PNEUMOLAB PROCEEDINGS
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Asthma is a chronic inflammatory disorder of the airways characterized by airway hyperresponsiveness and airflow limitation. Despite respiratory symptoms may be episodic, progressive changes occur in the structure of the airway, leading to its irreversible remodeling. Changes include mucus hypersecretion, injury to epithelial cells, smooth muscle hypertrophy, sub-basement membrane fibrosis and angiogenesis. The risk factors for developing asthma are a combination of genetic predisposition along with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways, such as in- and out-door allergens, tobacco smoke, chemical irritants in the workplace and air pollution. Asthma is a clinically heterogeneous entity due to the complexity of its pathogenetic substrate. Recent evidence suggests asthma to be associated with a sort of immunodeficiency accounting for an increased susceptibility to infection in asthmatic patients. The role of infections as triggers and promoters of disease progression is well established. Conversely, the impact of asthma as a predisposing condition to infection has not clearly been addressed. Such a topic will be the focus of the present review.

**KEY WORDS:** Asthma - Infection - Immunologic deficiency syndromes - Prevention and control - Etiology.

The World Health Organization (WHO) estimates that more than 200 million people are currently suffering from asthma, which is the most common non-communicable disease among children. The exact causes underlying asthma have not yet been fully elucidated. The strongest risk factors for developing asthma are represented by a combination of genetic predisposition to atopy and exposure to inhaled substances and particles that may provoke allergic reactions or airway irritation; these include in- and out-door allergens, tobacco smoke, chemical irritants in the workplace and air pollution. Asthma is a clinically heterogeneous entity owing to the high complexity of its pathophysiology. Asthma is widely recognized as a chronic inflammatory disease of the airways, whose initiation and progression may be triggered by a series of environmental factors, including infections. Regarding this issue, some debate has re-

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Susceptibility of asthma patients to infectious diseases

There is evidence that asthma influences patients’ susceptibility to infections. Up to now, research in this field has been limited and identifying asthmatics with frequent/recurrent infections is not currently considered a priority when addressing the different disease phenotypes. It is likely that more than one mechanism contribute to the increased susceptibility to infection in asthma patients; in fact, such condition is not equally represented in all patients. The greater susceptibility of asthmatics to infectious diseases seems to be independent of the entity of their asthma and not related to inhaled corticosteroid therapy. In a population who underwent screening for immunoglobulins, no higher rates of atopic reactions were reported among those with IgA deficiency. On the other hand, increased prevalence of allergy has been observed among several groups of pediatric patients with low IgA levels who mainly attended the hospital with respiratory complaints. The finding that local immune deficiency, i.e. at the mucosal level, may facilitate inflammation, bronchial hyperresponsiveness, and asthma development in patients with a concomitant primary immune defect, clearly indicates the protective role of secretory IgA against asthma and allergy in the early years of life. It has been shown that members of families with a patient affected by common variable immune-deficiency (CVID) have an alteration in humoral immunity, thus suggesting the need of their routine screening.

The hypothesis of a direct mechanism

Different hypotheses for explaining the close relationship occurring between microbial colonization/recurrent infections and atopic diseases/asthma have been proposed. As previously reviewed, compelling evidence suggests that such a bidirectional link encompasses 4 specific hypotheses: the hygiene hypothesis, the counter-hygiene hypothesis, the microbiome hypothesis, and the reverse causality. The hygiene hypothesis suggests that exposure to microbial colonization or infection during early childhood provides a protective effect on the development of atopic conditions; on the opposite side is the counter-hygiene hypothesis, which suggests early childhood microbial colonization/infection (i.e., human rhinovirus infection) to be provocative for the development of atopic conditions later on. The more recent microbiome hypothesis suggests a contextual effect of such exposure on the development of atopic conditions depending on the diversity of the microbiome, while the reverse causality hypothesis argues for a causal role of atopic conditions in increasing susceptibility to microbial colonization or infections.

Respiratory viruses cause 85% of asthma exacerbations, and 62% of all virus-induced asthma exacerbations are caused by human rhinovirus. When polymerase chain reaction (PCR) is used in addition to or instead
The correlation between infectious diseases and asthma leads to the idea that no one single mechanism or single genetic variation is at the origin of the higher susceptibility of asthmatics to infections, but that it is the result of multiple mechanisms, with different genetic or epigenetic basis, and of an individual's exposures and interaction with the environment as well. This would bring the necessity to evaluate if these mechanisms exist in the individual asthmatic and to recognize their interconnections, thus ideally identifying the specific defect in the single patient, through the evaluation of a given individual risk factor.

A series of studies on airway biopsies and primary epithelial cell cultures have established that asthma is primarily an epithelial disease driven by increased susceptibility to environmental stimuli and an altered repair response as depicted by sustained activation of the epithelial mesenchymal trophic unit (EMTu), notably invoked in fetal branching morphogenesis. The activation of the EMTu connects the origins of asthma to its progression over time with involvement of epithelial susceptibility through impaired barrier and innate immune functions and altered mesenchymal susceptibility as exemplified by polymorphisms of the metalloprotease gene, ADAM33. In fact, while the biological activities of ADAM33 are as yet unknown, ADAM33 may play a possible role in airway remodeling because of its high expression in epithelium, myo/fibroblasts, and airway smooth muscle cells and its role in promoting angiogenesis and stimulating cell proliferation and differentiation. Thus, ADAM33 represents a promising target for asthma.

Taken together these observations have led to a fundamental re-evaluation of the pathogenesis of asthma. Rather than considering allergic inflammation as the driving abnormality, it has been proposed that the airway epithelium lies at the center of asthma pathogenesis, and that, in conjunction with the underlying mesenchyme, it is the main orchestrator of both the induction of asthma and its evolution during lifetime. This concept has provided the basis for a
new preventive and therapeutic approach more focused on increasing airways resistance to environmental insults rather than on suppressing downstream inflammation once it is established. In this there is no contrast with the observation that the immune response in atopic and asthmatic patients is greatly influenced by Toll-like receptors (TLRs), belonging to a large family of pattern recognition receptors known as the ancient gatekeepers of the immune system. TLRs are located at the first line of defense against invading pathogens as well as aero-allergens, which makes them an interesting target to modulate the natural history of respiratory allergy. Furthermore, the "lesion" leading to asthma appeared to lie in impaired signaling downstream to microsomal toll-like receptor 3 after binding of viral RNA, thus failing to induce endogenous primary IFNs (step 1), while the step 2 (the IRF-7-dependant induction of the anti-viral response via autacoid activation of the common IFNα/β receptor) appears to remain intact. In a separate transcriptomic study and pathway analysis of circulating mononuclear cells before, during and after asthma exacerbations a distinct systemic innate Type 1 IFN response was observed, together with a weaker B-cell antigen receptor and IL-4 adaptive immune response, as compensation for the defect in mucosal innate immunity (agonists of TLRs have been widely employed in therapeutic or prophylactic preparations useful for asthma/allergic rhinitis patients. Targeting the TLRs can indeed enhance the efficacy of specific allergen immunotherapy, currently the only available curative treatment for respiratory allergies). 

Clinical implications and hygiene hypothesis

Compelling evidence suggests a bidirectional causal relationship between exposure to microbial colonization or infection and risk of atopic conditions, which encompasses 4 specific hypotheses: the hygiene hypothesis, the counter-hygiene hypothesis, the microbiome hypothesis, and reverse causality. The hygiene hypothesis suggests exposure to microbial colonization or infection during early childhood provides a protective effect on the development of atopic conditions, whereas the counter-hygiene hypothesis suggests a provocative effect of exposure to microbial infection during early childhood on the development of atopic conditions (e.g., human rhinovirus infection). The recent microbiome hypothesis suggests a contextual effect of such exposure on the development of atopic conditions depending on the diversity of the microbiome. Although these hypotheses address a causal direction for the influence of exposure to microbial organisms on the development of atopic conditions, the reverse causality hypothesis argues for a causal direction that atopic conditions alter susceptibility to microbial colonization or infections. Overall, harnessing current mechanistic studies for translational research on microbiota composition and function in relation to atopy have potential for the design of therapeutics that could moderate these diseases. Thus new perspectives arise in terms of disease understanding and management, because all stages of microbial infection, from colonization to severe invasive disease, are likely to occur in atopic patients.

Besides the well-known association between asthma and viral infections, asthma patients also have a significantly increased risk of bacterial infection, invasive pneumococcal diseases and pneumococcal pneumonia. Similarly, higher rates of upper respiratory infection by Streptococcus pyogenes along with colonization by Staphylococcus aureus have been reported. Infection by Gram-negative bacteria is also an issue of concern, on which we still have few available data along with an increased susceptibility to infection by intracellular pathogens, like Mycoplasma pneumoniae and Chlamydia pneumoniae. Non-respiratory infections have been described as well. Different mechanisms, including innate (as previously discussed) and adaptive immune incompetence (that is, a
Asthma is associated with increased susceptibility to infection. Patients with a clinical history of recurrent infections, either respiratory or not, may improve counseling through more precise answers to patients' needs and doubts. In addition, the need that both clinicians and patients take into consideration and meet the routine vaccination guidelines also represents a topic of concern. Such an effort may significantly contribute to increase the rate of influenza and pneumococcal vaccination among atopic patients. To date, vaccination with PPV23 should be offered in all instances, regardless of asthma severity or poor therapy control, in consideration of the increased infection susceptibility even in patients with mild disease. Conversely, type-2 predominant immune environment, sub-optimal IgG responses), along with both genetic and epigenetic abnormalities and environmental factors, may account for this association, as recently reviewed by Young. Altogether, these features contribute to the definition of a more appropriate epidemiology and their implications on health-related costs open new perspectives in terms of disease understanding and management. From a clinical point of view (and focusing on asthma patients), searching for risk factors accounting for increased infection susceptibility may help to characterize phenotypes and endotypes needing distinct diagnostic and therapeutic strategies. Therefore, the efficient differentiation of atopic conditions from immunodeficiency in patients with a clinical history of recurrent infections, either respiratory or not, may improve counseling through more precise answers to patients' needs and doubts. In addition, the need that both clinicians and patients take into consideration and meet the routine vaccination guidelines also represents a topic of concern. Such an effort may significantly contribute to increase the rate of influenza and pneumococcal vaccination among atopic patients. To date, vaccination with PPV23 should be offered in all instances, regardless of asthma severity or poor therapy control, in consideration of the increased infection susceptibility even in patients with mild disease. Conversely,

![Flowchart of Diagnostic Approach](image)

**Flowchart of Diagnostic Approach**

- **Asthma without Infections**
  - **Asthma with Infections**
    - **Atopy Hyperactivity**
      - Exclude alternative diagnosis: aspiration syndromes including foreign body dysfunctions, swallowing congenital abnormalities chronic lung disease of prematurity congenital lung malformation congenital heart disease
    - **Viral infection**
      - Exclude alternative diagnosis (e.g., pulmonary tuberculosis, suppurative lung disease).
    - Immunodeficiency

**to characterize phenotypes and endotypes needing distinct diagnostic and therapeutic strategies**

**Asthma Endotypes**

- **Endotype 1 Allergen**
  - Treatment: Inhaled GCS, LABA, anti-LTs, anti-ige
- **Endotype 2 Severe eosinophilic non-allergic**
  - Treatment: Inhaled GCS, LABA, oral GCS
- **Endotype 3 Aspirin sensitive**
  - Treatment: Inhaled GCS, LABA, anti-LTs, intense treatment often required
- **Endotype 4 Bronchopulmonary mycosis**
  - Treatment: Inhaled GCS, LABA, anti-ige
- **Endotype 5 Endotypes not yet identified or rare**
  - Treatment: conventional or new approaches

*Modified by Lötvall et al., JACI 2011*
both the risks and cost-benefits ratio of antibiotic therapy and/or prophylaxis need to be further evaluated as at present there are no clear recommendations for their use in this setting. Future research effort should be focused on the identification of individual risk factors accounting for infection susceptibility, in order to better understand mechanisms that link them to inflammation. This is a crucial point, as such knowledge would also help addressing why some atopic patients recover from infection while others do not. In addition to its biological meaning and its possible contribution to characterize the pathogenesis of asthma, acquisition of this information may significantly challenge current clinical management strategies for patients, thanks to the identification of new predictive variables and outcome measures. Similarly, the development of new target-specific therapeutic approaches may be pursued as well. Finally, epidemiology studies are also requested. It is widely accepted that viruses such as syncytial virus and human rhinovirus are actively involved in asthma development and disease progression. Conversely, up to now the influence of any atopic condition, and in particular of asthma, on the microbial infection trend in a given geographic setting has not been investigated. Reports on health-related costs and quality of life should also be generated.

The development of a disease management flow-chart accounting for the infection risk stratification of patients may represent a valid clinical approach for improving asthma phenotype characterization. An example of a similar step-by-step diagnostic algorithm is reported in Figure 1. Even though more studies are needed in the field, in our opinion it may offer a provisional guide to tailor both preventive and therapy strategies.

Conclusions

The role of infections in the initiation and progression of asthma is well characterized. Conversely, the impact of asthma on infection susceptibility has only recently been arisen as a concern and needs more research to be fully addressed. Due to the dual relationship between these two conditions, assessment of the infection risk (low vs. high) should be included in the diagnostic algorithm of patients affected by allergic diseases, including asthma, as it may have a significant clinical impact in terms of both prevention and treatment. Indeed, improving our knowledge in this field may offer the opportunity to re-modulate the management of these patients through a more comprehensive approach aimed at deciphering the close cross-talk between individual susceptibility and environment.

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Emphysema: coiling up the lungs, trick or treat?

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Lung volume reduction coil (LVRC) treatment is a minimally-invasive technique planned to achieve an improvement of exercise capacity and pulmonary function in subjects with advanced emphysema and hyperinflation. It has been proposed together with other bronchoscopic lung volume reduction approaches to reduce lung hyperinflation in emphysema as less invasive alternatives to LVRS and are currently under clinical investigation. Following the successful early experiences in previous pilot trials, recent studies allow further investigation into the feasibility, safety and efficacy of LVR coil treatment in a multi-center setting in a larger group of patients. According to this studies we can state that LVR coil treatment results in significant clinical improvements in patients with severe emphysema, in multicenter analysis, with a good safety profile and sustained results for up to 1 year. The literature on endobronchial coils continues to look promising with an acceptable safety profile, and positive long-term follow-up data are certainly more and more available. However, further well-designed, blinded, placebo (or sham) controlled trials, and even randomized trials against LVRS (lung volume reduction surgery), are needed before routine clinical use can be recommended. This is true not only for endobronchial coils, but also for the whole field of bronchoscopic lung volume reduction.

**Key Words:** Clinical trials - Emphysema - Pneumonectomy.

Many subjects suffering from chronic obstructive pulmonary disease (COPD) experience worsening of health related quality of life due to debilitating breathlessness and exercise limitation. Such effects are particularly evident in COPD patients with predominant emphysema.

The emphysematous lungs are characterized by tissue damage with reduction of elasticity. As a consequence, the lungs do not expand and recoil efficiently to drive air through the bronchi to the alveoli and back as the patient inhales and exhales.¹

Moreover, the decreased lung elastic recoil in emphysema increases expiratory airflow resistance and leads to dynamic hyperinflation.²

During exercise, dynamic hyperinflation grows rapidly, decreasing chest wall compliance, impairing respiratory muscle func-

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tion, and increasing the work of breathing.3,4

Pharmacological therapy does little to restore these effects of emphysema, even when associated with pulmonary rehabilitation which, improving muscle strength, can increase exercise capacity without changing though pulmonary pathophysiology.

Treatment options beyond conventional medical therapies are limited to a minority of patients.

**Lung volume reduction surgery**

Lung volume reduction surgery (LVRS) has been proposed to attain lung volume reduction, mainly by removing the grossly impaired areas of the emphysematous lung.

The rationale of LVRS is to increase lung elastic recoil and decrease end-expiratory lung volume, thereby improving lung and respiratory muscle mechanics and overall exercise tolerance.5,6 These approaches showed short-term benefit in pulmonary function and dyspnea in highly selected patients7 with increased survival, one of the most important unmet need in COPD patients, but with safety concerns about mortality and morbidity.

More recently, in selected patients, LVRS has become more widely accepted and bilateral LVRS procedures appear to result in greater short-term improvement than unilateral LVRS.8

**Bronchoscopic lung volume reduction**

Nonetheless a number of bronchoscopic lung volume reduction approaches for emphysema have been proposed as less invasive alternatives to LVRS and are currently under clinical investigation. Such treatments include endobronchial one-way valves, aimed at achieving lobar atelectasis through the unidirectional occlusion of the lobar or segmental bronchi. To date, this has been the most extensively investigated technique in this field.

Endobronchial valves appear to have a very acceptable safety profile. However, successful clinical outcomes from valve therapy can only be achieved in patients with no interlobar collateral ventilation and when the one-way valves are placed to entirely block all the airways into the target lobe. This can be technically difficult due to local anatomy and in the absence of significant experience with these devices.9 It is estimated that only about 33% of patients with severe emphysema have no collateral ventilation between the target and adjacent lobe and can thus potentially be treated using one-way valves.10 This clearly shows the need for alternative bronchoscopic treatments that work independently of the presence of collateral ventilation.

Among these lung volume reduction coils (LVRCs) are for sure the most studied non-blocking device.

**Coils**

Following the successful early experiences in previous pilot trials, a recent study allows further investigation into the feasibility, safety and efficacy of LVRC treatment in a multi-centre setting in a larger group of patients. This is the largest LVRC study to date of its publication, and also evaluated longer-term results of LVRC treatment in a multicentre setting.11 The key question here is: “Is LVRC treatment feasible and does it sustainably improve quality of life and clinical outcomes in a broad group of patients with severe emphysema treated in a multicentre setting?”

Endobronchial coils certainly appear to have a very good short-term safety profile.

As in previous trials, safety was evaluated by recording all adverse events, efficacy by the St George’s Respiratory Questionnaire (SGRQ) as primary endpoint, and pulmonary function testing, modified Medical Research Council dyspnoea score (mMRC) and 6-min walk distance (6MWD). The novelty here is that these data were collected up to 12 months after the final treatment.

According to this study we can state that LVRC treatment results in significant clinical improvements in patients with severe emphysema, in a multicenter analysis, with a good safety profile and sustained results for up to 1 year.
Moreover, post-hoc analysis of CT scan heterogeneity showed significant responses in both heterogeneous and homogeneous emphysema, suggesting that, in contrast with results from other similar devices, LVRC treatment may benefit patients with both heterogeneous and homogeneous disease distribution.

State of the art

LVRC treatment is a minimally-invasive technique planned to achieve an improvement of exercise capacity and pulmonary function in subjects with advanced emphysema.

The Coils work by a mechanical action, specifically the compression of diseased lung parenchyma, due to the physical elastic properties of the Nitinol wire of which the coils are made.

The desired effects of coil treatment are elicited by a reduction of lung volume, similarly to what observed with lung volume reduction surgery. Such benefits are related to the improvement of mechanical properties of the remaining tissue, that may expands following the compression produced by the coils. As a consequence, to obtain the such results, the coils require some minimal amount of lung tissue to compress. Differently from one-way valves, collateral ventilation do not interfere with coils treatment outcomes. Nitinol combines strength and memory shape properties with great elasticity, thereby improving tissue strength and elastic recoil, potentially further reducing the dynamic hyperinflation that occurs easily in these patients.

Few studies have explored the role of endobronchial coils in bronchoscopic lung volume reduction of patients with severe COPD. Preliminary experiences mostly focused on the safety profile of this treatment. In the first pilot study, Herth et al. enrolled and treated 11 patients with severe emphysema (GOLD stage 3 or 4) who were followed-up for three months after the last intervention. Ten subjects received a second treatment, in the contralateral lung (6 patients) and in the same lung as the first treatment (4 subjects). The mean number of coils implanted per procedure was five. The primary endpoint was safety, and only in secondary analysis functional data were analyzed.

The procedures resulted well tolerated in all cases. A total of 33 adverse events were registered and none of them were judged as severe. The Authors reported an increase in dyspnoea (6 cases), cough (5 patients), exacerbations of COPD (3 events) and thoracic pain in one subject.

Efficacy data showed meaningful improvements only in patients with heterogeneous emphysema without any significant benefit for subjects with homogeneous emphysema. Slebos et al. firstly focused on the efficacy of the lung volume reduction treatment with coils. Twelve patients with severe heterogeneous emphysema were treated bilaterally in two sequential procedures, while in four subjects coils were implanted in one lung only. In 28 procedures, 260 endobronchial coils (median ten per lung) were placed and none had to be replaced or removed. All the procedures were performed under general anesthesia with an endotracheal tube and flexible bronchoscope under fluoroscopy guidance.

Follow-up data were available at one, three and six months after the final treatment.

Compared with baseline, after six months, the Authors registered a significant improvement in FEV1 (+14.9%), FVC (+13.4%), 6MWT (+84.4 m) with a significant reduction in RV (-11.4%). Quality of life, evaluated with SGRQ, significantly improved (-14.9 points) as well. Bilateral treatment further improved the initial single lung 1-month results.

Furthermore, more than 50% of the patients responded to above the accepted minimal clinical important difference (MCID) for FEV1, 6MWT and SGRQ.

No life-threatening adverse events occurred. The observed complications were represented by one pneumothorax, mild hemoptysis in 12 patients (all resolved spontaneously during the first day) and chest pain...
in four cases. At one to six months follow-up, 16 patients experienced a total of 14 COPD exacerbations.

Only one randomized controlled trial compared the efficacy and safety of bronchoscopic lung volume reduction with coils with the best medical care.17

At three centers in United Kingdom, 47 patients with severe emphysema were randomized 1:1 to either coils treatment or usual care. The primary endpoint was the difference between change in SGRQ from baseline to 90 days after the final procedure, from treatment and usual medical care. Secondary endpoints were changes from baseline of some functional parameters.

In the coils group, 21 of 23 patients completed the planned bilateral treatment.

Notably, 38 (86%) of 44 procedures were done under local anesthesia and conscious sedation (intravenous midazolam and fentanyl) and only six procedures (in three patients) under general anesthesia.

In 23 treatment patients, 410 coils were implanted with a mean procedure time of 44.9 minutes and a mean number of coils per bilaterally treated patient of 18.5.

Efficacy data showed remarkable superiority of bronchoscopic lung volume reduction arm over the medical treatment group.

The Authors registered a greater improvement of SGRQ, 6MWT and FEV1 from baseline in the coils treatment group than in the usual care arm. The reduction of residual volume was significantly greater in patients treated with coils than in the medical care arm.

On the contrary, no between groups difference in change were detected in mMRC dyspnea score and total lung capacity.

Safety data showed no between-arm differences in serious adverse events.

During the initial treatment recovery period (within the initial 30 days), six serious adverse events were reported in the coils group and one in the usual care group. These events comprised exacerbations of COPD, pneumothoraces and lower respiratory tract infections.

During days 30 to 90 of follow-up, three serious adverse events were recorded in both study arms (exacerbations of COPD and lower respiratory tract infections).

In this study, patients with upper lobe-predominant, lower lobe-predominant and homogeneous disease were all included and, in the opinion of the authors, all had beneficial effects from treatment. Actually, it is worth noting that no specific subgroup analysis was conducted.

Follow-up efficacy data

The presence of collateral ventilation due to incomplete fissures is the major limiting factor in lung volume reduction with endobronchial valves. Treatment with coils overcomes this factor and may serve as an alternative choice in this specific group of patients. Kontogianni et al. successfully treated 26 patients with predominantly unilateral heterogeneous emphysema and bilaterally incomplete fissures. Treating unilaterally upper or lower lobes, they demonstrated an improvement in several functional parameters (FEV1, VC, RV, 6MWD, SGRQ) at six months of follow-up in these patients as well.18

One study specifically focused on the efficacy of LVR coil treatment in patient with exclusively homogeneous emphysema. Klooster et al. enrolled 10 patients with homogeneous disease, placing a maximum of 12 coils in each upper lobe in two sequential procedures. After six months of follow-up, 6MWD, FVC, RV, Raw (airways resistance) and SGRQ resulted significantly improved from baseline. Only two COPD exacerbations and one pneumothorax were recorded as serious adverse events, confirming the safety profile of the treatment also in these patients.19

In this literature context, the study by Deslee et al.20 comes out as the largest LVRGstudy to date, firstly reporting data of efficacy and safety after six months and one year of follow-up.

The Authors enrolled 60 patients in 11 European centers. 58 subjects were evaluated at six months (German cohort) and 34 at twelve months. A total of 1125 coils were placed with a median of 10 coils per
EMPHYSEMA

al. reported data of effectiveness and safety at two and three years of follow-up of 38 patients who participated in two previous pilot study and were invited, after the study completion, for a voluntary annual follow-up.21

Safety data fully confirmed the results by Deslee et al., with only two post-treatment pneumothoraces and mild hemoptysis being the early most frequent complication (74% of the patients). Despite of some lower respiratory tract infections throughout the whole follow-up period, no late pneumothoraces, coil migrations, major infectious complications or treatment-related deaths were registered.11 At 2-year follow-up, 27 patients showed RV, mMRC and the SGRQ score significantly improved when compared with baseline, while at 3-year follow-up, 22 subjects revealed only mMRC being significantly improved compared with baseline values. It is worth noting that the rate of decline of FEV1 did not change after the coil treatment. Nevertheless, the treatment increased FEV1 to the extent that return to pre-treatment baseline levels only after approximately 3 years.

Limits of the recent studies

The results of the prospective multicentre study of LVRC treatment in patients with severe emphysema11 show an acceptable safety profile associated with a significant and sustained improvement over 12 months in relevant clinical and functional parameters including FEV1, RV, 6MWD and SGRQ. Significant mean improvements in pulmonary function, exercise performance and symptoms at 1 year were also seen in a subsequent study by Hartman et al.21 with a longer extension of follow up to 3 years, but in that more recent trial there did appear to be a waning of benefit over time, with only the modified Medical Research Council dyspnoea score significantly different at 3 years.

This had already happened before in a similar setting investigating the results of BLVR through airway bypass.22

Longer follow up and data verification
Table I.—Summary of studies assessing efficacy and safety of lung volume reduction coil treatment of patients with severe emphysema.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study protocol</th>
<th>Patients enrolled (N.)</th>
<th>Primary outcome</th>
<th>N. of coils implanted (median)</th>
<th>Type of emphysema</th>
<th>Functional parameters significantly improved from baseline after follow-up completion (median values)</th>
<th>Follow-up duration (months)</th>
<th>Severe complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herth (2010) 14</td>
<td>PCS</td>
<td>11</td>
<td>Safety</td>
<td>5/lobe</td>
<td>Heterogeneous and homogeneous</td>
<td>FEV1 (+14.9%) FVC (+13.4%) 6MWT (+81.4 m) RV (-11.4%) SGRQ (-14.9 points)</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>Slebos (2012) 16</td>
<td>PCS</td>
<td>12</td>
<td>Efficacy (SGRQ) Safety</td>
<td>10/lobe</td>
<td>Heterogeneous</td>
<td>SGRQ , 6MWT, FEV1 and RV in LVRCs arm Vs medical care arm</td>
<td>6</td>
<td>20 AECB 1 PNX</td>
</tr>
<tr>
<td>Shah (2013) 17</td>
<td>RCT</td>
<td>47</td>
<td>Efficacy (SGRQ) 18.5/patients</td>
<td>Heterogeneous and homogeneous</td>
<td>SGRQ , 6MWT, FEV1 and RV in LVRCs arm Vs medical care arm</td>
<td>3</td>
<td>5 AECB 2 PNX 2 LRTI</td>
<td></td>
</tr>
<tr>
<td>Kontogianni (2014) 18</td>
<td>RCS</td>
<td>26</td>
<td>Efficacy (FEV1) 10/lobe</td>
<td>Unilateral heterogeneous with bilateral incomplete fissures</td>
<td>FEV1 (+0.06 l) FVC (+0.11 l) FVC (+0.32 l) VC (+12%) RV (-0.42) RV (-14%) RV/TLC (-3%) 6MWD (+46 m) SGRQ (-6 points)</td>
<td>6</td>
<td>7 AECB 2 PNX</td>
<td></td>
</tr>
<tr>
<td>Klooster (2014) 19</td>
<td>PCS</td>
<td>10</td>
<td>Efficacy (6MWD) 11/lobe</td>
<td>Homogeneous</td>
<td>FVC (+0.38 l) RV (+ 0.60 l) RV (-22%) TLC (-0.12 l) RV/TLC (-6) 6MWD (+61 m) SGRQ (-15 points) Raw (-0.01 Kpa/l/s)</td>
<td>6</td>
<td>2 AECB 1 PNX</td>
<td></td>
</tr>
<tr>
<td>Deslee (2014) 20</td>
<td>PCS</td>
<td>60</td>
<td>Efficacy (SGRQ) safety</td>
<td>10/lobe</td>
<td>Heterogeneous and homogeneous</td>
<td>FEV1 (+15.3%) FEV1 (+0.11 l) FVC (+0.20 l) RV (-0.65) RV (-11.3%) RV/TLC (-4.5) 6MWD (+29.7 m) SGRQ (-12.1 points) mMRC(-0.6)</td>
<td>6 (German cohort) 12 (French and Dutch cohort)</td>
<td>25 AECB 7 PNX 1 Haemoptysis</td>
</tr>
<tr>
<td>Hartman (2015) 21</td>
<td>RCS</td>
<td>38</td>
<td>Efficacy safety 10/lobe</td>
<td>Heterogeneous and homogeneous</td>
<td>mMRC (-0.05)</td>
<td>36</td>
<td>2 PNX 1 haemoptysis</td>
<td></td>
</tr>
</tbody>
</table>

PCS: prospective cohort studies; RCS: retrospective cohort studies; RCT: randomized controlled trial; SGRQ: Saint George’s Respiratory Questionnaire; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; RV: residual volume; LVRC: lung volume reduction coils; TLC: total lung capacity; 6MWD: 6 minute walking distance; mMRC: modified Medical Research Council Dyspnea Scale; AECB: acute exacerbation of chronic bronchitis; PNX: pneumothorax; LRTI: lower respiratory tract infection; Raw: airway resistance; l: liters; s: seconds; Kpa: kilopascal.

Over time is needed is needed to confirm these optimistic preliminary results. Efforts to do so are underway and data putting together longer follow up outcomes of patients enrolled in different cohorts from previous trials will be available soon. Also, drawing conclusions from small cohorts of patients can be difficult, and the data are inevitably subject to bias.

Survival bias are almost impossible to avoid in such follow-up studies, with some of the more severely affected patients dy-
velop patient selection pathways to prospectively predict who may benefit.

Given the preliminary nature of most data available, more trials are still needed. Research should be designed as a comparative effectiveness research model, trials need to involve a larger number of participants, with a much longer duration of follow-up.

Possibly different markers of improvement than the ones traditionally used have to be considered in order to measure clinical but more importantly functional benefit.

The literature on endobronchial coils continues to look promising with an acceptable safety profile, and positive long-term follow-up data is certainly more and more available. However, further well-designed, blinded, placebo (or sham) controlled trials, and even randomized trials against LVRS, are needed before routine clinical use can be recommended. This is true not only for endobronchial coils, but also for the whole field of bronchoscopic lung volume reduction.

The purpose of future research trials in this field is twofold: first, to demonstrate sustainable clinically significant benefits, and second to determine those patient characteristics that predict response to each individual technique.

We need more about long term survival, by innovative trials designed with a comparative effectiveness research model, involving a larger number of participants, with a much longer duration of follow-up, and, what is more important, with different markers of improvement than the ones traditionally used in these trials as in earlier studies.

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Idiopathic pulmonary fibrosis landscapes: looking glass from pathology to therapy

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Idiopathic pulmonary fibrosis (IPF) is a rare chronic and ultimately fatal disease resulting in an aberrant scarring and thickening of lung tissue. Molecular pathogenetic mechanisms of IPF are still unknown and till now no effective therapy is known to really improve disease's outcome. A deeper understanding of IPF biology is now mandatory to clarify IPF origin in order to identify action-able targets. Here we discuss and analyze the data presented by a recent paper published by De Pianto et al. on the prestigious respiratory journal Thorax. The work is focused on how gene expression analysis can be ap-plied to stratify IPF cases based on their risk of disease progression. Moreover they tried to match genetic and phenotypic profiles in order to predict therapeutic response and pa-tients' prognosis.

Key words: Biological markers - Biopsy - Genes - Idiopathic pulmonary fibrosis - Immunohistochemistry.

Idiopathic pulmonary fibrosis: etiopathology and diagnosis

Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal lung disease of un-known etiology that is still lacking of ef-fective targeted therapy. The disease mainly affects middle-aged and older adults, most often smoker subjects. It is thought to arise following the aberrant execution of the biological process activated in response to alveolar epithelial cell injury characterized by secretion of excessive amounts of extracellular matrix components, resulting in scarring of the lung, architectural distortion, and irreversible loss of function. Al-though advances in disease definition and diagnostic criteria, the biological features and the genomic makeup of IPF remain largely unexplored. It is well known that IPF is defined, as key pathological features, by a microscopic spatial and temporal het-erogeneity. Indeed microscopy analysis of IPF histological samples demonstrates a heterogeneous appearance with areas of subpleural and paraseptal fibrosis and honeycombing alternating with areas of less affected or normal parenchyma (spatial heterogeneity). Small areas of active fibrosis (fibroblast foci) are present in the background of collagen deposition, and they reflect the temporal heterogeneity of the

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process and indicate current ongoing disease. Inflammation is usually mild and consists of a patchy lymphoplasmacytic interstitial infiltrate. These features are reflected in CT scan images as reticular opacities, often associated with traction bronchiectasis, with little or no ground-glass opacifications. Honeycombing, mainly affecting subpleural areas, clustered cystic airspaces with well-defined walls, is common and is critical for making a definite diagnosis.\(^2\)-\(^6\) Importantly, a plethora of resident and infiltrating cell types are involved and/or responsible for activation of repair and remodelling processes. How the match among cell genotype and phenotype can be deciphered in such a complex scenario? Does spatial and temporal histological inter-cellular heterogeneity reflect a molecular and intra-cellular one? A deeper understanding of IPF biology at local level is now mandatory to clarify pathogenetic mechanisms of a still elusive diseases as well as to prelude the design of hypothesis-driven therapies. The paper by DePianto et al. recently published on Thorax\(^7\) is timely focused on this issue. The key question proposed by authors is if gene expression analysis can be applied to stratify IPF cases with the aim to link a genetic signature to pathology and clinical manifestations of the disease. The challenge is thus to exploit molecular expression patterns, in other words molecular heterogeneity, to identify actionable targets on one hand and to identify patients at higher risk of disease progression on the other.

**Gene expression**

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product, namely proteins or transfer or small nuclear RNA, which ultimately gives rise to phenotype. Thus the gene expression analysis is determination of the pattern of genes expressed at the level of genetic transcription, under specific circumstances or in a specific cell. DePianto et al. applied transcriptomic approach to IPF by evaluating – on a series of lung biopsies - gene expression microarrays in parallel with conventional immunohistochemistry. Concomitant measure of blood levels of some biomarkers was established as well. The aim of the study was to assess a relationship between transcription patterns, morphological features and circulating biomarkers in order to identify a signature predictive of disease severity and progression. IPF is characterized by a temporally heterogeneous appearance with areas of normal appearing lung intermixed with older collagenized fibrosis, microscopic honeycombing, and small focal areas of younger, myxoid-appearing matrix with aggregates of actively proliferating and collagen-producing myofibroblasts termed “fibroblast foci”.\(^8\) In particular these foci are often identified in a transition zone between the more normal uninvolved lung and the abnormal fibrotic lung and identify the biological key player of disease progression.\(^2\) \(^9\) The relevant challenge of the study by DePianto et al. is to decipher how phenotype-genotype interactions define disease onset and progression. The ultimate goals of the study are to contribute to clarify IPF pathogenesis and to identify a predictive/stratification signature, useful for both targeted therapeutic approach and molecular diagnostics. Use of molecular research techniques in large populations of well-phenotyped patients is a leading approach in understanding of IPF. In this perspective, authors designed a unique multilevel approach. They hypothesize that a systematic gene expression profiling study matched with morphology could potentially be useful to unveil non-invasive biomarkers of IPF outlook. This is among the most relevant studies on system biology applied to IPF. The latter is a novel discipline translating biology into medicine and gives the opportunity to think about patient stratification at diagnosis. This approach is of extremely importance in order to design future trials with target therapy and revise their outcomes or in alternative to early refer patients for transplant. Thanks to these advances, the idea of giving a patient a drug tailored to the genetic makeup of the disease is landing the clinical arena.
**IDIOPATHIC PULMONARY FIBROSIS**

Recently, MMP3 has been reported to be elevated in IPF. Overexpression of MMP3 leads to lung fibrosis in animal models, whereas mice lacking MMP3 are protected. Notably, in vitro analysis demonstrated that MMP3 may play a role in activating beta-catenin signaling and in the induction of the epithelial-to-mesenchymal transition, a process which is known to be activated during IPF development.\(^{20, 21}\) Moreover, cytokines have been shown to play a major role in the progression of IPF, both in animal models and in human patients. Indeed, many mechanisms that are exploited by these cells to sustain their survival and proliferation require activation of cytokines. Among the interleukins (e.g., IL6), cytokine receptors (IL6R1) and chemokines and chemokine receptors are known to play a key role.\(^{22}\) Among chemokines, the C-X-C motif chemokine 13 (CXCL13) is known to act as a prognostic marker in patients and notably, CXCL13 mediates B-cell trafficking and is increased, proportionally to disease activity, in many antibody-mediated syndromes. CXCL13 overexpression is intrinsic to IPF, thus suggesting that the chemokines and B cells may directly play a role in IPF pathogenesis.\(^{23}\)

Previous application of gene expression in IPF tissue has led to the identification of up-regulated pathways of molecular mechanisms involved in cell motility, fibroblast differentiation and inflammation in relation to the different disease behavior, slow or rapid progression of the disease.\(^{3, 24, 25}\) More recently, expression of cilium genes was identified in a subgroup of patient and associated with microscopic honeycombing, MUC5B and MMP7 gene expression.\(^{26}\) Importantly, there is emerging evidence that immune markers may provide useful information for patient stratification and prognostication. In fact, recent data indicate that activation of blood mononuclear cells might provide interesting markers for IPF outcome, as the level of expression of genes associated with the costimulatory signal during T cell activation (including CD28, ICOS, LCK and ITK) was shown to predict prognosis in two IPF cohorts.\(^{27}\) Circulating autoantibodies targeting...
periplakin and HSP70, a chaperone protein, were proposed as promising prognostic markers for IPF.28, 29

**IPF and genetic factors**

Based on this observation it is clearly evident that a number of different cellular elements are activated and cooperate in inducing IPF. The paper by DePianto et al. properly underlines, already in the manuscript title, that IPF is a heterogeneous disease and that this point is reflected in patients’ outcome. Although a systematic approach to IPF is not a novel one, authors correlate gene expression to specific histological features of IPF identifying two prevalent patterns of disease: abnormal bronchiolization and lymphoid aggregates, which could be targetable by new therapies. More important they identified soluble mediators that reflect these molecular signals and can be easily measured and longitudinally sampled for stratification of this heterogeneous population.

Thus phenotypic heterogeneity in IPF cell populations, despite creating an opportunity for selectivity, is one of the most important cause of therapeutic failure. It is conceivable that IPF arises from an intricate interplay between genetic and non-genetic factors. Multiple genes have been evaluated using a candidate gene approach with limited success, with results suggesting a disease modifier effect rather than a disease causing effect. Among them, specific polymorphisms in genes encoding interleukin-1 receptor antagonist, tumor necrosis factor-α, and complement receptor 1. More recently research on familial pulmonary fibrosis cohorts documented a mutation in the gene encoding surfactant protein C was identified as the cause of pulmonary fibrosis in this family. Subsequently, another individual with idiopathic pulmonary fibrosis was identified with a different mutation in surfactant protein C.30-33 Moreover the concept of diversity in IPF cell population is consistent with the hypothesis that IPF active lesions - namely fibroblast foci - may derive from a cell of origin which is responsible for the maintenance and progression of the lesions themselves. This idea is directly mediated from the concept of cellular diversity and hierarchy that has been demonstrated in tumoral masses.34, 35 In cancer somatic evolution - which drives tumor progression - is characterized by complex mechanisms that arise from the Darwinian nature of the neoplastic process itself.36 The cancer stem cells, bring about the maintenance and progression of the tumor, are capable of both self renewal and multilineage differentiation. Most of the tumor mass therefore consists of differentiated cells with less proliferative potential. Thus, in tumor, phenotypic and genetic heterogeneity associated with stem-cell differentiation is almost irrelevant for tumor progression because the stem-cell compartment is not affected.37 Does IPF behave as cancer? It is a crucial point to clarify the findings published by DePianto et al. Growing evidence sustains that IPF progression can be assimilated to that of cancer, and, indeed several signaling patterns appear to be disrupted in both diseases.38-40 A main point that requires be underlined is that IPF is characterized by policlonality. As discussed above, IPF arises from the crosstalk of a number of cellular players which contribute to define its gene expression signatures. Furthermore the data in the current study support those prior observations and together highlight the potential contribution of innate and adaptive
idiopathic pulmonary fibrosis STELLA

...copy, explanted lungs) and detailed clinical and demographic features of each corresponding patients. On the contrary few data are available from histological features of each samples and from which areas of them RNA was really extracted. It is conceivable that signatures found corresponded to wide fibrotic areas, since signatures markers are finally localized to specific histological areas, namely normal alveolar tissue, “transition zones” of active fibrogenesis and advanced scar tissue characterized by dense fibrosis. Readers do not really know which specific gene expression signature is associated to fibroblast foci, the key histological IPF feature. Another key point is related to data analysis. The gene expression clustering allows an open-ended exploration of the data, without getting lost among the thousands of individual genes. Beyond simple visualization, there are also some important computational applications for gene clusters. Overall the goals of gene clustering are: i) discovering the underlying structure of the data; ii) discovering groups of co-expressed genes/tissues. Importantly clustering is an unsupervised method. The hierarchical clustering (as in DePianto et al. paper) does not require the number of clusters to be known in advance and offers an intuitive visual clue of the distribution of the data. As opposite clustering methods should be used with the goal to pursue pattern discovery or dimensionality. In the work by DePianto et al. the class label (bronchiolar and lymphoid) are already well known categories and thus hierarchical clustering is not properly applied. Notably the authors does not really clarify this point and no statistical analysis is associated to data interpretation. A third point - and authors properly stressed is - that may act as potential confounder is that almost a quarter of patients in the biomarker cohort were taking immunosuppressants at time of samples collections. This data could potentially affect systemic biomarkers level as well as survival time. Finally, the authors detect these soluble secreted factors produced by pathological structure as biomarkers to study clinical assessment and disease pro-

immunity to IPF pathogenesis and in particular the involvement of adaptive response in IPF progression. The finding of lymphoid follicles in IPF biopsies has been reported previously. The presence of B-cell aggregates in injured tissues suggests a possible direct contribution of the significant pathogenetic potential of B cells to the fibrotic process. Xue et al. observed an increase in circulating antigen-differentiated plasma cells in IPF patients and overexpression of a specific B-cell trophic factor (plasma B lymphocyte-stimulating factor), associated with poor short-term outcome. It seems likely that both the innate and adaptive immune systems participate in lung fibrosis progression. However, the question of whether the immune system is directly causative of lung fibrosis rather than a consequence of the loss of the normal lung architecture and therefore of natural defenses is more difficult to resolve. The finding of a lymphoid signature together with the increased serum marker of inflammation (CXCL13 overexpression) linked to IPF progression may arise to think of the possibility of an interaction between the different pathogenetic mechanisms including immune activation.

Oncogenes are defined by the acquisition of mutations, which results in a dominant gain-of-function of the targeted protein. In this situation a single mutated allele is sufficient to induce malignant transformation. Notably, tumor becomes addicted to those particular genetic alterations that cause oncogenic activation and the continue expression of signalling. Moreover an increasing amount of evidence sustains the rationale for targeting oncogenic pathways rather than single mutated gene. In the IPF context, gene expression analysis is frankly related to cellular heterogeneity. Thus, due to its policlonality, genome-wide analysis in IPF may not be interpreted under the focus of the oncogenic addiction phenomenon. Coherently, DePianto et al. did not perfomed DNA sequence analysis, but a transcriptomic test by analysing a cohort of 40 IPF cases. The authors properly detailed the characteristics of each sample analyzed (obtained through video-assisted thoracos-
Progression. However, it should be noted that histological evaluation has been obtained in end stage population, in other word in a different time point of the cohort of patient collected for blood samples (first evaluation at ILD center). About the end stage population clinical and functional data were not available and it might be possible that different course of the disease (slow or accelerated) might influence the final histological evaluation.

**Prognosis**

Due to intrinsic study limitation, study findings lose relevance under the therapeutic perspective, which is a real urgent unmet clinical need in IPF. Although two different signatures corresponding to bronchiolar and lymphoid structures clearly emerge from the analysis, very few data are really actionable. More information can be exploited to understand IPF pathogenesis. Nevertheless results confirm already available and known data. The pathogenetic role of MMPs is confirmed by the study: in particular MMP3 emerges as a key player in the chronic and pathological wound repair and tissue remodelling characteristic of advanced IPF. Moreover the bronchiolar signature is similar to a set of genes reported to be upregulated in lungs from patients affected by concomitant pulmonary fibrosis and pulmonary artery hypertension. Thus, it is “molecularly” conceivable that advanced scarring honeycombing and bronchiolization is associated with increased pulmonary vascular resistance and hypertension. On the other hand the lymphoid signature clearly confirms that an inflammatory component is associated to chronic lesions in IPF, although the exact role of the immune lymphoid aggregates in IPF onset and progression is still unclear. In this setting authors confirm that CXCL13 overexpression may be considered as a biomarker of advanced disease thus suggesting that lymphoid aggregates may arise as late manifestation of the disease. In conclusion, can gene expression analysis really impact IPF management? The paper by DePianto et al. clearly points out that this approach is feasible and reliable. Nevertheless some issues should be improved firstly, by a better and deeper characterization of the cytohistological fibrotic areas. A more informative and actionable profile should be obtained by selective transcriptome analysis from fibroblast foci. A second point is related to the fact that no analysis have been applied to verify how immunosupression can impact gene expression. If MMPs (namely MMP3) and chemokines (CXCL3) emerge as potential biomarkers of disease progression, a clarification of how the immune response can modulate protein expression should be mandatory. From this perspective it has been reported by *in vitro* experiments that gelatinase B (MMP-9) activity is increased in untreated IPF patients, whereas it is normal in patients treated with steroids. Moreover, it is well documented that steroids treatments alters chemokines’ expression in a number of pathological conditions. Again serum markers were obtained at the first evaluation and correlated with functional parameters at this point of the course of disease. The prognostic significance and correlation with clinical disease severity need further evaluation since the authors in the study considered the association with FVC and DLco at one visit different probably from diagnosis and before lung transplant. Survival could be a good measure to investigate the prognostic role of biomarkers but it can be affected by significant comorbidities or acute fatal event, whereas FVC change is traditionally the measure of choice of greater sensitivity and specificity useful to examine the possible association with disease progression. Finally, data obtained by the group of DePianto require a validation in additional cohorts.

**Conclusions**

The concept that “precision medicine” can be exploited to properly manage IPF has now acquired an unprecedented clinical value. Many advances have been
reached to identify molecular markers of disease onset and progression which will also provide a rationale to set up molecular classification of IPF stages. Thanks to these efforts, the idea of giving a patient drugs tailored to the genomic makeup of the disease is now becoming commonplace. New targeted agents (pirfenidone and nintedanib) have now reached the clinical scenario and several molecules are under design and development. Future works will be thus addressed to capitalize and integrate clinical and molecular biomarker data to develop and validate models that predict disease progression and enable evaluation of potential endotypes. The integration of genomic information arising from both biological and clinical studies is mandatory to uncover genetic and epigenetic mechanisms driving IPF and to stratify patients’ outlooks by refining their diagnosis and treatments.

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