Original Article

Possible relevance of epidermal growth factor as a biomarker of inflammation in severe COVID-19

Posible importancia del factor de crecimiento epidérmico como biomarcador de inflamación en COVID-19 grave

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ABSTRACT

Background: EGFR plays a critical role in inflammation at the pulmonary level. Data exploring the role of its canonical ligand are scarce. Exploration of new and potential biomarkers expands diagnostic and therapeutic options, providing resilience in complex healthcare settings.

Aim: To explore the variability of serum EGF levels according to sex, age and clinical status in subjects with COVID-19 vs. control, as well as to determine possible notable correlates.

Methods: Controlled cross-sectional exploratory study with quota sampling in patients with COVID-19 admitted to the Saturnino Lora Hospital. The commercial

kit UMELISA EGF from the Cuban Immunoassay Center was used for EGF determinations.

Results: Significant differences were observed with respect to serum EGF values in COVID-19 vs. controls (g = 0.6603, *p* = 0.0021). Serum EGF values < 200 pg /mL were associated with the risk of worse clinical status (OR = 30.8, HR = 11.19 CI95%: 1.82-10.79, X2 = 17.42, p = 0.000*). No biologically relevant differences were observed between sexes (p=0.7694); Regarding age, slight differences in the effect were observed (g = 0.4232, *p* = 0.0527). A higher probability of obtaining serum EGF values > 200 pg /mL was identified in patients ≤ 65 years (HR = 2.5 CI95 %: 1.06-3.03, p = 0.0308). Serum EGF in relation to NLR (β = 0.2232, *p* = 0.0353) and PLR (β = 0.2117, p = 0.0411) behaved as a predictor of inflammation in that condition.

Conclusions: Serum EGF determination may be a presumed independent marker of inflammation with implications for the pathophysiology of SARS-CoV-2 pneumopathy.

Keywords: serum biomarkers/inflammation; epidermal growth factor; COVID-19.

RESUMEN

Introducción: El factor de crecimiento epidérmico juega un papel crítico en la inflamación a nivel pulmonar. Los datos que exploran el papel de su ligando canónico son escasos. La exploración de biomarcadores nuevos y potenciales amplía las opciones diagnósticas y terapéuticas, proporcionando resiliencia en contextos sanitarios complejos.

Objetivo: Explorar la variabilidad de los niveles séricos de EGF según sexo, edad y estado clínico en sujetos con COVID-19 *vs.* control, así como determinar posibles correlatos notables.

Métodos: Estudio exploratorio transversal controlado con muestreo por cuotas, en pacientes con COVID-19 ingresados en el Hospital Saturnino Lora. Para las determinaciones de EGF se utilizó el kit comercial UMELISA EGF del Centro de Inmunoensayo de Cuba.

Resultados: Se observaron diferencias importantes respecto a los valores séricos de EGF en COVID-19 vs. controles (g = 0,6603, *p* = 0,0021). Valores séricos de EGF < 200 pg /mL se relacionaron con el riesgo de un peor estado clínico (OR = 30,8, HR = 11,19 IC95 %: 1,82-10,79, X2 = 17,42, p = 0,000*). No se observaron diferencias biológicamente relevantes entre sexos (p = 0,7694); Respecto a la edad, se observaron ligeras diferencias en el efecto (g = 0,4232, *p* = 0,0527). Se identificó una mayor probabilidad de obtener valores séricos de EGF >200 pg /mL en pacientes ≤65 años (HR = 2,5 IC95%: 1,06-3,03, *p* = 0,0308). El EGF sérico en relación con NLR (β = 0,2232, *p* = 0,0353) y PLR (β = 0,2117, *p* = 0,0411) se comportó como predictor de inflamación en dicha condición.

Conclusiones: La determinación de EGF sérico puede ser un presumible marcador independiente de inflamación, con implicaciones en la fisiopatología de la neumopatía por SARS-CoV-2.

Palabras clave: biomarcadores séricos/inflamación; factor de crecimiento epidérmico; COVID-19.

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Introduction

COVID-19 is an atypical form of pneumonia that in 2020 achieved widespread dissemination worldwide, rapidly becoming a pandemic that marked a turning point in the history of public health.[1] The virus responsible, called severe acute respiratory syndrome type 2 coronavirus (SARS-CoV-2), belongs to the group of coronaviruses, generally encapsidated single-stranded RNA positive (ssRNA positive) viruses, and specifically to the betacoronavirus subgroup. with a history

of health concern for its ability to cause Adult Respiratory Distress Syndrome (ARDS).[2]

One of the critical proteins is the Spike (S) protein, which is responsible for binding to surface receptors, preferably through angiotensin II converting enzyme receptors,[3] causing a rearrangement of cellular metabolism, production of new viruses and progressively altering functionally and structurally infected cells, leading to programmed cell death.[4]

In the context of cancer-associated chronic inflammation, a complex molecular relationship between angiotensin II-converting enzyme (ACE2), transmembrane protease, serine 2 (TMPRSS2) and epidermal growth factor receptor (EGFR) has been demonstrated.), with effects leading to the promotion of a sustained state of tyrosine residue phosphorylation independent of receptor heterodimerization in EGFR tyrosine kinase arm mutations, despite the use of specific inhibitors.[5,6]

Likewise, the relationship between ACE2 expression dependent on EGFR activity in the acute and chronic inflammatory context is known and well documented.[7,8] In the inflammatory environment, ACE2 and EGFR are overexpressed with a direct relationship to the presence of tumor necrosis factor alpha (TNF-a), interleukin (IL) 1 and IL-6.[9,10,11] EGFR activation itself stimulates the positive regulation of proinflammatory cytokines.[12]

Epidermal growth factor (EGF) triggers crucial molecular events for EGFR activation. Its known function as a restricted and canonical ligand of the EGFR receptor has made it a therapeutic target in certain oncological pathologies such as lung cancer, although little is known about its basic biology and the existence of other functions beyond those dependent on its HER-1 interaction.[13]

Associated with the severity of COVID-19, a series of molecular phenomena, colloquially called cytosine storm, have been identified. Evidence for the role of proinflammatory molecules, such as interferon gamma (IFN- γ), TNF-a, IL-1 β, IL-6 and IL-8, among others, in the evolution of the severity of SARS-CoV -2 infection is robust.[14]

In a proteomics study that explored the relationship of certain molecules and their contribution to disease severity, compared to sepsis and influenza as an alternative model of pneumoinflammation, in a nodal network analysis model, a relationship was found between higher serum EGF values in patients with severe COVID-19; although the differences were in the shared circulating proteomic response, the specific differences involved EGF values.[15]

Although EGFR has been reported to play a critical role in SARS-CoV-2 infection[16,17] and modulation of its signals has been observed to be associated with an effective therapeutic response in the disease[18,19,20,21] data on the behavior of its canonical ligand are scarce in the published literature. Therefore, the aim of this study is to describe the variability of serum EGF levels according to sex, age and clinical status in subjects with COVID-19 vs. Control, as well as to determine possible notable correlates, allowing us to evaluate in an initial approximation its possible importance as a biomarker of inflammation in the context of COVID.

Methods

Study design

Controlled cross-sectional exploratory study with quota sampling, which enrolled 79 subjects, 53 patients with COVID-19, attended at the Saturnino Lora Hospital between the months of August-September 2021, and 27 apparently healthy subjects, recruited at the Renato Guitar blood bank between the months of November-December 2019. Minimum recruitment quotas per group were determined as a n ≥ 25 units of analysis.

The diagnostic criteria used were those declared by MINSAP based on WHO recommendations. The selection of subjects and/or patients was made by simple random sampling. In the case of healthy donors, periodic tests and a thorough physical examination certified their status as apparently healthy subjects.

Clinical and analytical data

Clinical and analytical data were collected by the professional patient care teams at the corresponding medical institutions. The information used was collected from medical records and included: age, sex, and comorbidities. It was verified that the acquisition of analytical parameters was carried out in strict compliance with the standardized procedures of the clinical laboratory of the Institutions and in accordance with the current CECMED regulations.

EGF detection in serum

EGF detection in serum was performed using the commercial kit UMELISA-EGF from the Cuban Immunoassay Center. Sampling was performed within the first 72 hours of admission. In all cases, 5 mL of blood was collected by puncture of the cephalic vein in the flexure of the arm using disposable syringes of 10 mL capacity, with 21 G hypodermic needles, deposited in a dry test tube, obtaining the serum by the clot technique. retraction for 4 hours and centrifugation (according to the manufacturer's recommendations at 1500 rpm for 10 minutes at 24°C). The serum obtained was dosed by means of eppendorf micropipettes in 1.5 mL eppendorf vials, after which it was stored frozen at -20 0C, until its processing in the SUMA laboratory, certified by CECMED, at the Juan Bruno Zayas Hospital. The results were expressed in picograms per milliliter (pg /mL). For the alternative procedure, the serum processing guidelines were modified, according to the recommendations for obtaining platelet-rich serum, and lysis was performed using the freeze/thaw procedure.

A value of 200 pg/mL of EGF is assumed *a priori* as a value to make comparisons between groups, based on a graphical analysis of the distributions of possibilities on a case-by-case basis.

Bias control

To mitigate the possible influence of confirmation bias and/or selective thinking, activities were carried out to unify criteria and delimit the responsibilities of the specialists in the intensive care services with respect to the classification of patients based on the recommendations of the Ministry of Public Health. Biases in the means of observation were mitigated by guaranteeing that the studies were performed in laboratories certified by CECMED, and by professionals with an experience curve of more than 5 years, and with technological equipment of the same commercial brands. Statistical processing was carried out based on the constitution of a single database previously filtered and purified. No extremely atypical quantitative data were observed, so no data were eliminated from the analysis. The operations were triplicated, confirming the reproducibility of the results.

Statistics Analysis

A digital database created using the technical facilities of the Excel software of the Microsoft office 2010 platform (Microsoft, USA), on a Hewlett-Packard laptop computer, **was used to record the data**. We proceeded to work with 100% of the data in the database (no missing data). Data processing was performed on the same technological platform. In the statistical analysis, measures of central tendency (arithmetic mean), dispersion (standard deviation and confidence interval) and Pearson's correlation coefficient were used as summary parameters. Graphical tests of normality QQ and the Jarque-Bera (JB) statistic were performed; in addition, the statistical significance of observable differences between groups was explored with the chi-square test (categorical variables) or Welch's t-test (continuous variables), the effect size was estimated using Hedge's g-formula. A multiple regression analysis was performed, with a univariate strategy (a single dependent variable).

Ethical Statement

The study was designed and conducted according to the general principles established in the documents adopted by the international community in relation to biomedical research on human subjects, included in the Declaration of Helsinki (update of the World Medical Assembly held in Brazil, 2013). The research also complied with the current state regulations according to the requirements of the national regulatory authority (CECMED Regulation 165/2000), as well as the Good Clinical Practice Guidelines of the International Conference on Harmonization (ICH E6). The research was approved by the Research Ethics Committee of the Saturnino Lora Hospital, and the corresponding certification by the Regional Ethics Committee of the southeastern region of Cuba. Prior to the inclusion of each subject in the study, Informed Consent was requested and obtained. No funding was received from third parties to carry out this research, nor did the subjects involved in the study receive any payment. No conflicts of interest are declared.

Results

We analyzed 52 patients with COVID-19, 1:1 ratio according to sex (stochastic relationship), 15 patients with attention report, 37 with severe report. Adjustments were made to the samples analyzed, eliminating 4 extreme outliers from the analysis. The control group consisted of 27 subjects with apparent health history.

Comparatively between sexes, no biologically relevant differences were observed (t = 0.2954, p = 0.7694). There is a tendency to reduce the probability of obtaining serum EGF values higher than 200 pg/mL in men with respect to women (HR = 0.83 CI95 %: 0.48-1.54, p = 0.6324).

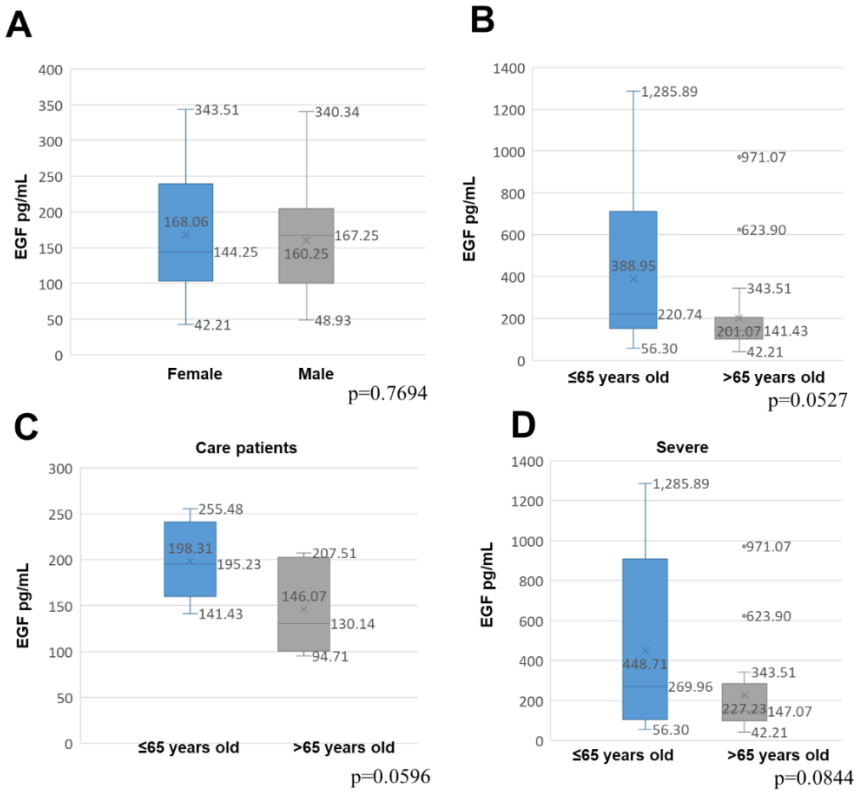
With respect to biological age, slight differences in effect are observed (g = 0.4232, t = 2.0225, p = 0.0527) presumably attenuated given the greater discrepancy of data in the group of patients ≤65 years. There is a significant increase in the probability

of obtaining serum EGF values greater than 200 pg/mL in patients ≤ 65 years with respect to those > 65 years (HR = 2.5 CI95 %: 1.06-3.03, *p* = 0.0308).

Depending on the reporting status, no significant differences were observed with respect to the groups (g = 0.0671, t = 0.4079, *p* = 0.6856), although a subtle trend was observed in terms of obtaining serum EGF values > 200 pg/mL in patients. with care report with respect to patients with severe report (HR = 0.84 CI95%: 0.44-1.47).

An age-based analysis shows in the group of patients with care report large differences in effect in favor of the group of patients ≤ 65 years with respect to (g = 0.7317, t = 2.0992, *p* = 0.0596), with a trend increase in serum values in this subgroup (HR=1.6 CI95%: 0.56-3.89).

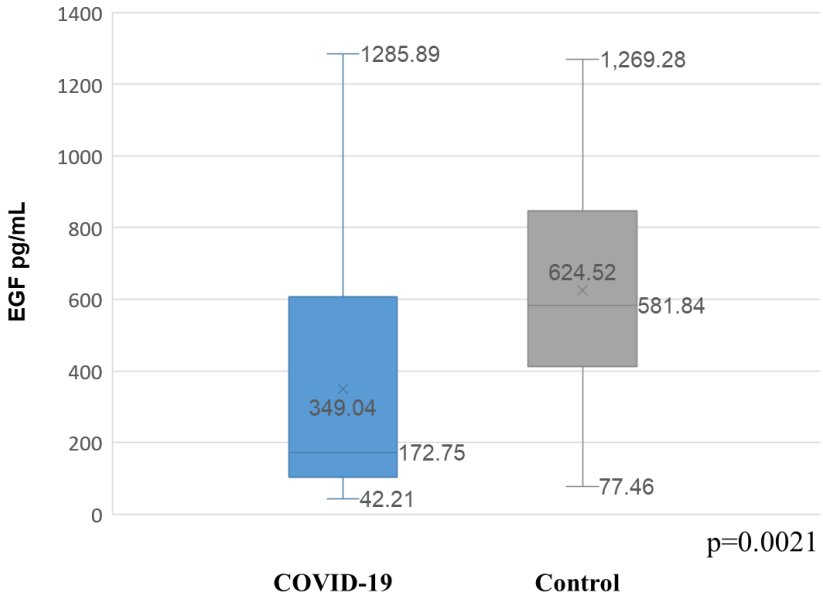
In patients with severe report, the observed difference was less pronounced (g = 0.4413, t = 1.8067, *p* = 0.0844), with greater dispersion of data in the group of patients ≤65 years, and replicating the trend towards an increase in serum values (HR=1.9 CI95%: 0.94-3.22). These differences and trends can be seen in figure 1.



Source: Database.

Fig. 1 – Distribution of EGF values according to sex (A) and age (B) in patients with COVID-19; according to age in relation to patients' reporting status: (C) care and (D) severe.

Particularly in the group of severely ill patients, compared to a control of healthy subjects, significant differences were observed with respect to serum EGF values (g = 0.6603, p = 0.0021), where in relation to serum EGF values > 200 pg/mL, the specificity was 96.4% with a sensitivity of 46.6%. The analysis with the control group showed that serum EGF values < 200 pg/mL were related to a higher risk of worse clinical status (OR = 30.8, HR = 11.19 95 %CI: 1.82-10.79, X^2 = 17.42, p = 0.000*), as can be seen in figure 2.



Source: Database.

Fig. 2 – Serum EGF levels: COVID-19 vs. control.

Particularly with respect to sex, the analysis against the control group reflected the behaviors previously observed between COVID-19 vs. healthy subjects, with moderate differences with respect to female sex (g = 0.5756, t = 2.0649; p = 0.0490) and serum EGF. values < 200 pg/mL being related to a higher risk of worse clinical status (OR = 21.6, HR=2.87 CI95%: 1.46-9.86, X2 = 10, *p* = 0.0016). In the case of the male sex, the differences were more marked (g = 1.5889, t = 2.3187; *p* = 0.038), with a protective effect of EGF values > 200 pg /mL (OR = 0.01, HR = 0.1 CI95%: 0.03-0.45, X2 = 14.9, *p* = 0.0001).

Regression analysis after adjusting for EGF in relation to clinically relevant cellular and molecular parameters (only performed in patients with report of severe), showed that serum EGF concentrations in relation to neutrophil lymphocyte ratio (NLR) (β = 0.2232, p = 0.0353) and platelet lymphocyte ratio (PLR) (β = 0.2117, *p* = 0.0411) are significant predictors of inflammation in this condition.

Discussion

The study described the behavior of serum EGF levels in patients with COVID-19 as a function of age and sex, comparatively with respect to a group of healthy subjects and internally with respect to COVID-19 subgroups as a function of reporting status. In COVID-19 patients, the sex differences observed in the control group are not observed, although there is a trend towards a reduced probability of obtaining serum EGF values above 200 pg/mL in men relative to men. women. In terms of biological age, slight marginally significant effect differences are observed; Despite which, a significant increase in the probability of obtaining serum EGF values greater than 200 pg /mL was identified in patients ≤ 65 years compared to those > 65 years.

Depending on the reporting status no significant differences were observed with respect to the groups, although a subtle trend was observed in obtaining serum EGF values > 200 pg/mL in patients with care report with respect to patients with care report. severe. An analysis based on age shows in the group of patients with care report large differences in effect in favor of the group of patients ≤ 65 years, with a trend toward increased serum values in this subgroup.

In the group of severely ill patients, with respect to the control of healthy subjects, significant differences were observed with respect to serum EGF values, with a specificity of 96.4 % and a sensitivity of 46.6% for serum EGF values > 200 pg/mL. Analysis with the control group showed that serum EGF values < 200 pg/mL were associated with an increased risk of worse clinical status. Regression after adjusting for EGF in relation to clinically relevant cellular and molecular parameters showed that serum EGF concentrations in relation to NLR and PLR are significant predictors of inflammation in that condition.

The EGF molecule is a protein with an important and known function in the biology of fibroblasts and epithelial cells.[22] Its biodistribution has commonly been associated mainly with platelets, although this hypothesis lacks solid experimental support.

Transcriptomic analyses point to a very intense activity at the renal level (30 pTPM) and more balanced in the rest of tissues and cells of our body (3 pTPM) with small peaks slightly higher than 3 pTPM at the level of skeletal muscle and ganglia. ciliary, triseminal and upper cervical; all this in basal conditions of the organism.[23]

It should be noted that there are no formal studies that define a solid relationship between EGF and inflammation, so even in studies that explore the role of EGFR in certain pathologies, it is systematically rare to find data on EGF behavior; In most studies that explore the involvement of EGFR in dissimilar contexts, the association with other ligands such as amphiregulin and TGF-a is explored and established, negligently ignoring the possible role of its canonical ligand.

Recent studies in the context of COVID-19 have reported positive correlations between plasma EGF concentration and several cytokines, particularly with classical proinflammatory cytokines such as IL-1 β , IL-8, MIP-1a (CCL3), as well as cytokines associated with Th2 (IL-4, IL-5 and IL-13) and Th17 (IL-17A, IL-17F, IL-17E/IL-25 and IL-22) polarization. Plasma EGF levels have been reported to correlate positively with both CRP levels ($r > 0.5$) and lung injury due to computed tomography (CT)-characterized pneumonia ($r > 0.4$) and, to a lesser but notable extent, with platelet concentration ($r > 0.3$), which in turn were significantly related to important inflammatory cells and cytokines, highlighting the positive correlations with monocytes and neutrophils ($r>0.6$) and to a lesser extent with lymphocytes, similar to the correlation of EGF with CRP; a negative correlation with IL-10 concentrations is notable.[24]

Studies exploring serum EGF values are scarce in the international literature in general, and especially in relation to apparently healthy subjects. *Sarang Meybosch et al.*,[25] have reported a progressive decrease in EGF values with advancing age, with sex differences gaining in category from adolescence onwards; reporting for subjects in an age range of 30-50 years (n = 16) average values of 209 pg /mL [2-314 pg /mL] and in those over 50 years (n = 16) average values of 76 pg /mL [17-337 pg /mL].

These results are very different from those reported by *González et al*,[26] who report for subjects in similar age ranges average values of 1012.00 ± 41.85 pg /mL (n =

37). The data from both studies also differ from what was observed in our control subjects with an average of 624.57 pg/mL (n = 27) with a range of values from 77.46-1269.28 pg/mL Although a priori there is no consistent reason to explain the remarkable differences observed between these apparently comparable subjects, our results, in addition to responding to a normal distribution, are very close (without differences) to that reported by the Human Protein database for EGF (680 pg /mL),[27] which provides external validity to our control based on a value recognized internationally by the scientific community, and indirectly to the evaluation method used.

However, the results of *Madé et al.*,[28] have reported different results from those already mentioned, showing differences (p < 0.005) in plasma samples from subjects with COVID-19 between critical and non-critical patients, with higher values in relation to lower severity (452 ± 43.67 pg /mL vs. 258 ± 25.99 pg /mL). Our results in critically ill patients show values different from those reported by Madè A *et al*, despite which they are commonly lower than those reported for healthy subjects, taking as standard the results of our control comparable to those of the average value reported in the Atlas of human proteins.

According to the authors, the differences observed in the EGF values, even though they bridge the reproducibility of the trends observed in a general context according to age and sex, according to the studies consulted, may be due to variations in the procedure for obtaining the sample, and not to differences in the technologies used, which were compatible both in the technical platform itself and in the system for expressing the results used.

The general superficial exploration in the literature consulted regarding the concentrations and variability of EGF values, based on possible correlates with other molecules and cells, in the context of healthy subjects; in addition to the lack of in-depth studies in specific pathological conditions, constitutes a major limitation for a better understanding of the role of EGF beyond the existing unillustrative reports and the links inferred from the analysis of existing preclinical information.

Although the sample size is a limitation of the study, the observed differences are statistically supported, so this study constitutes a valid initial approach and serves as a reference for the design of subsequent interventions that achieve a greater degree of robustness.

Based on the data obtained, added to the available experimental and clinical evidence, it is valid to sustain as a logical hypothesis that EGF may play a critical role in the development of severe forms of pneumonia, particularly SARS CoV-2, despite the fact that randomized, controlled studies are required for conclusive confirmation.

Serum EGF determination may be a putative independent marker of inflammation, with implications for the pathophysiology of SARS-CoV-2 pneumopathy.

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Conflict of interest

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