CLINICAL UTILITY OF SERUM PERIOSTIN LEVEL TO DIAGNOSE THE SEVERITY OF SARS-COV-2: A NOVEL APPRROACH FOR DEVELOPMENT OF COV-2 SERUMMARKES

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Abstract: COVID-19 has emerged as multifarious lung disease with various clinical presentations in addition with common cold and flu. The airway inflammation, bronchial hyper responsiveness and mucus secretion leading to airflow obstruction is characterized as cause of inconvenient breathing. Currently the CoV-2 is diagnosed with polymerase chain reactions and some biochemical techniques employed in virology but lung function tests are ignored. No one is still established serum biomarker to assess the severity of inflammation and obstruction of airways. Present study was conducted to evaluate clinical utility of serum Periostin level in Cov-2 infected patients in term of significant correlation of serum Periostin levels with absolute Eosinophil count and lung function test. Total 760 CoV-2 infected participants were subjected to study. Infection was further classified into mild, intermittent, moderate and severe types and 100 patients for each groups were studied. Patients with COPD or other type of allergy were excluded from the study. Patients from 18-60 years of age, diagnosed with asthma by a Pulmonologist and age and gender matched non-asthmatic adults were included in the study bearing CoV-2 infections. Absolute eosinophil count were determined by XN-1000 Sysmex hematology analyzer and serum Periostin level were measured with manual Elisa method. Lung function test was performed with spirometer (FEV1) at Ayub Teaching hospital. The data was entered and analyzed using SPSS 20.0. Comparison between two groups were calculated by sample t-test and correlation were calculated by person correlation coefficient. Study concluded that Serum periostin level and absolute eosinophil count were significantly correlated with severity of COVID-19 infections and can be used as surrogate markers for diagnosis and severity of disease.

Keywords: SARS-CoV-2, Periostin, Eiosinophil, Respiratory volumes, Serum markers

1: Introduction

The start of 20th century has lead us to emergence of novel viruses causing worldwide health hazards, damaging international economies and demolishing economic growth. The seafood market at Wuhan, China has witnessed the appearance of deadly Corona Virus that has halted the growth of worldwide economies [1]. The SARS-CoV-2 infections generally accompany respiratory damage, inflammation of mucosa and airway hindrance with signs and symptoms of fever, nasal discharge, cough and sore throat. Fatal cases involved acute respiratory distress, pneumonia, and multi-organ failure. The severity and fatality associated with the SARS-CoV-2

virus infection appears to be milder when compared with other human corona viruses (SARS and MERS) [2]. To date, millions of cases with more than couple of million deaths of SARS-CoV-2 infection have been reported from the globe. Initially, there were limited evidences of virus human to human transfer because no health care personals in the hospitals were infected [3]. After few days, as the cases starts to increase officials admits the horizontal transfer of SARS-CoV-2, spread through nasal secretions of the infected person [4, 5]. The coronavirus belongs to the family Coronaviridae, based on their genetic properties devided into four genera including Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. The coronaviruses are enveloped, non-segmented, single stranded positive sense RNA genome. The genome size is ranging from 26 kbp to 32 kbp, largest genome in all RNA viruses. The corornaviruses are 80 to 120 nm in diameter and spherical or pleomorphic in shape [6,7].

Some surrogate markers are currently used for diagnosis of lung function include: serum IgE, peripheral blood eosinophils, serum periostin, FeNO, and and Eosinophil Derived Neurotoxin [8]. Eosinophils are circulating granulocytes produced in the bone marrow with other blood cells and present at relatively low level in the bloodstream, making up 1 to 3% of white blood cells. These are the main types of cells that can be recruited from immunological or inflammatory response [9]. The association of Peripheral blood eosinophils with asthma was first time described in 1908 [10] and have been widely studied as useful biomarkers for the diagnosis of severity of lungs inflammation. Blood eosinophils are considered as a key mediator of airways inflammation because, when they are activated, they release harmful granular proteins and pro-inflammatory cytokines, which damage epithelial tissue of airway and eosinophils mediate remodeling and an hypertrophy of smooth muscle, leading to airflow obstruction in asthma [11]. Elevated blood eosinophils count is associated with higher frequency of symptom, lower force expiratory volume (FEV1) values, and greater variation in maximum daytime peak expiratory flow, this can increase the risk of severe exacerbations lungs diseases [12]. Although the measurement of blood Absolute eosinophil count (AEC) is an easily accessible and cheap test, but its use has drawbacks. First, peripheral eosinophils can be elevated in other than asthma, such as parasitic infections, allergies and autoimmune diseases. Second, as eosinophils are primarily tissue-living leukocytes, AEC may not accurately reflect bronchial activity of eosinophils [13].

The fractional exhaled nitric oxide (FeNO) test measures the nitric oxide (NO) in exhaled breathing and provides an indication of eosinophilic inflammation in the lungs. FeNO levels correlates with the severity of eosinophilic inflammation in airway with eosinophils of bronchial washings bronchial biopsies and sputum samples [14]. IgE is produced by plasma cells (usually) in response to an immunogen. The presence of IL-4 and IL-13 from th2 cells in asthma induce switching in plasma cells to produce IgE instead of other immunoglobulin's. It is present at the lowest concentration of all immunoglobulins, normal human serum concentrations being approximately 50 ng / mL [15]. Periostin is a protein expressed in the extracellular matrix of fibroblasts or epithelial cells. Periostin expression is induced in fibroblasts by T helper cell type 2 (Th2) cytokines and can cause subepithelial fibrosis [16]. There is significant periostin expression in bronchial epithelial cells of asthmatic children, and serum periostin levels have been reported to be a potential biomarker of airway eosinophilic inflammation in adults [17]. Eosinophil derived neurotoxin (EDN) is one of the four main proteins present in the cytoplasmic granules eosinophilic leukocytes [18]. In addition to eosinophils, EDN is also found in the liver, lungs and spleen [19]. The eosinophil-derived neurotoxin (EDN) has been suggested as a useful marker for of eosinophilic inflammation of airways. Inspired by the above literature the present study was focused to find the potential surrogate markers associated with SARS-COV-2 that will be required to predict and diagnose the severity, onset and help the clinical utilities. The study was also designed to evaluate the utility of serum Periostin level SARS-COV-2 patients in term of significant correlation of serum Periostin levels with absolute Eosinophil count and lung function test.

2: Materials and Methods

It was a comparative cross sectional study conducted to evaluate clinical utility of serum periostin level in asthmatic patients. The project was conducted at Department of Medical Laboratory Technology, The University of Haripur, KPK Pakistan. Samples were processed and analyzed at Ayyub teaching hospital, Abbottabad Pakistan. The sample size was calculated by the following formula of sample size calculation for comparison of two sample means which is out first objective.

$$n_1 = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2(\sigma_1 2 + \sigma_2 2)}{(\mu 1 - \mu 2)^2}$$

α = Desired Level of Significance (5%) = 1.96

1-β = Power of study (90%) = 1.28

μ1= Mean levels of Serum Periostin in normal individuals = 197.0 ng/ml

μ2= Mean levels of Serum Periostin in Asthmatic patients =391 ng/ml

σ1= Standard deviation of Serum Periostin in normal individuals = 49.7

σ2= Standard deviation of Serum Periostin in patients =142.1 [20]

As we use ELISA plate of 96x10 wells for determination of Serum pariostin levels that's why we increase our sample size up to 360 for infected and 360 for non-infected to get more valuable results. Remaining 160 wells were used for controls group. Patients from 18-60 years of age, diagnosed with severe persistent asthma by a Pulmonologist and age and gender matched non-asthmatic adults were included in the study. Patients with mild and moderate asthma, COPD patients and patients with any other type of allergy were excluded from the study. Random sampling techniques were used in present study. Demographic detail including age and gender, clinical information and consent were obtained from Severe persistent asthmatic and non-asthmatic adults.

3: Data and Sample collection procedure

Severe persistent asthmatic and non-asthmatic adults were selected under supervision of pulmonologist. Their medical history, Lung function test data and demographic data were obtained on approved proforma. From all the selected participants of the study, two ml venous blood in EDTA tube for AEC and three ml in clotted tube for analysis of Serum periostin was collected. After clot formation, the gel tubes were centrifuged to obtain serum. Tow aliquots of each serum sample were made and 0.7ml serum was dispensed in each aliquot. These aliquots were used for the measurement of Serum periostin. Serum samples were stored at -20C till the analysis of serum periostin. The analysis of EDTA blood samples for ACE was performed on the same day of blood

collection. Lab measurement of AEC were performed on same day of sample collection at Asia Diagnostic Center, Blue Area, Islamabad by 5 part differential, Mindray BC-5000, Auto Hematology Analyzer, while periostin level were performed at pathology department of Ayyub Teaching Hospital, by ELISA method on Bio-Rad Elisa reader. Mindray BC-5000 Auto Hematology Analyzer was used to measure AEC. This instrument work with different technologies for analyses of 5-part differential analysis WBC and complete blood count (CBC). Electrical Direct current (DC) Impedance is used for RBC and PLT counting. Hemoglobin is measured with Cyanide free reagent. WBC 5-part differential analysis is performed by combination of Flow Cytometry (FCM), Tri-angle laser scatter and Chemical dye method [20].

4: Measurement of Serum Periostin level

Serum samples aliquots store at -20C were thawed to room temperature. Enzyme Immunoassay kit for the quantitative analysis of periostin level in serum by International Immuno Diagnostics was used on Bio-Rad Elisa Reader to determine the Serum periostin. Bring wash buffer (WASHBUF) concentrate to room temperature to liquefy for analysis of periostin assay. All crystals in buffer solution will dissolved at room temperature. WASHBUF concentrate was diluted with 1:2. To perform assay take 50 mL WASHBUF concentrate into 950 mL deionized water. Use only diluted wash buffer in serum periostin assay determination. Standard and control was reconstitute with 200 µL of distilled water or deionized water as per labeled on vials. Vertex and leave on room temperature for 20 °C. Prior to use standard and control were diluted with 1+50 with ASYBUF (assay buffer).

5: Results and Discussions

A standard curve from the absorbance read-outs of the standards using commercially available software capable of generating a four-parameter logistic (4-PL) fit. Alternatively, plot the standards' concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. Curve fitting algorithms other than 4-PL have not been validated and will need to be evaluated by the user. The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot at production date. Data for OD obtained by customers may differ due to various influences including the normal

decrease of signal intensity throughout shelf life. However, this does not affect validity of results as long as an OD of 1.50 or higher is obtained for the standard with the highest concentration and the values of the CTRLs are within the target range.

The data was entered and analyzed using SPSS 20.0. Mean ± SD were given for quantitative variables like serum periostin level, absolute eosinophil count and FEV1. Comparison between two groups were calculated by sample t-test and correlation were calculated by person correlation coefficient Data was presented in the form of graphs, tables and charts.

In total participants (n=720), 360 were infected patients and 360 were non-infected. Infected patients were further classified into mild, intermittent, moderate and severe types and 100 patients for each groups were studied. In infected participant, 200 patients were males and 200 were females. In non- infected (control group), 180 individuals were males and 180 were females. Consolidates results was shown in table 1. SARS-CoV-2 is a multifarious lung disease with various clinical presentations. The most important characteristics of asthma are airway inflammation, bronchial hyper responsiveness, mucus secretion, and collagen deposition leading to airflow obstruction. No one is still established serum biomarker to assess the severity of inflammation and obstruction of airways in SARS-CoV-2 infections. Present study was conducted to evaluate clinical utility of serum Periostin level in asthmatic patients in term of significant correlation of serum Periostin levels with absolute Eosinophil count and lung function test. Absolute eosinophil count were determined by XN-1000 Sysmex hematology analyzer and serum Periostin level were measured with manual Elisa method. Lung function test was performed with spirometer (FEV1) at Ayub Teaching hospital. The data was entered and analyzed using SPSS 20.0. Comparison between two groups were calculated by sample t-test and correlation were calculated by person correlation coefficient. Respectively serum Periostin level count were found significantly high in infected group (75.88ng/mL, $0.233x10^9$/L) than non-infected group (43.33ng/mL, $0.700x10^9$/L) that can establish a basis for the surrogate marker development [14]. The samples were also analyzed for eosinophil count that were recorded as 0.07 10^3/uL in non-infected individuals that was significantly lower than that of infected group having the value 0.23 10^3/uL. Forced expiratory volumes of participants were also compared and it was found that infected group had an average volume of 49.30% that was significantly lower than non-infected group with 92.80% expiratory volume on average. Higher Periostin levels, lower forced respiratory volumes and higher

eosinophil counts suggest that Serum Periostin level and absolute eosinophil count were significantly correlated with severity of SARS-CoV-2. This study concludes that serum Periostin level are high and have significant correlation with force expiratory volume and absolute eosinophil count in infected patients with clear cutoff values. So it is now suggested to be used as a surrogate marker for the diagnosis of SARS-CoV-2 and its severity

Figures and Tables

Figure 1. Protocol for satanderd, controls, balanks and sample by ELISA strip.

Table 1: Gender wise frequency distribution of participants

	Infected		**Non Infected**	
	Males	**Females**	Males	Females
Mild	30 (8.3%)	50 (13.8%)	180(25%)	180(25%)

Intermittent	50 (13.8%)	40 (11.1%)	
Moderate	50 (13.8%)	40(11.1%)	
Severe	50 (13.8%)	50 (13.8%)	
Total	360 (100%)		360 (50%)

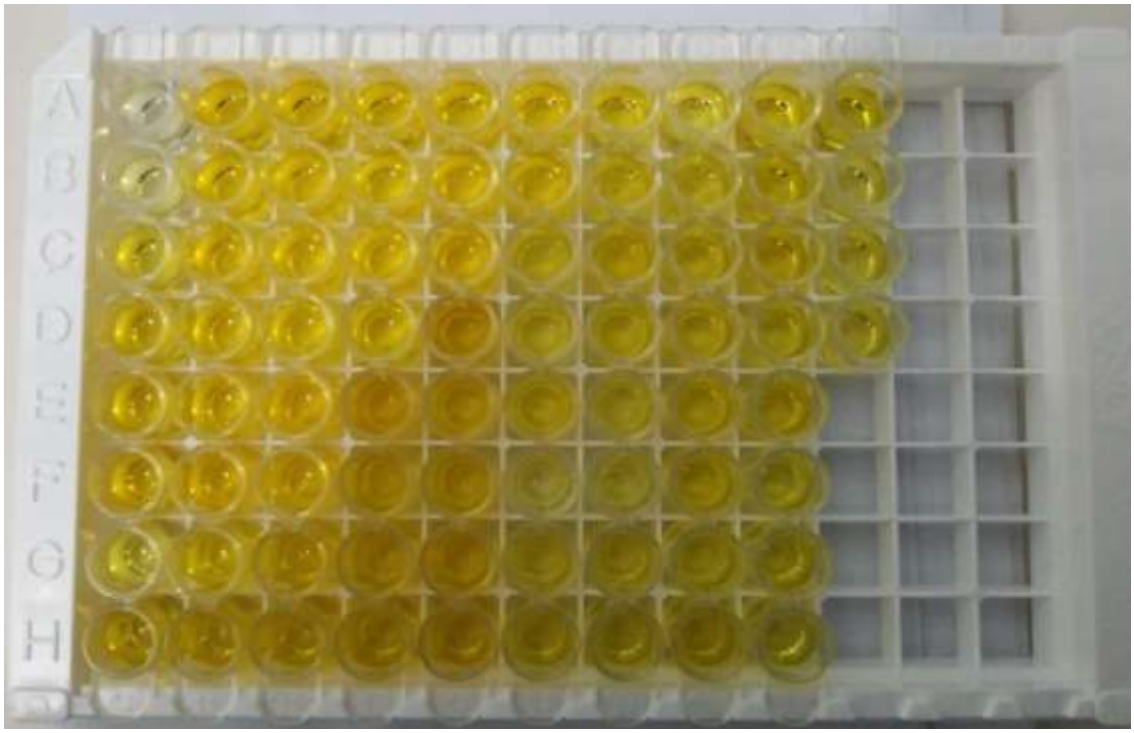

Figure 2. Elisa test for one sample of 72 participatants with 36 infected and 36 non-infected samples

Table 2. Clinical investigations for serum biomarker Periostin, Eosinophil count and Forced Expiratory Volumes

ISSN: 0034-8082

	Asthmatic, Non-asthmatic	N	Mean	Std. Deviation
Serum Periostin ng/ml	Non-infected	360	43.33	5.74
	Infected	360	75.88	12.26
AbsoluteEosinophil Count 10^3/ul	Non-infected	360	0.07	0.02
	Infected	360	0.23	0.10
Forced Expiratory Volume (L)	Non-infected	360	92.80	5.90
	Infected	360	49.30	14.47

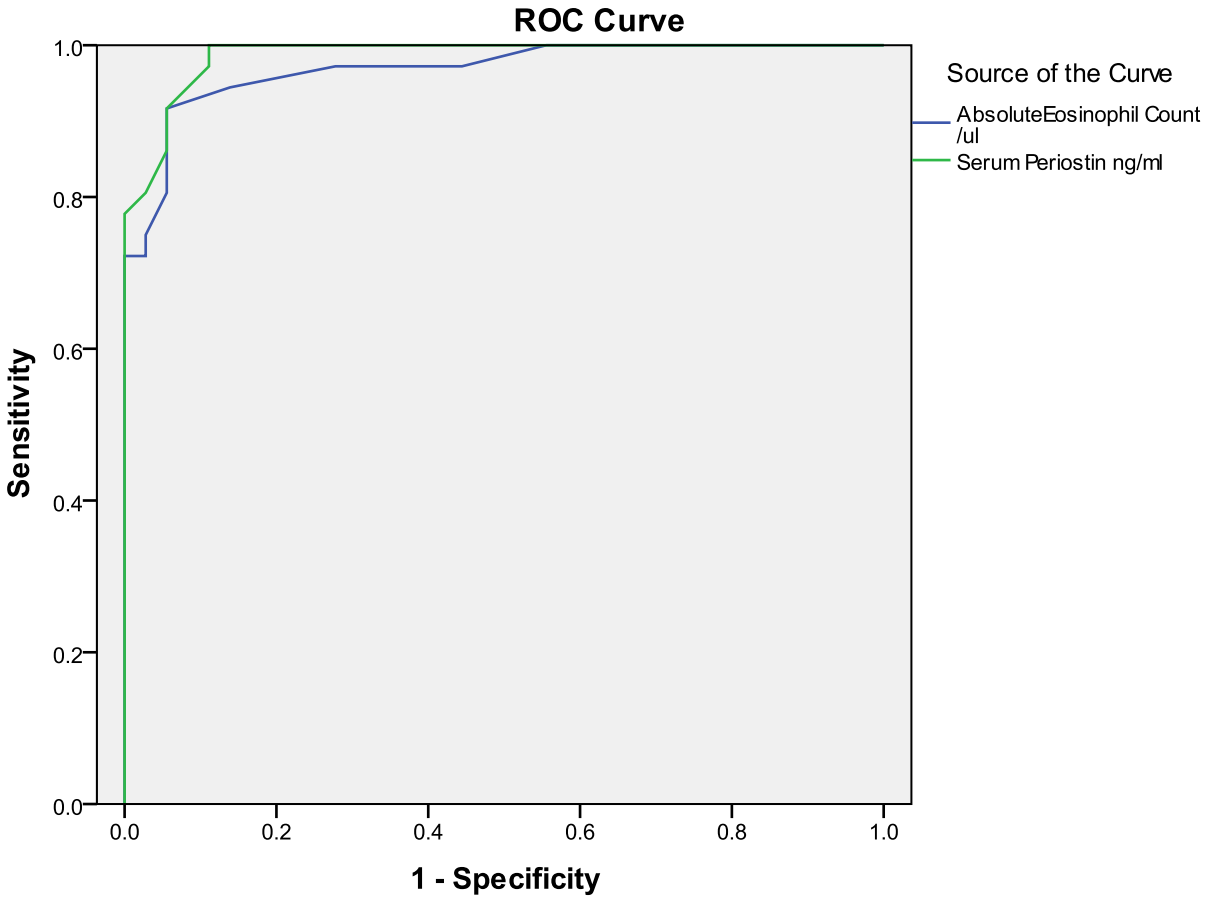

Figure 3. Analysis of ties between Absolute Eosinophil .968 Count /ul, Serum Periostin .986ng/ml showing at least one tie between the positive actual state group and the negative actual state group.

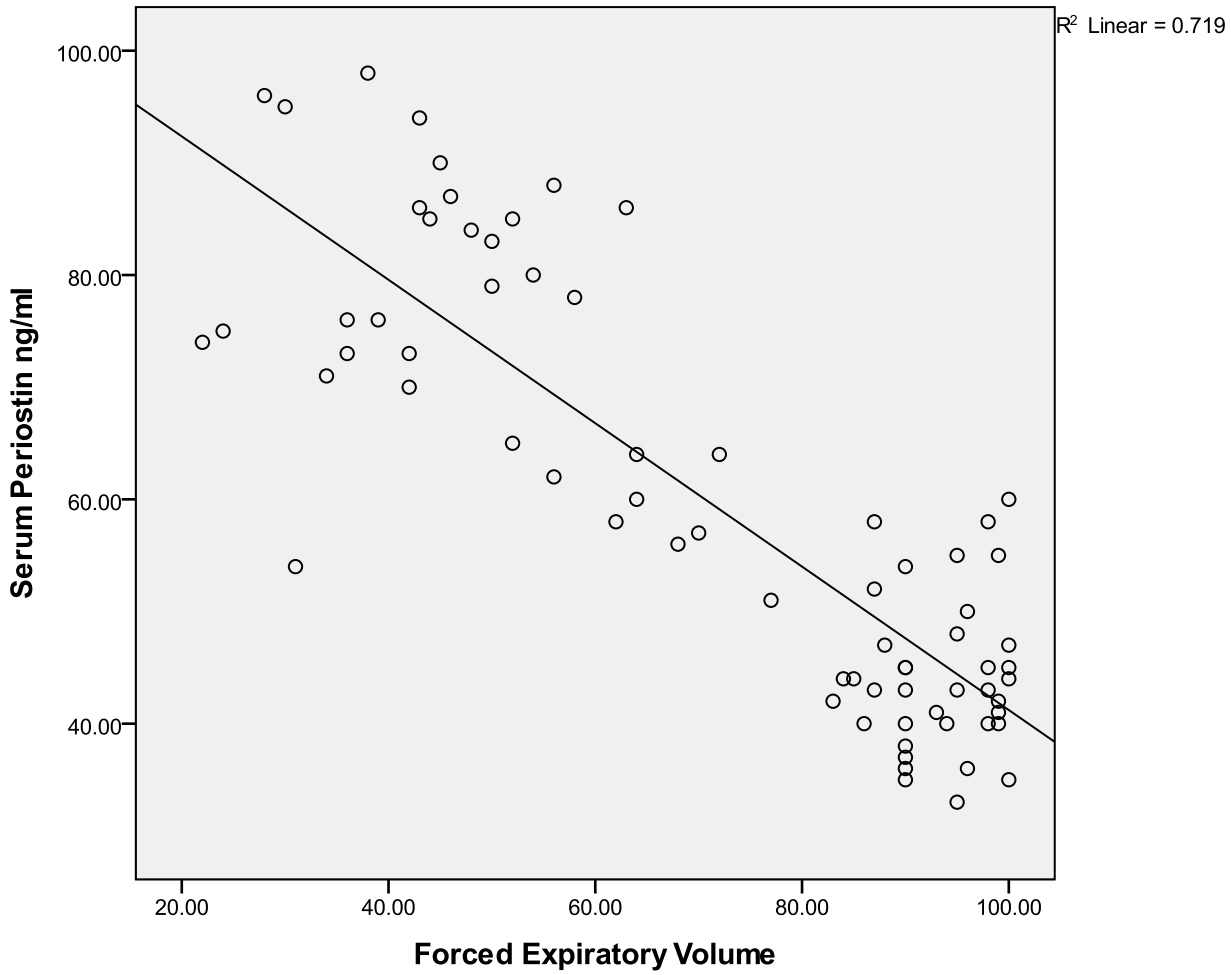

Figure 4. Analysis of forced expiratory volumes indicating a positive correlation of it with serum Periostin levels

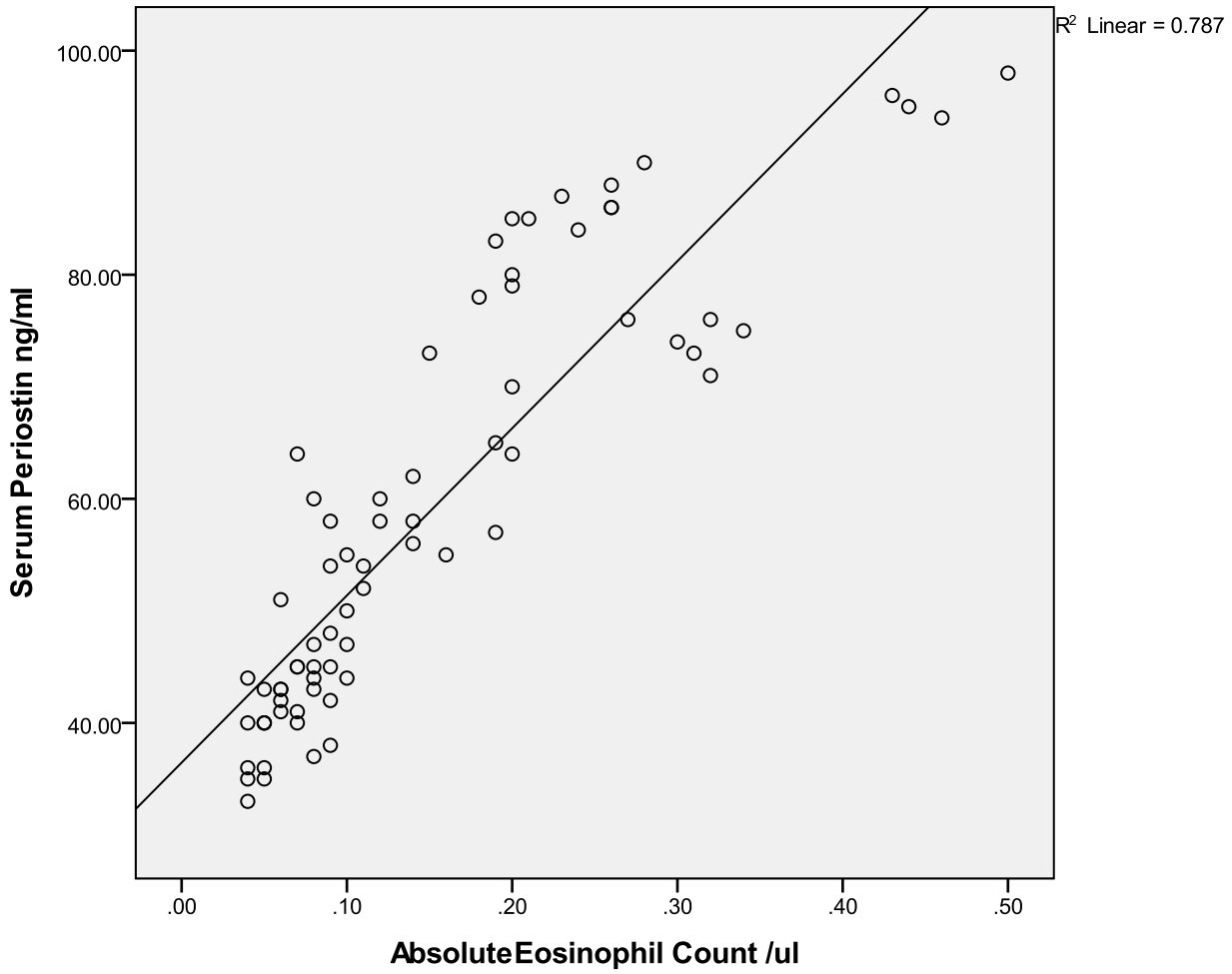

Figure 5. Serum Periostin levels showing positive correlation with absolute eosinophil count

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