Introduction:

ARDS is a complex syndrome which can be initiated and prorogated by a diverse array of precipitating factors with variable phenotypes. A multitude of mechanisms are involved in the pathogenesis of ARDS, and each mechanism or set of mechanisms applies primarily to a specified ARDS subgroup. The definition and phenotyping of ARDS is still imprecise.The ARDS phenotype is likely to be a result of multiple independent or interactive mechanisms. There is complex interplay of various cytokine, humoral and complement cascade. This advantage in defining/diagnosing ARDS is also a disadvantage in terms of individual pathophysiology and treatment. The application of bioinformatics enables us to understand ARDS better. One specific pathological finding of ARDS is diffuse alveolar damage. In 2012, in an effort to increase diagnostic specificity, a revised definition of ARDS was published in JAMA. However, no new parameters or biomarkers were adopted by the revised definition. The genetic and biomarker approach to ARDS in clinical setting is still in an early stage. The bioinformatics need help characterize and risk stratify the patients. We get clinical information including symptoms, physical signs, medical history, lung function or images from the patients. On the other hand, we draw bioinformation from their blood, BALF or tissue. Then, clinical information and bioinformation should be validated and complemented with each other in order to detect effective and powerful biomarkers.

Utility of biomarkers and genetic determinants in ARDS:

For an intensivist in ICU, the pertinent concerns regarding bioinformatics in ARDS are

- Can a genetic marker/biomarker identify patients susceptible to ARDS
- Risk stratification
- Holy grail for diagnosis
- Response to therapy
- Individualized therapies
- “Pharmacogenomics” referred to the application of whole-genome scanning for the discovery of new drug targets
- “Theragnostic study” referred to biomarker level guided interventional studies
- Prognsotication
- Disease course

- Potential new insight into physipathogenesis and therapy
- Newer therapeutic targets

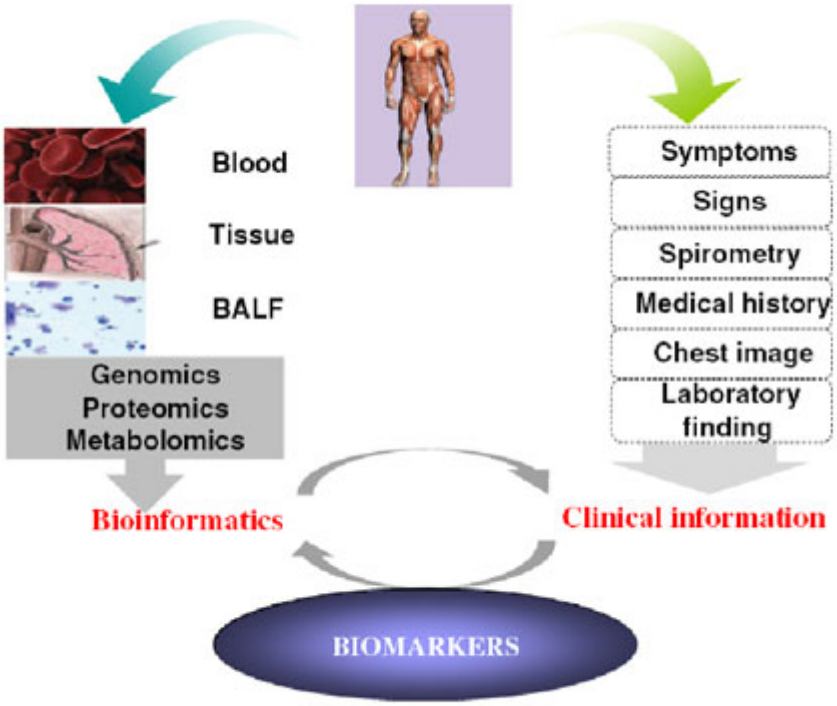

Figure 1. The optimum pattern of clinical bioinformatics in ALI/ARDS.

Genetic markers:

In ARDS multiple biologically plausible candidate genes have been identified. However, ARDS can not be explained with single gene alterations alone. The basic assumption remains that all diseases have an underlying genetic contribution. The major difference among diseases with regard to genetic contribution is the overall percent of this contribution, the number of genes that make this contribution, and whether and how the contributing genes interact. ARDS genetics has multigenic/multifactorial etiology and it increases in magnitude as the number of contributing genetic factors increases and the percent of genetic contribution

decreases. Moreover, gene interactions may lead to synergistic or epistatic effects, and these may yet add another layer of complexity in the study of disease pathogenesis. Furthermore, the overall genetic contribution may be subgroup and/or disease stage dependent, where a greater number of genes and environmental factors play a role in at risk and mild phenotype of disease, whereas more severe phenotypes may be due to a prominent genetic contribution of fewer genes.

Accurate definition of phenotype is quintessential to genetic studies. ARDS like most other critical illness remains a very heterogeneous syndrome. Different sets of genetic markers may underlie susceptibility to ARDS due to infections or trauma.

Candidate genetic markers:

It seems likely that there are genetic determinants that increase an individual's risk of developing ALI/ARDS, since only a small proportion of the patients who are exposed to typical insults actually develop ALI/ARDS. There are several publications reviewed the application of genomics in the study of ALI. In 2002, Marshall and his colleagues provide the first evidence of a genetic influence in ARDS, suggesting an important role for angiotensin converting enzyme (ACE).Insertion-deletion polymorphisms associated with the angiotensin converting enzyme (ACE) gene have also been suggested as a possible risk factor for ALI/ARDS. Studies that link mutations in the surfactant protein B (SP-B) gene to an increased risk of ALI/ARDS support this notion. Gong, et al demonstrated that -308GA promoter polymorphism in the TNF-a gene was associated.

with the mortality of ARDS. SNPs of 43 Toll like receptor-related genes were identified and one of these genes was demonstrated to be associated with susceptibility to organ dysfunction and death in patients with sepsis.

Genes	Technologies	Relationship with ALI
NADPH oxidase	NOX2 subunit knockout mice	NOX-2(-/-) mice exhibited diminished TNF alpha-induced acute inflammatory responses in the lungs but not other tissues, as evidenced by decreased activation of the redox-sensitive transcription factor NF-kappa B, and decreased gene expression of IL-1 beta, IL-6, TNF alpha, E-selectin, and other cellular adhesion molecules.
ANGPT	SNP array performed in ALI and non-ALI patients	An ANGPT2 region is associated with both ALI and variation in plasma angiopoietin-2 isoforms.
Fas (CD95)	Genptyped 14 SNPs in FAS in healthy white volunteers and patients with ALI	Common genetic variants in FAS are associated with ALI susceptibility
Surfactant protein B (SPB)	Genotyping was performed on seven linkage isequilibrium-tag SNP in the surfactant protein B gene	SPB are associated with more severe lung injury as indicated by the association of specific SNP genotypes and haplotypes with the need for mechanical ventilation in African American children with community-acquired pneumonia.
Acvr1, Arhgap15, Cacnb4, Cacybp, Ccdc148,Fancl, Mycn, Mgat4a, Rfwd2, Tgfbr3, and Tnn	haplotype association mapping, microarray/ qRT-PCR analyses, in silico SNP	11 candidate genes are associated with acrolein-induced acute lung injury in 40 different inbred strains of mice
IRAKs	tagging SNPs array	common SNPs in IRAK3 gene might be determinants for sepsis-induced ALI association with ALI development among septic patients
nmMLCK	nmMLCK knockout mice, nmMLCK silencing RNA	nmMLCK knockout mice were significantly protected from VILI

Abbreviations: *Ref*Reference; *ANGPT*:angiopoietin; *NADPH*:glyceraldehyde-3-phosphate dehydrogenase; *NOX*:*NADPH* oxidases; *TNF*-alpha:tumor necrosis factor alpha; *IL-1*:interleukin-1; *IL-6*:interleukin-6; *SPB*:Surfactant protein B; *Acvr1*: activin A receptor, type 1; *ARHGAP15*:Rho GTPase activating protein 15; *CACNB4*:calcium channel, voltage-dependent, β 4 subunit; *CACYBP*:calcyclin binding protein; *CCDC148*:coiled-coil domain containing 148; *FANCL*: Fanconi anemia, complementation group L; *MYCN*:v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian); *MGAT4A*: mannoside acetylglucosaminyltransferase 4, isoenzyme A; *RBMS1*:RNA binding motif, single stranded interacting protein 1; *RFWD2*:Ring finger and WD repeat domain 2; *TGFBR3*:transforming growth factor-β receptor III; *TNN*: tenascin N; *IRAKs*: interleukin-1 receptor-associated kinase genes; *nmMLCK*: non-muscle myosin light chain kinase isoform; *VILI*:ventilator induced lung injury.

Table 1.Genomics application in ARDS

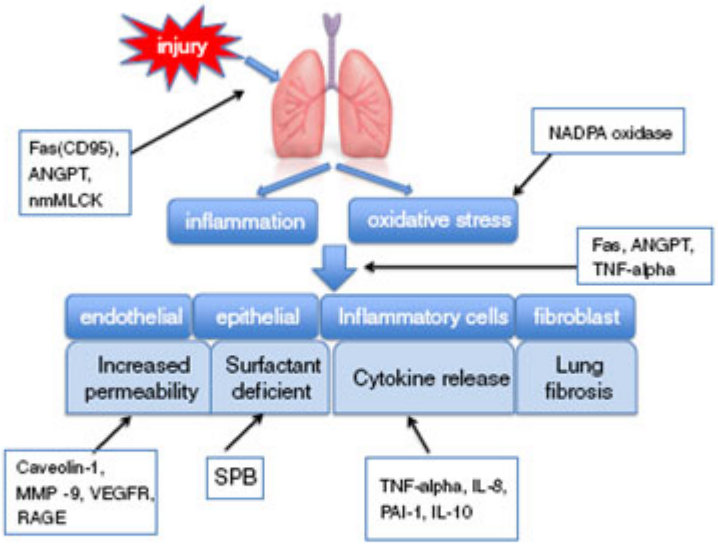

Figure 2. The potential mechanisms of these identified genes or proteins in the process of ALI/ARDS.

Biomarkers:

In parallel with progress in the understanding of ARDS pathophysiology, several molecules have been shown to be candidate biomarkers of this disease, with the clinical usefulness of some being confirmed by large-scale or multicenter studies. However, none of these candidates have been clinically applied for diagnosis or prediction of disease severity, response to therapy, and prognosis in patients with ARDS. Future progress, along with a search for new biomarker candidates, need to determine the potential application(s) of each candidate will be discussed here. This will lead to improved diagnosis and treatment strategies for patients with ARDS.

Potential Biomarkers:

Various humoral factors induced by inflammation and molecules derived from activated cells or injured tissues have been shown as potential biomarkers that may be applied in clinical practice. The following classification explains the potential biomarkers.

The innate immune system and inflammation

The pathogenic role of neutrophil mediated acute inflammation in ARDS development is well researched. Numerous proinflammatory cytokines play major roles in acute inflammation and the development of inflammatory lung diseases, including ARDS. Among these, tumor necrosis factor alpha (TNF α) and interleukin 1beta (IL-1β)

can induce ALI when administered to animals, and their levels are also elevated in the lungs of ARDS patients. Recently, pattern recognition receptors (PRRs) were demonstrated to play a key role in innate immunity. PRRs are cell-surface or cytosolic proteins expressed by innate immune cells, and each is activated by a specific molecule

(s). PRR ligands are divided into two categories, namely, pathogen-associated molecular patterns (PAMPs) and damage (danger)-associated molecular patterns (DAMPs).

Humoral factors as biomarkers of ARDS

Many humoral factors have been identified as candidate biomarkers of ARDS.

Among the proinflammatory cytokines, TNFα, IL-1β, interleukin 6 (IL-6), and IL-8 are elevated in the bronchoalveolar lavage fluid (BALF) of ARDS patients, and higher

levels correlated with outcome. Several growth factors have been determined to be

candidate biomarkers of ARDS. In this regard, the lung levels of vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF) are noteworthy.Ang-2, a competitor of Ang-1 and a regulator of vascular permeability could predict

the prognosis of ARDS. Leptin induced the expression of transforming growth factor beta

(TGF-β) and the production of collagen types I and II in the presence of TGF-β, and

leptin-deficient mice were resistant to the development of ALI.

Regulation of vascular permeability

The dysregulation of vascular permeability leads to a change in vascular permeability; therefore, these factors may represent potential biomarkers for ARDS.

Substances derived from activated cells or injured tissues

Pathogenic role of HMGB1 in ARDS, it was shown to be a candidate biomarker of ARDS, along with soluble RAGE. Excessive formation and ineffective clearance of neutrophil extracellular trap in alveolar space would be responsible in pathogenesis of ARDS. Among endothelial cell-derived molecules, plasma levels of soluble P-selectin and soluble intercellular adhesion molecule (sICAM-1) were reported as candidate biomarkers.

Currently available biomarkers in clinical practice

Differentiating similar diseases or conditions from ARDS remains to be a matter of great importance. Currently,only a few biomarkers are clinically available for this purpose. For example, brain natriuretic peptide (BNP) is often used for differentiation between ARDS and hydrostatic pulmonary edema. Procalcitonin is increased in bacterial infection, but not in viral or fungal infection; it may be useful for discriminating between bacterial pneumonia and ARDS. However, no ARDS-specific biomarkers are currently available commercially.

Name	Change in ARDS	Clinical prediction
Humoral mediators		
Cytokines, growth factors		
TNFα	BALF↑	Poor outcome
IL-1β	BALF↑	Poor outcome
IL-2	Blood↑	Development
IL-4	Blood↑	Development
IL-6	Blood↑, BALF↑	Poor outcome
IL-8	Blood↑, BALF↑	Development and severity (BALF), poor outcome
IL-18	Blood↑	Poor outcome
VEGF	ELF↑	Better outcome
KGF	BALF↑	Poor outcome
GDF-15	Blood↑	Poor outcome
Ang-2	Blood↑	Development, poor outcome
Neutrophil elastase	Blood↑	Development and severity
Leptin	BALF↑	Poor outcome
Coagulation/fibrinolysis factors		
PAI-1	Blood↑	Poor outcome
Thrombomodulin	Blood↑	Poor outcome
von Willebrand factor	Blood↑	Development
Protein C	Blood↓	Poor outcome
Substances released from injured or activated tissues		
DAMPs		
HMGB-1	Blood↑	Poor outcome
DNA	BALF↑	Poor outcome
Endothelial cells		
Soluble P-selectin	Blood↑	Poor outcome
Soluble ICAM-1	Blood↑	Poor outcome
Epithelial cells		
Soluble RAGE	Blood↑	Poor outcome
SP-B	Blood↑	Development
SP-D	Blood↑	Poor outcome
CC-16	Blood↑	Poor outcome
Laminin γ2	ELF↑	Poor outcome
KL-6	Blood↑, BALF↑	Poor outcome

BALF bronchoalveolar lavage fluid, *ELF* epithelial lining fluid, *TNFα* tumor necrosis factor alpha, *IL* interleukin, *VEGF* vascular endothelial growth factor, *KGF* keratinocyte growth factor, *GDF-15* growth differentiation factor-15, *Ang-2* angiopoietin-2, *PAI-1* plasminogen activator inhibitor 1, *DAMPs* damage (danger)-associated molecular patterns, *HMGB-1* high-mobility group box 1, *ICAM-1* intercellular adhesion molecule 1, *RAGE* receptor for advanced glycation end products, *SP* surfactant protein, *CC-16* Clara cell specific protein 16, *KL-6* Krebs von den Lungen-6.

Table 2.Potential Biomarkers in ARDS

Conclusion:

The importance of biomarkers is underscored by the fact that they can also be utilized to predict response to therapy and prognosis. The genetic and proteomic approach to study ALI/ARDS in clinical setting is still in an early stage, while some important data have been generated. There is a great need of further exploratory studies to better understand the molecular mechanisms and pathophysiology of ALI/ARDS.

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