in understanding the roles of blood platelets in the pathogenesis of allergic inflammation and bronchial asthma. *Allergology International* **67**, 326–333 (2018).
18. Shah, S. A., Page, C. P. & Pitchford, S. C. Platelet–Eosinophil Interactions As a Potential Therapeutic Target in Allergic Inflammation and Asthma. *Front. Med.* **4**, 129 (2017).
19. Shah, S., Riffo Vasquez, Y., Page, C. & Pitchford, S. Platelets roll and adhere to the vascular bed in response to allergen challenge in allergic mice. in *Allergy and immunology* OA4845 (European Respiratory Society, 2017). doi:10.1183/1393003.congress-2017.OA4845.

20. Luo, L., Zhang, J., Lee, J. & Tao, A. Platelets, Not an Insignificant Player in Development of Allergic Asthma. *Cells* **10**, 2038 (2021).
21. Cognasse, F. et al. Platelets as Key Factors in Inflammation: Focus on CD40L/CD40. *Front. Immunol.* **13**, 825892 (2022).
22. Luo, L., Zhang, J., Lee, J. & Tao, A. Platelets, Not an Insignificant Player in Development of Allergic Asthma. *Cells* **10**, 2038 (2021).
23. Johansson, M. W. et al. Platelet Activation, P-Selectin, and Eosinophil β_1 -Integrin Activation in Asthma. *Am J Respir Crit Care Med* **185**, 498–507 (2012).
24. Johansson, M. W. et al. Platelet activation, P-selectin, and eosinophil β_1 -integrin activation in asthma. *Am J Respir Crit Care Med* **185**, 498–507 (2012).
25. Tian, J., Zhu, T., Liu, J., Guo, Z. & Cao, X. Platelets promote allergic asthma through the expression of CD154. *Cell Mol Immunol* **12**, 700–707 (2015).
26. Takeda, T. et al. Platelets constitutively express IL-33 protein and modulate eosinophilic airway inflammation. *Journal of Allergy and Clinical Immunology* **138**, 1395-1403.e6 (2016).
27. Johansson, M. W. et al. Platelet Activation, P-Selectin, and Eosinophil β_1 -Integrin Activation in Asthma. *Am J Respir Crit Care Med* **185**, 498–507 (2012).
28. Foer, D. et al. Novel Methods for Studying Platelet-Mediated Inflammation in Asthma. *Journal of Allergy and Clinical Immunology* **149**, AB129 (2022).
29. Jiang, D., Wang, L., Bai, C. & Chen, O. Association between abdominal obesity and asthma: a meta-analysis. *Allergy Asthma Clin Immunol* **15**, 16 (2019).
30. Santilli, F., Vazzana, N., Liani, R., Guagnano, M. T. & Davì, G. Platelet activation in obesity and metabolic syndrome. *Obesity Reviews* **13**, 27–42 (2012).

31. Cazzola, M., Rogliani, P., Ora, J., Calzetta, L. & Matera, M. G. Asthma and comorbidities: recent advances. *Pol Arch Intern Med* **132**, 16250 (2022).
32. Asthma and risk of coronary heart disease - Annals of Allergy, Asthma & Immunology.
[https://www.annallergy.org/article/S1081-1206\(17\)30210-7/fulltext](https://www.annallergy.org/article/S1081-1206(17)30210-7/fulltext).
33. Schanen, J. G. *et al.* Asthma and incident cardiovascular disease: the Atherosclerosis Risk in Communities Study. *Thorax* **60**, 633–638 (2005).
34. Cazzola, M., Segreti, A., Calzetta, L. & Rogliani, P. Comorbidities of asthma: current knowledge and future research needs. *Current Opinion in Pulmonary Medicine* **19**, 36 (2013).
35. Hazarika, S., Van Scott, M. R. & Lust, R. M. Myocardial ischemia-reperfusion injury is enhanced in a model of systemic allergy and asthma. *Am J Physiol Heart Circ Physiol* **286**, H1720-1725 (2004).
36. Hazarika, S., Van Scott, M. R., Lust, R. M. & Wingard, C. J. Pulmonary allergic reactions impair systemic vascular relaxation in ragweed sensitive mice. *Vascul Pharmacol* **53**, 258–263 (2010).
37. Knoflach, M. *et al.* Allergic rhinitis, asthma, and atherosclerosis in the Bruneck and ARMY studies. *Arch Intern Med* **165**, 2521–2526 (2005).
38. Levy, B. D. *et al.* Lipoxin A₄ stable analogs reduce allergic airway responses via mechanisms distinct from CysLT1 receptor antagonism. *The FASEB Journal* **21**, 3877–3884 (2007).
39. Serhan, C. N. & Levy, B. D. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *Journal of Clinical Investigation* **128**, 2657–2669 (2018).

40. Donovan, C., Barnes, J. L. & Kim, R. Y. Back to the Eosinophil: Resolvin Spatiotemporal Regulation. *American Journal of Respiratory Cell and Molecular Biology* **69**, 608–609 (2023).
41. Spite, M. *et al.* Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature* **461**, 1287–1291 (2009).
42. Barr, R. G. *et al.* Aspirin and Decreased Adult-Onset Asthma: Randomized Comparisons from the Physicians' Health Study. *Am J Respir Crit Care Med* **175**, 120–125 (2007).
43. Kurth, T., Barr, R. G., Gaziano, J. M. & Buring, J. E. Randomised aspirin assignment and risk of adult-onset asthma in the Women's Health Study. *Thorax* **63**, 514–518 (2008).
44. Menzies, D., Nair, A., Meldrum, K. T., Hopkinson, P. & Lipworth, B. J. Effect of aspirin on airway inflammation and pulmonary function in patients with persistent asthma. *Journal of Allergy and Clinical Immunology* **121**, 1184-1189.e4 (2008).
45. Tomos, I. *et al.* Pulmonary hemorrhage in patients receiving anti-platelets- a case series and a suggested therapeutic algorithm. *Respiratory Medicine Case Reports* **40**, 101782 (2022).
46. GINA 2024 Strategy Report.

Aknwlodgements. The authors thank Dr. Danila Madonna for her support during the study enrollment phase

Author contributions statement. F. S., R. L. and I. A. contributed to the experimental design and methodology. R. L., M. A., M. M., S. L., and A. R. performed the experiments. F. S. and R. L., analyzed the data. R. L., I. A., A. M. D., A. D. S., P. D. M., F. P. and F. S. participated in sample collection. F. S., R. L., P. G. S., M. A., A. R. drafted the manuscript. E. C. and F. C. reviewed and all authors approved the final manuscript.

Additional Infomation

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Funding

This research received no external funding.

Competing interests

The authors declare no competing interests.

Supplementary Information The online version contains supplementary material available at

Correspondence and requests for materials should be addressed to F.S.

Reprints and permissions information is available at www.nature.com/reprints.

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

Figure 1. Platelet Activation Markers Are Elevated in Severe Persistent Asthma and Correlate with Poor Disease Control

Flow cytometric analysis of platelet P-selectin (CD61+CD62p+, A–B) and CD40 (CD61+CD40+, C–D) expression in patients with severe persistent asthma (step 5; n=16), moderate asthma (steps 3–4; n=23) and in the Control Group (CG: n=12). An inverse correlation was observed between platelet CD40 levels (CD61+CD40+) and Asthma Control Test (ACT) scores (E–F). Data are presented as median (25th–75th percentile). Statistical significance was assessed with the Mann–Whitney U test and correlation Spearman.

Figure 2. Platelet activation markers P-selectin and CD40 across control subjects and asthma inflammatory endotypes.

Flow cytometric analysis of platelet P-selectin (CD61+CD62p+) and CD40 (CD61+CD40+) expressed as percentage (%) (A, C) and mean fluorescence intensity (MFI) (B, D) in the Control Group (CG: n=12) and in the inflammatory endotypes: allergic type 2 (GT2A; n=20), non-allergic type 2 (GT2NA; n=13), and non-type 2 (GNT2, n=6).

Data are presented as median (25th–75th percentile). Statistical significance was assessed with the Mann–Whitney U test.

Flow cytometric analysis of platelet P-selectin (CD61+CD62p+) and CD40 (CD61+CD40+) expressed as percentage (%) (E, G) and mean fluorescence intensity (MFI) (F, H) across inflammatory endotypes: allergic type 2 (GT2A; n=20), non-allergic type 2 (GT2NA; n=13), and non-type 2 (GNT2, n=6).

Data are presented as median (25th–75th percentile). Statistical significance was assessed with the Mann–Whitney U test.

Figure 3. Platelet activation markers in distinct endotypes across clinical severity steps.

Flow cytometric analysis of platelet P-selectin (CD61+CD62p+) and CD40 (CD61+CD40+) expressed as percentage (%) (A–C, G–I) and mean fluorescence intensity (MFI) (D–F, J–L), across inflammatory endotypes: allergic type 2 (GT2A), non-allergic type 2 (GT2NA), and non-type 2 (GNT2).

Data are presented as median (25th–75th percentile). Statistical significance was assessed with the Mann–Whitney U test.

Figure 4. Enhanced platelet activation in patients receiving high-dose ICS/LABA therapy.

Flow cytometric analysis of platelet CD40 (CD61+CD40+) and P-selectin (CD61+CD62p+) expression shown as percentage (%) (A–B) and mean fluorescence intensity (MFI) (C–D) in patients treated with high-dose Inhaled Corticosteroids/Long-Acting β_2 -agonists (ICS/LABA) (YES) compared with those not receiving high doses therapy (NO).

Data are presented as median (25th–75th percentile). Statistical significance was determined using the Mann–Whitney U test.

Figure 5. Reduced RvD1 and 17(R)-RvD1 levels in severe persistent asthma.

Resolvins D1(RvD1) levels (A), and its epimer (17(R)-RvD1 (B) in a subgroup of patients (n=15) with severe persistent asthma (step 5; n=9) compared with those with moderate disease (steps 3–4; n=6). Table showing levels of D-series Resolvins (RvD1, 17(R)-RvD1, RvD2, RvD3 and RvD4) in severe (step 5) and moderate steps (step 3-4) of clinical severity of bronchial asthma (C). Data are presented as median (25th–75th percentile). Statistical significance was assessed using the Mann–Whitney U test.

Figure 6. Lower RvD2 levels in the type 2 allergic endotype.

Resolvin D2 (RvD2) levels in a subgroup of Control Group (CG, n=5) and patients across inflammatory endotypes (n=12): allergic type 2 (GT2A, n=5), non-allergic type 2 (GT2NA; n=4), and non-type 2 (GNT2; n=3) (A-B). Table showing levels of D-series Resolvins (RvD1, 17(R)-RvD1, RvD2, RvD3 and RvD4) across inflammatory endotypes: allergic type 2 (GT2A, n=5), non-allergic type 2 (GT2NA; n=4), and non-type 2 (GNT2; n=3) (C). Data are presented as median (25th–75th percentile). Statistical significance was assessed using the Mann–Whitney U test.

Table 1: Clinical characteristics of patients in the control group (CG) and patients stratified by the clinical severity of bronchial asthma [moderate (**step 3–4**) and severe (**step 5**)].

Table 1	CG	step 3-4	step 5	P
N	12	23	16	-
Male, n (%)	4 (33.3)	7 (30.4)	5 (31.3)	0.985
Age (years)	0 (0.0)	51.0 (44.5-58.0)	51.0 (43.3-58.0)	0.546
Weight (kg)	0 (0.0)	78.0 (69.0-90.0)	73.5 (59.3-80.8)	0.072
Obesity, n (%)	67.5 (58.0-74.5)	6 (26.1)	3 (18.8)	0.156
BMI (kg/m ²)	24.0 (22.2-24.7)	25.7 (23.3-30.0)	24.8 (21.4-28.9)	0.068
Waist Circumference (cm)	84.5 (74.5-95.8)	99.0 (91.8-105.3)	99.0 (85.0-103.0)	0.021
SAP (mmHg)	112.5 (107.3-120.0)	123.0 (104.0-140.0)	127.5 (112.0-133.5)	0.186
DAP (mmHg)	70.0 (60.2-72.8)	80.0 (70.0-92.0)	80.0 (75.3-87.0)	0.006
Red Blood Cell (*10 ⁶ /mmc)	4.6 (4.3-4.9)	4.9 (4.5-5.0)	4.9 (4.7-5.0)	0.092
Hemoglobin Count (g/dL)	13.6 (12.8-14.7)	14.0 (12.8-15.7)	14.1 (13.2-15.2)	0.663
Hematocrit (%)	41.3 (38.9-44.0)	42.3 (41.0-45.5)	42.3 (40.6-45.5)	0.582
Mean Corpuscular Volume (fL)	90.9 (89.2- 91.9)	88.3 (84.3-90.8)	86.4 (83.3 -91.2)	0.121
Mean Corpuscular Hemoglobin (pg)	30.0 (28.4-30.8)	29.4 (27.7-30.9)	28.7 (27.6-30.1)	0.359
Mean Corpuscular Hemoglobin concentration (%)	33.2 (32.9-30.8)	33.0 (32.3-34.1)	33.1 (32.0-33.8)	0.857
Platelet count (*10 ³ /mmc)	228.5 (182.8-258.0)	254.0 (228.0-286.0)	265.0 (237.0-323.8)	0.108
Mean Platelet Volume (fL)	10.2 (9.9-10.8)	10.3 (9.8-10.9)	10.3 (9.9-10.7)	0.993
Red Cell Distribution Width coefficient of Variation (%)	12.6 (12.2-13.1)	13.1 (12.5-13.6)	13.4 (12.8-14.2)	0.018
White Blood cell (*10 ³ /uL)	5.5 (4.3-6.5)	6.7 (5.6-7.0)	6.5 (5.9-8.2)	0.076
Neutrophil (*10 ³ /uL)	2.7 (2.2-3.5)	3.7 (2.9-4.4)	3.7 (3.1-4.8)	0.041
Lymphocyte (*10 ³ /uL)	2.1 (1.7-2.4)	1.9 (1.6-2.3)	2.0 (1.5-2.2)	0.837
Monocyte (*10 ³ /uL)	0.4 (0.3-0.5)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.031
Eosinophil (*10 ³ /uL)	0.1 (0.0-0.2)	0.2 (0.1-0.3)	0.2 (0.1-0.5)	0.104
Basophil (*10 ³ /uL)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.013
Dermatitis [(n (%))]	0.0 (0.0)	1 (4.3)	1 (6.3)	0.694
Rinite/CRsNP [(n (%))]	0.0 (0.0)	16 (69.6)	7 (43.8)	0.000
CRSwNP [(n (%))]	0.0 (0.0)	5 (21.7)	4 (25.0)	0.180
ASA Sensitivity [(n (%))]	0.0 (0.0)	3 (13.0)	2 (12.5)	0.426
Food Allergies [(n (%))]	1.0 (8.3)	1 (4.3)	2 (12.5)	0.366
GERD [(n (%))]	1.0 (8.3)	6 (26.1)	10 (62.5)	0.007
OSAS [(n (%))]	0.0 (0.0)	0 (0)	1 (6.3)	0.328
Autoimmune Disease [(n (%))]	1.0 (8.3)	3 (13.0)	4 (25.0)	0.436
Previous Neoplasia [(n (%))]	0.0 (0.0)	1 (4.3)	1 (6.3)	0.694
T2DM [(n (%))]	0.0 (0.0)	2 (8.7)	0 (0)	0.282
Hypertension [(n (%))]	1.0 (8.3)	7 (30.4)	4 (25.0)	0.338
Dyslipidemia [(n (%))]	2.0 (16.7)	4 (17.4)	4 (25.0)	0.211
Therapy				
ICS/LABA AB, n (%)	0.0 (0.0)	1.0 (4.3)	1.0 (6.3)	0.694
ICS/LABA low dose [(n (%))]	0.0 (0.0)	13.0 (21.7)	0.0 (0.0)	0.000
ICS/LABS medium dose [(n (%))]	0.0 (0.0)	10.0 (73.9)	2.0 (12.5)	0.007
ICS/LABA high dose [(n (%))]	0.0 (0.0)	0.0 (0.0)	14.0 (87.5)	0.000
LTRA [(n (%))]	0.0 (0.0)	1.0 (4.3)	4.0 (25.0)	0.044
LAMA [(n (%))]	0.0 (0.0)	0.0 (0.0)	5.0 (31.3)	0.002
Corticosteroid Nasal Spray [(n (%))]	0.0 (0.0)	7.0 (26.1)	6.0 (37.5)	0.058
Antihistamine [(n (%))]	0.0 (0.0)	8.0 (34.8)	4.0 (25.0)	0.070
β-blockers [(n (%))]	0.0 (0.0)	0.0 (0.0)	2.0 (12.5)	0.103
Diuretics [(n (%))]	1.0 (8.3)	3.0 (13.0)	0.0 (0.0)	0.144
ACE-I [(n (%))]	0.0 (0.0)	4.0 (17.4)	0.0 (0.0)	0.195
CCA [(n (%))]	0.0 (0.0)	2.0 (8.7)	3.0 (18.8)	0.249
Antiplatelet drugs [(n (%))]	0.0 (0.0)	1.0 (4.3)	0.0 (0.0)	0.537
Metformin [(n (%))]	0.0 (0.0)	2.0 (8.7)	0.0 (0.0)	0.282
sGLT2-i [(n (%))]	0.0 (0.0)	1.0 (4.3)	0.0 (0.0)	0.537
GLP-1 [(n (%))]	0.0 (0.0)	1.0 (4.3)	0.0 (0.0)	0.537
Thiazolidinediones [(n (%))]	0.0 (0.0)	1.0 (4.3)	0.0 (0.0)	0.537
Ezetimibe [(n (%))]	0.0 (0.0)	2.0 (8.7)	0.0 (0.0)	0.282
Statins [(n (%))]	0.0 (0.0)	4.0 (17.4)	0.0 (0.0)	0.071
Other Antihypertensives [(n (%))]	0.0 (0.0)	0.0 (0.0)	1.0 (6.3)	0.328
ARBs [(n (%))]	0.0 (0.0)	0.0 (0.0)	2.0 (12.5)	0.103
Proton Pump Inhibitors [(n (%))]	0.0 (0.0)	4.0 (17.4)	4.0 (25.0)	0.189

Abbreviation: BMI: Body Mass Index; SAP: Systolic Arterial Pressure; DAP: Diastolic Arterial Pressure; CRsNP: Chronic Rhinosinusitis without Nasal Polyps; CRSwNP: Chronic Rhinosinusitis with Nasal Polyps; ASA: AcetylSalicylic Acid; GERD: Gastroesophageal Reflux Disease; OSAS: Obstructive Sleep Apnea Syndrome; T2DM: Type 2 Diabetes Mellitus; ICS/LABA: Inhaled Corticosteroid / Long-Acting Beta-Agonist; LTRA: Leukotriene Receptor Antagonist; LAMA: Long-Acting Muscarinic Antagonist; ACE-I: Angiotensin-Converting Enzyme Inhibitor; CCA: Calcium Channel Antagonist; sGLT2i: Sodium-Glucose Cotransporter-2; GLP-1RA: Glucagon-Like Peptide-1receptro agonist, ARBs: Angiotensin II receptor blockers.

Table 2: Clinical characteristics of patients stratified by the clinical severity of bronchial asthma [moderate (steps 3–4) and severe (step 5)].

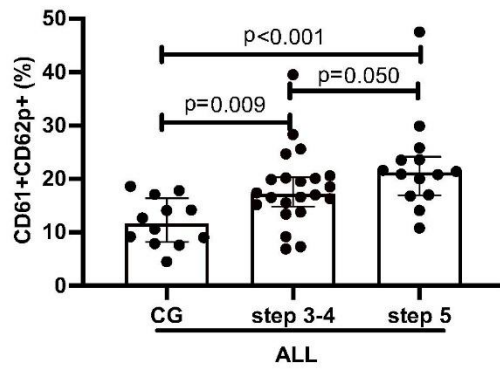
Table 2	step 3-4	step 5	P
N	23	16	-
Male, n (%)	7 (30.4)	5 (31.3)	0.909
Age (years)	51.0 (44.5-58.0)	51.0 (43.3-58.0)	0.921
Weight (kg)	78.0 (69.0-90.0)	73.5 (59.3-80.8)	0.143
Obesity, n (%)	6 (26.1)	3 (18.8)	0.593
BMI (kg/m ²)	25.7 (23.3-30.0)	24.8 (21.4-28.9)	0.373
Waist circumference (cm)	99.0 (91.8-105.3)	99.0 (85.0-103.0)	0.472
SAP (mmHg)	123.0 (104.0-140.0)	127.5 (112.0-133.5)	0.703
DAP (mmHg)	80.0 (70.0-92.0)	80.0 (75.3-87.0)	0.767
Red Blood Cell (*10 ⁶ /mmc)	4.9 (4.5-5.0)	4.9 (4.7-5.0)	0.724
Hemoglobin Count (g/dl)	14.0 (12.8-15.7)	14.1 (13.2-15.2)	0.966
Hematocrit (%)	42.3 (41.0-45.5)	42.3 (40.6-45.5)	0.855
Mean Corpuscular Volume (fl)	88.3 (84.3-90.8)	86.4 (83.3-91.2)	0.489
Mean Corpuscular Hemoglobin (pg)	29.4 (27.7-30.9)	28.7 (27.6-30.1)	0.489
Mean Corpuscular Hemoglobin Concentration (g/dL)	33.0 (32.3-34.1)	33.1 (32.0-33.8)	0.944
Platelet Count (*10 ³ /mmc)	254.0 (228.0-286.0)	265.0 (237.0-323.8)	0.420
Mean Platelet Volume (fl)	10.3 (9.8-10.9)	10.3 (9.9-10.7)	0.907
Red Cell Distribution Width Coefficient Of Variation (%)	13.1 (12.5-13.6)	13.4 (12.8-14.2)	0.168
White Blood Cell (*10 ³ /ul)	6.7 (5.6-7.0)	6.5 (5.9-8.2)	0.746
Neutrophil (*10 ³ / ml)	3.7 (2.9-4.4)	3.7 (3.1-4.8)	0.832
Lymphocyte (*10 ³ / ml)	1.9 (1.6-2.3)	2.0 (1.5-2.2)	0.877
Monocyte (*10 ³ / ml)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.855
Eosinophil (*10 ³ / ml)	0.2 (0.1-0.3)	0.2 (0.1-0.5)	0.810
Basophil (*10 ³ / ml)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.832
Dermatitis [(n (%))]	1 (4.3)	1 (6.3)	0.439
Rinite/CRsNP [(n (%))]	16 (69.6)	7 (43.8)	0.001
CRSwNP [(n (%))]	5 (21.7)	4 (25.0)	0.174
ASA Sensitivity [(n (%))]	3 (13.0)	2 (12.5)	0.320
Food Allergies [(n (%))]	1 (4.3)	2 (12.5)	0.257
GERD [(n (%))]	6 (26.1)	10 (62.5)	0.015
OSAS [(n (%))]	0 (0)	1 (6.3)	0.430
Autoimmune Disease [(n (%))]	3 (13.0)	4 (25.0)	0.318
Previous Neoplasia [(n (%))]	1 (4.3)	1 (6.3)	0.439
T2DM [(n (%))]	2 (8.7)	0 (0)	0.231
Hypertension [(n (%))]	7 (30.4)	4 (25.0)	0.272
Dyslipidemia [(n (%))]	4 (17.4)	4 (25.0)	0.184
Therapy			
ICS/LABA Low Dose [(n (%))]	1.0 (4.3)	1.0 (6.3)	0.791
ICS/LABA Medium Dose [(n (%))]	13.0 (21.7)	0.0 (0.0)	0.000
ICS/LABA High Dose [(n (%))]	10.0 (73.9)	2.0 (12.5)	0.039
LTRA [(n (%))]	0.0 (0.0)	14.0 (87.5)	0.000
LAMA [(n (%))]	1.0 (4.3)	4.0 (25.0)	0.058
Corticosteroid Nasal Spray [(n (%))]	0.0 (0.0)	5.0 (31.3)	0.005
Antihistamine [(n (%))]	7.0 (26.1)	6.0 (37.5)	0.715
β-Blockers [(n (%))]	8.0 (34.8)	4.0 (25.0)	0.515
Diuretics [(n (%))]	0.0 (0.0)	2.0 (12.5)	0.082
ACE-I [(n (%))]	3.0 (13.0)	0.0 (0.0)	0.133
CCA [(n (%))]	4.0 (17.4)	0.0 (0.0)	0.078
Antiplatelet Drugs [(n (%))]	2.0 (8.7)	3.0 (18.8)	0.356
Metformin [(n (%))]	1.0 (4.3)	0.0 (0.0)	0.398
sGLT2-i [(n (%))]	2.0 (8.7)	0.0 (0.0)	0.226
GLP-1RA [(n (%))]	1.0 (4.3)	0.0 (0.0)	0.398
Thiazolidinediones [(n (%))]	1.0 (4.3)	0.0 (0.0)	0.398
Ezetimibe [(n (%))]	1.0 (4.3)	0.0 (0.0)	0.387
Statins [(n (%))]	2.0 (8.7)	0.0 (0.0)	0.226
Other Antihypertensives [(n (%))]	4.0 (17.4)	0.0 (0.0)	0.078
ARBs [(n (%))]	0.0 (0.0)	1.0 (6.3)	0.225
Proton Pump Inhibitors [(n (%))]	0.0 (0.0)	2.0 (12.5)	0.082
ICS/LABA Low Dose [(n (%))]	4.0 (17.4)	4.0 (25.0)	0.563

Abbreviation: BMI: Body Mass Index; SAP: Systolic Arterial Pressure; DAP: Diastolic Arterial Pressure; CRsNP: Chronic Rhinosinusitis without Nasal Polyps; CRSwNP: Chronic Rhinosinusitis with Nasal Polyps; ASA: AcetylSalicylic Acid; GERD: Gastroesophageal Reflux Disease.; OSAS: Obstructive Sleep Apnea Syndrome; T2DM: Type 2 Diabetes Mellitus; ICS/LABA: Inhaled CorticoSteroid / Long-Acting Beta-Agonist; LTRA: Leukotriene Receptor Antagonist; LAMA: Long-Acting Muscarinic Antagonist; ACE-I: Angiotensin-Converting Enzyme Inhibitor; CCA: Calcium Channel Antagonist; GLP-1: Glucagon-Like Peptide-1.

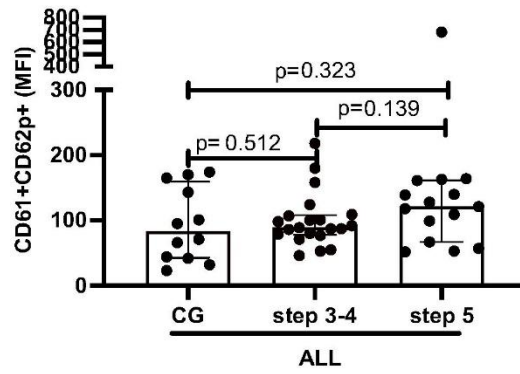
Figures

Figure 1

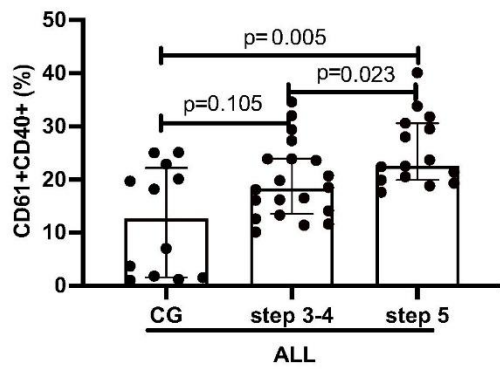
A



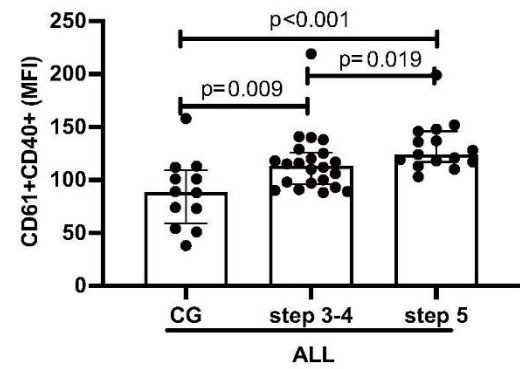
B



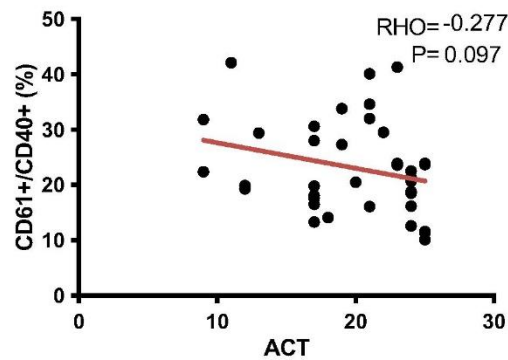
C



D



E



F

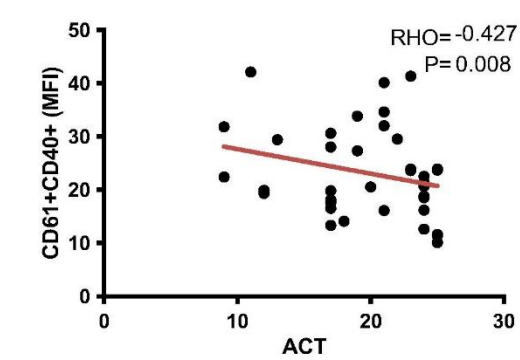


Figure 2

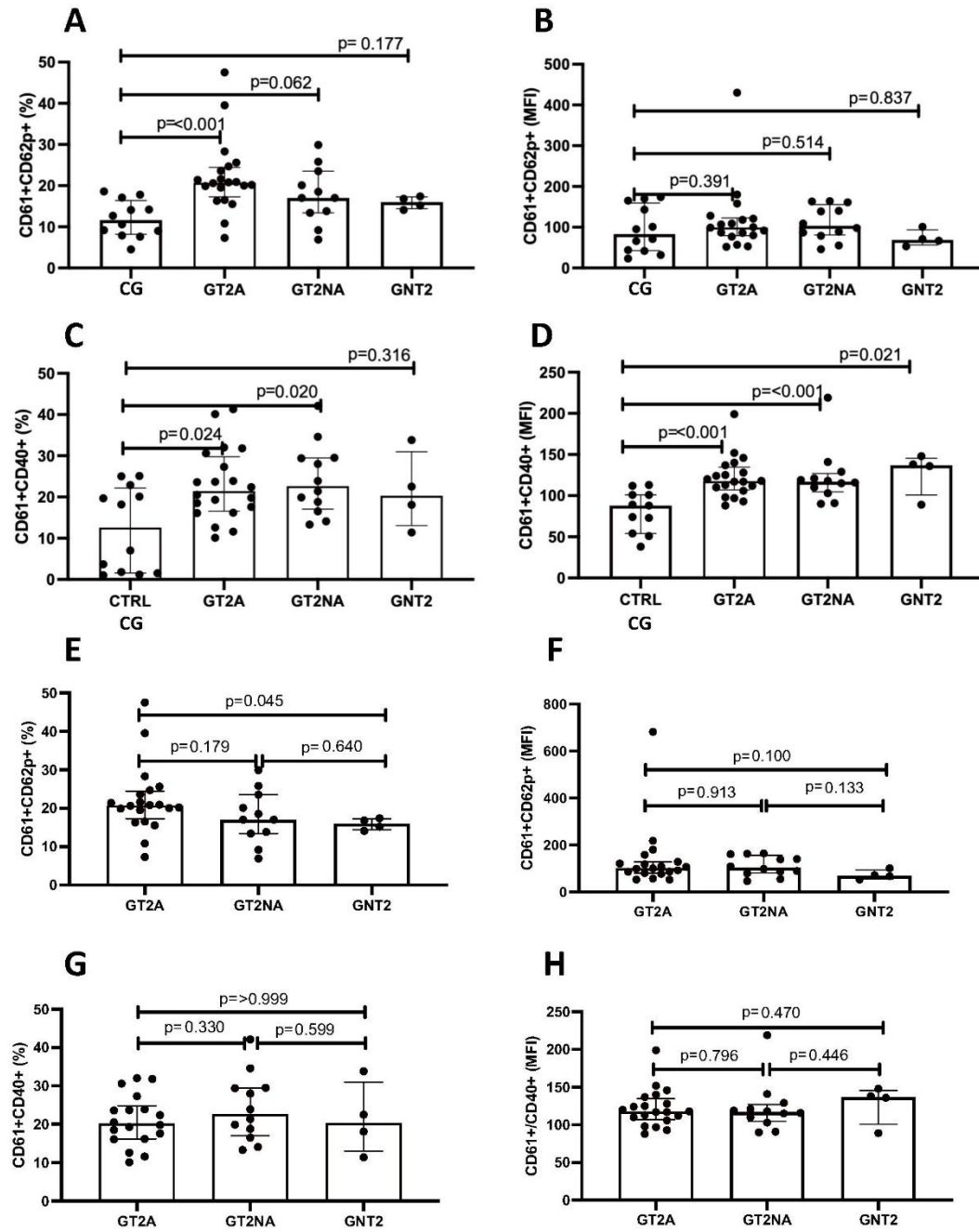


Figure 3

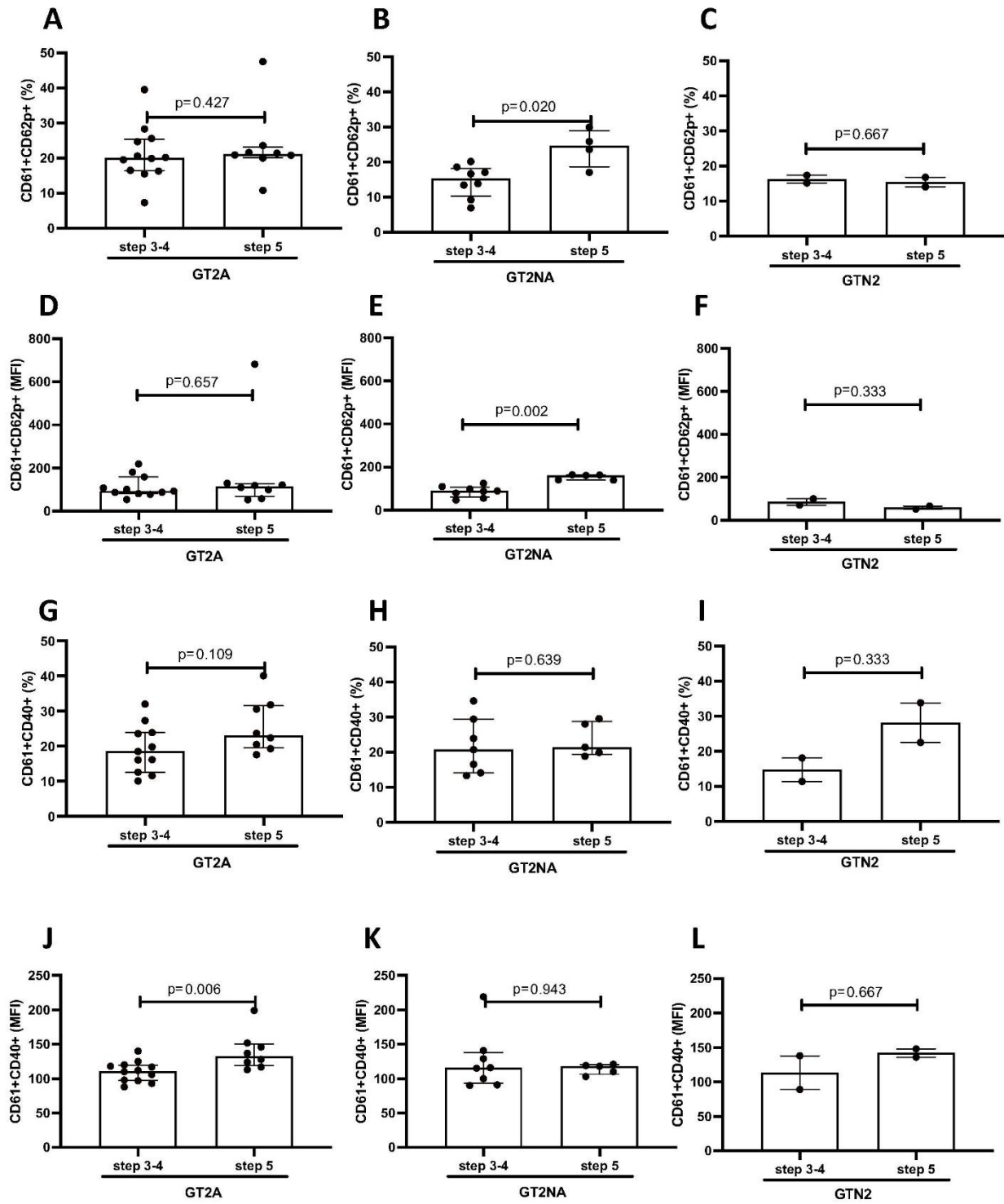


Figure 4

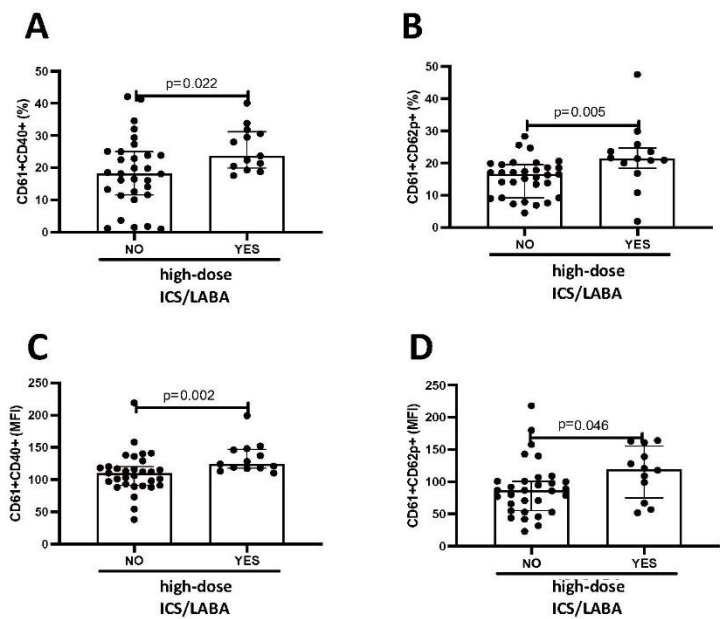


Figure 5

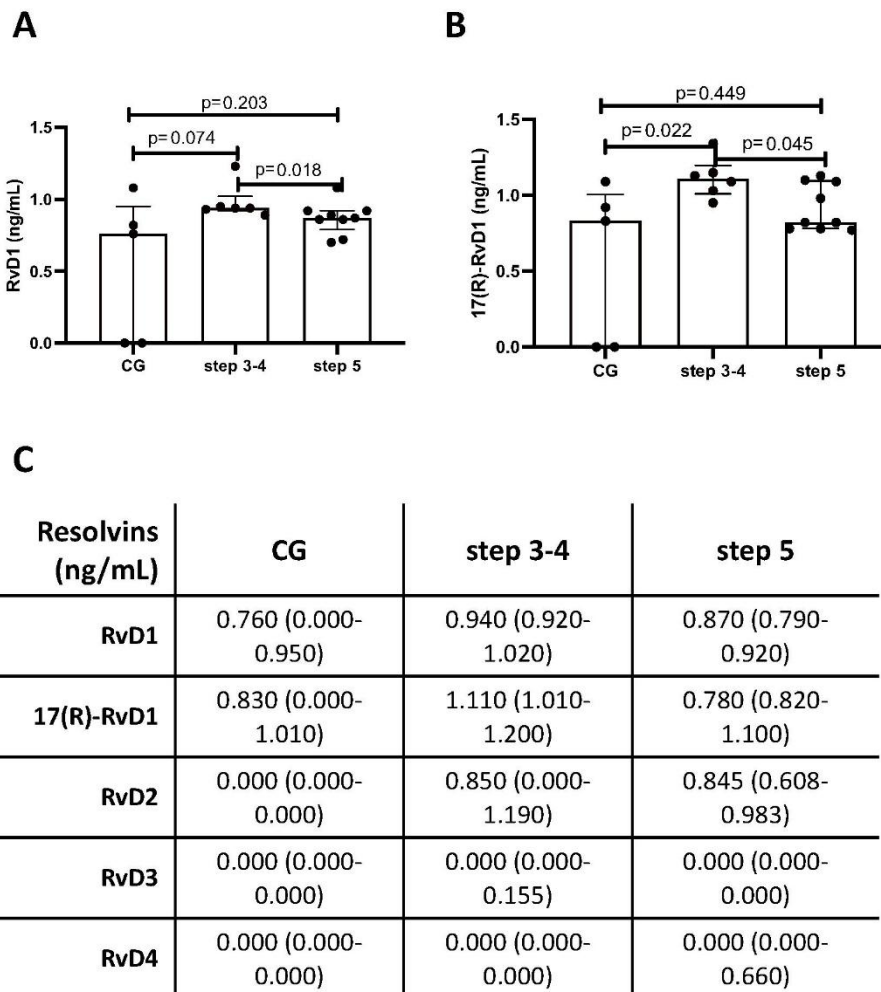
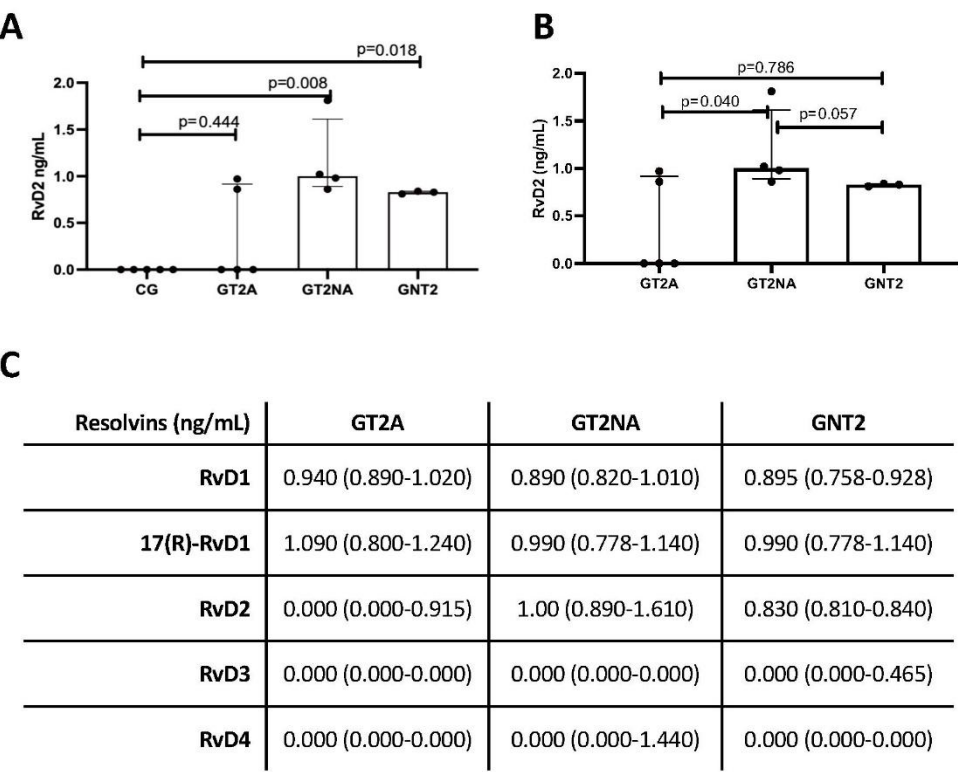


Figure 6



Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)
- [Table2.docx](#)
- [SupplementaryInformation.docx](#)