Immunoglobulin Free Light Chains in Severe Asthma Patient: could they be a new biomarker?

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Abstract

Background: Increasing evidence are available about the presence of increased serum concentration of Immunoglobulin (Ig) Free Light Chains (FLCs) in both atopic and non-atopic inflammatory diseases, including severe asthma, providing a possible new biomarker of disease, disease severity and also an alternative approach to the treatment. **Methods:** We analyzed clinical and laboratory data, including FLCs, obtained from a cohort of 79 asthmatic subjects, clinically classified into different GINA steps. A control group of 40 age-matched healthy donors (HD) was considered. Particularly, HD have been selected according to the absence of monoclonal components (in order to exclude paraproteinemias), were tested for total IgE (that were in the normal ranges) and were negative for aeroallergens specific IgE. Moreover, no abnormality of common inflammatory markers (i.e. erythrocyte sedimentation rate, C-reactive protein) was detectable. **Results:** FLC-k levels were significantly increased in the asthmatic population, compared to the control group. Despite the absence of statistically significant differences in FLC-λ levels, the FLC-k/FLC-λ ratio displayed remarkable differences between the two groups. A positive correlation between FLC-ϰ and FLC-λ levels was found. FLC- λ level displayed a significant negative correlation with the FEV1 value. Moreover, the FLC-ϰ /FLC- λ ratio was negatively correlated with the SNOT-22 score and a positive correlation was observed between FLCs and *Staphylococcus Aureus* IgE enterotoxins sensitization. **Conclusions:** Our findings confirmed the role of FLCs in asthma as a potential biomarker in an inflammatory disease characterized by different endotypes and phenotypes. In particular, FLC-ϰ and FLC-k/FLC-λ ratio could be a qualitative indicator for asthma, while FLC-λ levels could be a quantitative indicator for disease severity.

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Abstract

Background: Increasing evidence are available about the presence of increased serum concentration of Immunoglobulin (Ig) Free Light Chains (FLCs) in both atopic and non-atopic inflammatory diseases, including severe asthma, providing a possible new biomarker of disease, disease severity and also an alternative approach to the treatment.

Methods: We analyzed clinical and laboratory data, including FLCs, obtained from a cohort of 79 asthmatic subjects, clinically classified into different GINA steps. A control group of 40 age-matched healthy donors (HD) was considered. Particularly, HD have been selected according to the absence of monoclonal components (in order to exclude paraproteinemias), were tested for total IgE (that were in the normal ranges) and were negative for aeroallergens specific IgE. Moreover, no abnormality of common inflammatory markers (i.e. erythrocyte sedimentation rate, C-reactive protein) was detectable.

Results: FLC-k levels were significantly increased in the asthmatic population, compared to the control group. Despite the absence of statistically significant differences in FLC-λ levels, the FLC-k/FLC-λ ratio displayed remarkable differences between the two groups. A positive correlation between FLC-ϰ and FLC-λ levels was found. FLC- λ level displayed a significant negative correlation with the FEV1 value. Moreover, the FLC-ϰ /FLC- λ ratio was negatively correlated with the SNOT-22 score and a positive correlation was observed between FLCs and *Staphylococcus Aureus* IgE enterotoxins sensitization.

Conclusions: Our findings confirmed the role of FLCs in asthma as a potential biomarker in an inflammatory disease characterized by different endotypes and phenotypes. In particular, FLC-ϰ and FLC-k/FLC-λ ratio could be a qualitative indicator for asthma, while FLC-λ levels could be a quantitative indicator for disease severity.

Keywords: biomarker, free light chains, severe asthma, type 2 inflammation.

Background

Inflammation is an important component of cancers and chronic diseases and many inflammatory mediators have shown to have potential prognostic roles. Healthy individuals produces five classes of immunoglobulins: IgG, IgM, IgA, IgD and IgE. Immunoglobulins, normally, consist of two identical heavy chains and two identical light chains. The heavy chains select which class the immunoglobulin belongs to. Different conditions are characterized by the overproduction of monoclonal immunoglobulins. In some patients, only light chains are produced. More studies have investigated the role of immunoglobulin free light chains (FLCs), that can trigger mast cell activation in an antigen-specific manner. Increased expression of FLCs has been observed within the stroma of many human cancers including breast, colon, lung, pancreas, kidney and skin. Increased serum concentration of polyclonal FLCs in inflammatory diseases has been correlated with the degree of inflammation (1).

The ability of free light chains to activate mast cells and then to become an active part of the pathogenetic mechanisms of chronic inflammatory diseases led to an increase of interest in their clinical use, both as an attractive therapeutic target and as a biochemical marker of disease evolution or remission (2). It has been hypothesized that differences in serum immunoglobulins, FLCs and secretory IgA (sIgA) could exist between subjects with asthma of varying severity and non-asthmatic subjects; moreover, the circulating FLCs levels could correlate with lung function, symptoms and airway inflammation (3).

FLCs mediate antigen-specific hypersensitivity responses in the presence of a not yet identified FLCs receptor on mast cells, independently of complement activation. γ-chain associated receptors, such as FcεRI and FcγRIII, are not involved in FLC-triggered mast-cell activation. Redegeld at al. have isolated a mast-cell membrane associated protein that interacts with FLCs (4).

Mast cells and neutrophils are fundamental cells in the sensitization and effector phases of chronic inflammatory immune responses in the lung, therefore a possible link between mast cells and FLCs suggest their potential role in the pathophysiology of asthma and might be a novel biomarker involved in the humoral immune response to antigens (4-6).

FLCs extend neutrophil lifespan suggesting an effective contribution to chronic neutrophilia associated with chronic obstructive pulmonary disease (COPD). Increased levels of FLCs in serum and lung tissues have been associated with increased blood neutrophil count in COPD patients. FLCs binding to neutrophils induces CXCL8 inflammatory chemotactic mediator, increasing neutrophils recruitment into the airways with enhanced blood neutrophilia in COPD (7,8).

In the mammalian immune system, two isotypes, k and λ, of FLCs are produced. The k/ λ ratio significantly varies among species. Serum FLCs levels are elevated in autoimmune diseases as systemic lupus erythematosus, rheumatoid arthritis and Sjögren syndrome and changes in their levels are associated with disease activity (9).

Both k and λ FLCs share a common binding site on Tamm–Horsfall protein (THP), a monomeric glycoprotein produced by cells in the ascending limb of Henle of the kidney. The responsible of this binding is F99, a 9-mer peptide derived from the amino-acid sequence in THP (10). Kraneveld et al. have shown that the highly selective FLCs antagonist (F-991) could be used to inhibit the development of airway hyperreactivity and inflammation (6) and has demonstrated to have remarkable biological activity in a number of animal models of allergic diseases, representing a potential treatment of allergic diseases in humans (11). Therefore, the presence and the characterization of FLCs in atopic patients could be of interest as they may provide an alternative approach to the treatment.

Recent studies evaluated the presence of FLCs in SARS-CoV-2 infected patients. SARS-CoV-2 affects the upper respiratory tract, preferentially nasal ciliated cells, mucus-producing cells and ciliated cells in the bronchial epithelium.

The damage of the epithelial barrier is the basis of chronic inflammatory diseases, including the most severe forms of asthma. Firstly, Malecka-Gieldowska et al. (12) observed that FLCs levels were markedly elevated in COVID-19 patients in comparison to non COVID-19 intensive care unit (ICU) patients. Importantly, the k/ λ ratio was similar in those groups, but λ concentration was higher and the k/ λ ratio decreased in SARS-CoV-2-infected but non-hospitalized in ICU group, compared to the non-infected patients from ICU. There was also a difference in the k concentration and k /λ ratio between tested groups with the highest values in COVID-19 patients.

Recently, Napodano et al. (13) compared salivary levels of immunoglobulin A subclasses (IgA1 and IgA2) FLCs in a cohort of 29 SARS-CoV-2 patients and 21 healthy subjects, describing the role of λFLC as an ideal indicator of patient conditions, that effectively could monitor patients' fluctuation in real-time.

It is well known that healthy airway epithelium produces inducible Nitric Oxide Synthase (iNOS) (14), but its expression is significantly upregulated in asthmatic patient's airways, mainly in epithelial and inflammatory cells such as eosinophils, neutrophils, and macrophages. Nitric oxide (NO) acts as a messenger molecule and its activity depends on factors such as oxidative stress, antioxidants, and the amount and activity of NOS. NO has a role in muco-ciliary function and ciliary movement frequency, in epithelial ion transport, in restoring barrier dysfunction by damage repair processes after barrier injury and in modulating inflammation by regulating epithelial production of inflammatory mediators, contributing to the patient's innate defense.

Increased NO levels contributes to bronchial hyperreactivity and mucus hypersecretion, increases vascular permeability, reduces ciliary heartbeat, and promotes free radical production, airway inflammation, and tissue damage (15). Fractional exhaled nitric oxide (FeNO) has gained great clinical importance as a biomarker of type 2 inflammation in chronic airway diseases such as asthma and it is also very useful to identify those severe asthma patients that might benefit from personalized therapies with monoclonal antibodies.

Aim of this study was to describe clinical and laboratory characteristics of a population of asthmatics patients and evaluate the presence and isotypes distribution of FLCs in asthmatic and healthy subjects, aiming to investigate their potential role as quantitative and objective serum biomarkers of this condition. Additionally, the study seeks to evaluate any clinical correlations with disease severity.

Methods

Clinical evaluation, based on patient reported outcomes (Asthma Control Questionnaire 5 (ACQ5) and Asthma Control Test (ACT), was performed. Pulmonary function tests and FeNO have been used to assess airway function and inflammation, respectively. Sino-nasal Outcome Test 22 (SNOT-22) has been used to score upper airway involvement. Sensitization to *Staphylococcus aureus* enterotoxins was detected by ImmunoCAP (ThermoFisher Scientific/Phadia, Uppsala, Sweden).

FLCs Laboratory Testing

Each sample was tested on the OPTILITE (The Binding Site, Birmingham, UK) analyzers, according to the manufacturer's instructions.

Statistical analysis

Results

The clinical and baseline parameters of the patients are summarized in Table 2. Interestingly, no gender-based differences were observed in the studied parameters (Table S1-S2).

In figure 3, a correlation matrix of Spearman's correlation coefficients is shown. As expected, FLC-k and FLC-λ strongly and positively correlated. Consequently, also their linear combinations show positive correlations. Very interestingly, FLC- λ levels, and not FLC-k levels, display a significant and negative correlation with the FEV1 parameter, which provides information on the disease severity. A moderate positive correlation is observed for FLC-k and FLC-k +FLC-λ and*Staphylococcus Aureus* enterotoxins Ultimately, the FLC-k/FLC-λ ratio negatively correlates with the SNOT-22 score.

A linear regression analysis of FLC-λ (figure 4A) as a function of FEV1 was performed, obtaining a significant slope of 1.42±0.13 (p=0.027) and an intercept of 28.5±4.8 (p=1.35e-6), confirming that FLC-λ levels could provide a quantitative indicator of disease severity. This hypothesis is further strengthened by the results of figure 4B, which show a significant decrease in FLC-λ levels in patients treated with

Figure Legends

Figure 2. FLC-k levels as a function of FLC-λ levels in asthmatic patients (cyan dots) and (red dots).

Figure 4: FLC-λ levels as a function of the FEV1 values (A). FEV1 data are reported as a percentage. FLC-λ levels in patients systemic corticosteroids (B).

Table 1: Age, free light chain (FLCs) and FeNO levels in patients and healthy controls.

Variable	**Asthma**, N = 79[1]	**Ctrl**, N = 40[1]
age	56 (49, 63)	63 (34, 71)
k	17 (13, 21)	10 (8, 13)
λ	14.5 (12.4, 16.4)	15.6 (9.1, 20.4)
k/ λ	1.21 (1.06, 1.40)	0.70 (0.61, 0.82)
k+ λ	31 (26, 37)	26 (17, 33)
FeNO	40 (24, 82)	8 (6, 13)
[1]Median (IQR) [2]Wilcoxon rank sum test	[1]Median (IQR) [2]Wilcoxon rank sum test	[1]Median (IQR) [2]Wilcoxon rank sum

Table 2: Clinical and baseline parameters of the asthmatic population.

Variable	**N = 79**[1]
Gender	
F	46 (58%)
M	33 (42%)
Age (years)	56 (49, 63)
Disease Duration (Years)	16 (10, 27)
Chronic Rhinosinusitis	
Without Nasal Polyps	7 (8.9%)
With Nasal Polyps	52 (66%)
Absent	20 (25%)
Atopy	46 (58%)
FEV1 (%)	73 (59, 89)
GINA-STEP	
3	2 (2.5%)
4	9 (11%)
5	68 (86%)
OCS	29 (53%)
SNOT-22	66 (51, 88)
Blood Eosinophils	510 (400, 900)
Exacerbations *(in 12 months)*	
0	7 (8.9%)
1	8 (10%)
2	29 (37%)
3	17 (22%)
4	9 (11%)
5	3 (3.8%)
>6	6 (6.3%)
Hospitalization	12 (25%)
Smoking habits	30 (38%)
m80	0.05 (0.01, 0.15)

m81	0.05 (0.02, 0.38)
m223	0.12 (0.02, 0.36)
m226	0.20 (0.06, 0.52)
[1]n (%); Median (IQR)	[1]n (%); Median (IQR)

Authors' contribution

CC, IB, SC, DF, DB, RI, MC, MS, CC conceptualized the idea and collected clinical data about patients, contributed to write and revise the paper. VB and MM performed laboratory assessments, GC e RDS performed statistical analysis of data. AG, GP, GWC, EH, and SDG critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest for this manuscript.

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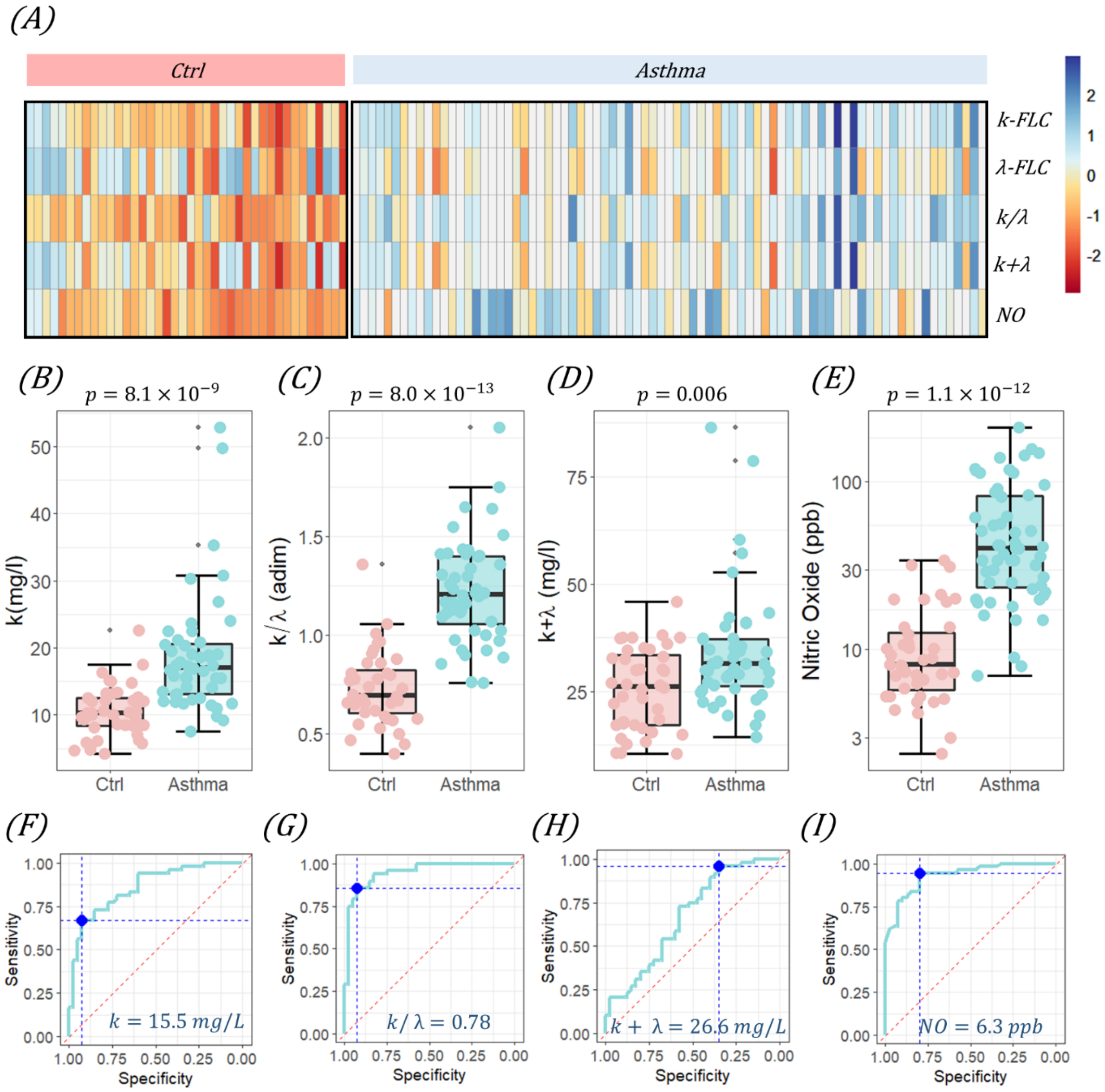
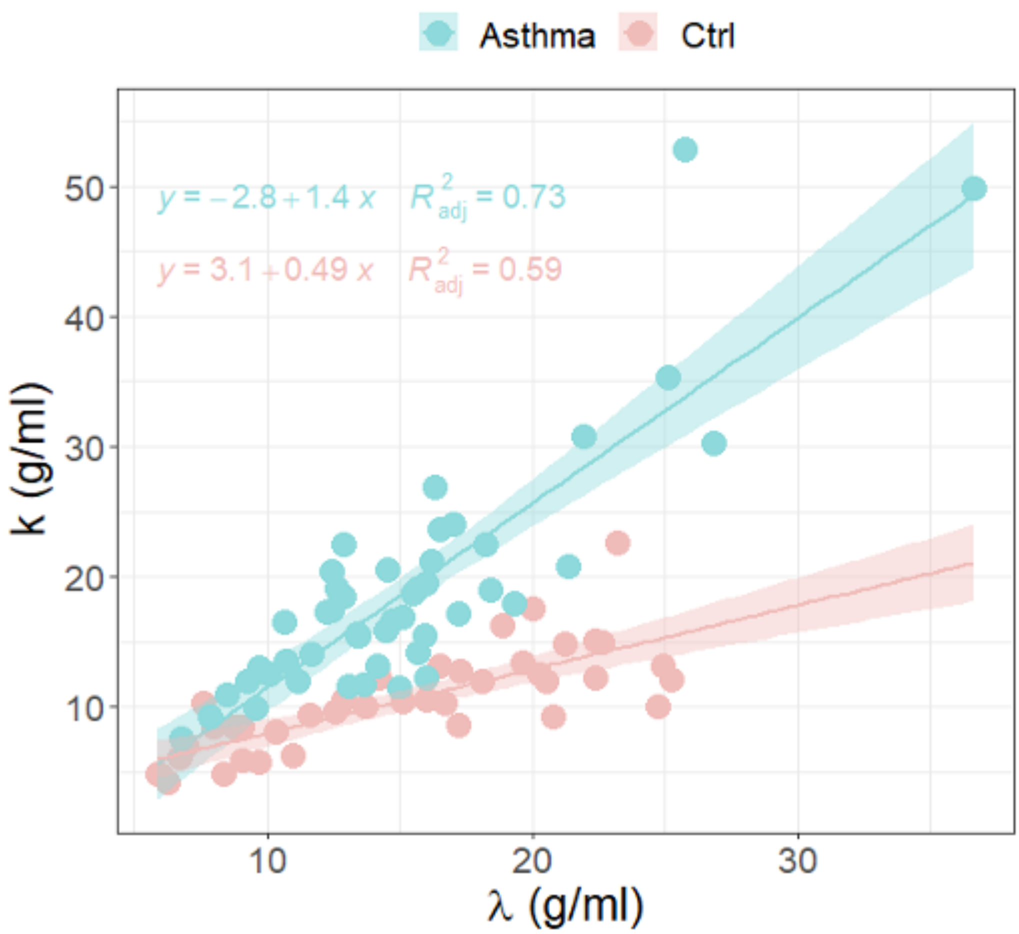
Figure 1



(A)
Ctrl
Asthma
k-FLC
λ-FLC
k/λ
k+λ
NO
2
1
0
-1
-2
(B)
p = 8.1 × 10⁻⁹
k(mg/l)
Ctrl
Asthma
(C)
p = 8.0 × 10⁻¹³
k/λ (adim)
Ctrl
Asthma
(D)
p = 0.006
k+λ (mg/l)
Ctrl
Asthma
(E)
p = 1.1 × 10⁻¹²
Nitric Oxide (ppb)
Ctrl
Asthma
(F)
Sensitivity
Specificity
k = 15.5 mg/L
(G)
Sensitivity
Specificity
k/λ = 0.78
(H)
Sensitivity
Specificity
k + λ = 26.6 mg/L
(I)
Sensitivity
Specificity
NO = 6.3 ppb

Figure 2



Asthma
Ctrl
$y = -2.8 + 1.4\,x \quad R^2_{adj} = 0.73$
$y = 3.1 + 0.49\,x \quad R^2_{adj} = 0.59$
50
40
30
20
10
k (g/ml)
10
20
30
λ (g/ml)

Figure 3

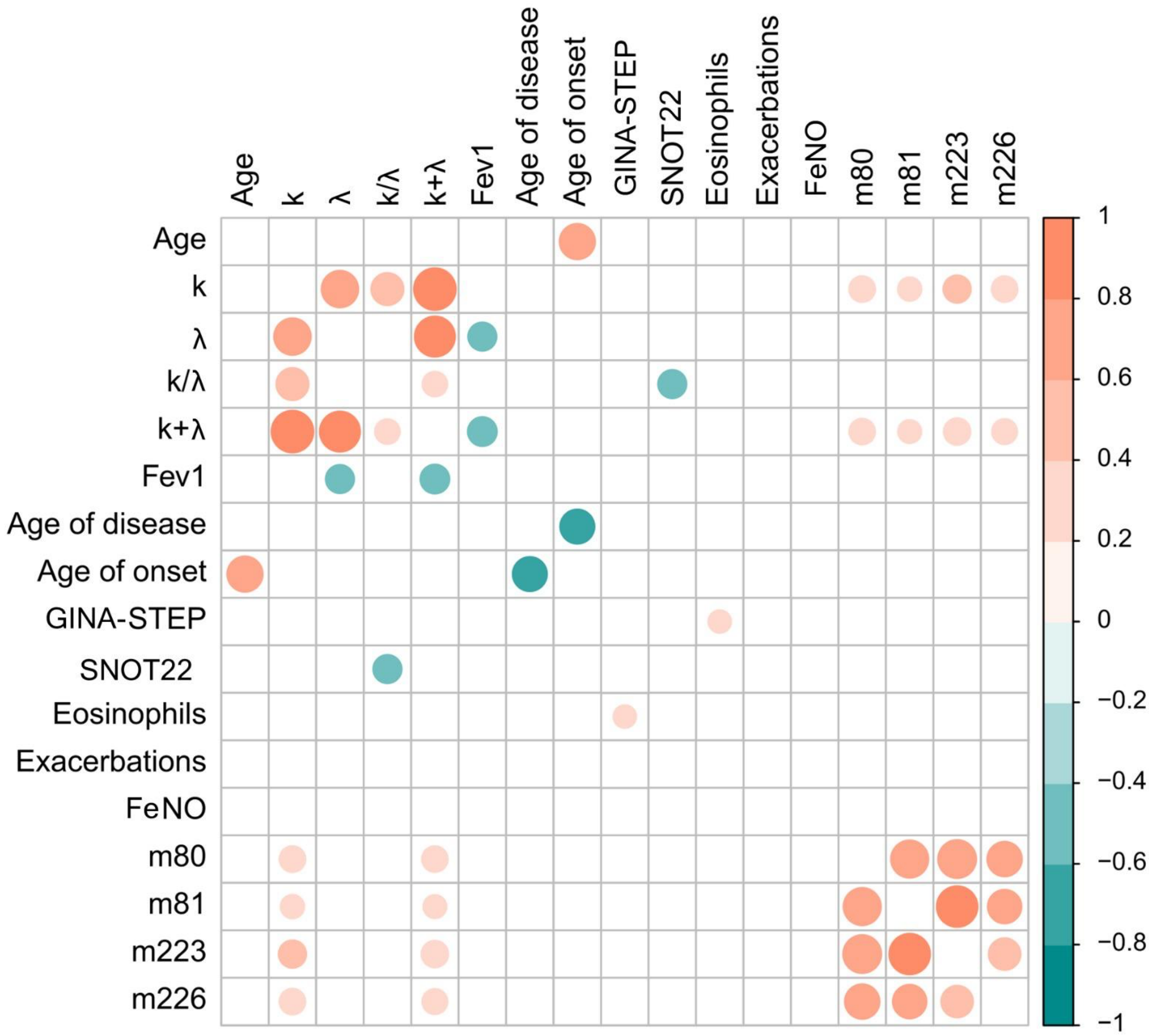
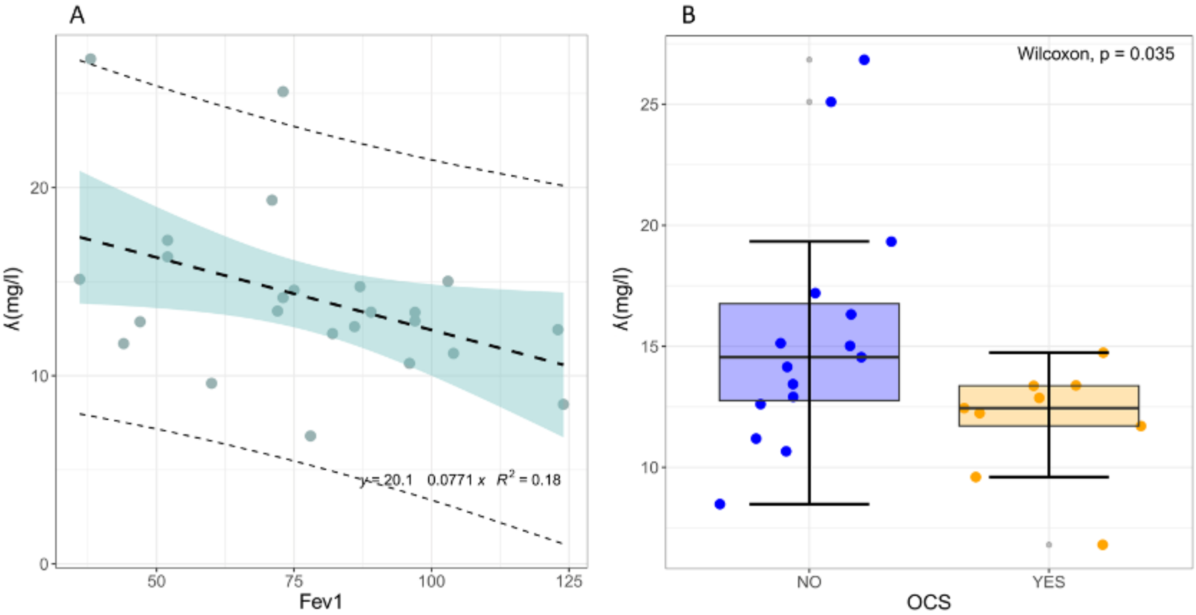

Age
k
λ
k/λ
k+λ
Fev1
Age of disease
Age of onset
GINA-STEP
SNOT22
Eosinophils
Exacerbations
FeNO
m80
m81
m223
m226
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 4



A
Λ(mg/l)
0
10
20
50
75
100
125
Fev1
y = 20.1   0.0771 x   R² = 0.18
B
Wilcoxon, p = 0.035
Λ(mg/l)
10
15
20
25
NO
YES
OCS