



## ANGIOPOIETIN 2 AS PREDICTIVE FACTOR FOR INTENSIVE CARE UNIT ADMISSION OF COVID-19 PATIENTS.

### Internal Medicine

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| <b>Dr. Pericherla Hemanth Varma</b> | Resident, Department of General Medicine, Amrita Institute of Medical Sciences and Research Centre.  |
| <b>Dr. Vasant P K</b>               | Professor, Department of General Medicine, Amrita Institute of Medical Sciences and Research Centre. |
| <b>Dr. MGK Pillai</b>               | Professor, Department of General Medicine, Amrita Institute of Medical Sciences and Research Centre. |

### ABSTRACT

**Background-** COVID-19 is caused by SARS CoV-19 group of viruses. COVID-19 causes excess inflammatory responses and causes activation and recruitment of leukocytes. Activated leukocytes damage endothelium and causes release of E-selectin, or Angiopoietin-2. It has been associated with thrombo- inflammatory events progressing to DIC, MODS and death. Angiopoietin-2 is used as marker of endothelial activation in COVID 19 patients. This helps in assessing the severity of the disease and need for ICU admission. Various studies have proved that serum Angiopoietin-2 levels were higher in moderate to severe COVID pneumonia patients. **Methods** – All patients admitted in COVID ward or ICU in Department of General Medicine department at Amrita Hospital were enrolled in the study. After obtaining informed consent, serum angiopoietin-2 levels are checked. Angiopoietin-2 levels are compared with ward and ICU patients and they are used to assess the correlation of angiopoietin-2 levels with severity of infection. Angiopoietin-2 levels were compared with CRP, ferritin, D-Dimer, creatinine. Pneumonia scoring systems (CURB-65, APACHE 2, MuLBSTA) were compared in assessing the severity of infection. **Results-** Among 19 patients who have angiopoietin  $\geq 4781$  pg/mL, 16 (84.2%) had moderate to severe outcome compared to those who had angiopoietin  $< 4781$  pg/mL (n=20), 3 (15%) had moderate to severe outcome which is statistically significant (p value  $< 0.001$ ). **Conclusion-** Angiopoietin 2 is a reliable biomarker to predict the severity of COVID-19 infection. This study showed a cut off value of 5247 for ICU admission. It has a sensitivity of 84.2% and specificity of 90%. This biomarker can be used to triage the patients to ward and ICU at the time of admission for timely and appropriate management. Similarly, APACHE 2 score is better predicting the severity of COVID 19 pneumonia.

### KEYWORDS

### INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an important emerging infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In December 2019, this virus was identified as the cause of a cluster of pneumonia cases in Wuhan, China [1]. COVID-19 was declared as a pandemic by the World Health Organization (WHO) in March of 2020 [2].

COVID-19 patients may present with a spectrum of symptoms ranging from asymptomatic, mild upper respiratory tract symptoms, to severe pneumonia and multiorgan failure. The lung is the most common organ affected in SARS-CoV-2 infection. The predominant pattern of lung abnormalities during illness is ground-glass opacity. Furthermore, many patients have residual opacity on the chest CT scans, in which the main pattern is ground-glass opacity at the time of discharge [3]. The pathology of the lung in COVID-19 patients includes diffused alveolar damage [4], bronchiolitis, alveolitis and interstitial fibrosis [5]. Thus, patients who are infected with SAR-CoV2 may have a restrictive or obstructive defect on a spirometry during recovery. Previous studies [6] in severe acute respiratory syndrome (SARS) showed that patients had an abnormal pulmonary function test up to 20% after recovery from SARS. Few studies, mainly in China [7] have reported abnormal lung function and six-minute-walk test (6MWT) in patients who were infected with SARS-CoV-2 after recovery. Most of these previous studies were conducted only in patients with pneumonia and those with mild symptoms were not included.

COVID-19 has an impact on multiple organs such as the heart, kidneys, and liver but the primary system affected has been the respiratory system with most of the clinical manifestations including cough, sputum production, dyspnoea, fever, fatigue, and in severe cases acute respiratory distress syndrome (ARDS) and respiratory failure [8]. The route of transmission of SARS-CoV-2 is through the respiratory system, the virus invades type 2 alveolar cells in the lung, it attaches to specific angiotensin-converting enzyme 2 (ACE2) receptors, multiplies inside the cells, and elicits inflammatory response with vasodilatation, increased pulmonary capillaries permeability, necrosis of endothelial cells, and in the severe cases this could progress to acute systemic inflammatory responses and cytokine storm. The virus may enter the circulation and cause systemic multiple organ failure especially in tissues having ACE2 receptors such as the heart and kidneys [9].

COVID-19 causes excess inflammatory responses and causes activation and recruitment of leukocytes. Activated leukocytes damage endothelium and causes release of E-selectin, or Angiopoietin-2. It has been associated with thrombo- inflammatory events progressing to DIC, MODS and death. Angiopoietin-2 is used as marker of endothelial activation in COVID 19 patients. This helps in assessing the severity of the disease and need for ICU admission. Various studies have proved that serum Angiopoietin-2 levels were higher in moderate to severe COVID pneumonia patients (HemSyn 1,2).

### METHODS

#### Selection and description of Participants

This is an observational cohort study with the setting being the General Medicine Department of Amrita Institute of Medical Sciences, conducted during the year 2021 - 2022. This research was approved by both the dissertation review committee and ethical committee. Subjects admitted in COVID ward and ICU in Amrita Hospital were enrolled in the study. The selected patients were studied in detail, history was taken and physical examination was done. Required blood and imaging investigations were done.

#### The inclusion criteria were:

All patients were confirmed COVID-19 positive using specimens derived from nasopharyngeal or oropharyngeal swabs prior to or during the hospitalization

#### The exclusion criteria were :

- 1-Age  $< 18$  years;
- 2- Patients with pneumonia due to non COVID causes.
- 3- Patients on nitric oxide trial
- 4- Patients with active malignancies
- 5- Patients with traumatic spinal cord injury and recent fractures.
- 6- Patients with Kaposi's sarcoma

### OBJECTIVES

**Primary Objective-** To analyze the association of serum angiopoietin-2 levels with the severity of COVID 19 infection and need for direct ICU admission.

**Secondary Objective-** To compare serum angiopoietin 2 levels with CRP, ferritin, D-Dimer and creatinine in COVID19 patients.

To compare pneumonia scoring systems (CURB-65, APACHE 2, MuLBSTA) in assessing severity and mortality rate in COVID 19 patients.

### SAMPLE SIZE AND STATISTICAL ANALYSIS

Based on the Odds ratio of Angiopietin 2 for finding the association of ICU admission. Odds ratio= 9.3 in COVID 19 patients observed in an earlier publication ("Angiopietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID -19 patients" published in Springer nature- 17 May 2020) and with 95% confidence and 80% power, the minimum sample size comes to 30, 15 each. In the current study 19 ICU and 20 ward patients were taken.

### Statistical Analysis

Statistical analysis was performed using IBM SPSS version 20.0 software. Categorical variable was expressed as frequency and percentage. Continuous variables were presented by mean and standard deviation. ROC curve analysis was used to find an ideal cut-off for Angiopietin-2 as a predictor for ICU admission of COVID-19 patients. To test the statistical significance of association of angiopietin 2 cut off with ICU admission Chisquare test was used. Diagnostic measures such as sensitivity, specificity, predictive value positive, predictive value negative and accuracy was calculated. ROC curve analysis was used to compare pneumonia scoring systems using Area under the curve in assessing severity and mortality, followed by test statistic De Long method was applied. To find the linear relationship of angiopietin 2 with blood parameters and Pneumonia severity scores, Spearman rank correlation coefficient was computed and its statistical significance was tested using Linear Regression t test.

### RESULTS

Among the 39 patients, mean age of the patients was  $60 \pm 17.67$  years ranging from 20 to 88 years. 64% were, males and 36% were females with male to female ratio 1.8: 1. Out of 39 patients, majority, 19 (48.7%) were admitted in ICU and remaining 20 (51.3%) in ward. Majority, 30 (76.9%) were discharged and 9 (23.1%) patients died. 20 (51.3%) were Mild, 9 (23.1%) Moderate and 10 (25.6%) had Severe infection. 20 (51.3%) had mild outcome and 19 (48.7%) patients had Moderate to Severe outcome. For prediction of mild to moderate outcome, AUC for angiotensin 2 was 0.939 (p value <0.001) with Sensitivity of 84.2% and specificity of 85%. Among 19 patients who have angiopietin  $\geq 4781$  pg/mL, 16 (84.2%) had moderate to severe outcome compared to those who had angiopietin < 4781 pg/mL (n=20), 3 (15%) had moderate to severe outcome which is statistically significant (p value <0.001). All the variables were significant to predict severity of outcome (all p values <0.001) but AUC was maximum for APACHE 2 and Angiopietin-2 (0.962 and 0.939 respectively). CURB score shows sensitivity of 78.9 with cut off value of 1.50, APACHE 2 shows sensitivity of 89.5 with cut off value of 13.50, MULBSTA score shows sensitivity of 84.2 with cut off value of 6.00. There was statistically no significant difference in the scores. APACHE 2 score has maximum Sensitivity (89.5%) followed by Angiopietin 2 and MULBSTA (84.2% and 84.2%) respectively. APACHE 2 score and Angiopietin 2 had maximum Specificity (90%) for prediction of severity of outcome. CURB score showed a cut off value of 1.50 with sensitivity of 88.9%, APACHE 2 showed a cut off value of 17.50 with sensitivity of 77.8%, MULBSTA score showed cut off value of 10.00 with sensitivity of 77.8%.

That all the variables were significant to predict mortality (all p values <0.001) but AUC was maximum for APACHE 2 and Angiopietin (0.896 and 0.852 respectively)

CURB Score has maximum Sensitivity (88.9%) APACHE 2 had maximum Specificity (80%) for prediction of mortality. There was statistically significant positive good correlation between angiopietin level and with laboratory parameters as CRP ( $r=0.846$ ,  $p<0.001$ ), D-Dimer ( $r=0.876$ ,  $p<0.001$ ), Creatinine ( $r=0.510$ ,  $p<0.001$ ), Ferritin ( $r=0.867$ ,  $p<0.001$ ). There was statistically significant positive good correlation between angiopietin 2 level and with different scores CURB Score ( $r=0.673$ ,  $p<0.001$ ), APACHE  $r=20.847$ ,  $p<0.001$ ), MULBSTA ( $r=0.698$ ,  $p<0.001$ ).

### DISCUSSION

SARS-CoV-2 infection can be complicated by the development of an acute respiratory distress syndrome (COVID-19 ARDS) associated with high mortality rate. The severity of the lung injury often requires

mechanical ventilation and recently, some morphological pathways of the COVID-19-related ARDS have been elucidated in a series of autopsies. The histologic analysis of pulmonary vessels showed widespread thrombosis with microangiopathy, diffuse alveolar damage, capillary congestion, necrosis of pneumocytes, interstitial and intra-alveolar edema and platelet-fibrin thrombi. These results suggest that the profound hypoxemia that these patients might experience can be due to both epithelial and endothelial injury.

Angiopietin-2 (Ang-2), soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), P-selectin, E-selectin are used as biomarkers of endothelium injury, whereas receptor for advanced glycation end-products (RAGE) is considered a marker for alveolar epithelial injury. The pathophysiology of severe COVID-19 had been reported to be characterized by severe inflammation and vascular injury. However, the extent to which these findings are specific to COVID-19 as opposed to critical illness in general is unclear. To our knowledge, this is the first study of a cohort of ICU patients admitted in which circulating biomarkers had been compared between patients that ultimately ruled in or out for COVID-19.

Distribution according to the age of the patient: In the present study, mean age of the patients was  $60 \pm 17.67$  years ranging from 20 to 88 years. 64% were, males and 36% were females with male to female ratio 1.8: 1. In the present study, out of 39 patients, majority, 19 (48.7%) were admitted in ICU and remaining 20 (51.3%) in ward.

Among COVID 19 survivors, 80% required ICU admissions while 20% were outpatient [10]. In a study, out of 40 enrolled patients, Half of them were admitted in conventional wards without any ICU transfer during hospitalization; whereas the 20 others were directly transferred to ICU. COVID-19 patients directly admitted to the ICU had a significant severity of infection [11]. In another study, 40 ICU and 9 non-ICU admissions [12]. Above mentioned research had consistent findings with our study.

**Distribution according to outcome of patients:** In the present study, majority, 30 (76.9%) were discharged and 9 (23.1%) patients died.

**Distribution according to severity of patients:** In present study, 20 (51.3%) patients were Mild, 9 (23.1%) Moderate and 10 (25.6%) had Severe infection.

**Distribution according to laboratory parameters of the patients:** In present study, Majority of the values Angiopietin ( $4777.85 \pm 3746.061$  pg/mL), CRP ( $46.10 \pm 59.132$  mg/dL), D-dimer ( $2.378 \pm 3.5385$   $\mu$ mL), Creatinine ( $1.722 \pm 2.0521$  mg/dL) and Ferritin levels ( $638.06 \pm 706.011$  mg/dL) were raised.

COVID-19 patients admitted in ICU had higher D-dimer levels at entrance ( $p=0.008$ ), CRP level ( $p=0.002$ ) and serum creatinine levels ( $p=0.001$ ), sE-selectin and angiopietin-2 levels were significantly increased (p value at 0.009 and 0.003, respectively) [11].

**Distribution according to severity of Outcome:** In present study, 20 (51.3%) and 19 (48.7%) patients had mild and Moderate to Severe outcome respectively. For prediction of mild to moderate outcome, AUC for angiotensin 2 was 0.939 (p value <0.001) with Sensitivity of 84.2% and Specificity of 85%.

As compared to previous literature, ROC curve analysis identified an Angiopietin-2 cut-off of 5000 pg/mL as the best predictor for ICU outcome (Sensitivity=80.1%, Specificity=70%, PPV=72.7%, NPV=77%) [11].

**Association between Angiopietin 2 Level and Response to treatment:** Among 19 patients who have angiopietin  $\geq 4781$  pg/mL, 16 (84.2%) had moderate to severe outcome compared to those who had angiopietin < 4781 pg/mL (n=20), 3 (15%) had moderate to severe outcome which is statistically significant (p value <0.001)

In previous literature, ROC curve analysis identified an Angiopietin-2 cut-off of 5000 pg/mL as the best predictor for ICU outcome [11]. In various other studies, markers of endotheliopathy—angiopietin-2, follistatin, and PAI-1—were elevated in ICU patients who died compared to those who survived [13].

**In present research** all the variables were significant to predict

severity of outcome (all p values <0.001) but AUC was maximum for APACHE 2 and Angiotensin (0.962 and 0.939 respectively). APACHE 2 score has maximum Sensitivity (89.5%) followed by Angiotensin 2 and MULBSTA (84.2% and 84.2%) respectively. APACHE 2 score and Angiotensin 2 had maximum Specificity (90%) for prediction of severity of outcome. In another study, APACHE II, (mean  $\pm$  SD) were  $14 \pm 5$  in survivors, and  $16 \pm 4$  in nonsurvivors, p-value 0.2 [14]

**Correlations of angiotensin-2 with CRP, D-Dimer, Creatinine and Ferritin and Correlations of angiotensin with CURB Score, APACHE 2 and MULBSTA:** In present study, there was statistically significant positive good correlation between angiotensin level and with laboratory parameters as CRP ( $r=0.846$ , p value <0.001), D-Dimer ( $r=0.876$ , p value <0.001), Creatinine ( $r=0.510$ , p value <0.001), Ferritin ( $r=0.867$ , p value <0.001) and with different scores as CURB Score ( $r=0.673$ , p value <0.001), APACHE  $r=20.847$ , p value <0.001), MULBSTA ( $r=0.698$ , p value <0.001).

In another study, there was a strong association between angiotensin-2 and CRP, creatinine and D-dimers (with p value at 0.001, 0.001 and 0.003, respectively) in ICU COVID-19 patients [11].

**ROC curve for predictability Mortality:** In present study, all the variables were significant to predict mortality (all p values <0.001) but AUC was maximum for APACHE 2 and Angiotensin (0.896 and 0.852 respectively). CURB Score has maximum Sensitivity (88.9%) APACHE 2 had maximum Specificity (80%) for prediction of mortality.

In another study, Area under the receiver operating characteristic curve (AUROC) was used to assess the accuracy of 3- and 10-day angiotensin-2 for in hospital mortality and nonresolving pulmonary condition, respectively. Three-day angiotensin-2 increase of at least twofold from baseline was significantly associated with in-hospital mortality by multivariate analysis (hazard ratio [HR], 6.69; 95% confidence interval [CI], 1.85-24.19; P 5 .004) with AUROC 5 0.845 (95% CI, 0.725-0.940) [15].

In another study [10], biomarker performance done to predict severe COVID-19 outcomes, it was observed that most biomarkers had modest predictive value, with suPAR (AUC 0.81) and IL-6 (AUC 0.77) having the best performance. Many biomarkers had weak performance (CRP, neutrophils, lymphocytes, KL-6), with AUC figures between 0.5 and 0.65.

From all the previous literature mentioned here and present study findings, angiotensin-2 levels were significantly higher in patients admitted to the ICU by comparison to those admitted to conventional wards, confirming the importance of angiotensin-2 pathway in endothelial inflammation. Angiotensin-2 levels were associated to CRP and D-dimers, the latter reflecting the link between the Covid-19 coagulopathy and endothelial dysfunction.

## CONCLUSION

Angiotensin 2 is a reliable biomarker to predict the severity of COVID-19 infection. This study showed a cut off value of 5247 for ICU admission. It has a sensitivity of 84.2% and specificity of 90%. This biomarker can be used to triage the patients to ward and ICU at the time of admission for timely and appropriate management. Similarly, APACHE 2 score is better predicting the severity of COVID 19 pneumonia.

## LIMITATIONS

This biomarker can be elevated in sepsis, bacterial pneumonia and in subjects with underlying undetected malignancy and multiple inflammatory diseases. Thus leading to falsely elevated results. This biomarker is not available routinely in most of the laboratories in the country.

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