"Soluble form of the receptor for advanced glycation end products (sRAGE) as a marker of inflammation in pediatric cystic fibrosis population, a pilot study."

Cristina De Manuel[1], Verónica Sanz-Santiago[1], Alejandro López-Neyra[1], África González Murillo[1], José Villa[1], and Alberto García-Salido[2]

[1]Hospital Infantil Universitario Nino Jesus
[2]Hospital Infantil Universitario Nino Jesus Servicio de Cuidados Intensivos Pediatricos

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Abstract

The receptor for advanced glycation end products (RAGE) has been studied in several respiratory diseases described as an important inflammatory mediator. The RAGE-axis is activated by multiple endogenous ligands related to pro-inflammatory states, upregulate the RAGE expression. The function of soluble RAGE (sRAGE) is not completely understood, it has been hypothesized an anti-inflamatory role as RAGE decoy receptor. Few studies have explored the RAGE-axis in Cystic Fibrosis (CF) with contradictory results. Based on previously, we present this pilot study with the aim of describe the plasma sRAGE levels in children with cystic CF (CFp), compare with the sRAGE levels in a healthy cohort and study its possible correlation with CFp clinical features. We conducted a single-center, cross-sectional observational study. We included 35 clinically stable CF patients (aged < 18 years). The median plasma sRAGE level in CFp was 1494,75 pg/ml [interquartile range (IQR) 708,75pg/ml], compared with 714,20 pg/ml (IQR 490,50 pg/ml)) in the historical cohort of healthy controls (p < 0,001). A positive correlation was found between plasma sRAGE level and forced expiratory volume in 1 second/forced vital capacity ratio (FEV1/FVC) (p 0,004) and forced expiratory flow between 25% and 75% (FEF25%-75%) (p 0,032). In this preliminary study, the plasma sRAGE level were higher in CFp than in healthy controls. Also, we described a positive correlation between FEV1/FVC and FEF25%-75% and plasma sRAGE. To our knowledge, our study is the largest to describe plasma sRAGE values in CFp and the only one carried out in pediatric CF population.

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AUTHORS:

Cristina de Manuel Gómez [a1] (ORCID: 0000-0001-6224-1561), Verónica Sanz-Santiago[a] (ORCID: 0000-0003-3368-8393), Alejandro López Neyra[a] (ORCID: 0000-0002-4032-1429), África González Murillo[b], José Ramón Villa Asensi[a], Alberto García-Salido[c] (ORCID: 0000-0002-8038-7430).

[a] Sección Neumología Infantil, Hospital Infantil Universitario Niño Jesús, Madrid, Spain.[b] Laboratorio oncohematología, Hospital Infantil Universitario Niño Jesús, Madrid, Spain.[c] Sección Cuidados Intensivos Pediátricos, Hospital Infantil Universitario Niño Jesús, Madrid, Spain.[1] Present address: Sección Neumología Infantil, Hospital Universitario La Paz, Madrid, Spain.

**Correspondance author: Cristina de Manuel Gómez*Telephone: +34 620 443 540 Email:*cristina6490@gmail.com*

All authors have made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be submitted.

DECLARATION OF INTEREST:

All the authors declare to have no conflict of interest directly or indirectly related to the manuscript contents.

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ABSTRACT

The receptor for advanced glycation end products (RAGE) has been studied in several respiratory diseases described as an important inflammatory mediator. The RAGE-axis is activated by multiple endogenous ligands related to pro-inflammatory states, upregulate the RAGE expression. The function of soluble RAGE (sRAGE) is not completely understood, it has been hypothesized an anti-inflamatory role as RAGE decoy receptor.

Few studies have explored the RAGE-axis in Cystic Fibrosis (CF) with contradictory results. Based on previously, we present this pilot study with the aim of describe the plasma sRAGE levels in children with cystic CF (CFp), compare with the sRAGE levels in a healthy cohort and study its possible correlation with CFp clinical features.

We conducted a single-center, cross-sectional observational study. We included 35 clinically stable CF patients (aged < 18 years). The median plasma sRAGE level in CFp was 1494,75 pg/ml [interquartile range (IQR) 708,75pg/ml], compared with 714,20 pg/ml (IQR) in the historical cohort of healthy controls (p < 0,001). A positive correlation was found between plasma sRAGE level and forced expiratory volume in 1 second/forced vital capacity ratio (FEV1/FVC) (p 0,004) and forced expiratory flow between 25% and 75% (FEF25%-75%) (p 0,032).

In this preliminary study, the plasma sRAGE level were higher in CFp than in healthy controls. Also, we described a positive correlation between FEV1/FVC and FEF25%-75% and plasma sRAGE. To our knowledge, our study is the largest to describe plasma sRAGE values in CFp and the only one carried out in pediatric CF population.

LETTER:

To the Editor;

The receptor for advanced glycation end products (RAGE) is a protein member of the immunoglobulin superfamily. It is constitutively expressed in the lungs and localized in the basal membrane of type 1 and 2 alveolar epithelial cells. Also, the RAGE has been described in vascular smooth muscle cells, endothelial cells, and immune cells.

Due to its main expression in the lungs the has been studied in several respiratory diseasesdescribed as an important inflammatory mediator in allergic airway inflammation, asthma, pulmonary fibrosis, lung cancer, chronic obstructive pulmonary disease, acute lung injury, pneumonia, cystic fibrosis, bronchopulmonary dysplasia, and COVID-19[1].

The RAGE-axis is activated through the presence of a wide variety of endogenous ligands like the advanced glycation end products (AGEs), S100/calgranulin proteins or high mobility group box 1 protein (HMGB1). Those RAGE-ligands, expressed in pro-inflammatory states, upregulate the RAGE expression and induce the upregulation of soluble isoforms. The soluble RAGE or sRAGE is the predominant RAGE isoform in plasma. It is derived from membrane-bound RAGE or full-length RAGE (mRAGE or fl-RAGE) by proteolytic cleavage[2]. Others soluble RAGE forms are consequence of RNA alternative splicing, its main example is the endogenous secretory RAGE (esRAGE)[3]. The function of those soluble isoforms is not

completely understood, and it has been hypothesized that they have an anti-inflamatory role as RAGE decoy receptor. Because of its similarity with mRAGE, the sRAGE may capture and inactivate circulating ligands. This may prevent RAGE-axis activation and therefore, stop or downregulate the proinflammatory status[4].

Cystic fibrosis (CF) is the most common life-threatening genetic condition in Caucasian population. It is associated with bronchial infection and airway inflammation due to an impaired mucociliary clearance. Different inflammatory pathways play a critical role in CF lung disease progression, making it an attractive area of research and important therapeutic target.

Few studies have explored the RAGE-axis in CF. These studies have been focused on CF related diabetes (CFRD) and have shown contradictory results. Mulrennan et al[5] assessed RAGE in healthy controls, CF patients, CFRD patients and diabetics no-CF. They concluded that sRAGE did not differ significantly among any group. In the other side, sputum sRAGE level were significantly lower in CF and CFRD patients compared to healthy and diabetics subjects, despite a markedly overexpressed of mRAGE mRNA in CF and CFRD sputum. In addition, they found a significantly positive correlation with sputum sRAGE level and forced expiratory volume in 1 second percent predicted (FEV_1pp). In contrast, Hunt et al[6] did not find any significant relationships between plasma sRAGE levels and absolute values of FEV_1. But the plasma advanced glycation end products (AGEs) were significantly elevated in CFRD and correlate negatively with FEV_1. This report found that CFRD patients had high plasma levels of AGEs and S100A12, but sRAGE was not significantly different among CF, CFRD and healthy controls.

Based on previously explained, our group present this preliminary study. The first objective was to describe the plasma sRAGE levels in children with cystic CF (CFp). Later we compare these values with the sRAGE levels in a healthy cohort. Finally, we study its possible correlation with CFp baseline clinical features.

We conducted a single-center, cross-sectional observational study in the Pediatric CF Unit of a tertiary hospital in Madrid (Spain). The study was carried out according to the principles of the Declaration of Helsinki and current legislation and approved by the ethic committee. We included consecutive clinically stable CF patients (aged $<$ 18 years) since November 2018 to October 2019. Blood tests and clinical data collection were performed at the same time as the annual review. We include clinical and demographic data such age, sex, pancreatic status, CF liver disease (CFLD), diabetes related to CF (CFRD), airway colonization-infection, pulmonary function test, CFTR mutations, body mass index, current treatments, respiratory support, history of hemoptysis, history of allergic bronchopulmonary aspergillosis (ABPA). Plasma sRAGE level were measured by Human RAGE Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN), using an enzyme-linked polyclonal antibody specific for human RAGE (extracellular domain). We collected 3 ml of whole blood via venipuncture in EDTA blood tube and spun at 1500 rpm for 10 min. After that, plasma was stored at -80 degC until use with the commercial kits. Serum sRAGE level was compared to a historical cohort of healthy controls.

Thirty-five CFp were included, their clinical characteristics are provided in Table 1. The healthy cohort differs significantly from our CFp in age, with a median age of 9 and 12,04 years respectively ($p = 0.003$) (Figure 1). The median plasma sRAGE level in CFp was 1494,75 pg/ml [interquartile range (IQR) 708,75pg/ml], compared with 714,20 pg/ml (IQR) in the historical cohort of healthy controls, being statistically significantly different (Wilcoxon-Mann-Whitney test, $p < 0{,}001$). Univariate analyzes were performed (Spearman's Rho) to correlate clinical characteristics with plasma sRAGE levels. A positive correlation was found between plasma sRAGE level and the following spirometry values: forced expiratory volume in 1 second/forced vital capacity ratio (FEV_1/FVC) (0.488, p 0,004) and forced expiratory flow between 25% and 75% ($FEF_{25\%-75\%}$) (0.38, p 0,032).

In this preliminary study, the plasma sRAGE level were higher in CFp than in healthy controls. To our knowledge, our study is the largest to describe plasma sRAGE values in CFp and the only one carried out in pediatric CF population. Also, in contrast with previous papers, we described a positive correlation between FEV1/FVC and $FEF_{25\%-75\%}$ and plasma sRAGE[5,6].

As said, the sRAGE levels were higher in CFp than in healthy controls. This observation may be due to a proinflammatory status in the respiratory airway of CFp. The sRAGE cleavaged from lung cells may act as a decoy receptor for the different RAGE ligands. Related with this observation we also described a positive correlation between FEV1/FVC and $FEF_{25\%-75\%}$ and plasma sRAGE. Both spirometric values inform about airway obstruction and early lung damage in CF. In our cohort, those children with higher sRAGE levels showed lower values. The possible sRAGE protective role in these children should be studied and confirmed in future studies. Finally, we did not observe correlation between sRAGE and FEV_1. Most our patients have a normal FEV_1 baseline value and this may influence our results which are similar to the observations done by Hunt et al[6].

Our preliminary study has several limitations. It was cross-sectional and do not allow to study sRAGE temporal dynamic in the children included. Related to the comparison with healthy children we observed that they were younger than our CFp group. We compared the plasma sRAGE level with an historical cohort, not matched controls. It may affect the results, but we found a large difference in the absolute value of sRAGE, hardly explainable only by age. About the RAGE-axis, we didn't assess RAGE-ligands or sRAGE levels from the respiratory airway so we cannot evaluate if sRAGE showed intrinsic respiratory airway upregulation.

In conclusion, our pilot study shown that CFp have higher plasma sRAGE level than healthy controls. Also, plasma sRAGE level is positively correlated with spirometry less obstruction. Further multicenter studies are needed to determine the true role of sRAGE in CFp with a larger population and more clinical and molecular variables.

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Table 1. Clinical and demographic characteristics of recruited patients (n = 35).

Sex Male – n (%)
Age (years) – median (SD)
CFTR mutation – n (%) F508del/F508del F508del/other Other/other
BMI (kg/m2) – median (SD)
FEV_1pp (%)* – median (SD)

Pancreatic insufficiency – n (%)
Liver disease related to CF – n (%)
Diabetes related to CF – n (%)
History of ABPA – n (%)
History of hemoptysis – n (%)
Respiratory support – n (%)
Chronic airway infection-colonization**– n (%) *Pseudomonas aeruginosa* Methicillin-sensitive *Staphylococcus aureus* Methic

Abbreviations: n: absolute number; %: percentage; SD: standard deviation; CFTR: gen of cystic fibrosis transmembrane conductance regulator, BMI: body mass index; FEV1pp: forced expiratory volume in 1 second percent predicted; CF: cystic fibrosis; ABPA: allergic bronchopulmonary aspergillosis.

*Spirometry was only performed in 33 patients for reasons of age.

**Defined according to: T.W.R Lee, K.G. Brownlee, S.P. Conway, M. Denton, J.M. Littlewood. Evaluation of a new definition for chronic*Pseudomonas aeruginosa* infection in cystic fibrosis patients.*J Cystic Fibros* . 2 (2003) pp. 29–34. Doi: 10.1016/S1569-1993(02)00141-8

Figure 1. Soluble receptor for advance glycation end products (sRAGE) plasma levels in our cystic fibrosis patients and historical healthy control cohort.

Boxes represent the 25th to 75th percentiles; median values are represented by the solid line within the box, whiskers represent the 10th and 90th percentile; and the single points and asterisks represent outliers.