# Recent insight into potential acute respiratory distress syndrome

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## ABSTRACT

متلازمة الضائقة التنفسية الحادة (ARDS) هو إصابه الرئة الالتهابي الحاد، ويتميز بزيادة الخلايا البطانية الشعرية الرئوية و الخلايا السنخية الظهارية النفاذية مما يؤدي إلى فشل الجهاز التنفسي في ظل غياب فشل عضلة القلب . على الرغم من التطورات الحديثة في العلاج، لا يزال العدد الإجمالي للوفيات بسبب ARDS مرتفع . المؤشرات الحيوية قد تساعد على تشخيص المرض والتنبؤ بشدته و تطوره ونتائجه من أجل تحسين رعاية المرضى وخفض معدلات الاعتلال والوفيات . يركز هذا البحث على مستقبلات الذوبان للنواتج النهائية لبالغليكوزيل المتقدم و عامل نخر الورم مستقبل قابل للذوبان و انترلوكين 6 (IL-6)و 8- IL و منشط البلازمينوجين المانع1– والذي لديه إمكانيات أكبر بناء على الدراسات الأخيرة .

Acute respiratory distress syndrome (ARDS) is an acute inflammatory lung injury, characterized by increased pulmonary capillary endothelial cells and alveolar epithelial cells permeability leading to respiratory failure in the absence of cardiac failure. Despite recent advances in treatments, the overall mortality because of ARDS remains high. Biomarkers may help to diagnose, predict the severity, development, and outcome of ARDS in order to improve patient care and decrease morbidity and mortality. This review will focus on soluble receptor for advanced glycation end-products, soluble tumor necrosis factor-receptor 1, Interluken-6 (IL-6), IL-8, and plasminogen activator inhibitor-1, which have a greater potential based on recent studies.

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A biomarker is a biological variable that may help to identify patients at a higher risk of developing disease, assess response to therapy, predict outcomes, and optimize enrollment in clinical trials.[1] Multiple biomarkers have been studied to assess the severity and prognosis of acute respiratory distress syndrome (ARDS). Acute respiratory distress syndrome is an acute inflammatory lung injury with increased permeability of pulmonary capillary endothelial cells and alveolar epithelial cells resulting in hypoxemia that is refractory to usual oxygen therapy.[2] However, the mortality due to ARDS remains high despite advances in treatment.[3]

The course of ARDS is characterized by 2 phases that may sometimes overlap: exudative and fibroproliferative phases. The exudative phase is the acute inflammatory stage of ARDS marked by alveolar injury and the release of various proteins in the blood and the alveolar compartment. The Fibroproliferative phase is developed due to an imbalance between profibrotic and antifibrotic mediators.[4] Among all the biomarkers in acute respiratory distress syndrome, soluble receptor for advanced glycation end-products (sRAGE), soluble tumor necrosis factor-receptor 1 TNFR-1 (sTNFR-1), Interluken (IL)-6, IL-8, and plasminogen activator inhibitor-1 (PAI-1) appear to have a greater potential use based on recent literature from Baron et al[5] and Ware.[6] Compared with other biomarkers, these biomarkers have been studied with a greater number of patients with good diagnostic and prognostic values.[7]

*Soluble receptor for advanced glycation end-products (RAGE).* The RAGE belongs to the immunoglobulin superfamily of cell surface molecules that can act as a transmembrane pattern recognition receptor. Receptor for advanced glycation end-products is a multiligand-binding protein that can interact with advanced

glycation end products (AGEs), amphoterin, or high mobility group box-1 protein (HMGB1), amyloid, fibrils, and members of the S100/calgranulin family.[8]

Although RAGE is expressed in many cells, it is highly expressed on basal membranes of alveolar type I (ATI) cells.[9,10] It activates the pathways responsible for innate immunity and alveolar inflammation leading to the activation of nuclear transcription factor NF-κB.[11] RAGE can be measured in biological fluids such as bronchoalveolar lavage fluid (BALF), and plasma as soluble forms such as sRAGE and endogenous secretory RAGE (esRAGE).[11] Soluble RAGE comprises the extracellular domain of membrane RAGE and is generated through the cleavage of full-length RAGE by proteinases.[12] Soluble receptor for advanced glycation end-products is considered as a marker of AT1 cell injury.[13,14] Meanwhile, esRAGE is produced by alternative splicing of the AGER gene.[15] These RAGE isoforms may act as decoy receptors; thus, preventing interaction between ligands and transmembrane RAGE.[16]

Higher sRAGE levels in arterial, central venous, and alveolar fluid have been reported during the course of ARDS, compared to mechanically ventilated controls without ARDS.[17,18] Soluble RAGE levels in Bronchoalveolar lavage fluid were much higher than those in plasma in patients with ARDS, suggesting that the alveolar type I cell might be the primary source of plasma sRAGE. Higher levels of sRAGE were also associated with more impaired oxygenation and alveolar fluid clearance.[19-21]

Soluble RAGE also acts as an endothelial adhesion receptor that mediates interactions with the leukocyte integrin Mac-1 (CD11b/CD18).[22] Soluble RAGE levels might reflect the expression of RAGE on pulmonary microvascular endothelium, leading to the inflammatory cell accumulation into the alveolar space.[23]

Based on the meta-analysis conducted by Terpstra et al[23] in 317 patients, elevated sRAGE levels had good diagnostic values for ARDS in at-risk patients, with OR 3.48 (95% CI, 1.69-7.15).[7] Jabaudon et al[24] also reported that arterial sRAGE could be used to diagnose ARDS with an area under the curve (AUC) of 0.99 (95% CI, 0.99-1). Better than other markers implicated in RAGE pathway such as S100A12 (AUC 0.94; 95% CI, 0.87-1), AGEs (AUC 0.73; 95% CI, 0.59-0.88), HMGB1 (AUC 0.65; 95% CI, 0.49-0.81), and esRAGE (AUC 0.65; 95% CI, 0.49-0.81). Arterial sRAGE could also be used to characterize lung morphology, as assessed by lung CT scan and higher levels of sRAGE were associated with nonfocal ARDS (OR 0.79; 95% CI, 0.6-0.92).[24] A cut-off value of 3494 pg/mL had a sensitivity of 82% (95% CI, 60-95) and a specificity of 75% (95% CI, 35-97) for predicting non-focal ARDS. When combined with other markers, the AUC increased to 0.82 (95%CI, 0.59-1).[17,24-27]

Levels of sRAGE may also be useful to assess severity in ARDS, with significant correlations between arterial sRAGE and ratio of arterial oxygen partial pressure to inspired oxygen fraction ($PaO_2$/$FiO_2$) (Spearman's: -0.36; 95% CI, -0.54 to -0.16; $p$=0.0008).[28] In addition, although future validation studies are warranted, sRAGE levels might be useful in assessing response therapy, namely, to recruitment maneuvers and or ventilator strategy.[29] However, in a meta-analysis of 756 patients conducted by Terpstra et al,[23] sRAGE was not associated with increased mortality in ARDS patients (OR 2.54; 95%CI: 0.75-8.63).[7] The study by Gu et al[30] also reported that there is no significant difference between survivor and non-survivors, although plasma sRAGE levels were higher in non-survivors. However, some inconsistencies in the available data may result from technical issues, namely poor detection of circulating RAGE isoforms due to the technique used to measure sRAGE.[30]

*Interluken-6.* Interluken-6 is a cytokine produced by various cell types that exhibit pro-inflammatory and anti-inflammatory properties in response to stimulation by endotoxin, IL-1ß, and TNF-α.[31] Interluken-6 serum level is elevated during infectious, traumatic, or inflammatory stress. Interluken-6 primarily correlates with the pro-inflammatory properties in ARDS. Usually, IL-6 is already present in both plasma and broncoalveolar lavage before the onset of ARDS due to the prior infection and is extremely elevated during the course of ARDS.[32]

Due to the complex nature of IL-6, which has both pro- and anti-inflammatory properties, studies examining the role of IL-6 in ARDS yielded conflicting results. The pro-inflammatory effects of IL-6 are produced by signaling through the soluble IL-receptor (trans-signaling). Meanwhile, anti-inflammatory effects are produced only by a macrophages, neutrophils, T cells, and hepatocytes, which express the IL-6 receptor (classic signaling).[33] In an in-vitro study, IL-6 demonstrated a pro-inflammatory by inducing vascular leakage via trans-signaling through endothelial cells which reflected in a high protein level in BAL. The opposite result was found in an in-vivo study. Interluken-6–/– mice have higher protein in BAL compared to wild type (WT) mice which may indicate that IL-6 exhibits anti-inflammatory activity to resolve neutrophilic inflammation which is then reflected in low protein levels in the presence of IL-6.[34] However, it

is still possible that the increase in BAL protein seen in IL-6 -/- mice was due to an increase in other cytokines or inflammatory mediators in the absence of IL-6. Further study is needed to evaluate the role of IL-6 in vascular leakage. In contrast with vascular leakage, the role of IL-6 in recruitment of inflammatory cells is well known. IL-6 plays an important role in the recruitment of immune effector cells such as neutrophil into the lung in the acute stages of inflammation.[35] This migration of neutrophil into alveoli will activate lung epithelial cells and augments the expression of adhesion molecules such as vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) to stimulate production of chemokine.[36] Based on the meta-analysis carried out by Terpstra et al,[23] in the at-risk population, IL-6 was associated with ARDS diagnosis with OR 2.4 (95% CI,1.32-4.26).[7] Ware et al[37] also found that altered levels of plasma biomarkers may be useful for discriminating sepsis patients with ARDS from those without ARDS. When used alone, IL-6 have AUC 0.61 (95% CI, 0.53-0.69) and 0.63 (95% CI, 0.53-0.72) on severe cases only. However, if IL-6 is combined with other biomarkers (includes Surfactant protein D , RAGE, IL-8, Clara Cell Protein 16), the AUC increases to 0.78 (95% CI, 0.74-0.87) and 0.82 (95% CI, 0.77-0.9) on severe cases.[37] As reported from the meta-analysis of 1480 patients conducted by Terpstra et al,[23] high plasma IL-6 levels are independently associated with morbidity and mortality (OR 3.38; 95% CI, 1.81-6.31).[7] The same result also reported by Calfee et al[38] with OR 1.24 (95% CI, 1.14-1.35).[38] The clinical outcomes also correlate with tidal volume ventilation. Patients who received low-tidal-volume ventilation (6 mL/kg) had a 26% reduction (95% CI, 12-37%) of IL-6 levels compared with patients with 12mL/kg tidal volume ventilation.[39] In low risk population, IL-6 was also significantly associated with decreased ventilator-free days, and poorer outcomes.[36]

*Interluken-8.* Interluken-8 is a pro-inflammatory cytokine, which acts as a potent neutrophil chemoattractant. It is secreted by multiple cell types in response to an inflammatory stimulus. Interluken-8 is mainly found in pulmonary edema fluid and BAL fluid of ARDS patients.[40] Terpstra et al[23] reported in the meta-analysis that IL-8 was associated with diagnosis of ARDS with OR 3.21 (95% CI, 1.41-7.29).[7] Interluken-8 also has AUC 0.63 (95% CI, 0.55-0.7) to distinguish severe sepsis patients with ARDS from those without ARDS.[37] The high serum level of IL-8 is associated with severe illness and mortality.[41] Tseng et al[42] found that higher levels of IL-8 was observed in ICU non-survivors. However, it is not statistically significant in multivariate

analysis. This may be due to the small sample size (n=56).[42] In contrast, on meta-analysis carried out by Terpstra et al,[23] IL-8 was independently associated with the outcome of ARDS with OR 3.35 (1.96-5.71).[7] This result was replicated in the multicenter study of 853 patients by Calfee et al[38] with OR 1.41 (95% CI, 1.27-1.57).[38] Similar with IL-6, IL-8 also correlated with decreased VFDs and poorer outcomes. This study also showed that the presence of a detectable IL-8 level alone may identify higher-risk patients in a generally low-risk population.[43] Ware et al[41] also suggested the major prognostic value of IL-8 when combined with clinical risk factors.[41] The clinical outcome was also associated with volume tidal ventilation. There was a 12% reduction (95% CI, 1-23%) in IL-8 levels in the patients who received 6 mL/kg tidal volume ventilation compared with 12 mL/kg tidal volume ventilation.[39] From a practical standpoint, only 250 µL of plasma which can be obtained from excess plasma drawn, can be used to measure IL-8 levels. The result will be available in 6-8 hours and then it could be used in combination with Acute Physiology and Chronic Health Evaluation (APACHE) score to calculate the prognosis of patients.

*Soluble tumor necrosis factor-receptor 1.* Tumor necrosis factor-receptor 1 acts as a TNF-α receptor. This receptors are transmembrane proteins with extracellular, intracellular, and transmembrane domain which are present on almost all nucleated cells.44 TNFR-1 is mainly associated with inflammation and tissue degeneration.[45] It is responsible for neutrophil apoptosis, down-regulates chemokine (C-X-C motif) receptor 2 (CXCR2) expression in neutrophils, and reduces neutrophil infiltration and migration to the infectious site.[46] During inflammation, soluble TNFR-1 (sTNFR1) is shed from the cell surface and binds to circulating TNF-α.

In a multicenter study of 377 patients with ARDS, sTNFR1 was associated with morbidity and mortality with OR 5.76 (95% CI, 2.63-12.6) for 10-fold increase in plasma sTNFR1. This study also reported that low-tidal volume ventilation was associated with a decrease of sTNFR1 levels.[47] A similar result was also reported in the study by Frank et al,[48] which reported a 2-fold decrease of sTNFR1 in the patients receiving low-tidal volume ventilation and higher positive end-expiratory pressure, levels.[48]

*Coagulation and fibrinolysis.* Plasminogen activator inhibitor-1 is a member of the serine protease inhibitor (serpin) family. Plasminogen activator inhibitor-1 is a key regulator between activation of coagulation and activation of fibrinolysis.[49] It also serves as acute-phase proteins (APPs), which were elevated following injuries

**Table 1 -** Potential biomarkers of acute respiratory distress syndrome (ARDS).[54, 55]

| Patophysiologic feature | Biomarker | Change in ARDS | Clinical prediction |
|---|---|---|---|
| *Exudative phase* | | | |
| *Epithelium damage* | | | |
| Alveolar type 1 cells | Soluble RAGE | Blood↑ | Poor outcome |
| *Lung matrix alteration* | | | |
| Inflammatory cascade | IL-6 | Blood↑, BALF↑ | Poor outcome |
| | IL-8 | Blood↑, BALF↑ | Poor outcome |
| Anti-inflammatory | sTNF-RI/sTNF-RII | Blood↑ | Poor outcome |
| *Coagulation and fibrinolysis* | PA-1 | Blood↑ | Poor outcome |

RAGE - receptor for advanced glycation end-products, IL - interluken, sTNF-RI - soluble tumor necrosis factor α-receptor I (sTNF-RI), PA-1 - plasminogen activator-1, BALF - bronchoalveolar lavage fluid

and related to poor outcomes.[50] Plasminogen activator inhibitor-1 regulates fibrinolysis through the conversion of plasminogen to plasmin by covalently binding to the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA).[49] Both the plasminogen activator (PA), and PA-1 are secreted by various cells including macrophages, fibroblasts, and lung endothelial and epithelial cells. On unstimulated alveolar macrophages, PA activity is higher than PAI-1 activity (pro-fibrinolytic), but when stimulated by endotoxin (LPS), alveolar macrophages will inhibit fibrinolysis through releasing higher amounts of TNF-α, IL-1ß, and PAI-1. Plasminogen activator inhibitor-1 promotes inflammation through the autophagy modulation.[51,52] Accumulated fibrin enhances inflammatory processes by activating endothelial cells to produce pro-inflammatory mediators and therefore increasing vascular permeability.[53]

Plasminogen activator inhibitor-1 was associated with ARDS as reported by Terpstra et al[23] with OR 1.78 (95% CI, 1.32-2.39)[7] In ARDS, the alteration in coagulation (as measured by plasma protein C) and fibrinolysis (as measured by plasma PAI-1) are not only prevalent, but also associated with clinical outcomes including death and fewer ventilation-free and organ-failure-free days. ARDS patients have decreased urokinase activity in the air spaces due to increased levels of PAI-1. Agrawal et al[43] found that plasma PAI-1 was significantly associated with increased oxygenation index. This study was carried out in an homogenous population that has a low mortality rate by excluding patients with sepsis and who have APACHE score of ≥25 (259 patients).[43] This result is supported by a multicenter study of 779 patients carried out by Ware et al[40] with OR 1.84 (95% CI, 1.08-3.12).[40] However, this result is different with meta-analysis conducted by Terpstra et al,[23] which stated that PAI-1 was not associated with ARDS mortality (OR 3.87 (95% CI, 0.75-8.63).[7]

In conclusion, biomarker study can help us to understand complex pathophysiology of ARDS. Soluble RAGE, IL-6, IL-8, sTNFR, and PAI-1 may provide valuable diagnostic and prognostic information that could help to tailor treatment strategies in patients with ARDS and ultimately to improve patient outcomes. However, further study is still needed before these biomarkers can be routinely applied in daily practice.

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* Only 1-2 up to date references should be used for each particular point in the text.

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http://www.nlm.nih.gov/bsd/uniform_requirements.html