I BIOMARCATORI

Marialuisa BOCCHINO

«UOS dedicata allo studio e cura della fibrosi polmonare idiopatica e delle altre interstiziopatie polmonari»

Dipartimento di Medicina Clinica e Chirurgia
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Diagnosis of Idiopathic Pulmonary Fibrosis
An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline

Question 8: Should Patients with Newly Detected ILD of Unknown Cause Who Are Clinically Suspected of Having IPF Undergo Serum Biomarker (MMP-7, SPD, CCL-18, KL-6) Measurement for the Purpose of Diagnosis?

- We recommend NOT measuring serum MMP (matrix metalloproteinase)-7, SPD (surfactant protein D), CCL (chemokine ligand)-18, or KL (Krebs von den Lungen)-6 for the purpose of distinguishing IPF from other ILDs (strong recommendation, very low quality of evidence).

- Samples for serum testing are easily obtained with few complication
- Biomarkers testing is costly and not widely available
- High false-positive and false-negative results rates

Am J Respir Crit Care Med Vol 198, Iss 5, pp e44-e68, Sep 1, 2018
Definition of biomarker

A characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic process, or of pharmacological responses to therapeutic intervention.

Ideally, a biomarker is normal in the absence of disease, dysregulated in disease and normalized with effective treatment.

Also, a biomarker should be easy and widespread measurable in non invasively collected body samples.
Searching for the optimal biomarker in IPF: critical issues

- Low incidence disease (<10 cases/100,000/yr)
- High morbidity and mortality
- Lack of a diagnostic gold standard (*working diagnosis*)
- Clinical heterogeneity
- Comorbidities
- Unmet therapy needs
Why we need a biomarker?

Ameliorate the diagnostic process (early case identification, no/less need of invasive procedures)
Facilitate clinical phenotyping
Discriminate patients according to disease severity and behaviour
Improve accurate disease monitoring
Predict responsiveness to pharmacological therapies
Identify potential therapeutic targets
Stratify patients for clinical trails
Alveolar epithelial injury due to an initial stimulus

**Old Paradigm of IPF**
- **Step 1:** Initial stimulus
- **Step 2:** Recruitment and Activation of Immune Cells causes injury and tissue damage
- **Step 3:** Fibroblast activation and ECM accumulation

**New Paradigm of IPF**
- **Step 2:** Fibroblast activation and ECM accumulation
- **Step 3:** Recruitment and activation of immune cells modulates existing fibrotic response
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Diagnosis of IPF/disease susceptibility</th>
<th>Differential diagnosis from other ILDs</th>
<th>Disease prognosis (progression/mortality)</th>
<th>Treatment response</th>
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<tbody>
<tr>
<td>Associated with alveolar epithelial cell dysfunction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL-6</td>
<td>+</td>
<td>-</td>
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</tr>
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<td>SP-A</td>
<td>+</td>
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<tr>
<td>SP-A genetic variants (SFTPA2)</td>
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<td>SP-C genetic variants (SFTPC)</td>
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<td>+ as part of a biomarker index</td>
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<td>CA 19-9</td>
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<td>Associated with ECM remodeling and fibroproliferation</td>
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<td>MMP-7</td>
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<td>Fibrocytes</td>
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<td>Periostin</td>
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<tr>
<td>Osteopontin</td>
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<tr>
<td>CCL-18</td>
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<td>YKL-40</td>
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<td>S100A12</td>
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<td>Anti-HSP70</td>
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<td>α-Defensins</td>
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<td>Anti-vimentin Abs</td>
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<td>CD4 + CD28+</td>
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<td>Tregs</td>
<td>+</td>
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<tr>
<td>Microbiome</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>miRNA</td>
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<tr>
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## Multiple biomarker signature

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Use</th>
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<tr>
<td>SP-D, MMP-7, Osteopontin</td>
<td>diagnostic accuracy (IFS vs ILDs other than IPF)</td>
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<tr>
<td>MMP-7, MMP-1, MMP-8, IGFBP-1, TNFRSF1A</td>
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<td>KL-6, CCL18, ICAM1, SP-D, SP-A, MMP-7, HE-4, prostatin</td>
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<td>Degradation products of ECM</td>
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<td>miRNA-302, miRNA-423, miRNA-210, miRNA-376C, miRNA-185</td>
<td>disease phenotyping and behaviour (slow vs fast)</td>
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<td>SP-D, CA19.9, CA125</td>
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<tr>
<td>Degradation products of ECM</td>
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<tr>
<td>KL-6, SP-D</td>
<td>prognostic accuracy (progression and mortality)</td>
</tr>
<tr>
<td>SP-A, SP-D</td>
<td></td>
</tr>
<tr>
<td>Gender, FVC, DLCO, MMP-7</td>
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</tr>
<tr>
<td>MMP-7, SP-A, KL-6, FVC, DLCO, age, ΔFVC₆ₘ</td>
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<tr>
<td>Degradation products of ECM</td>
<td></td>
</tr>
<tr>
<td>52-gene signature (Scoring Algorithm for Molecular Subphenotypes)</td>
<td></td>
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</table>
Oxidative stress-linked biomarkers in idiopathic pulmonary fibrosis: a systematic review and meta-analysis

### Executive summary

**Idiopathic pulmonary fibrosis & oxidative stress**
- The idiopathic pulmonary fibrosis (IPF) is characterized by increased systemic oxidative stress (OS).
- We conducted, for the first time, a systematic review and meta-analysis of studies investigating the relationship between the OS biomarkers and presence of IPF.

**Studies selected**
- Fifteen studies were included in the meta-analysis, involving 293 IPF patients (191 males and 102 females, mean age 48.1 ± 24.3 years) and 234 healthy controls (149 males and 85 females, mean age 62.9 ± 8.7 years).
- Two studies evaluated thiobarbituric acid reactive substances, hydroperoxides and isoprostanes in blood, two isoprostanes in expired breath condensate, three glutathione in epithelial lining fluid and four protein carbonyls in bronchoalveolar lavage fluid.

**Results**
- Pooled systemic hydroperoxides and thiobarbituric acid reactive substances concentrations were significantly higher in IPF patients when compared with controls.
- A significant decrease in epithelial lining fluid-glutathione concentrations was observed in IPF patients compared with controls.
- Bronchoalveolar lavage fluid carbonyl proteins concentrations were significantly higher in IPF than in controls.
- Isoprostane expired breath condensate levels were significantly higher in IPF than in controls.

**Conclusion**
- This meta-analysis demonstrated a significant reduction in antioxidant markers and a consistent increase in the concentrations of OS markers in IPF, independent of the biological sample examined.

*Biomark. Med.* (Epub ahead of print)
Breath biomarkers in idiopathic pulmonary fibrosis: a systematic review

Table 2 Biomarkers reported to discriminate between IPF patients and healthy controls. Direction of discrimination and reported p-value. a) C₅₋₇-NO, b) FeNO₅₋₇/FeNO₁₂₋₁₅/FeNO₁₅₋₂₀/C₅₋₇-NO

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sample Medium</th>
<th>Discrimination</th>
<th>p-value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric Oxide</td>
<td>Exhaled breath</td>
<td>Higher in IPF</td>
<td>0.0001, &lt; 0.001</td>
<td>[62]³, [63]³</td>
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<td>8-isoprostane</td>
<td>EBC</td>
<td>Higher in IPF</td>
<td>0.02, &lt; 0.05</td>
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<td>Hydrogen Peroxide</td>
<td>EBC</td>
<td>Higher in IPF</td>
<td>0.003</td>
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</tr>
<tr>
<td>Nickel</td>
<td>EBC</td>
<td>Higher in IPF</td>
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<td>[58]</td>
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<td>Silicon</td>
<td>EBC</td>
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<td>Cobalt</td>
<td>EBC</td>
<td>Lower in IPF</td>
<td>&lt; 0.05</td>
<td>[58]</td>
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<tr>
<td>Iron</td>
<td>EBC</td>
<td>Lower in IPF</td>
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<td>[60]</td>
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<td>Copper</td>
<td>EBC</td>
<td>Lower in IPF</td>
<td>&lt; 0.05</td>
<td>[58]</td>
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<tr>
<td>Selenium</td>
<td>EBC</td>
<td>Lower in IPF</td>
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<td>[60]</td>
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<td>Molybdenum</td>
<td>EBC</td>
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<td>&lt; 0.05</td>
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<td>EBC</td>
<td>Higher in IPF</td>
<td>&lt; 0.01</td>
<td>[60]</td>
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<td>Nitrate</td>
<td>EBC</td>
<td>Lower in IPF</td>
<td>&lt; 0.01</td>
<td>[60]</td>
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<tr>
<td>2,2,4 LPA</td>
<td>EBC</td>
<td>Higher in IPF</td>
<td>0.001</td>
<td>[63]</td>
</tr>
<tr>
<td>Unidentifiable metabolite</td>
<td>EBC</td>
<td>Higher in IPF</td>
<td>0.01</td>
<td>[64]</td>
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<tr>
<td>p-cymene</td>
<td>Exhaled breath</td>
<td>Lower in IPF</td>
<td>&lt; 0.001</td>
<td>[66]</td>
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<td>Acetoin</td>
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<td>&lt; 0.001</td>
<td>[66]</td>
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<tr>
<td>Isoprene</td>
<td>Exhaled breath</td>
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<td>&lt; 0.001</td>
<td>[66]</td>
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<td>Ethylbenzene</td>
<td>Exhaled breath</td>
<td>Higher in IPF</td>
<td>&lt; 0.001</td>
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<tr>
<td>Unidentified VOC</td>
<td>Exhaled breath</td>
<td>Higher in IPF</td>
<td>&lt; 0.001</td>
<td>[66]</td>
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</tbody>
</table>

IPF: idiopathic pulmonary fibrosis, EBC: exhaled breath condensate, 2,2,4 LPA: Docosatetraenyl hypophosphatidic acid, VOC: volatile organic compound, C₅₋₇-NO: alveolar nitric oxide concentration, FeNO: fractionated exhaled nitric oxide at 50 ml/100 ml/150 ml per second.

Respiratory Research
Serum amyloid A: A potential biomarker of lung disorders
Peripheral frequencies of CD1c⁺ dendritic cells and serum levels of interleukin-6 are prognostic biomarkers in idiopathic pulmonary fibrosis patients.

Circulating DC subsets, peripheral levels of oxidative stress and serum concentrations of IL-6 are not modulated by currently used anti-fibrotic drugs.

M Bocchino, et al., submitted manuscript
**Homeostasis**

**Low biomass, high diversity**

**Gut-lung axis**
- Dysregulated bacterial killing
- IL-17 production
- Translocation?

**High biomass, high diversity**

**Disease Exacerbation**

**Trigger**
- Viral infection, aspiration, allergic response

**Change in microbial growth conditions**

**Dysbiosis**
- Change in community structure

**Altered immunity**

**Antibiotic use**

**IPF Microbiome**
- Increased bacterial burden
- Potential pathogens present

**Increased microaspiration from GERD**
- Activated innate host response

**Impaired mucociliary clearance**

**Progressive scar formation**

**KEY POINTS**

- Advances in molecular sequencing technology in the last decade have allowed study of the role of the microbiome in health and disease.

- The lung contains a dynamic community of microbes in health, and patients with interstitial lung disease may have systematic derangements in bacterial community composition.

- Existing evidence suggests that knowledge of lung microbiome composition in IPF may serve as a prognostic biomarker, a therapeutic target, or provide an explanation for disease pathogenesis.
Unanswered questions regarding the immune and inflammatory cells in idiopathic pulmonary fibrosis (IPF).

- To what extent do data obtained from mouse models reflect the situation in the fibrotic human lung? Can mimetics be developed that more accurately simulate the IPF disease state?
- Do events in the peripheral blood truly reflect events occurring in the diseased lung?
- Do the innate immune abnormalities seen in IPF represent a unique form of immunosenescence?
- Can therapies targeting macrophage activation stabilize or restore lung function in patients with IPF?
- Does the altered microbiome cause pathogen-associated molecular pattern-driven innate immune activation in IPF and are antimicrobial therapies efficacious in IPF?
- Does perpetuated microinjury cause danger-associated molecular pattern (DAMP)-driven innate immune activation in IPF and are therapies targeting DAMPs and their receptors efficacious in IPF?
- Are neutrophil extracellular traps an important part of IPF pathogenesis?
- What is the role of fibrocytes and myeloid-derived suppressor cells in IPF?
- Do innate lymphoid cells participate in IPF?
- How does the relative balance of T-helper cells participate in IPF and can this contribution be targeted in a safe and efficacious manner?
- Are B cells involved in the development of IPF?
- Can immune events detected in the circulation be used to guide personalized therapies in IPF?
Is IPF a genetic disease?
Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive

Chad A. Newton¹,², Kiran Batra³, Jose Torrealba⁴, Julia Kozlitina⁵, Craig S. Glazer⁶, Carlos Aravena⁷, Keith Meyer⁸, Ganesh Raghu⁹, Harold R. Collard⁵, and Christine Kim Garcia¹,²

A

IPF  Chronic HP  PPFE  NSIP

B

Family members with variant HPF mutation

Family members with variant TOP1 mutations

Chronic hypersensitivity pneumonitis

Eur Respir J, 2016
The ORs for heterozygous and homozygous individuals are 6.8 and 20.8 for FIP and 9.0 and 21.8 for sporadic IPF, respectively.

**MUC5B promoter polymorphism (rs 35705950):**

- seems to be specific to IIPs
- is associated with radiographic evidence of ILA (Framingham cohort) and their progression (age and copy number)
- is associated with the CT *UIP pattern* in the setting of fibrotic IIPs
rs3750920 (TT and CC genotype)
WARNING!

At this time there are no clinical guidelines suggesting genetic testing in the routine care and counseling of IPF patients
Genetic testing in FIP/IPF: a proposed flowchart

Kropski JA et al., Am J Respir Crit Care Med, 2017
Epigenetics: a new frontier in IPF
Exosomal miRNAs in Lung Diseases: From Biologic Function to Therapeutic Targets

Julien Guiot, Ingrid Struman, Edouard Louis, Renaud Louis, Michel Malaise, and Makon-Sébastien Njock

<table>
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<tr>
<th>Characteristics</th>
<th>Exosomes</th>
<th>MVs</th>
<th>ABs</th>
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<td>Budding and scission of plasma membrane</td>
<td>Cell fragmentation /blebbing</td>
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</tbody>
</table>

References: [9-12], [9,13,16,17], [9,14,15]

Abbreviations: ABs, apoptotic bodies; MVs, microvesicles; MVBs, multivesicular bodies.
<table>
<thead>
<tr>
<th>Lung Diseases</th>
<th>Biofluids</th>
<th>EVs</th>
<th>miRNAs</th>
<th>Expression in Lung Disease (vs. Controls)</th>
<th>References</th>
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<td><strong>COPD</strong></td>
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<td>Circulating miRNAs</td>
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<td>Exosomes</td>
<td>miR-223-3p, miR-223-5p, miR-338-3p, miR-1469, miR-204-5p, miR-618</td>
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<td>let-7d, miR-191, miR-126, miR-125a</td>
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<td>Let-7a, miR-24, miR-26a, miR-99a, miR-200c</td>
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<td><strong>IPF</strong></td>
<td>Sputum</td>
<td>Exosomes</td>
<td>miR-142-3p, miR-33a-5p, Let-7d-3p</td>
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</table>

Abbreviations: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; EVs, extracellular vesicles; IPF, idiopathic pulmonary fibrosis; miRNAs, microRNAs; MVs, microvesicles.
Liquid biopsy, that allows the isolation of circulating cell-free (ccf) DNA from blood, is an emerging non-invasive and convenient tool for cancer biomarker discovery. Specifically, ccfDNA in patient-derived plasma/serum samples contains DNA fragments released from the tumor cells that carry both genetic and epigenetic information.

Recent ccfDNA-based studies have provided promising results with potential clinical application in early cancer detection, diagnosis and prognosis.

In 2010, Casoni et al. first suggested that serum ccfDNA may help discriminate patients affected by idiopathic pulmonary fibrosis (IPF) from those with other interstitial lung diseases.

M. Bocchino, et al., ERS International Congress 2019, Madrid manuscript in preparation
The «biomarker pipeline» in IPF: conclusions and perspectives

Evidence for the definite use of a specific biomarker in real life clinical practice is inconclusive

Critical issues include single center studies, retrospective design, small number of patients, heterogeneity of sample collection and analytic procedures

Better understanding of disease pathogenesis and disease behaviour

Identification of new players and drug targets

Standardization of sample collection and analytical procedures

Need of prospective longitudinal studies (combination with clinical parameters and genotyping)

Best assessment of timing and benefit of therapies

Personalized medicine in IPF