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AIRWAY INFLAMMATION AND INFLAMMATORY BIOMARKERS

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SUMMARY

Keywords: inflammation, airway inflammation, biomarkers.

Introduction. Airway inflammation is a common feature of many diseases. Its repercussions can be discovered in many aspects of the disease, therefore finding the biomarker or combination of biomarkers to define it continues to be challenging.

The scientific **material and methods** used are the review of current literature data, controversies, discordances and peculiarities of respiratory tract inflammation and biomarkers capable to assess it.

The **results** are centered on those biomarkers capable to capture inflammation from as many angles as possible, with impact not only on the pathogenesis of the disease but also with clinical, evolution, therapeutic and monitoring implications.

Conclusions. For airway inflammation many biomarkers are useful, some define the disease as a whole, some are defining for certain aspects or endotypes. The winning combination proposed by many authors appears to be a panel of biomarkers which captures as many aspects as possible for each entity, but is usually difficult to find.

Tables and figures

Introduction

Despite all its negative traits, the inflammation is a remarkable reaction of the body to injurious factors, designed to restore homeostasis. The inflammatory process is very complex, implicating numerous cells, mediators, immune system and regulatory pathways. Two forms of inflammation are classically described, acute and chronic, based on its duration. Chronic inflammation lasts more than 4 weeks and is usually preceded by an acute form. These two forms may also coexist. In order to keep a prolonged duration, the inflammatory response lingers and the injurious agent acts continuously. Chronic inflammation includes several events, involving recruitment of cells and chronic cellular infiltrate as a result, tissue destruction, resolution or scarring in the attempt to repair the damaged tissue. Repair is a very complex process, far from perfect, involving proliferation of the fibroblasts and fibrosis, collagen deposition and remodeling of the tissues.

Biomarkers to define both acute and chronic inflammation are continually discovered. These biomarkers are far more important for chronic inflammation, where they define many endotypes for each disease and have impact on its evolution, treatment and prognosis.

Chronic airway diseases are defined by chronic local inflammation and a state of low-grade systemic inflammation.

Biomarker – definition

According to the Food and Drug Administration (FDA), a ***valid biomarker*** is defined as "measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results." [1]

Classification of biomarkers

Molecular biomarkers can be classified based on many criteria, such as objective, source, origin, chemical composition, genetics and molecular biology.

Based on their *objective,* biomarkers can be: diagnostic biomarkers, prognostic biomarkers and biomarkers for monitoring clinical response.

If the *the measured parameter* is taken into consideration, the biomarkers can be divided into three distinct groups:

- Biomarkers of exposure – measuring in an internal compartment an exogenous molecule or metabolite resulting from an extrinsic exposure. They assess the exposure and are useful for biomonitoring.
- Biomarkers of effect – physical, biochemical, molecular parameters correlated with a certain dysfunction or disease;
- Biomarkers of susceptibility – refers to the intrinsic ability of the body to react in a predictable way to a extrinsic exposure, reflect the features that make an organism more susceptible.[8]

Biomarkers are classified in three categories, based on *genetics* and *molecular biology methods*:

- Type 0 – natural history biomarker – correlates the natural history of the disease with the clinical features.
- Type 1- drug activity biomarker- which capture the effects of an action, act as marker of responsiveness to the treatment and can be further divided into efficacy biomarkers (therapeutic effects of a drug), mechanism biomarkers (mechanism of action of a drug) and toxicity biomarkers (toxicological effects of a drug)
- Type 2 – surrogate marker, indicating a risk. [5]

Sources of biomarkers correlated to airway inflammation are very divers. Some of them are easier to approach, others are reserved only to selected cases.

- Bioptic fragments obtained by bronchoscopy are considered the gold standard but difficult to obtain, costly, therefore not accessible;
- Bronchoalveolar lavage is reliable for the underlying inflammatory process and obtained with minimum invasiveness;
- Induced sputum is also reliable for the inflammation and noninvasive, but difficult to obtain for pediatric patient;
- Exhaled breath condensate contains a group of biomarkers and great variability in composition;
- Serum is a rich source of divers biomarkers and reliable for the disease;
- Urine has limited value for airway inflammation, even though many biomarkers have been discovered in the past years.

Origin of biomarkers can be the resident cells of the lung and airway or the cells infiltrating at inflammation site. The cells themselves and their products can be considered biomarkers. Some of the biomarkers assess the inflammation overall while others give information about certain aspects, closely related with the underlying immune processes.

Biomarkers of chronic airway inflammation

There is a large variety of biomarkers assessing the airway inflammation. The panel includes cellular biomarkers, proteic molecules, lipidic derivates, oxidative stress products, enzymes, hormones, receptors, nucleic acids in different combinations and evaluated in varied conditions such as: stable disease, exacerbation, association of infection.

Biomarkers that assess globally the inflammatory process are acute phase reactants- fibrinogen, ferritin, "C" reactive protein, complement, haptoglobin, serum amyloid A, plasminogen and white blood cells. They give a measure to the inflammation, without giving information of the immune dyregulations underlying it. Together with other biomarkers can build an endotype of a disease. Among these biomarkers hs-CRP, serum amyloid A, haptoglobin were highly studied.

Table 1. ***Types of biomarkers assessing the airway inflammation [1,11,24]***

Source	*Type of biomarker*
Bronchoalveolar lavage	**Cells**:macrophages, neutrophils, eosinophils, lymphocites,epithelial cells, **Proteic molecules**: cytokines (IL-1RA, IL-1α, IL-1β, IL-2Rα, IL-5, IL-6, IL-7, IL-8, IL-37, G-CSF, RANTES). **Enzymes**: matrix metalloprotease 9 (MMP-9)
Induced sputum	**Cells**: Eosinophils, neutrophils, NK, lymphocites **Proteic molecules:** cytokines, eosinophil cationic proteins, granulocyte macrophage colony stimulator factor, neurokinin A, claudins, HMGB1 **Lipidic derivates**: Cys-LT, lipoxin A4, isoprostan **Oxidative stress products**: NO derivates, aldehides. **Receptors**: IL17R **Enzymes**: plasminogen activator, MMPs, tissue inhibitors of metalloproteinase (TIMPs), ADAMTS, ADAM-8, ADAM-7, ADAM-33
Exhaled breath condensate	**Oxidative stress products**: eNO, hydrogen peroxide, nitric oxides **Lipidic derivates:** LT, PGE2,TXB2, Cys-LT, isoprostanes **Proteic molecules**: cytokines, endotelin-1. **Nucleic acids**: micro-ribonucleic acid (miRNA). **Enzymes**: matrix metalloprotease 9 (MMP-9)

Serum	**Cells**: Eosinophils, IgE total and specific, **Proteic molecules**: cytokines, eosinophil cationic protein, eosinophil- derived neurotoxin, surfactant protein D, fibrinogen, hsCRP, growth factors- fibroblast, hepatocyte, stem cell, periostin, YKL-40, leptin, lactoferin, desmosine, natriuretic peptide type b, copeptin, DDP-4, defensins, collectins, osteopontin, galectin 9, TLRs, complement fractions serum Amyloid-A, pancreatic stone protein/ regenerating protein, neopterin **Nucleic acids**: micro-ribonucleic acid (miRNA), DNA methylation profile **Other molecules**:, LT antagonists, MAMPS, DAMPS
Urine	**Proteic molecules**: eosinophil protein X, bromotyrosine, lactate, taurine, trimethylamine N oxide (TMAO), myoinozitol, β2 micro-globulin **Lipidic derivates**: Cys-LT- LTC4, LTD4, LTE4, PGD2, PGF2, F2- isoprostane, 8-hydroxyguanosine

IL-interleukin, G-CSF- Granulocyte colony-stimulating factor, RANTES- regulated on activation, normal T cell expressed and secreted, NK- natural killer, HMGB1- high mobility group box type 1, Cys-LT- Cysteinyl leukotrienes, NO- nitric oxide, LT- leucotrien, PG- prostaglandin, TX-thromboxan, IgE- imunglobulin E, YKL-40- Chitinase-3-like protein 1, hs-CRP-highly sensitive C reactive protein,TLR-Toll-like receptor, MAMP- microbe-associated molecular pattern, DAMPs -Damage-associated molecular patterns

Biomarkers that assess globally the inflammation

Highly sensitive "C" reactive protein is elevated in patients with asthma, especially allergic asthma, COPD, cystic fibrosis highly related with stage and severity of the disease. Higher levels are associated with the decline of pulmonary function. [2,12,19]

Recent studies attest that there is a significant positive correlation between the hs-CRP values and the exacerbation rate, that the level of hs-CRP is significantly higher during exacerbations than in stable asthmatics, and the treatment with inhaled corticosteroids may reduce hs-CRP values. [18]

The association with other conditions that induce low grade systemic inflammation, such as anemia, obesity or malnutrition interferes with hs-CRP values. [19]

Hs – CRP levels are elevated for the pre-term infants with bronchopulmonary dysplasia and Ureaplasma spp. colonization. [21]

Biomarkers related with a certain subtype of disease, endotype or particular condition

Cytokines are small, soluble, molecules responsible for intercellular communication. In airway inflammatory diseases, cytokines are produced by both residents cells as well as the inflammatory cells recruited at the inflammation site. They have an important role in maintenance and prolongation of inflammatory process.[3]

Table 2. ***Types of interleukines and their connection with airway inflammation [3,15]***

Type of interleukin	Origin	Effects related with airway inflammation	Asthma COPD	Cystic fibrosis	Broncho-pulmonary dysplasia
IL-1α,β	Macrophages, monocytes, lymphocites, neutrophils, fibroblasts	Induction of proinflammatory proteins, Th17 differentiation	√	√	√
IL-2	CD4, CD8 activated cells, mast cells, NK	Proliferation of T and B cells, NK cells	√		√
IL-3	Macrophages, mast cells, T cells, NK eosinophils	Activatory for eosinophils and basophils, differentiation of dendritic cells	√		√
IL-4	Basophils, eosinophils, mast cells, NK cells,Th2 cells	Differentiation of Th2 Inflammatory and tissue adhesion, IgE synthesis, eosinophil recruitment	√		
IL-5	Eosinophils, mast cells, NK cells,Th2 cells	Chemotactic for eosinophils, remodelling	√		
IL-6	Mast cells, eosinophils, Endothelial cells, fibroblasts, macrophages, monocytes	Induction of acute phase reactants T and B diffetentiation	√		
IL-7	Epithelial cells, dendritic cells, monocytes, macrophages, B cells	Induction of proliferation of pre-B cells			

Il-8	Monocytes, eosinophils, lymphocites, neutrophils	Chemoattractant for neutrophils, basophils, NK cells, T cells		√	
IL-9	Eosinophils, mast cells, Th2,Th9,Th17	Growth factor for T cells and mast cells Inhibition Th1 cytokines Stimulatory for mucus production	√		
IL-10	Dendritic cells, macrophages, T and B cells	Imunosuppressive effect	√		√
IL-11	Endothelial cells, epithelial cells, fibroblasts	Induction of acute phase proteins, chronic remodeling	√		
IL-12	Dendritic cells, neutrophils, macrophages, B cells	Development of Th1 Activatory for NK cells			
IL-13	Basophils, eosinophils, mast cells, T cells	Activator and recruitment for eosinophils	√		
IL-15	Macrophages, monocytes, T cells	T cell activation Th2 differentiation	√		
IL-16	Macrophages, monocytes, T cells	Modulatory for T cells responses	√		
IL-17A	Th17, NK cells	Induction of proinflammatory cytokines	√		√
Il-17B	Mucosal epithelial cells, lung cells	Induction of proinflammatory cytokines	√		√
IL-18	Macrophages, epithelial cells, dendritic cells	Promoting Th1, Th2 cell responses IFN γ induction	√		
IL-21	Th9, Th17, NK cells	Stimulatory for B cells proliferation			
IL-22	Th17, NK cells	Tissue remodeling			
IL-23	Macrophages, dendritic cells	Stimulatory for production of IL-17 Proliferation T cells, NK cells			
IL-25	Eosinophils, Th2	Induction Th2 Inhibition for Th1, Th17 Stimulatory for IgE production	√		
Il-27	Dendritic cells, epithelial cells, macrophages	Differentiation of Th1 Inhibition of Th17	√		
IL-28	Dendritic cells	Downregulation Th2, upregulation Th1	√		
IL-31	Monocytes, mast cells	Eosinophils production of IL-6, Il-8	√		
Il-33	Epithelial cells, fibroblasts	Induction Th2 inflammation Proinflammatory cytokines release	√		
IL-36					
IFN α, β	Dendritic cells, nucleated cells	Activation T cells	√		
IFN γ	Macrophages, NK cells, T and B cells	Th 1 differentiation			√
TGF-β	Various cells	Balance of proinflammatory and antiinflammatory actions Imuntolerance			
TNF-α	Macrophages, monocites, T and B cells, mast cells	Both proinflammatory (strong host defense) and antiinflammtory actions (protection against autoumunity)		√	√

Asthma

The immune response can be divided in type 1 and type 2 and has in the center the CD4+ T- T helper 1 (Th1) and T helper 2 (Th2) cell. Th1 cells produce IL-2 and IFNγ being responsible by the type 1 immune response, and Th2 cells secrete IL-4, IL-5 and IL-13, responsible for type 2 immunity. Eosinophilia and high antibody titres characterize type 2 immunity. [10]

In asthmatic inflammation there is a Th1/Th2 imbalance, predominantly Th2, and probably a relative Th1 cytokine production deficit, which will direct the immune response by proinflammatory cytokines that stimulate the synthesis of IgE and eosinophils (IL-5). [14,30]

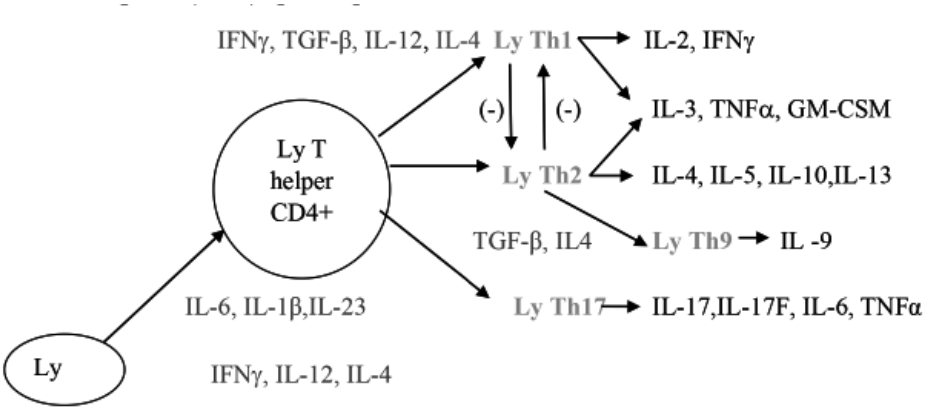

Figure 1. Lymphocite T polarization: type 1 and type 2 immune responses

Therefore, asthma biomarkers can be divided into biomarkers related with type 2 asthma and eosinophilic inflammation and non-type 2 asthma.

Biomarkers related with type 2 asthma:

- *Eosinophils* continue to be a valuable biomarker for asthma as a risk factor, severity marker and as an indicator for disease control. Eosinophils can be determined in both sputum and blood. The controller medication, especially ICS, can be assessed by evaluating the sputum and blood eosinophilia- persistence of peripheral blood eosinophils indicating that anti-IL-5 therapy can be effective. The relation with asthma exacerbations is not yet totally defined, the sputum eosinophilia appears to be more predictable for exacerbation, comparing to blood eosinophilia. [5] Some surface biomarkers have been directly correlated with severe asthma: CD13, CD25, CD29,CD32, CD44 and eosinophilic TGFβ expression.[28]
- *Interleukins* such as IL-4, IL-5, IL-10, IL-13 have high levels in sputum and serum. In a recent research, Agache I. and colleagues proposed a combination of biomarkers- IL-5, IL-13 as "best predictor for blood eosinophilia in adult asthmatics".[1]
- *Periostin* – protein encoded by a gene induced by IL-4 and IL-13 is a marker of type 2 immune response and therefore can be used as a biomarker for Th2 asthmatic phenotypes along with IL-13. High levels of periostin were found in bronchoalveolar lavage fluid, having as a source the epithelial cells of the airways. [5] It is involved in the amplification and extension of chronic inflammation, recruitment and tissue infiltration by eosinophils. [15,28]The limitations in the use of periostin are related to the other sources such as osteoblasts and tumors, nevertheless it can be considered as a surrogate marker not only for type 2 immunity, but also for the assessment of of treatments targeting IgE and IL-13. [5,15]
- *Dipeptidyl peptidase 4* (DDP-4, adenosine deaminase complexing protein 2, CD26) is a glycoprotein with a molecular weight of 110 kDa encoded by DPP4 gene induced by IL-13. DPP4 is composed of 766 amino acid residues, and is found in both structural cells in their membrane and as a soluble protein in the blood. [27] Although the role in asthma is not yet completely elucidated, it seems to have a stimulating role in proliferation of bronchial smooth muscle cells and lung fibroblasts. [15] DPP-4 regulates immunological pathways in asthma. There is evidence that DDP-4 can be used as a surrogate marker for aspirin-exacerbated asthma. [15,26] Recent studies show that increased serum DPP-4 levels predicted a beneficial response to tralokinumab (anti-IL-13 monoclonal antibody). [7]
- *Osteopontin* – cytokine having the extracellular matrix, most the immune system cells and structural cells as source is found to be elevated in both serum and bronchoalveolar lavage fluid in asthmatic patients. This molecule is upregulated in the tissue of the bronchi of asthmatics, being related to basal membrane thickness. [28]
- *VEGF*- vascular endothelial growth factor – has elevated expression in the airways of asthmatic patients, responsible for the reduction of the caliber and has high levels in sputum. [28]
- *HMGB1* – high mobility group box type 1-group of proteins from the alarmins family, a highly conserved DNA-binding protein, is one important damage-associated molecular pattern (DAMP), acting as a "danger signal", responsible for both the initiation and perpetuance of the immune responses. HMGB1 is produced by dendritic cells, NK, macrophages and released by nectrotic cells. [16] HMGB1 interacts with DNA and histones to determine chromatin structure and regulate key processes such as transcription [17], upregulates the synthesis of inflammatory cytokines, chemotaxis of inflammatory cells, being associated with acute and chronic inflammation, immune and inflammatory diseases, such as rheumatoid arthritis, myositis, scleroderma, systemic lupus erythematosus, Sjogren syndrome or ankylosing spondylitis. [17]. HMGB1 is implicated in the pathogenesis of asthma,

inducing peribronchial collagen deposition. [9] Recent studies show that HMGB1 positively related to total serum IgE in children with asthma. [16]

Biomarkers correlated with non type 2 asthma:

– *Cellular biomarkers* are neutrophils and paucigranulocytic pattern. [5]

– *Interleukins* – IL-8, IL-12, IL-17, IL-18, IL-32 are associated with non type2 asthma. Among them, IL-17 have prognostic value for asthma severity. [1] IL-17 is produced by activated CD4+ T cells – the Th17 cell lineage, along with IL-6, IL-17F, IL-22, αTNF. IL-17 and IL-17F can induce lung structural cells to secrete proinflammatory cytokines. IL-17 expression is increased in the lung, sputum, bronchoalveolar lavage fluid (BALF), and serum of asthmatics. IL-17 has an important therapeutic role- the presence of Th17 cells may be responsible for the steroid insensitivity due to uncontrolled IL-17 inflammation induced by progressive doses of steroids. Recent studies show that a IL-17A contributes to airway fibrosis in asthma due to profibrotic cytokines, proangiogenic factors, and collagen production and also IL-17, promote airway smooth muscle cell proliferation and migration. [6]

Cystic fibrosis (CF)

Cystic fibrosis is a recessive genetic exocrine disorder caused by mutations affecting the cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation is the deletion of a phenylalanine at position 508 on chromosome 7, but there are over 2000 CFTR mutations.

In cystic fibrosis the biomarkers follow more than one direction: biomarkers of infection, biomarkers if inflammation, biomarkers for therapy assessment. [22]

Biomarkers of inflammation in cystic fibrosis demonstrate the neutrophilic inflammation which is the hallmark of the disease:

– *Neutrophils* accumulate in the airways of cystic fibrosis patients, playing a key role in the pathogenesis of the disease. The excess of neutrophils drive a normal and salutary immune process, ment to defend the host, towards a pathological one. Besides the neutrophils accumulated, a lot of neutrophic products IL-8, LTB4, C5a, IL-17 are found at the inflammatory site which contribute to the chemotaxis of new neutrophils. The clearance of these cells is defective in cystic fibrosis. Among the neutrophilic products, serine proteases: elastase, cathepsin G, and proteinase-3, have an important role. The proteases released at high concentrations in the airway overload natural defense systems, induce the histological changes of the airway walls: disrupting tight junctions between epithelial cells, destruction of elastin, bronchiectasis, impaired mucociliary clearance. [23]

– *Cytokines* such as the proinfammatory interleukins: IL-1β, IL-6, IL-8, IL-17, IL-33 have high concentrations in the airways of patients with cystic fibrosis, being responsible for the attraction of neutrophils at the site of inflammation. They stimulate acute phase responses, induce cachexia and muscle catabolism. On the other hand, in cystic fibrosis, the airways lack some counter-regulatory molecules, such as IL-10 and nitric oxide. All these factors contribute to the status of chronic inflammation present in the disease. [23]

– *Neutrophil elastase* (NE) – a very valuable sputum biomarker capable to monitor CF lung disease-correlating with bronchiectasis, with the ability to predict the lung function decline. It is related to pulmonary exacerbations, predicting time to next exacerbation and can offer data relating the response to the treatment. Other sputum biomarkers found in CF are calprotectin, myeloperoxidase, high-mobility group box 1 (HMGB-1) and YKL-40. [22] *High mobility group box 1* (HMGB1) levels in sputum may predict future exacerbation. Recent studies, – Sagel and colleagues – demonstrated a strong association between sputum *neutrophil elastase levels* and the high velocity in the decline of the lung function. [24]

– *Systemic inflammatory biomarkers* are serum C reactive protein (CRP), serum amyloid A (SAA) and calprotectin, neutrophil elastase antiprotease complexes (NEAPC), IL-6. *CRP* brings no valuable information on the severity of exacerbation when compared to clinical scoring. *IL-8* and neutrophil elastase antiprotease complexes at the onset of exacerbation were associated with increased response to therapy, *calprotectin* is a superior biomarker for exacerbation than CRP. Serum calprotectin levels are significantly associated with pulmonary exacerbations and lung function decline in patients with cystic fibrosis [13,20,22,24]

Bronchopulmonary dysplasia

The development of the lung takes place in several stages: embryonic, pseudoglandular, canalicular, saccular and alveolar. Premature birth leads to disruption of lung development in the saccular stage. The final stage in the development is the alveolar and vascular growth, requiring a complex network of signals, involving VEGF (vascular endothelial growth factor) and nitric oxide synthesis from the endothelium. [25]

Genetic and environmental factors act together on immature lung in its development leading to inflammation which is one of the main features of bronchopulmonary dysplasia. [4] Some other factors participate, such as infection, injurious factor associated with mechanical ventilation, hyperoxia along with immune dysregulation. [4]

The immune dysregulation can be proven by decreased expression of CD62L on CD4 T cells, suggestive for their activation, while CD54 has an increased expression. [4]

Biomarkers of bronchopulmonary dysplasia

- Cytokines- oxidation and cellular damage are responsible for the production of pro-inflammatory cytokines, such as *IL-1β, IL-6, IL-8*. These interleukins have high levels in broncho-alveolar lavage fluid, along with macrophages inflammation proteins and monocyte chemoattractant proteins.[4,25]
- VEGF- altered levels of *VEGF* constitutes a risk factor for bronchopulmonary dysplasia. [25]
- *Endostatin* is a molecule derived from collagen, an inhibitor of angiogenesis and an antagonist of VEGF, therefore aggressive for the lung. Studies demonstrated high levels of endostatin in the lung of very low birth weight infants who developd bronchopulmonary dysplasia.[25]
- *Endothelin-1* is a endogenous vasoconstrictor, with pro-inflammatory actions. High levels of endothelin-1 were demonstrated in the lung of infants with bronchopulmonary dysplasia. [25]
- The maternal blood can serve as a source of biomarkers for the infant. High levels of *alpha-fetoprotein*, *human chorionic gonadothrophin* and *unconjugated estriol* are associated with higher risk for bronchopulmonary dysplasia in the newborn [25]

Conclusion

For airway inflammation many biomarkers are useful. Some define the disease as a whole, some are defining for certain aspects or endotypes. There is no ideal biomarker for every disease. The winning combination proposed by many authors appears to be a panel of biomarkers which captures as many aspects as possible for each entity, but is usually difficult to find.

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