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Biomarkers in Acute Lung Injury: Insights into the Pathogenesis of Acute Lung Injury

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Abstract

Studies of potential biomarkers in experimental models of acute lung injury (ALI) and from clinical samples from patients with ALI have provided extensive information relating to the pathophysiology of the mechanisms of lung injury and repair. The utility of biomarkers remains still solely part of the research tools to investigate lung injury and repair mechanisms and due to lack of sensitivity and specificity cannot yet be used as a clinical decision tool in patients with either acute lung injury or ARDS. We have reviewed known biomarkers in context of their major biological activity such as inflammatory mediators, coagulation/fibrinolytic mediators, growth factors and the emerging use of proteomics. The continued interest in identifying and studying biomarkers is relevant as it continues to provide important information regarding the mechanisms involved in lung injury and repair and how this may be helpful in the identification and design of future therapeutic targets and strategies as well as hopefully to identify a sensitive and specific biomarker that would be of clinical relevance.

The ability of the lung to perform gas exchange is made possible in part by the effective relationship between the alveolar epithelium and the endothelium of the pulmonary microvasculature.[1, 2, 3, 4] When either barrier is injured, interstitial and alveolar edema may develop. Based on both experimental *in vitro* and *in vivo* models, dysfunction of the normal endothelial-epithelial barriers plays a fundamental role in the development of acute lung injury (ALI).[2, 5, 6, 7, 8, 9, 10, 11,12, 13] ALI is characterized by non-cardiogenic pulmonary edema and is associated with a high mortality and morbidity associated with several clinical disorders including pneumonia, non-pulmonary sepsis, aspiration syndromes, and major trauma and shock.[2, 14, 15, 16, 17] Despite several clinical trials no pharmacologic intervention has been show to be effective in reducing mortality. [18, 19, 20] The selection and design of novel therapeutic interventions target should be achieved by understanding of the

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pathophysiological mechanisms involved in ALI.[2, 19, 20] Studies of biomarkers of lung and systemic injury in patients with ALI (as well as in animal models) can provide more insight into the pathogenesis of ALI and potentially help in the design of novel therapeutic approaches as well as objectively assessing the response to new therapies as a surrogate marker. The ideal biomarker would also possess both high sensitivity and high specificity for predicting clinical outcomes (Table 1).[21]

Over the past two decades focus on biomarkers in ALI has yielded important information regarding the pathophysiology of the lung injury and repair and have highlighted what cells and their mediators have been involved.[22, 23, 24, 25, 26, 27, 28] Studies of biomarkers have also indirectly led to the generation of new ideas regarding potential novel therapeutic targets.[19, 20, 29] These biomarkers in ALI can reflect either cellular activation or cell injury, as well as ongoing acute activation of the inflammatory, coagulation and fibrinolytic systems. Some of the biological markers may possess pleiotropic effects and may have a role in the repair process. Some of these biomarkers have been investigated as potential surrogate markers for the development of ALI as well as clinical outcomes, such as ventilator free days, morbidity and mortality.[22, 24] Currently, biomarkers in ALI remain primarily within the domain of a research tool to aid in the delineation of the pathophysiologic mechanisms involved in acute lung injury and its subsequent repair although they have been shown to have prognostic value as well. The validation of readily measureable biomarkers that may have a role in the design of future clinical trials or in selecting subgroups of patients with ALI is an important longer term objective. In addition to clinical criteria for ALI, biomarkers could be of value in the evaluation and testing of new pharmacologic or cell-based therapies for ALI. [22, 24]

This article will review some of the biomarkers that have been investigated in ALI, with a focus on biomarkers in groups that reflect their primary function (Table 2). In ALI, there are at least two different phases.[2] Initially there is an exudative phase occurring early associated with diffuse alveolar damage, microvascular injury with subsequent pulmonary edema, type I pneumocyte necrosis and the influx of inflammatory cells and mediators release. This phase is followed by a fibro-proliferative phase, during which there is proliferation of fibroblasts and type II pneumocytes hyperplasia and lung repair.[2] To date the mechanisms of epithelial repair at the alveolar level has mostly focused on the role of the alveolar type II cell type and how these cells and the progenitor cells spread, migrate and proliferate and what mechanisms are involved in this process and if any of these mechanisms could lead to therapeutic targets in the future.[30] The mechanisms that are important in lung epithelial repair include interactions with the extracellular matrix components, structural components, cellular signalling pathways leading to spreading and or migration and other mediators that might be important in progenitor cell selection or proliferation, or recruitment.[30]

INFLAMMATION

The inflammatory responses in ALI can either be directly related to an ongoing primary infectious stimulus such as pneumonia or there may be systemic inflammation which is being amplified by the lung injury.[2, 31] The inflammatory cascade involves inflammatory cells and the release of inflammatory mediators.31 Comparing the ratio of cytokines in serum compared to BALF suggests that most of the inflammatory mediators have a pulmonary origin and not just solely the effect of the exudative phase of ALI with the flooding of the alveolar environment with serum mediators. There are both pro-inflammatory and anti-inflammatory mediators and therefore it maybe more the balance of these mediators and their biological inhibitors in the surrounding milieu that regulate much of the development of lung injury and repair which may have implications for their use as biomarkers in isolation or in combination.[30, 31] Most publications have focused on the

significance of antigenic levels of inflammatory biomarkers rather than determination of the net inflammatory balance (use of molar ratios) which maybe be of greater physiological and clinical importance. Inflammatory mediators have been measured both in the plasma or serum, or locally in bronchoalveolar lavage fluid (BALF) or undiluted pulmonary edema fluid.[2, 24, 32, 33] These mediators may be actively secreted from cells which have been recruited into the air spaces in response to the inflammatory cascade or may appear due to release from cellular death.[2, 24, 32, 33, 34] These inflammatory mediators include the pro-inflammatory cytokines interleukins (IL) such as IL-1β, TNFα, IL-6, and IL-8 which possess potent pro-inflammatory actions, as well as the anti-inflammatory interleukins including IL-1ra, IL-10 and IL-13 (Figure 1).

One of the most biologically active cytokines in the early phases of ALI is IL-1β, which is elevated in plasma and is predictive of clinical outcomes.[35, 36, 37] IL-1β is a potent inducer of lung fibrosis[38, 39, 40] and causes release of a variety of pro-inflammatory chemokine e e.g. monocyte chemotatic protein (MCP)-1, macrophage inflammatory protein (MIP) -1α, IL-6 and IL-8 with subsequent recruitment of inflammatory cells [41, 42, 43, 44, 45] into the air spaces as well as being able to alter endothelial-epithelial barrier permeability and fluid transport leading to edema (which is mediated in part via the αvβ5/β6 integrins pathway).[46] IL-1β is elevated in plasma and BALF in patients with ALI, but also is elevated in higher tidal volume and lower positive end-expiratory pressure (PEEP) ventilation.[47, 48] Due to these important clinical findings in patients with ALI and the understanding of IL-1 biology including the knowledge of the existence of the presence of the naturally occurring inhibitors of IL-1 signalling namely IL-1Ra or sIL-1RII,[49, 50] it is proposed that these could be putative novel therapeutic targets due to their potential to possess anti-inflammatory activities. The existence of polymorphisms of IL-1Ra gives some clinical insight into the relevance of IL-1 biology in the role of ALI.[51] IL-1Ra has been demonstrated to be elevated in BALF of patients with ALI.[52, 53] However, it may be important to investigate the ratio between the levels of proinflammatory IL-1β and anti-inflammatory IL-1Ra because this ratio determines the net inflammatory effect of IL-1β.[50] The ratio of IL-1β/IL-1Ra influences the production of other mediators that may be important in epithelial repair such as hepatocyte growth factor (HGF) via a COX-2/prostaglandin E_2 dependent mechanism.[54] However, one study demonstrated that there was a significant relationship between an IL-1β functional bioassay and the antigenic measurement of IL-1β, and the presence of the antagonists IL-1ra or sIL-1RII did not correlate with IL-1β biological activity.[50]

Another early pro-inflammatory cytokine which is in both plasma and BALF in the early phase of ALI is TNFα. It is postulated that resident alveolar macrophages stimulated by pathogen recognition generate much of the production of the early cytokines, IL-1β and TNF-α, which in turn stimulate neighbouring cells to produce a battery of chemokines that mediate the recruitment of neutrophils, monocytes and lymphocytes into the alveolar space.[55, 56, 57, 58] The alveolar macrophages may initially be important therefore in the pro-inflammatory stage but they also have been postulated to have important anti-inflammatory activity in the resolution/repair phase via phagocytosis of apoptotic neutrophils and cell debris as well as secreting epithelial growth factors in a TNFα dependent mechanism. TNFα also has been demonstrated to indirectly promote pulmonary edema by producing reactive oxygen species which subsequently decrease the expression of ENaC and the Na^+-K^+-ATPase.[59] The dysregulation of the microcirculation leading to increased permeability occurs in part by a RhoA/ROCK dependent destabilisation of the microtubules.[60] There is some evidence that TNFα is increased in plasma and BALF and is predictive of outcome in single center studies.[36, 61] The lack of correlation of TNFα with outcomes in multicenter studies may relate to the timing of measurement and the source of measurement as well as the effect that the presence of the soluble TNFα receptors (sTNFr) I and II, which can bind

TNF and compete with its binding to the cellular receptor thus reducing its bioavailability of TNFα.[62] sTNFr I and II are elevated in the plasma and have been demonstrated to be predictive of a poor outcome.62, 63 TNFα has been modulated by the use of pharmacological agents in experimental models including inhibitors of TNF-α mRNA transcription e.g. pentoxifylline, accelerators of TNF-α mRNA degradation e.g. thalidomide, inhibitors of TNF-α protein translation e.g. tetravalent guanylhydrazones and the metalloproteinase inhibitors that prevent the cleavage of the membrane-bound protein to the active moiety.20, [64, 65, 66, 67] In the last decade there has been substantive investigations of these inhibitors in preclinical models of acute lung injury with some of these inhibitors being taken into further drug development in the form of early clinical trials but with little evidence of outcome data.[18]

IL-6 and IL-8 are two other pro-inflammatory cytokines which have been demonstrated to be elevated in both plasma and BALF and are predictive of poor outcomes in ALI patients. [61, 70] IL-8 has a role in neutrophil and monocyte chemotaxis and inhibits neutrophil apoptosis and elevated levels correlates well with number of neutrophils and total protein (a surrogate marker for permeability of the alveolar barrier) in BALF and also are elevated in non-survivors compared to survivors in single center and multi-center studies.[61], 71 The BALF levels of IL-8 have yielded varying results in different studies, in which there have been significant differences between survivors and non-survivors,53, 57, 72 however other studies have not demonstrated this difference. 73, [74] This result is similar with a variety of other pro-inflammatory cytokines e.g. IL-6, MCP-1, perhaps reflecting the heterogeneous group of ALI patients utilised in these studies, the timing of the collection of the BALF and the uncertain relationship to the timing of the onset of ALI. BALF IL-8 levels have been recently demonstrated to be negatively correlated with lung compliance and positively correlated with changes in Sequential Organ Failure Assessment score (SOFA).33 Recently, BALF IL-8 levels have been demonstrated to be independently associated with mortality in a multivariate analysis of patients with infection-induced ALI in a single center study.33 Obviously BALF measurement requires an invasive procedure and the use of plasma IL-8 may be easier and safer for these critically ill patients, and indeed in children with septic shock plasma levels less than 220 pg/ml identify patients with a low risk for death with a negative predictive value of 94–95 % for 28 day mortality.75 However a recent paper investigating the role of plasma IL-8 in the adult population with ALI the correlation with 28 day mortality and the plasma IL-8 levels above 220 pg/ml was much weaker in the adult group than in the pediatric population.76 These differences probably can be explained in part by the heterogeneity of ALI patients in the adult population which were selected prospectively for septic shock, the differences in co-morbidities that occur in the adult population that will affect the mediators and clinical outcomes and finally the timing of the onset of sepsis was not recorded in the adult population, whereas within the pediatric population the IL-8 was recorded at 24 hours after admission to ICU. Another difference is that of adult obesity and elevated body mass index (BMI). One group of investigators reported that within the adult population with ALI that the plasma IL-8 levels fall with increasing BMI, but this did not correspond to improvement in clinical outcomes.77 Several studies have evaluated the role of the anti-IL-8 autoantibody/IL-8 immune complexes in ALI.78 These complexes are present in patients who have been just admitted to ICU and prior to the inflammatory cascade with influx of neutrophils or resultant lung injury.79 A mixture of IL-8 and IL-8 autoantibodies (in excess) have inhibitory actions on neutrophil recruitment and activation, however IL-8 autoantibody/IL-8 immune complexes from patients with ALI have the ability via the FCγRIIa to possess pro-inflammatory activities.80, 81, 82 It has also been demonstrated that blocking the FCγRIIa pathway suppresses the *in vitro* pro-inflammatory actions of this immune complex.[81] The immune complex evokes complement activation and interaction with human epithelial and endothelial cells, leading to loss of integrity via cellular dysfunction. The IL-8 autoantibody / IL-8 – FCγRIIa pathway

signalling molecules could act as potential therapeutic targets for future development, e.g. Src, Spleen Tyrosine Kinase (Syk), Phosphoinositide 3-kinases (PI3-K) and extracellular signal-regulated kinase (ERK).[81, 83] In one study the levels of these immune complexes were higher in those patients who developed ALI and there was a correlation with mortality in patients who already had developed ALI.[79]

IL-6 is another cytokine involved in ALI that signals through the ERK pathway[84] and can activate multiple signal transduction pathways including Janus kinase / signal transducer and activator of transcription (JAK/STAT), Rat sarcoma / extracellular signal-regulated kinase (Ras/ERK) and the PI3-K/Akt.[84, 85, 86] IL-6 is critical for B-cell differentiation and maturation with secretion of immunoglobulins, cytotoxic T cell differentiation, macrophage and monocyte function and production of acute phase proteins. Elevated levels of IL-6 in plasma and BALF have been reported in both single center studies as well as larger multi-center studies to predict mortality in ALI.[61, 70] Although IL-6 activates both pro-inflammatory and anti-inflammatory mechanisms, IL-6 primarily has a pro-inflammatory profile. The presence of the IL-6 agonist sIL-6R has also been investigated in ALI, and higher molar ratio of IL-6/sIL-6R is associated with a higher risk of death.[50] IL-6 has also been investigated in ALI as a candidate gene with studies that have focused on IL-6 single-nucleotide polymorphism (SNPs) in the -174 promoter region and other gene polymorphism of IL-6.[87, 88, 89] Currently there has been no uniform finding from these studies and further research is required as the IL-6 polymorphisms may be stimulus specific.[89, 90, 91] IL-6 is protective in hyperoxia induced lung injury in which IL-6 is cytoprotectve to endothelial cells via inactivation of types of pro-apoptotic proteins e.g. B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax) via phosphorylation via the PI3K/Akt pathway.[92] IL-6 has also been demonstrated to possess pleiotropic effects *in vitro* and *in vivo* and has been suggested to play an important role in the repair of the alveolar epithelium. These pleiotropic effects are reported to be due to the cross talk with other cytokines in the alveolar environment and the particular cell types present that express the receptor for IL-6.[93, 94]

There are other cytokines that possess anti-inflammatory actions, e.g. IL-10, Transforming Growth Factor-β (TGF-β), IL-13 and IL-4. Novel therapeutic targets could be designed around such mediators which can *in vivo* attenuate the injury to the lung. IL-10 has preventative value in lipopolysaccharide (LPS) induced ALI via its anti-inflammatory actions including its inhibition of T_H1 differentiation, suppression of neutrophil activation, and chemokine down regulation.[93] This effect may be achieved by the destabilisation of mRNA of pro-inflammatory chemokines and preventing degradation of Iκβ-α, therefore inhibiting the activation of NF-κβ a pro-inflammatory intracellular signal moiety.[94, 95, 96] The timing of IL-10 administration in relationship to the injury is influential on its ability to possess anti-inflammatory activities. In a multicenter trial, the presence of increased plasma levels of IL-10 was a negative predictor of a poor outcome.[70] IL-13 is another pleiotropic cytokine that possess potent anti-inflammatory properties. It is mostly produced from T_H2 cells and also from T_H1 cells to a lesser extent and suppresses TNF-α, IL-1β, IL-8 and chemokine production and like IL-10 inhibits the activation of NFκβ, but can also enhance the production of the anti-inflammatory moiety IL-1Ra.[96, 97, 98] In addition to the anti-inflammatory cytokines, there is also an important effect of endogenous anti-inflammatory soluble receptors and other moieties that may bind with the pro-inflammatory cytokine hence reducing its bioavailabilty and adjusting the net balance between pro and anti inflammatory effects. Within the local environment there will be an influence of the cells (that constitute the alveolar microenvironment) by the variable expression of cellular receptors which therefore bind and influence the bioavailabilty of the mediators, a process that may have differing effects depending on the timing of the ligand receptor interaction during ALI, especially if the cytokine possesses pleiotropic effects. The inflammatory cascade has an important effect on the inflammatory cells that are recruited into the alveoli

and via their mediator release can elicit injury to the epithelial and endothelial barriers, but in certain instances can also be important in the repair and resolution phase. as highlighted by IL-6. Additionally, the inflammatory cascade is also important in leading to hemorrhage within the alveoli, in part related to the interactions with the coagulation and fibrinolytic cascades and platelets.

Coagulation and Fibrinolysis

Activation of the inflammatory cascade results in the activation of the coagulation system which in turn can influence inflammatory responses by effecting expression of IL-1, IL-6 and IL-8 and migration of inflammatory cells across the endothelial and epithelial barriers into the alveoli.[99, 100, 101] The pro-inflammatory events may also inhibit fibrinolysis and also induce platelet activation.[100, 101, 102, 103, 104, 105] Several coagulation biomarkers have been demonstrated to be abnormal in ALI including protein C, plasminogen activator inhibitor (PAI-1) and thrombomodulin.[26, 99, 106, 107] Thromomodulin activates protein C leading to the formation of a thrombus. The alveolar epithelium contains thrombomodulin which can activate protein C leading to formation of activated protein C (APC), an important endogenous anticoagulant. APC can improve endothelial permeability via activation of the sphigosine-1-phosphate pathway and suppression of pro-inflammatory cytokines. In ALI, the plasma and BALF levels of protein C (part of the APC complex) are low and the plasma levels of PAI-1 are elevated, and both of these findings have been associated with increased mortality.[26, 99, 106, 107] Therefore there was a rationale for drug trials in patients with ALI with therapeutic interventions focused on administering pharmacologic doses of human recombinant APC.[108, 109, 110, 111] Based on *in vitro* studies, APC can protect the endothelial barrier via protease activated receptor-1 (PAR-1) dependent mechanisms. In the PROWESS trial of APC for severe sepsis, APC was anticipated to have anti-coagulant and anti-inflammatory effects which may have benefited patients with more severe sepsis.[110, 112] A recent phase II trial, in which patients with ALI from non-septic causes, APC was not effective in increasing VFDs but did decrease pulmonary dead space.[111] The mortality rate in this study was only 13 % compared to other ALI studies that included patients with sepsis in whom the mortality is higher, approximately 25 %. The results of this trial agree with the results of the ADDRESS and RESOLVE trials in which patients had no benefit from APC but their severity of illness was less than in the original trial of severe sepsis in PROWESS.[113, 114] PROWESS-SHOCK is a large phase III clinical trial that is testing the efficacy of APC in patients with persistent septic shock and high risk of death is predicted to complete recruitment by summer of 2011.[115, 116]

Fibrinolytic activity is decreased in patients with ALI which may in part be related to high BALF levels of PAI-1 as well as decreased fibrinolysis there is also the occurrence of increased fibrin production.[100, 101, 107, 117] PAI-1 is secreted by a variety of cells such as endothelial cells, epithelial cells, macrophages, and fibroblasts. The significantly higher plasma levels of PAI-1 in ALI patients who do not survive may reflect a greater impairment in the fibrinolytic system in these patients.

Platelets also play a crucial role in haemostasis and thrombosis as well as the coagulation and fibrinolytic system. However, over the last decade it has been documented that the platelets have a diverse and extended role via their ability to release mediators to recruit inflammatory cells and progenitor cells, release pro- and anti-inflammatory cytokines, as well angiogenic factors, all of which may contribute to the pathobiology of ALI. All of these pathways utilise a variety of active moieties including chemokines, P-selectins and other adhesion molecules as well as signalling molecules such as Src kinases, all of which may be future therapeutic targets. The selectins that have been studied in small single center studies are expressed on the vascular endothelium and platelets and along with adhesion molecules help facilitate the interactions between inflammatory cells, platelets and vascular

endothelium.[118, 119, 120] The contribution of these cells in the pathophysiology of ALI are influenced by a variety of the mediators already outlined above, but also are regulated by growth factors and components of the extracellular milieu which will be briefly discussion in the next sections.

Growth Factors

Growth factors appear to play a major role in the repair and resolution of ALI.121 The repair of the damaged alveolar epithelium is an incompletely understood process that involves hyperplasia of type II pneumocytes (and perhaps type I pneumocytes), migration along the basement membrane by the type II cells to form a new epithelial barrier, and complex interactions with extracellular matrix and other cells such as alveolar macrophages. 122, 123, 124 A variety of growth factors promote repair of the alveolar epithelium including keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), acidic fibroblast growth factor (FGF), retinoic acid, transforming growth factor-α (TGF-α) and Insulin like growth factor (IGF-1). Lung endothelial repair is promoted by vascular endothelial growth factor (VEGF). Repair can be accompanied by fibrosis that may be promoted by TGF-β, activin-A, platelet derived growth factor (PDGF), basic FGF, IGF-1 but inhibited by HGF and Interferon-γ (IFN-γ). There are at least two major pathways that growth factors utilise in ALI, either tyrosine kinase receptor mediation e.g. KGF, HGF, FGF, VEGF, EGF and PDGF, or serine-threonine kinase receptors such as TGFβ1 which tend to have an opposing effect on the upregulation that occurs when the tyrosine kinase receptor pathway is utilised.125, 126 Some of these growth factors have been studied as biomarkers in ALI.

KGF and HGF are potent mitogens for type II pneumocytes and are postulated to play an important role in the repair of the epithelium following lung injury.[127, 128] KGF and HGF are elevated in BALF from patients with ALI.[127] HGF can be produced by alveolar neutrophils, macrophages, endothelial cells and fibroblasts and upregulated by a variety of pro-inflammatory cytokines such as IL-1β and TNFα via a COX_2/PGE_2 dependent mechanism.128, 129 HGF can also be released by the proteolytic activity of proteases that cleave the HGF from the extracellular matrix. In a single center study, elevation of HGF was associated with worse clinical outcomes.[127] HGF mediates its effects via the c-Met receptor which initiates several downstream effects such as protecting cells from DNA damage (via PI-3K pathway) and enhancing epithelial cell motility Gab-1 recruitment (Ras/Rac and PI-3K).[130, 131] There is a need to investigate how the combined effects of these growth factors occur such as the synergistic effect of HGF with VGEF on endothelial cell activity and the combined effect of HGF with KGF in the repair of lung injury.

In the lung, VEGF is produced primarily by epithelial cells. VEGF seems to increase microvascular permeability in ALI, but also during the repair phase has an important role in increasing endothelial cell proliferation and survival. The levels of VEGF are increased in plasma from patients with ALI, but are decreased in BALF compared to healthy controls. Subsequently BALF VEGF increases during the resolution of the lung injury.[132, 133, 134, 135, 136] The role of VEGF in ALI is not fully delineated but the low levels of VEGF in BALF from ALI patients and patients with hydrostatic edema may indicate that the low levels are due to a dilutional effect from alveolar flooding in the exudative edema fluid.[136] Certain single studies have shown increased plasma VEGF and decreased BALF VEGF levels are associated with worse clinical outcomes.

Another type of vascular growth factor which have been demonstrated to be linked to outcome in ALI patients are the angiopoietin peptides; angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2) and in particular the ratio of Ang-2/Ang-1.[137, 138] These peptides bind to the Tie-2 receptor tyrosine kinase and act in an agonist/antagonist manner.139 Ang-1

via the Tie-2 receptor stabilizes the endothelium by dampening inflammation and inhibiting apoptosis of the endothelial cell, whereas Ang-2 promotes endothelial and epithelial apoptosis, phosphorylates myosin light chain and causes barrier dysfunction.138, [139] Both of these peptides work to modify the integrity of the microvascular endothelium. One recent study reported that a high ratio of Ang-2/Ang-1 was an independent predictor of mortality in patients with ALI, even after adjustment for other variables associated with poor outcomes. [138]

Biomarkers of Alveolar Epithelial / Endothelial Injury

Because alveolar epithelial injury plays an important role in determining the severity of ALI, investigators have studied biochemical markers that may reflect injury to either the type I or type II alveolar epithelial cell. Surfactant proteins are primarily secreted by type II pneumocytes and are amphiphilic lipoproteins which help maintain low alveolar surface tension preventing alveolar collapse and they have a role in the innate immune defense of the lung. Surfactant protein D is primarily a product of type II cells and the receptor for advanced glycation end-products (RAGE) is released primarily by type I cells.140, 141, 142 RAGE belongs to the immunoglobulin superfamily and binds calgranulin (also called EN-RAGE) or high mobility group box-1 (HMGB-1) which activates NFκβ leading to the production of pro-inflammatory cytokines, reactive oxygen species and protease production. 143, 144, 145, 146, 147 Both of these proteins are elevated in the plasma in ALI patients and have been associated with worse clinical outcomes.140, 141, [142]. Elevated levels of plasma RAGE early in ALI seem to identify patients with more alveolar epithelial injury and it is these patients who benefited most from low tidal volume ventilation in the randomized ARDS net trial. Another epithelial protein which has been studied as a potential biomarker is an epithelial mucin protein, called Kerbs von den Lungren-6 (KL-6), which is unregulated during injury on the surface of the type II pneumocytes.[148] KL-6 has been demonstrated to be increased in interstitial lung disease in which there is disarray of the alveolar structure and is correlated with increased barrier permeability. 149 Early pulmonary edema levels of KL-6 has been demonstrated to be elevated in patients with ALI who did not survive compared to patients with ALI who have better mortality but is not able to predict those patients who are at risk of developing ALI.148, 150, [151] As well as the important role the alveolar epithelium type I and II play in acute lung injury and repair there is one other cell type which produces a protein which may have an important role as a biomarker, namely the Clara cell and the protein called Clara Cell specific protein (CC-16).[152] This has been evaluated in a single center study in which elevated CC-16 serum level was associated with increased risk of mortality. This may prove to be a biomarker of interest in the future.

Not only is there epithelial injury but endothelial dysfunction plays a central role in lung injury and repair (see Figure 1) not only in contributing to the influx of inflammatory cells and mediators during the exudative phase but is an active source of growth factors and mediators that can affect vascular tone, cellular proliferation and angiogenesis. The endothelium is also the site in which the above systems of inflammation, coagulation and fibrinolysis cross talk can occur and therefore influences directly the outcome of these systems. Endothelial activity is detected again by the use of a variety of potential biomarkers including von Willebrand factor (VWF), angiotensin converting enzyme or tissue factor pathway inhibitor and many studies have focused on these in acute lung injury as has been reviewed recently.[153] VWF has been well documented both in multi centre trials and single centre trials to be increased in plasma and BALF of patients with acute lung injury as well as be associated with being predictive of outcome of these patients.154, 155, 156 VWF is a glycoprotein that is secreted by both the megakaryocytes and the vascular endothelium and has a pivotal role in haemostasis by being the carrier protein for clotting factors such as factor VIIII as well as acting as a mechanical bridging component between the platelets and

the endothelium. There have been 2 major studies which have indicated that the plasma levels of VWF are not predictive of the onset of acute lung injury.[154, 155] However more recently[156] a study with patients in the early phase of ALI the pulmonary edema fluid and the plasma levels of VWF were associated with clinical outcome demonstrating that the endothelium activation is associated with increased mortality.

The study of proteins in the alveolar compartment may be a useful research tool to help delineate mechanisms that are involved in the lung injury and repair process. Proteomics is useful as it not only permits the identification and quantification of novel proteins that are present in the process but also can identify structural changes that may occur to some of these proteins that will directly effect the native biological activity. These are post-translational modification, such as glycation, glycosylation, phosphorylation or sulphation which can regulate the activity of the proteins, which can be missed when relying on identification of proteins simply by antigenic recognition.[157, 158, 159]

Conclusions

Studies of biomarkers in ALI have provided valuable insights into the pathogenesis of lung injury and repair. Currently, it has not been possible to identify a single biomarker that is specific or sensitive enough to be incorporated into routine clinical practice. However, in the future, measurement of plasma biomarkers may help stratify ALI patients for clinical trials. As illustrated in one recent study[24], the combination of clinical predictors and elevated levels of two to three plasma biomarkers may prove useful to predict prognosis in patients with ALI, as well as to select patients for clinical trials of new therapeutic modalities.

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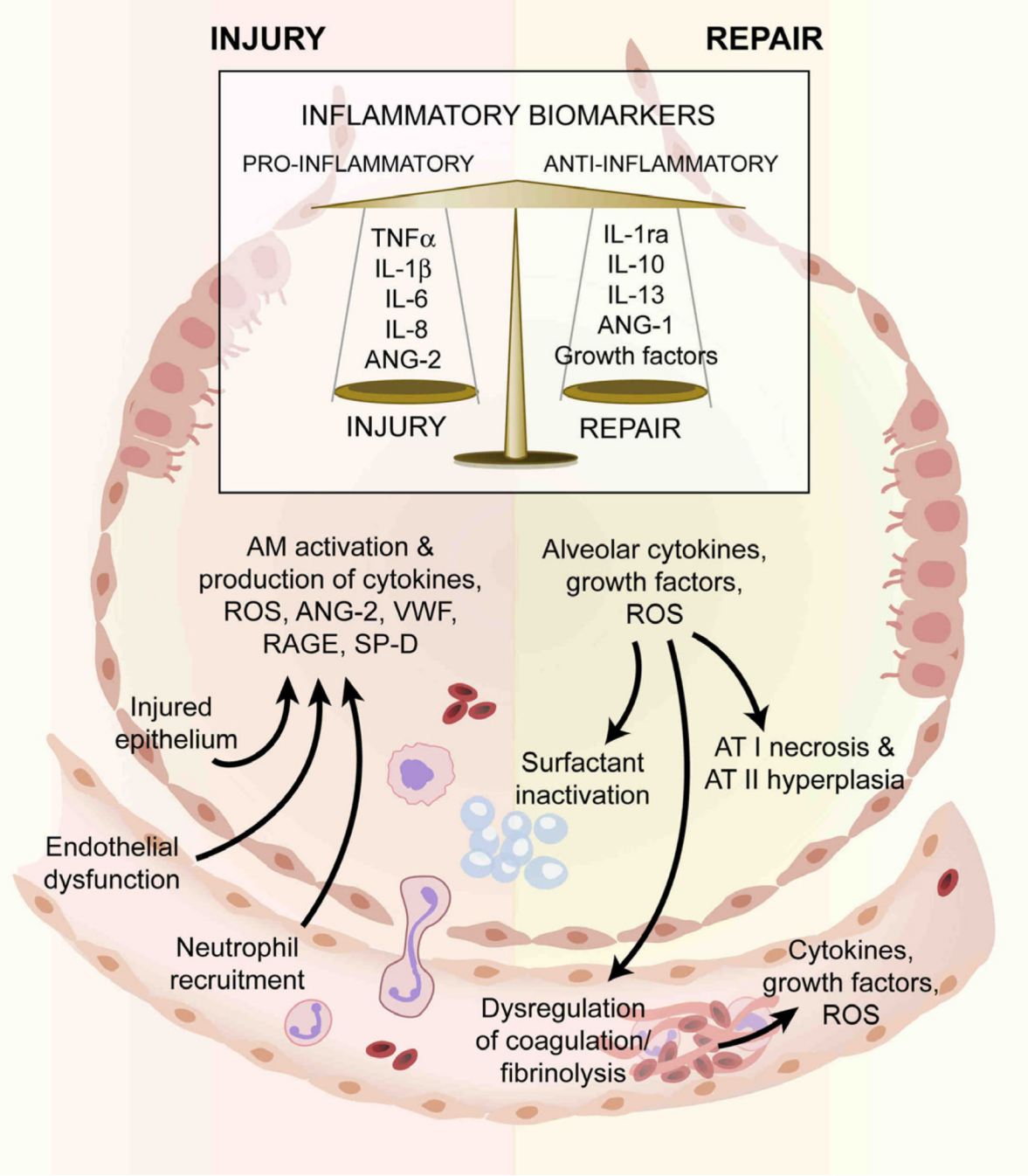

Figure 1.

The injured alveolus in the acute phase (left hand side) and the repair phase in acute lung injury. In the acute exudative phase, there is activation of resident alveolar macrophages which results in production of several pro-inflammatory molecules. These stimulate chemotaxis and activation of neutrophils which release a variety of mediators that further increase the pro-inflammatory environment of the injured alveolus and are associated with both alveolar endothelial and epithelial injury. Some of the mechanisms that play a role repair in ALI are illustrated (right hand side). Alveolar type II cells undergo hyperplasia and there is also recruitment of fibroblasts (not shown). There is release of growth factors and anti-inflammatory cytokines involved in repair. These mediators as well as cell specific

activation/injury can be measured as biomarkers. AM – alveolar macrophage; ANG - angiopoietin; AT – alveolar epithelial cell type; IL – interleukin; IL-1ra – interleukin 1 receptor antagonist, RAGE – receptor for advanced glycation end products, reactive oxygen species, SP=D – surfactant D; VWF – von Willebrand factor.

Table 1

Biomarkers for Acute Lung Injury: The Ideal Properties

➢ High sensitivity and specificity in predicting clinical outcome
➢ Sample has to be easily and safely collected from critically ill patients and the biomarker has to be able to be easily measured with reproducible results across multiple sites
➢ Has a defined role in the pathogenesis of acute lung injury and repair

Table 2

Summary table of biomarkers that have been investigated in Acute Lung Injury

Biomarker	System Classification	Predictive of Clinical outcome	Biological Sample Source	Evidence as role as biomarker from multicenter site studies	Summary of pathogenesis role in acute lung injury	Summary of cellular mechanisms – as potential therapeutic targets
IL-1β	Pro-inflammatory	Positive	Plasma BALF	No	➢ ↑ permeability of alveolar barrier ➢ Induction of inflammatory cytokines and chemokines ➢ Recruitment of inflammatory cells ➢ Pro-fibrotic ➢ Epithelial repair	➢ αvβ5/β6 integrins pathway ➢ IL-1R/Toll-like receptor activation via RhoA ➢ ↑ COX-2 producing PGE-2 which activates EP-3 receptors ➢ EGF/TGF-a pathway and MAPK
IL-1Ra / sIL-1RII and their ratio with IL-1β	Anti-inflammatory	Negative	Plasma BALF	No	Anti-inflammatory – antagonist of IL-1 β	↓ in IL-1β bioavailabilty
TNF-α	Pleiotropic	Positive	Plasma BALF	No	➢ Induction of inflammatory cytokines and chemokines ➢ Recruitment of inflammatory cells ➢ Indirectly promote pulmonary edema ➢ ↑ permeability of alveolar barrier ➢ Repair mechanisms	➢ Activation of TNF-α receptors TNF-R1, TNF-R2 and sTNF-R and subsequent pathways ➢ Production of ROS which ↓ ENaC and Na^+-K^+ ATPase ➢ RhoA/ROCK ➢ Phagocytosis of apoptotic neutrophils and debris
sTNFr-I and II	Anti-inflammatory	Positive	Plasma BALF	Yes	➢ Modulates TNF-α activity	➢ Binds TNF and reduces its bioavailabilty to bind to its cellular receptors
IL-8	Pro-inflammatory	Positive	Plasma BALF (not reliable predictor source)	Yes	➢ Recruitment of inflammatory cells ➢ Activation and priming of neutrophils ➢ Upregulation of adhesion molecules ➢ Activation of the endothelium	➢ CXCR1 and CXCR2 receptors via G_i ➢ CXCR1 and CXCR2 via Rho and Rac

Biomarker	System Classification	Predictive of Clinical outcome	Biological Sample Source	Evidence as role as biomarker from multicenter site studies	Summary of pathogenesis role in acute lung injury	Summary of cellular mechanisms – as potential therapeutic targets
Anti-IL-8 autoantibody / IL-8 immune complexes	Pro-inflammatory	Positive	Plasma BALF	No	➢ Complement activation ➢ Dysfunction of epithelial and endothelial ➢ Modulation of IL-8 bioactivity	➢ FCγRIIa pathway via ERK, Syk, Src, PI3-K
IL-6	Pleiotropic	Positive	Plasma BALF	Yes	➢ Lymphocyte differentiation and activation – both B cell and T cell ➢ Monocyte / macrophage activation ➢ Epithelial repair and endothelial cytoprotective	➢ ERK pathway via JAK/STAT, Ras/ ERK and PI3-K/Akt ➢ Cross talk with cytokines and inactivation of pro-apoptotic proteins via PI3-K/Akt
sIL-6R and ratio with IL-6	Pro-inflammatory	Positive	Plasma	No	➢ Enhancement of IL-6 effects	➢ IL-6 agonist activity binds with IL-6 to promote gp130 dimerisation
IL-18	Pro-inflammaory	Not determined	Plasma	Preclinical and experimental models	➢ Neutrophil recruitment and activation ➢ Cofactor for lymphocyte differentiation and activation	➢ Induction of IFN-γ via activation of the IL-1 receptor (R)/Toll receptor family
IL-10	Anti-inflammatory	Negative	Plasma	Yes	➢ Inhibition of T_H differentiation ➢ Inhibition of chemokine and cytokine release ➢ Neutrophil suppression	➢ Inhibition of NF-κβ
IL-4	Anti-inflammatory	No	Plasma BALF	No	➢ Epithelial repair ➢ Inhibits synthesis of pro-inflammatory cytokines ➢ Increases production of IL-1Ra ➢ Pulmonary fibrosis	➢ IRS-1 or -2
IL-13	Pleitropic	Negative	Plasma	No	➢ Suppresses pro-inflammatory cytokine production ➢ Increases production of IL-1Ra	➢ Inhibition of NF-κβ

Biomarker	System Classification	Predictive of Clinical outcome	Biological Sample Source	Evidence as role as biomarker from multicenter site studies	Summary of pathogenesis role in acute lung injury	Summary of cellular mechanisms – as potential therapeutic targets
TGF-β	Pleitropic	Negative	Plasma	No	➢ Inhibits expression of pro-inflammatory adhesion molecules	➢ Via Smad proteins
Protein C	Coagulation	Positive	Plasma	Yes	➢ Endogenous anticoagulant ➢ Improves endothelial permeability ➢ Suppression of pro-inflammatory cytokines	➢ Activation of the sphigosine-1-phosphate pathway ➢ Induced transcription of anti-apoptotic proteins
PAI-1	Coagulation	Positive	Plasma BALF	Yes	➢ Regulate fibrinolysis and dissolution of fibrin clots ➢ Directly inhibits integrin mediated cell migration	➢ Inhibits plasminogen activator
Thrombomodulin	Coagulation	Positive	BALF	No	➢ ↓ fibrin deposition ➢ ↓ leukocyte accumulation ➢ ↓ alveolar permeability and pulmonary edema	➢ Cofactor in thrombin induced activation of protein C
HGF	Growth factor	Positive	BALF	No	➢ Alveolar epithelial cell mitogen ➢ Epithelial repair	➢ C-Met receptor and activation of PI-3K pathway or Tyrosine kinase activation
KGF	Growth factor	Not determined	BALF	No	➢ Alveolar epithelial cell mitogen ➢ Epithelial repair	➢ FGF receptor and activation of tyrosine kinase
VGEF	Growth factor	Positive	Plasma BALF (decreased levels)	No	➢ ↑ vascular permeability ➢ ↑ endothelial cell survival and proliferation	➢ Via VEGF tyrosine kinase family of receptors
Ang-2/Ang-1	Growth factor	Positive	Plasma	No	➢ Promotes endothelial and epithelial apoptosis ➢ Causes barrier dysfunction	➢ Tie-2 receptor tyrosine kinase
Surfactant D	Epithelial cell - type II marker	Positive	Plasma BALF	Yes	➢ ↓ surface tension ➢ Confer innate immunity	➢ Physical properties of the molecule and induce phagocytosis

Biomarker	System Classification	Predictive of Clinical outcome	Biological Sample Source	Evidence as role as biomarker from multicenter site studies	Summary of pathogenesis role in acute lung injury	Summary of cellular mechanisms – as potential therapeutic targets
RAGE	Epithelial cell – type I marker	Positive	Plasma BALF	Yes	➢ Production of pro-inflammatory cytokines, ROS and protease production	➢ Binds EN-RAGE or HMGB-1 and activates NFκβ
VWF	Endothelial cell marker	Positive	Plasma BALF	Yes	➢ Haemostasis and role in platelet function	➢ Intrinsic activity