

## Predictive Value of sCD93 in The Diagnosis of Bronchial Asthma

Sahar M. Fayed<sup>1</sup>, Ola S. El-Shimi<sup>1</sup>, Lubna I. Fathi<sup>2</sup>, Rana A. Khashaba<sup>1,3</sup>

<sup>1</sup> Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University, Egypt

<sup>2</sup> Laboratory Medicine Department, Shebin Alkom Ophthalmology Hospital, MOH, Menofiya, Egypt.

<sup>3</sup> Faculty of Medicine, King Salman International University (KSIU), El Tur Branch, Egypt.

**Correspondence:** Dr. Ola El-Shimi, **E mail:** ola.samer@fmed.bu.edu.eg, **mobile:** +201225559623

### ABSTRACT

**Background:** Studying asthma is no longer focused on symptoms and pulmonary functions but it expands to the underlying cellular changes. The membrane-associated glycoprotein CD93 is an emerging biomarker for inflammation in many inflammatory and immune-mediated diseases including asthma.

**Objectives:** We aimed to evaluate the value of serum sCD93 and its mRNA expression level as a non-invasive biomarker for bronchial asthma.

**Patients and methods:** This case control study was conducted to assess serum and mRNA expression levels of sCD93 in 40 bronchial asthma patients and 40 age- and gender-matched healthy controls.

**Results:** Both serum and mRNA levels of sCD93 were significantly higher in asthmatic patients ( $18.4 \pm 2.4$  ng/ml and  $1.16 \pm 0.57$  folds) than healthy controls ( $11.2 \pm 1.3$  ng/ml and  $0.711 \pm 0.35$  folds) ( $P < 0.001$  each). Serum sCD93 was 95% sensitive and 80% specific in diagnosing asthma at cut-off value  $\geq 13.3$  ng/ml. While CD93 mRNA expression was 70% sensitive and 60% specific in diagnosing asthma at cut-off value  $\geq 0.81$  fold.

**Conclusion:** sCD93 is a valuable non-invasive biomarker for bronchial asthma.

**Keywords:** Asthma, Non-invasive biomarker, sCD93.

### INTRODUCTION

Asthma is a common chronic inflammatory airway disease that affects between 1 and 29% of the population in different countries<sup>1</sup>. It is a heterogenous disease characterized by wide range of respiratory symptoms including wheezes, shortness of breath (dyspnea), chest tightness and/or cough that could vary over time<sup>2</sup>. The diagnosis of bronchial asthma (BA) is based mainly on characteristic pattern of respiratory symptoms and variable expiratory airflow limitation by pulmonary function tests<sup>3</sup>.

However, these tests might be inconvenient for some patients requiring effort and cooperation of the patient beside carrying the risk of severe asthma attack<sup>4</sup>. Therefore, there is always a clinical need to non-invasive reliable biomarkers for asthma diagnosis, prognosis, and follow-up. These biomarkers are the molecules that undergo cellular, biochemical, or molecular changes in asthmatics rather than healthy subjects and can be measured in various specimens, such as lung tissue, bronchoalveolar lavage fluid, nasal fluid, or peripheral blood<sup>5</sup>.

CD93 (C1qRp) is a transmembrane glycoprotein expressed on different cells, including endothelial cells, epithelial cells, stem cells, platelets, and leukocytes<sup>6</sup>. It possess both angiogenic and growth-stimulating effects<sup>7</sup>. Inflammatory mediators associated with various inflammatory and immune-mediated diseases, including asthma, can lead to CD93 shedding in its measurable soluble form (sCD93)<sup>8</sup>. Thus, we aimed to investigate the value of sCD93 as a non-invasive biomarker in asthma patients' diagnosis, prognosis, and follow-up for treatment responsiveness.

### PATIENTS AND METHODS

**Study design:** The current case-control study included 40 bronchial asthma patients that were recruited from Chest Department, Benha University Hospitals, Egypt through the period from September 2021 to February 2022. Asthma was diagnosed according to the published criteria of the Global Initiative for Asthma 2019 (GINA 2019)<sup>9</sup>.

**Exclusion criteria:** Any case showed clinical, or laboratory signs of respiratory tract infection, any active inflammatory disease, adverse drug reaction and other lung diseases or under immunotherapy.

A detailed history was taken from all participants to explore their symptoms, medication history especially inhaled corticosteroids (ICS) and family history. A clinical examination by a pulmonologist was carried out. Asthma severity and control level were evaluated according to Khajotia<sup>10</sup>.

**Sampling:** Five milliliters venous blood were drawn from each subject and divided into: 2.5 ml in EDTA tube for total RNA extraction and 2.5 ml in standard serum separating tube and stored at  $-80^{\circ}\text{C}$  for subsequent testing for serum sCD93 levels.

**Laboratory investigations:** Serum level of sCD93 was assayed in all studied subjects using a commercial double-antibody sandwich ELISA Kit (Cat# EH0089, Fine Test, China) according to the manufacturer instructions. The assay sensitivity for sCD93 was 0.188 ng/ml.

Total RNA was extracted and purified using TriRNA Pure Kit (Cat# TRPD050, Geneaid, Taiwan). A complementary DNA (cDNA) was synthesized by TOPscript™ cDNA Synthesis Kit (Cat. # RT220, Enzymomics, Korea) on Applied Biosystem Thermal Cycler (Thermo Fisher, USA) at 50°C for 60 min then incubated at 95°C for 5 min to stop the reaction. A quantitative PCR was performed using TOPreal qPCR 2X PreMIX (SYBR Green with high ROX) (Cat. # RT 501S, Enzymomic, Korea) according to manufacturer instructions using CD93 primer pair [Forward: 5'-GCTGTTGCTCTTATCTGCAAGGTG-3' and Reverse: 5'-AGCAAGCCTTTCAGGGATCTA-3'] under the following reaction conditions; initial denaturation at 95°C for 10 min and 45 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 15 sec and elongation at 72°C for 20 sec. For accurate and reproducible results, the amount of CD93 mRNA was normalized by endogenous reference control (GADPH). Relative quantitation (RQ) and fold expression changes of CD93 mRNA were calculated using the equation ( $2^{-\Delta\Delta ct}$ )<sup>11</sup>.

**Ethical approval:** This study was conducted in accordance with the Declaration of Helsinki (2004) and approved by the Ethics Committee of Faculty of Medicine, Benha University (MS-23-11-2020). An informed consent was obtained from each subject before participation.

**Statistical analysis:**

Data management and statistical analysis were done using SPSS version 25 (IBM, Armonk, New York,

United States). Quantitative data were presented as mean ± SD and an independent t-test was used to compare between them. Categorical data were presented as numbers and percentages and compared using the Chi-square test. ROC analysis was done to evaluate the performance of serum sCD93 and CD93 mRNA levels in diagnosing bronchial asthma. Area Under Curve (AUC) with 95% confidence interval, best cut-off point, and diagnostic indices were calculated. All statistical tests were two-sided. P values ≤ 0.05 were considered significant.

**RESULTS**

This case-control study included 40 bronchial asthma cases 16 males and 24 females with mean age 43.5±10.1 years, and 40 apparently healthy subjects, 24 males and 16 females with mean age 41.8±13.3 years. All enrolled subjects in this study were age- and sex-matched. Thirty five percent of the studied bronchial asthma patients had positive family history of asthma and 60% of them used inhaled corticosteroid during their disease course.

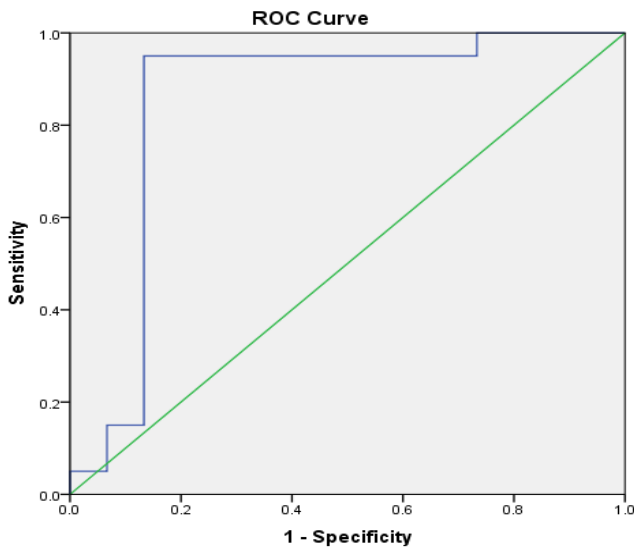
Evaluating asthma severity revealed that 80% of cases had mild disease and 20% had moderate disease. Regarding disease control by medication, treatment had controlled symptoms in 80% of studied patients. Serum sCD93 and CD93 mRNA expression were significantly elevated in asthma patients (18.4 ± 2.4 ng/ml and 1.16 ± 0.57 folds respectively) than control subjects (11.2 ± 1.3 ng/ml and 0.711 ± 0.35 folds respectively) (P 0.001 and 0.014 respectively) (Table 1).

**Table (1):** Demographic and laboratory characteristics of studied groups

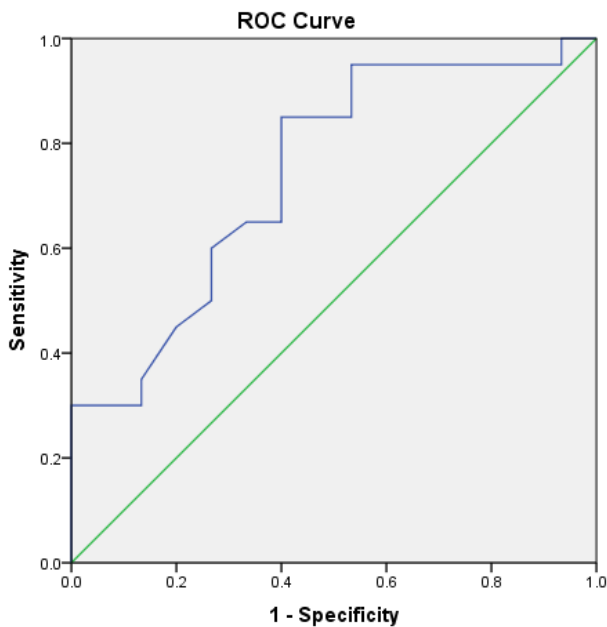
|                         |                      | HCV          | HCV & HCC    | Control      | Test                   | P      |
|-------------------------|----------------------|--------------|--------------|--------------|------------------------|--------|
|                         |                      | (N=25)       | (N=25)       | (N=25)       |                        |        |
| <b>Age (years)</b>      |                      | 53.04 ± 7.40 | 54.26 ± 6.97 | 51.48 ± 6.58 | F=0.995                | 0.375  |
| <b>Gender</b>           | <b>Male</b>          | 15 (60%)     | 13 (52%)     | 16 (64%)     | X <sup>2</sup> = 0.760 | 0.684  |
|                         | <b>Female</b>        | 10 (40%)     | 12 (48%)     | 9 (36%)      |                        |        |
| <b>AFP (ng/mL)</b>      |                      | 21 ± 18      | 1259.8 ± 119 | 4.0 ± 3.0    | F=2683.8               | <0.001 |
| <b>TLR4 (rs2149356)</b> | <b>GG</b>            | 12 (48%)     | 18 (72%)     | 10 (40%)     | X <sup>2</sup> = 22.96 | <0.001 |
|                         | <b>GT</b>            | 10 (40%)     | 5 (20%)      | 12 (48%)     |                        |        |
|                         | <b>TT</b>            | 3 (12%)      | 2 (8%)       | 3 (12%)      |                        |        |
| <b>IL-17 (pg/dL)</b>    |                      | 144.14±7.1   | 288.8±12.5   | 75.66±15.68  | F=47.121               | <0.001 |
| <b>IL-17 cut-off</b>    | <b>&lt;128 pg/dL</b> | 16 (64%)     | 1 (4%)       | 25 (100%)    | X <sup>2</sup> =47.09  | <0.001 |
|                         | <b>≥128 pg/dL</b>    | 9 (36%)      | 24 (96%)     | 0 (0%)       |                        |        |

Data represented as mean ±SD or number (percentage), AFP: alpha fetoprotein, IL-17 cut-off: the serum IL-17 concentration at which HCC could be predicted, Statistical tests used were ANOVA (F) and Chi square (X<sup>2</sup>) tests

Serum sCD93 showed a very good performance for diagnosing bronchial asthma at a cut-off level  $\geq 13.3$  ng/ml with 95% sensitivity, 80% specificity and 88% accuracy. (Figure 1). While, CD93 mRNA expression level showed a good performance for diagnosing bronchial asthma at a cut-off level  $\geq 0.81$  fold with 70% sensitivity, 60% specificity and 68% accuracy (Figure 2).



**Figure (1):** ROC analysis for serum sCD93 in diagnosis of bronchial asthma.



**Figure (2):** ROC analysis for CD93 mRNA in diagnosis of bronchial asthma.

Studying the relation between CD93 and disease severity and control, showed that higher levels of serum sCD93 and CD93 mRNA were significantly associated with increasing asthma severity (P 0.008 each). Furthermore, non-controlled symptoms were also significantly associated with higher levels of serum

sCD93 and CD93 mRNA (P 0.04 and 0.02 respectively) (Table 2).

**Table 2:** Correlation between HCC and studied parameters

|                     |        | HCC<br>(N=25)   | Non -<br>HCC<br>(N=50) | r     | P      |
|---------------------|--------|-----------------|------------------------|-------|--------|
| Age                 |        | 54.26<br>± 7.0  | 54.24<br>± 6.8         | 0.135 | 0.246  |
| Gender              | Male   | 13<br>(52%)     | 31<br>(62%)            | 0.096 | 0.414  |
|                     | Female | 12<br>(48%)     | 19<br>(38%)            |       |        |
| AFP (ng/mL)         |        | 1259.8<br>± 119 | 12.32<br>± 1.69        | 0.655 | <0.001 |
| TLR4<br>(rs2149356) | GG     | 18<br>(72%)     | 22<br>(44%)            | 0.265 | 0.022  |
|                     | GT     | 5<br>(20%)      | 22<br>(44%)            | -     | 0.236  |
|                     | TT     | 2 (8%)          | 6<br>(12%)             | -     | 0.061  |
| IL-17 (pg/dL)       |        | 288.8<br>± 12.5 | 109 ±<br>6.1           | 0.715 | <0.001 |

Data represented as mean ± SD or number (percentage)

AFP: alpha fetoprotein The statistical test used was Pearson correlation coefficient (r) test.

## DISCUSSION

In general, asthma diagnosis and management are based on symptoms, along with pulmonary function tests. The later requires a patient cooperation by blowing into a machine or expectorating sputum, which could be difficult or inconvenient to some of them. Also, these tests may not help to define the underlying airway inflammation. That's why recent asthma research is focusing on non-invasive and reliable cellular, soluble and genetic biomarkers <sup>12</sup>.

In our study, serum sCD93 levels were significantly higher in asthmatics than controls ( $18.4 \pm 2.4$  ng/ml vs.  $11.2 \pm 1.3$  ng/ml) (P 0.001). It showed a very good performance in diagnosing bronchial asthma at a cut-off level  $\geq 13.3$  ng/ml with 95% sensitivity, 80% specificity and 88% accuracy. CD93 mRNA was significantly upregulated in asthmatics than controls ( $1.16 \pm 0.57$  folds vs.  $0.711 \pm 0.35$  folds) (P 0.014). It showed a good performance in diagnosing bronchial asthma at a cut-off level  $\geq 0.81$  fold with 70% sensitivity, 60% specificity and 68% accuracy. These results agree with previous studies who demonstrated significant higher levels of serum sCD93 in asthma patients than in healthy controls <sup>12, 13</sup>. They used sCD93 to diagnose asthma with moderate sensitivity (71.4%) and specificity (82.4%) (AUC = 0.787, P < 0.001) <sup>12</sup>. The increased sCD93 levels in bronchial asthma could be due to the effect of proteinases, as matrix metalloproteinases (MMPs), on the transmembrane CD93 that leads to its cleavage and shedding in serum.

Tree pollen, fungi (*Aspergillus fumigatus*), cat, house dust mite, and cockroach allergens are allergic asthma triggers with protease activity that activate MMPs<sup>14</sup>.

Also, we found that higher levels of serum sCD93 and CD93 mRNA were significantly associated with increasing asthma severity (P 0.008 each). This was in line with other studies who revealed that serum CD93 level is significantly higher in patients with exacerbated asthma compared to those with stable asthma<sup>13, 15</sup>.

Regarding disease control by medication especially inhaled corticosteroids (ICS), our study showed that serum sCD93 and CD93 mRNA levels were significantly lower in controlled patients than in non-controlled (P 0.040 and 0.02 respectively). This comes along with **Park and colleagues**<sup>15</sup> findings, who showed that serum sCD93 levels in ICS-naïve BA patients were significantly higher than in healthy controls without BA and its level in high-dose ICS users were significantly lower than those in low- and medium-dose users. Also, a recent study reported significant reduction in serum sCD93 level post-treatment compared to pre-treatment<sup>16</sup>. The link between corticosteroid therapy and CD93 inflammatory action can be owed to the fact that corticosteroids inhibit cytokine synthesis, including IL-6<sup>17</sup>. IL-6 is claimed to mediate CD93 associated inflammation. Many studies investigated the correlation between CD93 and its soluble form with allergic and non-allergic asthma, however they could not confirm the exact mechanism by which CD93 affects bronchial asthma<sup>12</sup>.

In **Raedler et al.**<sup>18</sup>, the expression of the neutrophil-associated genes CD93, RGS13, and TREM1 were increased in non-allergic asthma compared to allergic asthma patients, with CD93 and RGS13 expression being associated with proinflammatory IL-17-shifted neutrophilic inflammation. CD93 and its soluble form may be involved in a non-T helper pathways of allergy and inflammation<sup>15</sup>.

Serum sCD93 levels can be affected by other diseases, such as ischemic heart disease<sup>19</sup>, skin sclerosis<sup>20</sup>, diabetes<sup>21</sup>, diabetic nephropathy<sup>22</sup>, metabolic dysregulation<sup>21</sup>, and in kidney transplant recipients with chronic inflammation<sup>23</sup>. Moreover, CD93 mRNA levels could be affected by other diseases such as coronary artery disease<sup>24</sup>. Thus, history of such diseases must be considered while interpreting both serum sCD93 and CD93 mRNA results.

## CONCLUSION

CD93 has a potential role in diagnosis of bronchial asthma. It could be used as well to assess disease severity and therapy responsiveness. The clinical usefulness of determination of sCD93 as a non-invasive biomarker of asthma requires further studies.

- **Acknowledgment:** The authors would like to thank all patients and healthy volunteers who participated in this study.

- **Funding and financial support:** Nil.
- **Conflict of interests:** Nil.

## REFERENCES

1. **Mortimer K, Lesosky M, García-Marcos L et al. (2022):** The burden of asthma, hay fever and eczema in adults in 17 countries: GAN Phase I study. *Eur Respir J.*, 60 (3): 1-12. doi:10.1183/13993003.02865-2021
2. **Global Strategy for Asthma Management and Prevention (2023):** <https://ginasthma.org/2023-gina-main-report/>.
3. **Reddel H, Bateman E, Becker A et al. (2015):** A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J.*, 46 (3): 622-639. doi:10.1183/13993003.00853-2015
4. **Kim S, Lee Y, Sung M, Bae H (2017):** Current Epidemiological Data on Asthma Management in South Korea from Qualitative Assessment of Asthma Management by Health Insurance Review and Assessment Service (HIRA). *Tuberc Respir Dis (Seoul)*, 80 (3): 221-225. doi:10.4046/trd.2017.80.3.221
5. **Sircar G, Saha B, Bhattacharya S, Saha S (2014):** Allergic asthma biomarkers using systems approaches. *Front Genet.*, 4: 308. doi:10.3389/fgene.2013.00308
6. **Løvik G, Vaage J, Disen E, Szpirer C, Ryan J, Rolstad B (2000):** Characterization and molecular cloning of rat C1qRp, a receptor on NK cells. *Eur J Immunol.*, 30 (12): 3355-3362. doi:10.1002/1521-4141(200012)30:12<3355::AID-IMMU3355>3.0.CO;2-1
7. **Kao Y, Jiang S, Pan W et al. (2012):** The epidermal growth factor-like domain of CD93 is a potent angiogenic factor. *PLoS One*, 7 (12): e51647. doi:10.1371/journal.pone.0051647
8. **Bohlon S, Silva R, Fonseca M, Tenner A (2005):** CD93 is rapidly shed from the surface of human myeloid cells and the soluble form is detected in human plasma. *J Immunol.*, 175 (2): 1239-1247. doi:10.4049/jimmunol.175.2.1239
9. **Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention (2019):** [www.ginasthma.org](http://www.ginasthma.org).
10. **Khajotia R (2008):** Classifying asthma severity and treatment determinants: national guidelines revisited. *Malaysian Fam physician*, 3 (3): 131-136.
11. **Livak K, Schmittgen T (2001):** Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25 (4): 402-408. doi:10.1006/meth.2001.1262
12. **Park H, Oh E, Han H et al. (2020):** Soluble CD93 in allergic asthma. *Sci Rep.*, 10 (1): 323. doi:10.1038/s41598-019-57176-2
13. **Sigari N, Jalili A, Mahdawi L, Ghaderi E, Shilan M (2016):** Soluble CD93 as a Novel Biomarker in Asthma Exacerbation. *Allergy Asthma Immunol Res.*, 8 (5): 461-465. doi:10.4168/aaair.2016.8.5.461
14. **Ingram J, Kraft M (2015):** Metalloproteinases as modulators of allergic asthma: therapeutic perspectives. *Met Med.*, 61. doi:10.2147/mnm.s63614
15. **Park H, Han H, Lee S et al. (2017):** Soluble CD93 in Serum as a Marker of Allergic Inflammation. *Yonsei Med J.*, 58 (3): 598-603. doi:10.3349/ymj.2017.58.3.598
16. **Hafez M, Abdelsalam S, Ahmed H, Rafea T (2021):** Serum soluble CD93 as a biomarker of asthma

- exacerbation in children. *Egypt J Hosp Med.*, 85 (2): 3673-3677. doi:10.21608/ejhm.2021.201987
17. **Barnes P (2001):** Molecular mechanisms of corticosteroids in allergic diseases. *Allergy*, 56 (10): 928-936. doi:10.1034/j.1398-9995.2001.00001.x
18. **Raedler D, Ballenberger N, Klucker E et al. (2015):** Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allergy Clin Immunol.*, 135 (1): 81-91. doi:10.1016/j.jaci.2014.07.046
19. **Youn J, Yu H, Jeon J et al. (2014):** Soluble CD93 levels in patients with acute myocardial infarction and its implication on clinical outcome. *PLoS One*, 9 (5): e96538. doi:10.1371/journal.pone.0096538
20. **Yanaba K, Asano Y, Noda S et al. (2012):** Augmented production of soluble CD93 in patients with systemic sclerosis and clinical association with severity of skin sclerosis. *Br J Dermatol.*, 167 (3): 542-547. doi:10.1111/j.1365-2133.2012.11020.x
21. **Strawbridge R, Hilding A, Silveira A et al. (2016):** Soluble CD93 Is Involved in Metabolic Dysregulation but Does Not Influence Carotid Intima-Media Thickness. *Diabetes*, 65 (10): 2888-2899. doi:10.2337/db15-1333
22. **Lee M, Park H, Choi M et al. (2020):** Significance of Soluble CD93 in Type 2 Diabetes as a Biomarker for Diabetic Nephropathy: Integrated Results from Human and Rodent Studies. *J Clin Med.*, 9 (5): 1-13. doi:10.3390/jcm 9051394
23. **Kielar M, Dumnicka P, Ignacak E et al. (2021):** Soluble Complement Component 1q Receptor 1 (sCD93) is Associated with Graft Function in Kidney Transplant Recipients. *Biomolecules*, 11 (11): 1-14. doi:10.3390/biom11111623
24. **Mälarstig A, Silveira A, Wågsäter D et al. (2011):** Plasma CD93 concentration is a potential novel biomarker for coronary artery disease. *J Intern Med.*, 270 (3): 229-236. doi:10.1111/j.1365-2796.2011.02364.