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Research article

# Role of C5aR2 in prognosis of patients with acute respiratory distress syndrome through negative modulation of C5a: A prospective observational study

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ABSTRACT

*Objective:* Diverse inflammatory pathology is involved in acute respiratory distress syndrome (ARDS). This study aimed to assess the role of complement component fragment 5a (C5a) receptor 2 (C5aR2) in prognosis of patients with ARDS.
*Methods:* A total of 64 adult patients diagnosed with ARDS were prospectively recruited to the study over a period of one year after obtaining the informed consent. The serum C5a and C5aR2were determined using ELISA Kit sandwich method. Area under receiver operating characteristic (AUROC) was used to analyse the prognostic performance of C5a, C5R2, and C5a/C5R2 ratio using MedCalc. The relationship of these biomarkers with the parameters of poor prognosis (non-recovery, hospitalization, ventilation and ICU admission) was analysed through regression using SPSSv20.
*Results:* The mean age of the included participants was 49.17 (SD:14.81) years. C5a/C5aR2 ratio had better discrimination (AUC: 0.707 vs 0.699 vs 0.511) and higher specificity (78.1 vs 71.9 vs 3.1) than C5R2 and C5a in predicting the poor prognosis among ARDS patients. The increased level of C5aR2 (OR: 0.225; $p = 0.009$) was significantly associated with better recovery and the high C5a/C5aR2 ratio (OR: 3.281; $p = 0.036$) was significantly associated with non-recovery in moderate to severe patients. Additionally, steroid treatment significantly associated with better recovery in patients with a high C5a/C5aR2 ratio (OR: 0.104; $p = 0.007$).
*Conclusion:* The current evidence indicates that a higher levels of C5aR2 significantly associated with better recovery, whereas high levels of C5a/C5aR2 significantly associated to poor prognosis

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**Abbreviations**

| | |
|---|---|
| ALI | Acute lung injury |
| APACHE II | Acute Physiology and Chronic Health Evaluation II |
| ARDS | Acute respiratory distress syndrome |
| AUC | Area under the curve |
| AUROC | Area under the receiver operating characteristic |
| CI | Confidence interval |
| C5a | Complement component fragment 5a |
| C5R2 | Complement receptor C5a receptor-like 2 |
| DAMA | Discharged against the medical advice/request |
| ELISA | Enzyme-linked Immunosorbent assay |
| GCS | Glasgow Coma Scale |
| GPCR | G-protein coupled receptor |
| MODS | Multiple organ dysfunction syndrome |
| OR | Odds ratio |
| pg/mL | picogram/millilitre |
| P/F | Partial pressure of oxygen in arterial blood to the fraction of inspiratory oxygen concentration |
| ROC | Receiver operating characteristic |
| SD | Standard deviation |
| SOFA | Sequential Organ Failure Assessment |
| T2DM | Type-2 diabetes mellitus |

in moderate to severe ARDS patients. However, adequately powered studies are required to confirm these findings in future.

## 1. Introduction

Acute respiratory distress syndrome (ARDS) involves heterogenous pathophysiology, associated with complex immunological response depending on numerous aetiologies, and other patient related factors such as comorbid illness, polypharmacy, and management strategies [1,2]. ARDS has multiple aetiological phenomena including sepsis, pneumonia, leptospirosis, scrub typhus, dengue, and trauma [3,4]. The inflammatory pathological basis of ARDS along with the variety of aetiologies contributes to its high severity, complications and mortality among these patients [3]. However, the role of specific immune mediation or the complement system is not elucidated, especially with respect to the Complement component fragment 5a (C5a) and it's second receptor (C5aR2) which is also called as C5a receptor-like 2 (C5L2).

C5a is a significant pro-inflammatory mediator derived from C5 and binds to G-protein coupled receptor (GPCR) CD88 and non-GPCR, C5a receptor-1 (C5aR1) [5]. Studies on ARDS patients indicate that C5a is extensively contributing to ARDS by the initiation of tissue factor expression of neutrophils and enhancing the neutrophil extracellular traps in alveoli [6,7]. C5a enhances the neutrophil extracellular traps in ARDS patients which contain constituents including chromatin, histones and proteins. This will increase the transforming growth factor -β production along with the platelets which further turn to tissue remodelling and all this together leads to the apoptosis and tissue injury [7–11]. The involvement of C5a in the pathogenesis and abolishing the effect of anti-human C5a des arg antibodies in ARDS has been reported in the literature since years and the elevated levels of C5a have been identified as a good predictor. Those primates treated with anti-human C5a des arg antibodies has shown a better survival and oxygenation, lesser incidence of oedema and hypotension [12,13]. The activation of C5a is known for its effect in predicting ARDS [9] and the complete inactivation of C5a among the recovered patients [14] since decade.

C5aR2, second receptor of C5a, containing 7 transmembrane domains shown to have negative signalling function of C5a. This has been evident through mice studies [15]. Human C5aR2 receptor clearly shown to exhibit an independent GPCR activity which shows C5aR2 may act through ways other than signalling [16]. Moreover, the internalization of C5aR2 and intracellular presence of C5a and C5a des Arg proposes the function of C5R2 as the removal of extracellular active complement fragments [16]. Wang et al., reported that C5aR2 shown to have an ability to inversely modulate the C5a activity, which is a new key that, enhanced or maintained expression of C5aR2 may control the severity of ARDS or acute lung injury (ALI). The absence of C5aR2 increases the inflammation and inflammatory biomarkers in cecal ligation and puncture induced inflammation [17]. Moreover, anti-C5aR blockade resulted in reversing of biomarkers such as TNF-α and IL-6 in bronchoalveolar lavage fluid in C5aR2 deficient mice [12]. Additionally, preclinical studies also showed the possible pro-inflammatory role of C5aR2 by inhibiting the inflammatory markers such as C3a, and C3aR [16].

While C5a-C5aR1 axes primarily mediates pro-inflammatory effects, C5a-C5aR2 has a more nuanced role, often functioning as a modulator of inflammation. This plays a pivotal role in ARDS through modulating inflammation, immune responses, and tissue damage. The C5a-C5aR1 axis predominantly promotes pro-inflammatory responses, including neutrophil recruitment, activation, and

cytokine release, contributing to pulmonary inflammation, vascular leakage, and alveolar injury in ARDS or ALI [11,18–20]. Whereas, the C5a-C5aR2 axis appears to exert regulatory and anti-inflammatory roles, potentially mitigating excessive immune activation and promoting resolution of inflammation. Dysregulation of these signalling pathways can exacerbate lung injury and worsen ARDS outcomes, highlighting their potential as therapeutic targets [21]. C5a-C5aR2 ligation initiates a β-arrestin-2–, PI3K-, and ERK-dependent signalling pathway in human mast cells that produce a better clinical benefit in immune response [22]. Previous studies underscore the importance of balancing these axes to modulate the inflammatory milieu in various diseases including ARDS [23]. Recent pre-clinical findings explore the potential role of C5a, C5aR1 and C5aR2 beyond the immunology and inflammatory diseases through its various mechanisms [24].

The aetiology of disease has significant role in the inflammatory responses due to antigenicity of the pathogens. This influences the type of inflammatory response involved, including the role of complement markers that influences the prognosis of ARDS [20,21,23]. However limited literature available on how inflammatory responses influence the prognosis and prediction of ARDS. The role of C5a and C5aR2 is very important in preventing the inflammatory disease process and pathology across all organ system based on the underlying etiology involved in it [25] along with other inflammatory modulations such as bacterial dissemination, tissue accumulation of neutrophils, and organ damages [26]. C5aR2 plays a key role in preventing cecal ligation and puncture induced inflammatory reactions and subsequent mortality in sepsis models [27] and the recent *in vivo* evidence indicating that maintaining or increasing the C5aR2 expression observed to have beneficial effects in ARDS/ALI through C5a-C5aR2 signalling [17]. Considering the above factors involved in pathology of ARDS, the C5a and C5aR2 can also be used to predict the etiological associated prognosis in future.

Existing literature evidence indicate that C5aR2 can act as an anti-inflammatory factor and is inversely correlated to C5a associated pathology of inflammatory diseases. However, there are no human studies assessing the role of C5aR2 in ARDS patients. Our study will be the first attempt to establish the role of C5a and C5aR2 in the prognosis of ARDS through C5a-C5aR2 signalling.

## 2. Methodology

### 2.1. Ethical consideration

Prior to the initiation of the study, ethical approval was obtained from Institutional Ethics Committee (IEC:638/2021) and the study was registered with Clinical Trials Registry of India (CTRI/2022/02/039954). A signed written informed consent was obtained from the patient or a legally acceptable representative of the patient before enrolling the patient into the study. All study procedures were carried out according to the biomedical and health research involving human participants, and Declaration of Helsinki. The study was reported in accordance with the strengthening the reporting of observational studies in epidemiology (STROBE) checklist as provided in Appendix 1.

### 2.2. Study design and participants

A single-centre prospective observational study was conducted from February 2022 to March 2023 in a critical care setting of an Indian tertiary care teaching hospital. Adult ($\geq$18 years of age) patients diagnosed with ARDS irrespective of aetiology during the study period were enrolled in the study. American/European Consensus Conference criteria [28] or Berlin Criteria [29] for ARDS was used to confirm the diagnosis of ARDS which includes the levels of hypoxia; the presence of bilateral infiltrates on chest radiograph and nonappearance of cardinal signs of left heart failure [27]. ARDS was categorized as mild, moderate, and severe if the partial pressure of oxygen in arterial blood to the fraction of inspiratory oxygen concentration (P/F) ratio is $\leq$ 300 millimetre of mercury (mm Hg), $\leq$200 mm Hg, and $\leq$100 mm Hg, respectively [29,30]. Patients with co-morbid illnesses like bronchial asthma, chronic obstructive pulmonary disease, immunocompromised patients including human immunodeficiency virus positive, history of bone marrow or solid organ transplantation, current malignancy, acute burn injury and pregnant woman were excluded.

### 2.3. Sample size and data collection

Purposive sampling method was used to recruit the participants as there was no pre-established literature evidence on humans. The collected patient data included the demographic characters (age, gender), the severity of disease (severity as per Berlin definition, P/F ratio, Sequential Organ Failure Assessment (SOFA) score, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Glasgow Coma Scale (GCS), comorbidities (type-2 diabetes mellitus [T2DM], kidney injury, liver disease), aetiologies (pneumonia, sepsis, septic shock, multiple organ dysfunction syndrome [MODS], leptospirosis, scrub typhus), corticosteroid treatment and prognosis.

### 2.4. Blood sample collection and analysis

The blood samples were collected within 24 h of ARDS diagnosis from the clinical laboratory and the samples were centrifuged (2500×*g*) at 4 °C for 10 min to collect the serum. The serum samples were collected in Eppendorf tube and stored at − 80 °C until further analysis. The C5a (CUSABIO, USA) and C5aR2 (MyBioSource, USA) analysis was performed by enzyme-linked Immunosorbent assay (ELISA) Kit Sandwich Method in accordance with the manufacturer's instructions. The concentrations of C5a and C5aR2 were standardised to picogram/millilitre (pg/mL). Leftover blood samples were used for the estimation of C5a/C5aR2 ratio. Samples were discarded as per the hospital guidelines once the study completed.

### 2.5. Outcome analysis

Patients were followed-up from admission to hospital discharge or death. The patients were categorized into two groups, in which those patients who are completely recovered and discharged with stable clinical improvement was considered as the recovery group and those who expired or discharged against the medical advice/request (DAMA) due to worsened outcome or poor prognosis were considered as the non-recovery group.

### 2.6. Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS)® version 20.0 developed by IBM [31]. Descriptive statistics were performed, and the results were presented as the frequency with percentages for categorical variables. For continuous variables, mean with standard deviation (SD) and median with range were used for normally distributed and non-normally distributed parameters, respectively. The characteristics were compared between the recovered and non-recovered participants. The Chi-square test, independent *t*-test, and Mann-Whitney *U* test were used for categorical, normally distributed, and non-normally distributed continuous variables, respectively. The prognostic performance of C5a, C5aR2 and C5a/C5aR2 ratio for non-recovery outcome was analysed through the area under the receiver operating characteristic (AUROC) analysis using MedCalc® statistical software [32]. Receiver operating characteristic (ROC) analyses were used for discrimination analysis, and to calculate the values for cut-off, sensitivity, specificity, positive likelihood ratio, negative likelihood ratio and area under the curve (AUC). The cut-off points were calculated by acquiring the best Youden index [33]. Additionally, the effect of severity and P/F ratio on the ROC analysis also explored through a pairwise comparison of ROC curves. Following the exploratory analysis, a univariate binary logistic regression was used for the identification of factors affecting the non-recovery in ARDS patients. Only the moderate to severe ARDS cases were considered for regression as all patients were recovered in the mild group. The results of univariate logistic regression

**Table 1**
The characteristics and biomarker expression among the included participants.

| | All (N = 64) | Recovered (n = 32) | Not recovered (n = 32) | p-value |
|---|---|---|---|---|
| Variable | n (%) | | | |
| Age (≤50 years) | 35 (54.7) | 17 (53.1) | 18 (56.2) | 0.802 |
| Age (>50 years) | 29 (45.3) | 15 (46.9) | 14 (43.7) | |
| | 44 (68.7) | 9 (28.1) | 11 (34.4) | 0.590 |
| Female | | | | |
| Male | 22 (31.3) | 23 (71.9) | 21 (65.6) | |
| | 7 (10.9) | 7 (21.9) | 0 (0) | < **0.001*** |
| Mild ARDS | | | | |
| Moderate ARDS | 29 (45.3) | 18 (56.2) | 11 (34.4) | |
| | 28 (43.8) | 7 (21.9) | 21 (65.6) | |
| Severe ARDS | | | | |
| | 29 (45.3) | 14 (43.7) | 15 (46.9) | 0.802 |
| AKI/CRF | | | | |
| Leptospirosis/Scrub typhus | 11 (17.2) | 8 (25) | 3 (9.4) | 0.098 |
| Liver disease | 3 (4.7) | 1 (3.1) | 2 (6.2) | 0.544 |
| MODS | 24 (37.5) | 14 (43.7) | 10 (31.2) | 0.302 |
| Pneumonia | 38 (59.3) | 17 (53.1) | 21 (65.6) | 0.309 |
| Sepsis | 28 (43.8) | 8 (25) | 20 (62.5) | **0.002*** |
| Septic shock | 22 (34.4) | 5 (15.6) | 17 (53.1) | **0.002*** |
| Steroid use | 28 (43.8) | 19 (59.4) | 9 (28.1) | **0.012*** |
| T2DM | 24 (37.5) | 14 (43.7) | 10 (31.25) | 0.302 |
| Recovered | 32 (50) | 32 (100) | 0 | NA |
| Expired | 29 (45.31) | 0 | 29 (90.62) | |
| Voluntary discharge | 3 (4.69) | 0 | 3 (9.38) | |
| **Variable** | **Mean (SD)** | | | |
| Age (in years) | 49.17 (14.81) | 48.09 (13.26) | 50.25 (16.35) | 0.632 |
| SOFA | 6.51 (3.05) | 6.45 (3.14) | 6.56 (3.00) | 0.591 |
| APACHE-II | 12.19 (6.58) | 11.00 (6.28) | 13.34 (6.76) | 0.855 |
| GCS | 14.19 (2.56) | 14.71 (1.17) | 13.66 (3.37) | < **0.001*** |
| P/F ratio | 124.63 (60.46) | 124.82 (60.19) | 124.45 (61.69) | 0.981 |
| **Variable** | **(Median; Range)** | | | |
| C5a (pg/mL) | 4488.22 (128.60–5229.44) | 4391.89 (2206–5152.33) | 4527.89 (135.40–5229.44) | 0.762 |
| C5aR2 (pg/mL) | 27.50 (6–2563) | 191 (6–2563) | 20 (10–1152) | 0.076 |
| C5a/C5aR2 | 106.10 (0.30–569.40) | 23.50 (1.30–569.40) | 215 (0.30–499.10) | 0.119 |

ARDS: Acute Respiratory Distress Syndrome; AKI: Acute kidney injury; APACHE: Acute Physiology and Chronic Health Evaluation; CRF: Chronic renal failure; C5a: Complement component fragment 5a; C5aR2 C5a receptor-2; GCS: Glasgow Coma Scale; mL: millilitre; MODS: Multiorgan dysfunction syndrome; NA: Not applicable; pg: picogram; SOFA: Sequential organ failure assessment; T2DM: Type-2 diabetes mellitus.
*indicate the statistical significance

(non-recovery and need of ventilation) and linear regression (duration of hospitalization, ventilation, and intensive care unit [ICU] admission) were presented in terms of odds ratio (OR) along with its 95 % confidence interval (CI) and regression (beta) coefficient estimate with standard error, respectively. Additionally, the treatment effect of steroid therapy on prognosis among the patients with high and low C5a/C5aR2 ratio were also assessed through regression analysis. A 2-sided p-value of less than 0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Characteristics of included participants

A total of 64 participants were enrolled for the study, out of 103 ARDS patients admitted during the study period based on the inclusion and exclusion criteria. There was a comparable proportion of participants age (≤50: 54.7 %; >50: 45.3 %) with an average age of 49.17 ± 14.81 years and male domination (68.7 %). Among the included participants, 45.3 % had moderate ARDS and 43.8 % had severe ARDS according to Berlin Criteria. The most observed comorbidities were renal disease (45.3 %), followed by T2DM (37.5 %). Pneumonia (59.3 %), sepsis (43.8 %), and MODS (37.5 %) were the major aetiological factors. The average (SD) SOFA score, APACHE-II scores, GCS score and the P/F ratio was 6.51 (3.05), 12.19 (6.58), 14.19 (2.56) and 124.63 (60.46), respectively. Steroids were used among 28/64 patients, out of which majority of patients belongs to recovery (n = 19) group. The non-recovered group had significantly more severe ARDS with sepsis and septic shock; and less steroid use compared to recovered patients. The complete recovery, death and the DAMA was documented among 32 (50 %), 29 (45.31 %) and 3 (4.69) among the included patients, respectively.

### 3.2. C5a and C5aR2 expression

The median levels of C5a, C5aR2 and C5a/C5aR2 was found to be 4488.22 (128.60–5229.44) pg/mL, 27.50 (6–2563) pg/mL, and 106.10 (0.30–569.40), respectively. Interestingly, there was a high level of C5aR2 (191 vs 20) among those who recovered than those who are not recovered. Whereas the levels of C5a (4527.89 vs 4391.89) and C5a/C5aR2 (215 vs 23.50) were high among those are not recovered than who recovered. A detailed characteristics and the biomarker expression among the included participants are provided in Table 1. The raw data for individual levels of C5a, C5aR2, and C5a/C5aR2 is presented in Supplementary file S1.

### 3.3. Prognostic performance of C5a, C5aR2 and C5a/C5aR2 ratio

C5a/C5aR2 ratio had a better discrimination (AUC: 0.707; p = 0.001) than the C5aR2 (0.699; p = 0.002) and C5a (0.511; p = 0.833) in predicting the prognosis among ARDS patients (Fig. 1). Though, C5a appeared to have a better sensitivity (87.50 %) than C5aR2 (62.50 %) and C5a/C5aR2 ratio (56.25 %), C5a/C5aR2 ratio had a higher specificity (78.12 %) than C5aR2 (71.87 %) and C5a (3.12 %). Severity of the disease (p = 0.7462) and P/F ratio (p = 0.8828) was not significantly affecting the ROC analysis (Fig. 2). A cut-off value of >2206, >36, and ≤25.8 were used to decide the higher or lower values of C5a, C5aR2 and C5a/C5aR2 with a Youden Index of 0·094, 0·344 and 0·344, respectively. The analysis findings on prognostic performance of C5a, C5aR2 and C5a/C5aR2 ratio in ARDS patients is provided in Table 2.

### 3.4. Clinical predictors of non-recovery

The univariate logistic regression analysis of clinical predictors of prognosis revealed that, the factors such as severity of ARDS (OR: 4·909; 95%CI: 1·574-15·314; p = 0·006), sepsis (OR: 6·667; 95%CI: 1·981-22·435; p = 0·002), and septic shock (OR: 5·950; 95%CI: 1·663-21·291; p = 0·006) were significantly associated with non-recovery. While the treatment with steroid (OR: 0·184; 0·059-0·576; p = 0·004) significantly increased the recovery among the moderate to severe ARDS patients. The result of analysis is provided in Table 3.

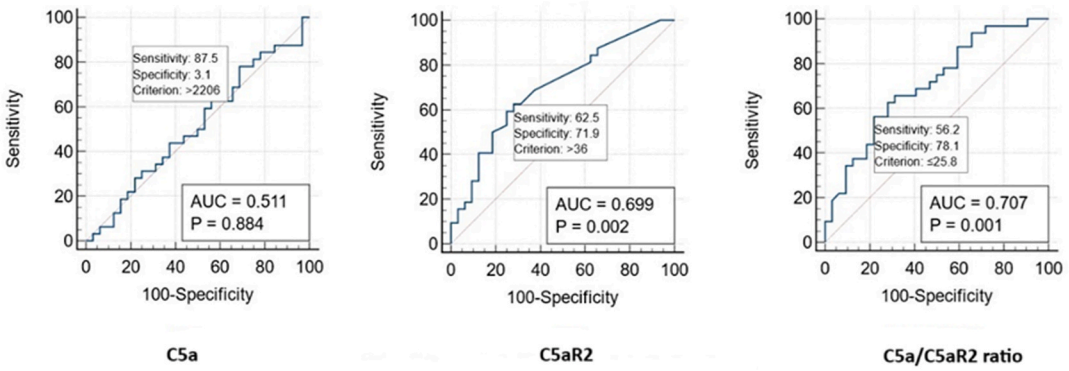


**Fig. 1.** Prognostic performance of C5a, C5aR2 and C5a/C5aR2 ratio for non-recovery in ARDS patients.

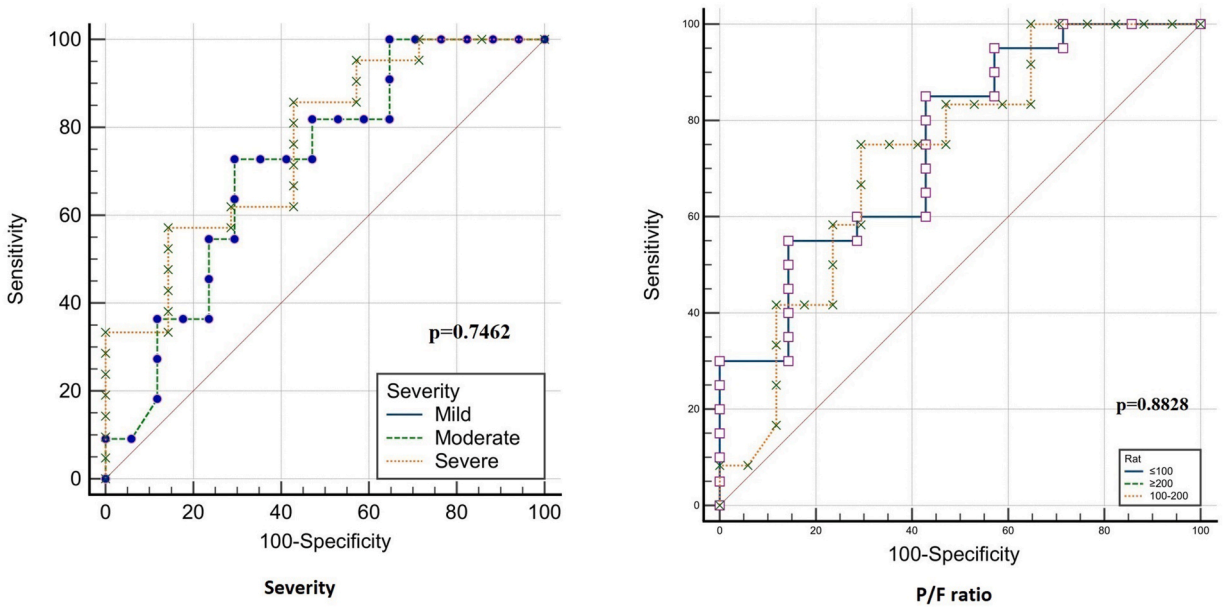


**Fig. 2.** Effect of severity and P/F ratio on the receiver operating curve (ROC) analysis.

**Table 2**
Prognostic performance of C5a, C5aR2 and C5a/C5aR2 ratio for non-recovery in ARDS patients (N = 64).

| Variable | AUROC (95%CI) | p-value of AUROC | Youden Index | Cut-off value | Sensitivity (95%CI) | Specificity (95%CI) | PLR (95%CI) | NLR (95%CI) |
|---|---|---|---|---|---|---|---|---|
| **Recovered: 32; Not recovered: 32** | | | | | | | | |
| C5a | 0.511 (0.383–0.638) | 0.833 | 0.094 | >2206 | 87.50 (71.0–96.5) | 3.12 (0.08–16.2) | 0.9 (0.78–1.04) | 4.0 (0.47–33.86) |
| C5aR2 | 0.699 (0.572–0.808) | **0.002*** | 0.344 | >36 | 62.50 (43.7–78.9) | 71.87 (53.3–86.3) | 2.22 (1.20–4.11) | 0.52 (0.32–0.86) |
| C5a/C5aR2 | 0.707 (0.579–0.814) | **0.001*** | 0.344 | ≤25.8 | 56.25 (37.7–73.6) | 78.12 (60.0–90.7) | 2.57 (1.25–5.30) | 0.56 (0.36–0.86) |
| **Effect of severity (Mild: 7; Moderate: 29; Severe: 28) on ROC** | | | | | | | | |
| Mild | – | – | – | – | – | – | – | – |
| Moderate | 0.714 (0.513–0.867) | 0.746 | – | – | – | – | – | – |
| Severe | 0.762 (0.564–0.901) | | – | – | – | – | – | – |
| Difference (moderate ~ severe) | 0.0480 (−0.243 to 0.339) | | – | – | – | – | – | – |
| **Effect P/F ratio (≤100; 28; 100–200: 29; ≥200: 7) on ROC** | | | | | | | | |
| ≥200 | – | – | – | – | – | – | – | – |
| 100–200 | 0.728 (0.532–0.875) | 0.883 | – | – | – | – | – | – |
| ≤100 | 0.750 (0.547–0.895) | | – | – | – | – | – | – |
| Difference (≤100–100–200) | 0.022 (−0.271 to 0.315) | | – | – | – | – | – | – |

AUROC: Area under receiver operating characteristic; CI: confidence interval; C5a: Complement component fragment 5a; C5aR2: C5a receptor-2; ROC: Receiver operating characteristic.
*indicate the statistical significance

The patients with severe ARDS, non-sepsis, non-septic shock and not on steroid treatment had a higher C5a. The patients with severe ARDS, sepsis, septic shock and not on steroid treatment had a higher C5aR2. This indicates the increased production of C5aR2 in case of higher severity or complications. The patients with moderate ARDS, non-sepsis, non-septic shock and steroid treatment had a higher C5a/C5R2. The level of C5a, C5aR2 and C5a/C5R2 was assessed based on the severity, status of sepsis, septic shock and steroid use as provided in Supplementary file S2.

**Table 3**
Factors affecting the non-recovery (N = 57).

| Variable | Odds Ratio | p-value |
|---|---|---|
| **Demographics** | | |
| Age (>50) | 0.843 (0.295–2.409) | 0.749 |
| Gender (Male) | 0.603 (0.187–1.947) | 0.398 |
| **Severity of disease** | | |
| SOFA | 0.993 (0.836–1.180) | 0.940 |
| APACHE-II | 1.050 (0.963–1.144) | 0.272 |
| GCS | 0.840 (0.640–1.102) | 0.208 |
| Severity (Severe) | 4.909 (1.574–15.314) | **0.006**[a] |
| **Comorbidities and medications** | | |
| AKI/CRF (Yes) | 0.814 (0.286–2.322) | 0.701 |
| Leptospirosis/Scrub typhus (Yes) | 0.266 (0.061–1.163) | 0.078 |
| MODS (Yes) | 0.682 (0.228–2.038) | 0.493 |
| Liver disease (Yes) | 1.600 (0.137–18.723) | 0.708 |
| Pneumonia (Yes) | 2.068 (0.708–6.038) | 0.184 |
| Sepsis (Yes) | 6.667 (1.981–22.435) | **0.002**[a] |
| Septic shock (Yes) | 5.950 (1.663–21.291) | **0.006**[a] |
| Steroid use (Yes) | 0.184 (0.059–0.576) | **0.004**[a] |
| T2DM (Yes) | 0.682 (0.228–2.038) | 0.493 |

AKI: Acute kidney injury; APACHE: Acute Physiology and Chronic Health Evaluation; CRF: Chronic renal failure; GCS: Glasgow Coma Scale; SOFA: Sequential organ failure assessment; T2DM: Type-2 diabetes mellitus.
[a] indicates the statistical significance.

### 3.5. Effect of biomarkers on the poor prognosis

High value of C5a/C5aR2 ratio (OR: 3·281; 95%CI: 1·078-9·989; p = 0·036) was significantly associated with a higher occurrence of non-recovery, whereas high levels of C5aR2 (OR: 0·225; 95%CI: 0·073-0·691; p = 0·009) significantly reduced the non-recovery among the moderate to severe ARDS patients. However, need of ventilation was not significantly associated with any of these biomarkers. Interestingly, the high levels of C5aR2 were reducing the duration of hospitalization, ventilation and ICU stay, when C5a, and C5a/C5aR2 was increasing these outcomes. However, all these effects were not statistically significant (p > 0.05). The detailed results are provided in Table 4.

### 3.6. Effect of corticosteroid on outcomes on patients with low or high C5a/C5aR2 ratio

Among 57 patients with moderate to severe ARDS, 24 patients had low (≤25.8) and 33 had high (>25·8) C5a/C5aR2 ratio. Corticosteroids treatment significantly reduced the poor prognosis among the patients with high C5a/C5aR2 ratio (OR: 0·104; 95%CI: 0·020-0·541; p = 0·007). This indicates that steroid treatment will be useful among moderate to severe ARDS patients with a C5a/C5aR2 ratio of >25·8 pg/mL. The effect of corticosteroids on patient outcomes with low or high C5a/C5aR2 ratio among moderate to

**Table 4**
Relationship of biomarkers with the parameters of poor prognosis.

| <!–Col Count:3–>**Non-recovery (Odds ratio, 95%CI, p-value)** | | |
|---|---|---|
| C5a (High) | 0.333 (0.033–3.418) | 0.355 |
| C5aR2 (High) | 0.225 (0.073–0.691) | **0.009*** |
| C5a/C5aR2ratio (High) | 3.281 (1.078–9.989) | **0.036*** |
| **Need of ventilation (Odds ratio, 95%CI, p-value)** | | |
| C5a (High) | 0.180 (0.014–2.295) | 0.187 |
| C5aR2 (High) | 0.321 (0.031–3.287) | 0.338 |
| C5a/C5aR2ratio (High) | 2.129 (0.208–21.843) | 0.525 |
| **Duration of hospitalization (beta coefficient, SE, p-value)** | | |
| C5a (High) | 5.844 (4.964) | 0.244 |
| C5aR2 (High) | −4.012 (2.511) | 0.116 |
| C5a/C5aR2ratio (High) | 2.095 (2.602) | 0.424 |
| **Duration of ventilation (beta coefficient, SE, p-value)** | | |
| C5a (High) | 3.288 (4.275) | 0.445 |
| C5aR2 (High) | −0.954 (2.192) | 0.665 |
| C5a/C5aR2ratio (High) | 1.061 (2.233) | 0.636 |
| **Duration of ICU stay (beta coefficient, SE, p-value)** | | |
| C5a (High) | 4.896 (4.497) | 0.281 |
| C5aR2 (High) | −1.578 (2.313) | 0.498 |
| C5a/C5aR2ratio (High) | 2.421 (2.344) | 0.306 |

CI: Confidence interval; C5a: Complement component fragment 5a; C5aR2: C5a receptor-2; SE: Standard error.
*indicates the statistical significance

severe patients is provided in Table 5.

## 4. Discussion

Globally, ARDS accounts for an overall burden of 10 % of all ICU patients, 23 % of ventilated patients and 5.5 cases per ICU bed annually [34]. The delayed diagnosis, onset from clinical presentation to hospital admission, lack of treatment protocol, categorization of severity, early treatment initiation, and lack of specific markers to predict the outcome [35] can contribute to the poor prognosis. Hence, there is a need for stratification models and prediction tools to tackle the present challenges [36]. The existing tools have lesser of clinical application and lack clinical validity in the hospital setting. Biomarker panels have various possible applications through molecular phenotyping in identifying patients who are at risk of developing ARDS, diagnosing and risk categorization of ARDS, treatment decision, monitoring, and outcome prediction [37]. This study aimed to develop possible markers which will help in predicting the prognosis in ARDS.

C5aR2 can produce an anti-inflammatory effect through controlling the C5a action [38]. However, there was no human studies conducted on role of C5aR2 in ARDS, although *in vivo* studies showed that sustained or increased levels of C5aR2 will be beneficial [17,39]. Hence, this study was an attempt to assess the role of C5aR2 and C5a/C5aR2 ratio in predicting the prognosis in ARDS patients using serum samples. There are some recent evidence supporting the usefulness of C5aR2 in regulating the inflammatory pathology such as odontoblastic differentiation of dental pulp stem cells [40,41], advanced human atherosclerotic plaques [42], and pro-inflammatory role of C5aR2 during the complement anaphylatoxin [43] and stimulating the mast cells [44].

We observed that high levels of C5aR2 can be an excellent biomarker to predict a better recovery among the ARDS patients, especially those with moderate to severe disease. The C5aR2 will be produced in a larger quantity in case of severe inflammatory conditions to suppress the C5a and it acts as a decoy receptor [45]. Additionally, C5aR2 is responsible for internalization and degradation of C5a and C5a-desArg pathway [16]. These findings were in accordance with the *in vivo* evidence by Wang R et al., which emphasised the significant protective and anti-inflammatory role of sustained or increased C5R2 expression among the ARDS patients [17]. To our best knowledge, this is the first study in the clinical settings which strongly support the beneficial role of C5aR2 in ARDS.

Additionally, increased level of C5a over C5aR2 (C5a/C5aR2) can be one of the important parameters to be considered in poor prognosis of ARDS. We observed that, a high level of C5a/C5aR2 ratio contributed to a significantly ($p = 0{\cdot}036$) higher risk of non-recovery (OR: 3·281) compared to those patients with a low C5a/C5aR2 ratio. C5aR2 can downregulate the inflammatory process through various mechanisms including the regulation of C5a [17], proinflammatory cytokine TNFα [40], dentinogenic marker dentin matrix protein-1 [40], acylation-stimulating protein [46], binds to C5a des-Arg [47], and ERK1/2 phosphorylation [15] depends on the pathology of the disease or condition, though an exact mechanism is still unknown [38]. Overall, higher levels of C5aR2 in ARDS patients may improve recovery by counterbalancing the pro-inflammatory effects of the C5a-C5aR1 axis, promoting resolution of inflammation, and protecting lung tissue from excessive immune-mediated damage through multiple mechanisms [21,48]. This can contribute to a better clinical benefit in case of higher C5aR2 level and worse prognosis with higher C5a/C5aR2 level in ARDS patients, respectively. This also highlights its potential as a therapeutic target to manage ARDS.

Emerging evidence has suggested the potential interaction between corticosteroids, such as prednisolone, and the complement protein C5a, which may influence its pro-inflammatory activity [49]. These findings hint at the possibility that corticosteroids may exert their therapeutic effects, at least in part, through modulation of the C5a-C5aR axis. This interaction could be particularly relevant in conditions like ARDS, characterized by excessive inflammation. While these insights are promising, further research is required to elucidate the exact mechanisms of this interaction, its clinical relevance, and its implications for corticosteroid-based therapies, particularly in moderate to severe ARDS patients with elevated C5a/C5aR2 ratio [49,50]. Corticosteroids seems to be a promising agent in the management of ARDS [51]. Thus, our findings will be helpful to explore the role of C5a-C5aR2 axis in predicting the prognosis and deciding the use of steroids in ARDS and other inflammatory diseases.

Though this study provides many insights to the clinical practice, following needs to be addressed through further research studies. We were unable to analyse the role of C5a, C5aR2, and C5a/C5aR2 based on the etiological associated prognosis of ARDS considering the less sample size and power. Adequately powered studies with a large sample size with respect to aetiologies are required to investigate in future. Also, effect of steroids on the C5a and C5aR2 values are unclear. The serum samples were collected and stored at −80 °C and all samples were tested at one point of time. We have not performed a longitudinal analysis at multiple time points to understand the influence of duration of the storage on the C5a and C5aR2 values. The severity of diseases on C5a and C5aR2 values needs to be investigated. Also, the prognosis of disease is not assessed at multiple time points. Additionally, the correlation between the expression of C5a, C5aR2 and C5a/C5aR2 and the levels of IL-1β, IL-6, and TNF-α, which are the biomarkers of lung injury should be assessed in future studies to understand how these markers affect the immunopathological changes in the ARDS patients. A comparison should be made between the C5a-C5aR1 and C5a-C5aR2 in future to have a better understanding of mechanism-based enhancing or inhibition of inflammatory pathways in ARDS. The statistical significance of performance metrices reported might have hampered by the limited sample size in this study. However, the findings of this study will serve as foundational evidence in the literature to support the development of the C5a-C5aR2 ratio as a potential prognostic marker or therapeutic target in ARDS. To the best of our knowledge, no prior studies have reported on this aspect, making our work a valuable contribution to the field. Further adequately powered well controlled studies are required to confirm our findings.

## 5. Conclusion

The findings of this study indicates that, increased level of C5aR2 was associated with a significantly better recovery and high C5a/

**Table 5**
The effect of corticosteroid on outcomes in patients with low or high C5a/C5aR2 ratio (n = 57).

| C5a/C5aR2 ratio | Steroid used/Not used | Recovered/Not recovered | OR (95%CI) | p-value |
|---|---|---|---|---|
| Low (n = 24) | 8/16 | 7/17 | 1.143 (0.205–6.366) | 0.879 |
| High (n = 33) | 18/15 | 18/15 | 0.104 (0.020–0.541) | **0.007*** |

CI: Confidence interval; C5a: Complement component fragment 5a; C5aR2: C5a receptor-2; OR: Odds Ratio.
*indicates the statistical significance

C5aR2 was associated with poor prognosis among the moderate to severe ARDS patients.

## CRediT authorship contribution statement

**Muhammed Rashid:** Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sreedharan Nair:** Writing – review & editing, Supervision, Investigation. **Pooja Gopal Poojari:** Writing – review & editing. **Vijetha Shenoy Belle:** Resources, Project administration, Formal analysis, Data curation. **Vijayanarayana Kunhikatta:** Writing – review & editing, Software, Formal analysis. **Daniel A. Vaz:** Project administration, Data curation. **Vishal Shanbhag:** Project administration, Formal analysis, Data curation. **Viji Pulikkel Chandran:** Writing – review & editing. **Shravya Chitrapady:** Writing – review & editing. **Girish Thunga:** Supervision, Software, Resources, Project administration, Investigation, Conceptualization.

## Ethical approval and consent to participate

Prior to the initiation of the study, ethical approval was obtained from Institutional Ethics Committee (IEC:638/2021) and the study was registered with Clinical Trials Registry of India (CTRI/2022/02/039954). A signed written informed consent was obtained from the patient or a legally acceptable representative of the patient before enrolling the patient into the study. All study procedures were carried out according to the biomedical and health research involving human participants, and Declaration of Helsinki. The study was reported in accordance with the Strengthening the reporting of observational studies in epidemiology (STROBE) Checklist.

## Data availability

All the supporting data used for the publication have been provided in the manuscript as a source data. Any additional data can be made available from the corresponding author on appropriate request.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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