Clinical update

Pulmonary hypertension: chapters of innovation and tribulation

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As can be seen by the mounting literature, there has been immense progress in the field of pulmonary hypertension (PH) over the last three decades, illustrated by several important milestones including improved understanding of disease pathogenesis, new classifications of disease, advances in screening and diagnostic techniques, and new rules for staging and follow-up, which have subsequently led to improvements in patient outcomes. The objectives of this manuscript are to not only highlight these very recent advances but also point out areas of deficiencies or gaps in our knowledge that may serve a focal point for future discussion and investigation.

Keywords
Pulmonary heart disease • Pulmonary hypertension • Thrombosis • Endarterectomy • Survival

Introduction

Pulmonary hypertension (PH) is a chronic and deadly disease characterized by reduction in pulmonary vessels leading to progressive increases in pulmonary vascular resistance. Pulmonary arterial hypertension (PAH), a subset of PH, can be further classified into either an ‘associated’ form, e.g. PH associated with connective tissue diseases, congenital heart disease, human immunodeficiency virus, cirrhosis with portal hypertension, or exposure to anorexiants or toxins, heritable PAH (HPAH) related to mutations in either bone morphogenic protein receptor type II (BMPR2), activin receptor-like kinase 1 (ALK1), or other genes, and idiopathic PAH (IPAH) with no identifiable cause. Significant heterogeneity exists among patients with PH, both in terms of disease development, including genetic and clinical predisposition, and clinical responses to available therapeutics. This heterogeneity makes both screening for the disease, initiating therapy, and choosing effective frontline therapy difficult. A better understanding of this variability through a more thorough investigation of genetic (germline and somatic), epigenetic, and post-translational mechanisms may increase our ability to accurately and reliably predict those who will develop disease and respond to therapy.

Pulmonary arterial hypertension may be looked at as a systematic loss of function of resistance arteries of the lungs. Medial hypertrophy, obstructive intimal proliferative and fibrotic changes, perivascular inflammatory infiltrates, and thrombotic lesions dominate the pathological pattern.1 Whether vessels disappear anatomically remains a matter of debate.

The identification of three key pathogenic pathways has led to the development of targeted therapies. The first evidence-based clinical practice guidelines in PAH were published in 20042 and updated in 2009.3,4 Guideline implementation that is updated by novel scientific insights should be of utmost importance as we prepare and strategize for the future.

While PAH remains a focus of recent advancements, understanding of non-PAH PH disease entities remains staggeringly slow, despite equally poor survival rates. Many of these forms of PH are much more common than PAH, but all of them have been less well studied, and comprise both pre- and post-capillary disease entities. Mechanistically, non-PAH PH shares certain pathological mediators with PAH. For example, recent research has illustrated that mast cells play a role in the pathogenesis of both pre- and post-capillary PH.5,6 However, what clearly distinguishes non-PAH PH from PAH are mechanical triggers, including high left atrial pressures and parenchymal lung damage with hypoxia or obstructive major vessel thrombus. Unfortunately, ignoring or failing to recognize these triggers often leads to these entities being misdiagnosed or treated similar to PAH. The bulk of
evidence today is pointing towards a lack of effect of PAH-targeted treatments in non-PAH PH or even the potential for increased patient morbidity, suggesting that financial resources may be wasted in these conditions unless more data are available to indicate that there is a benefit.

The objective of this manuscript prior to the approaching WHO conference in Nice 2013 is to highlight key recent advances and to point out gaps in knowledge.

**Beyond the gene: exploiting the role of epigenetics, somatic mutations, and environmental triggers**

Despite a difference in triggers, the histopathological changes of pulmonary vessels are very similar in all forms of PH, regardless of the suspected mechanism of disease, suggesting that the downstream regulatory pathways share common elements (Figure 1). Unfortunately, because PAH is an orphan disease, both the genetic causation (beyond the well-characterized BMPR2 mutations) and the genetic determinants of survival and response to therapy remain elusive. Until only recently, the role of epigenetic modification, somatic mutations, co-regulatory mutations, or polymorphisms as well as post-translational modifications and environmental triggers were not adequately explored.

It is well known that heterozygous germline mutations in the BMPR2 gene account for more than 80% of HPAH, with mutations in the ALK1 and endoglin genes associated with a much smaller number of cases. There are several important nuances to the BMPR2 story, however, that merit consideration at this point. First, disease penetrance is low, roughly 20%, in the presence of a mutation. Secondly, only 12–20% of IPAH and only rare instances of other PAH forms harbour BMPR2 mutations. Lastly, it was observed that expression of BMPR2 protein is reduced in pulmonary tissue from PAH patients even in the absence of a BMPR2 mutation and that this reduced expression is disproportionately low in those with BMPR2 haploinsufficiency. Therefore, other factors, genomic or environmental, may be required to alter this critical remodelling axis (secondary triggers) in order to confer the clinical phenotype on susceptible patients.

On a pure genetic level, outside of 12–20% of the patients with IPAH who harbour the BMPR2 mutation, very little is known about the genetic susceptibility to IPAH or other forms of PH. Several studies have suggested a potential relationship between genetic mutations or polymorphisms and clinical characteristics, particularly in scleroderma, sickle-cell anaemia, and portopulmonary hypertension. Unfortunately, these studies suffer from small sizes and lack validation in larger independent studies.

Epigenetics refers to all heritable changes in phenotype or gene expression states that are not involved in the modifications of the DNA sequence itself. Histone modifications, DNA methylation and genotype imprinting, and RNA-based mechanisms are examples of these modifications (Figure 2). The former two processes are particularly important in PAH as they can selectively activate or inactivate genes that control cell growth, proliferation, and apoptosis. Epigenetic modifications in superoxide dismutase-2 (SOD2), endothelial nitric oxide synthase, and BMPR2 pathways may be critically important in the development of PAH in susceptible individuals. A tissue-specific hypermethylation of a CpG island in an enhancer region within intron 2 and the promoter of the SOD2 gene was found to cause down-regulation of SOD2 leading to PH in a fawn-hooded rat model. These reports have not been duplicated in humans, although it is clear from pathological studies that SOD2 is down-regulated in the pulmonary vasculature of PAH patients. Epigenetic regulation is also important for endothelial nitric oxide synthase expression in the human pulmonary vasculature.

Somatic mutations offer an alternative explanation of secondary triggers and are of particular interest given the resemblance of PAH to a neoplastic disorder. Interestingly, and opposed to many cancer models, there is no evidence of somatic loss of the remaining wild-type BMPR2 allele in heterozygous mutation carriers that could account for the exaggerated loss of BMPR2 expression in these patients. An acquired somatic loss of chromosome 13 in a patient with PAH who had a germline mutation in BMPR2 illustrated a ‘second hit’ in the BMPR2 pathway by deleting Smad8.

RNA modifications, silencing, and disease-associated mutations that alter RNA structural assembly as well as unique environmental influences like oestrogen-related changes in N-nitrosoglutathione reductase activity in the lung, offer additional targets of exploration beyond simple genetic susceptibility.

MicroRNAs (miRNAs) are short non-coding RNA molecules that bind to complementary sequences in the 3′-untranslated regions of target messenger RNA transcripts, usually resulting in gene silencing. Bone morphogenetic protein-dependent miRNA processing is defective in PAH. In a hypoxic rat model of PAH, an early response of the pulmonary arterial smooth muscle appears to be down-regulation of one of the master miRNA regulators of the contractile phenotype. This may reflect a shift to more proliferative and secretory behaviour and thus contribute to vascular remodelling seen in PAH.

Id proteins (Id1–4), basic helix–loop–helix inhibitors of DNA binding, are major downstream transcriptional targets of BMP signalling (Table 1). A quantitative high-throughput screen of 3600 FDA-approved drugs and bioactive compounds revealed that FK-506 was the main activator of BMP signalling via Id1. A 3-week treatment with FK-506 in mice with a conditional deletion in BMPR2 in endothelial cells exposed to 3 weeks of hypoxia prevented the development of PAH and right ventricular hypertrophy. Rapamycin also emerged from these drug screens. These findings suggest that the same drugs that effectively inhibit coronary in-stent restenosis may be useful for preventing the vascular remodelling of PAH.

A collaborative group has formed between US and European investigators with participation from the international PH community at large to assemble the largest worldwide collection of banked DNAs (~2000 specimens; 58% IPAH) from patients with this orphan disease. Hopefully, meaningful genomic studies to identify both the genetic and epigenetic factors associated with PAH and response to therapy may result from these collaborations.
Despite the key information that BMPR2 is central to the disease process, its precise role in the pulmonary vascular changes of PAH remains to be defined.

Identification of modifying genetic or non-genomic factors is critical.

Survival and risk modelling: does it make a difference?

The ability to accurately and reliably predict prognoses at baseline and on treatment is of significant clinical value (Figures 3 and 4).\(^\text{45}\)

Although predictors of risk at diagnosis have been identified, no knowledge exists about their value over time. Today, there are several surrogate measures of efficacy after treatment for PAH. These include 6 min walking distance, World Health Organization functional class, Borg Dyspnea Scale, neurohormonal laboratory markers, imaging, and right heart catheterization haemodynamics. The first attempt to evaluate prognostic factors was by the National Institutes of Health (NIH) registry established in 1981.\(^\text{46}\) NIH registry data served to develop a prognostic equation that was useful for earlier implementation and individualization of treatment regimens.\(^\text{47}\) Today, the survival equation derived from NIH registry data is only applicable to patients who have not yet received treatment and does not reflect the current survival rates.\(^\text{45}\) Recently, the French Network on Pulmonary Hypertension registry (French registry) initiated in 2002,\(^\text{48,49}\) the Pulmonary Hypertension Connection registry (PHC registry) initiated in

**Figure 1** Updated diagram depicting mechanisms involved in the development of pulmonary vascular disease.\(^\text{17,77}\) All pathways converge to contraction, proliferation, and apoptosis inhibition of pulmonary artery smooth muscle cells (PASMC), illustrating that the downstream regulatory pathways share common elements (modified from Morrell et al.\(^\text{13}\)).
Figure 2  Mechanisms of epigenetic control: DNA methylation, histone modifications, and RNA-based mechanisms are examples of these modifications (modified from Matouk and Marsden74).

Table 1  List of candidate pathways and mediators of pulmonary vascular disease (updated from Hassoun et al.12)

<table>
<thead>
<tr>
<th>Cytokines and chemokines</th>
<th>RANTES (CCL5)</th>
<th>Monocyte chemotactic protein (MCP)-1 (CCL2)</th>
<th>IL-6</th>
<th>Tumor necrosis factor-α76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractalkine (CX3CL1) CXCR-475</td>
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<tr>
<td>Growth factors</td>
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<tr>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>Endothelin-1</td>
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<td>Transcription factors</td>
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<tr>
<td>Schistosoma mansoni inhibin/ activin</td>
<td>Oct-478</td>
<td>Id proteins (Id1–4)73</td>
<td></td>
<td>p5379</td>
</tr>
<tr>
<td>Infectious Agents</td>
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<tr>
<td>Human immunodeficiency virus, chimeric simian immunodeficiency virus (SIV) containing the HIV-nef gene SHIV-nef</td>
<td>Human gamma herpes virus 8</td>
<td>Staphylococcus aureus80</td>
<td>Lysosomal pathway via mammalian E3 ligase, Itch81</td>
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<tr>
<td>Autoantibodies directed to</td>
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<tr>
<td>Fibrillarin (anti-U3-RNP)</td>
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<tr>
<td>Receptors, transporters, and scaffolding proteins</td>
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<tr>
<td>Adenosine A2A receptor</td>
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<td>Apoptosis regulator proteins</td>
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<td>Survivin</td>
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<tr>
<td>Enzymes</td>
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<tr>
<td>Glycogen synthase kinase 3β85</td>
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<tr>
<td>Specific cell types</td>
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<tr>
<td>Mast cells</td>
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<tr>
<td>MicroRNAs</td>
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<tr>
<td>MicroRNA (miR)-20418</td>
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<tr>
<td>Other factors</td>
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<tr>
<td>Guanine nucleotide exchange factor-H1/RhoA/Rho kinase/p2799</td>
<td>Calcineurin</td>
<td>Tryptophan hydroxylase90</td>
<td></td>
<td>Ligand of G protein-coupled receptor APJ, apelin91</td>
</tr>
</tbody>
</table>
2004,50 and REVEAL, a 55-centre, observational, US-based registry initiated in 2006,51,52 have demonstrated improved survival of patients with PAH compared with those reported at the time of the NIH registry45,48,53 (Table 2). Prognostic formulas (Figure 3) and tools like the REVEAL calculator (Figure 4)54 are able to predict risk both at the time of diagnosis (French, REVEAL, PHC) and serially (REVEAL) to determine patient trajectory, and are being validated in both incident and prevalent cases.54

Compared with other contemporary algorithms, such as the Seattle and Michigan Heart Failure Scores, the REVEAL predictive formula and calculator performs well with c-indices exceeding 0.70 (Figure 5). Survival falls off sharply at a REVEAL score of 10, indicating the need for treatment step-up (parenteral prostacyclin or transplantation) in patients approaching scores of 8 (Figure 6).

Early detection will likely play a major role in future clinical practice. As can be seen in Figure 7, and in all contemporary registries,48,53,54 there is a steep attrition in survival in the first several months after diagnosis. Unfortunately, patients continue to be diagnosed late.52,55 Results from the EARLY trial have confirmed that treatment of mildly symptomatic patients results in clinical benefit.56 Recent data from the ItinerAir registry demonstrate that screened patients present in earlier disease stages and can be treated more effectively.57 But how can we diagnose PH earlier? Whether adaptive screening comes as part of improved detection of genetic susceptibility as noted earlier, identification of high-risk individuals through clinical risk factors gleaned from natural history studies (DETECT scleroderma Registry, European CTEPH Registry,58,59), use of functional tests in high-risk individuals (Cardio-Pulmonary Exercise Testing and exercise haemodynamics), or advanced imaging techniques (cardiac MRI60–62 novel biomarkers,63–69 CT fractal dimensions70,71) needs to be explored in proper clinical research arenas.

What will be the future trial methodology (e.g. disease modification vs. clinical endpoints) for randomized clinical trials in pulmonary arterial hypertension?

 Clinically relevant and sensitive endpoints and long-term data are important for future study designs in PAH. A significant barrier to conducting clinical trials in PAH is the lack of widely accepted and appropriately validated surrogate markers of survival. Six-minute walking distance is convenient and useful for short-term drug approvals, but limited by its lack of significance for long-term survival. Novel endpoints are required to assess clinical change in patients who are on multiple background therapies. Ongoing Phase III trials should be used to collect and validate the REVEAL and the French survival equations. In particular, trials of novel or combined pharmacotherapies that use measures of right ventricular structure and function as endpoints will be important. Others may argue though that until a relevant and stringently validated marker becomes available, the main endpoint in modern trials should be a ‘disease modification’ endpoint like mortality. Unfortunately, approved biomarkers are not on the immediate horizon and one of the major impediments to their development is the lack of large biorepositories connected to accurately phenotyped patients. ‘Omic’ studies are useless, unless the populations they are derived from are appropriately characterized. This is particularly true for patients with ‘metabolic syndrome’ and risk factors for left heart disease. Left heart disease with occult diastolic

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**Calculating PAH Survival Risk**

- **Pulmonary Hypertension Connection risk stratification equation**
  \[ P(t) = e^{-A(x,y,z)t} \]
  \[ A(x,y,z) = e^{(-1.270 \cdot 0.00148x + 0.0402y - 0.361z)} \] (equation for nonresponders to CCBs)
  \[ A(x,y,z) = e^{(1.012 \cdot 0.00148x + 0.0402y - 0.361z)} \] (equation for responders to CCBs)

- **French National Registry PAH risk prediction equation**
  \[ P(t|x,y,z) = H(t)A(x,y,z) = e^{(-0.02 \cdot 0.28t)\exp(-0.004x + 0.98y + 0.28z)} \]

  \[ P(t|x,y,z) = \text{probability of survival; } t = \text{number of years after diagnosis;} x = \text{mean PAP;} y = \text{mean RAP;} z = \text{Cl} \]

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**Figure 3** Formulas to calculate prognosis in pulmonary arterial hypertension.
dysfunction may be labelled as the ‘IPAH mimic’ of the twenty-first century. Pre-capillary disease comprises a pulmonary vascular disease affecting mainly the pre-capillary arteriolar compartment. Post-capillary disease originates distal to the pulmonary venules and entails morphological changes in the pre-capillary compartment only after a significant pressure increase in the venous compartment. More precise haemodynamic criteria for the diagnosis of true ‘out-of-proportion’ PH are needed.
Another key issue surrounding randomized clinical trials to investigate new drugs in PAH is the heterogeneity that exists as a result of background therapy and different standards of care. One solution is that patients are referred to specialist centres that have learned to maintain a ‘networked’ approach to patient care. A new drug has to be tested on top of best available therapy, which includes not only pharmaceuticals but also non-pharmaceutical interventions like exercise, all of which may vary between different countries and programmes. Ethical issues will certainly play a role in some clinical trial design if certain ‘standard of care’ interventions are restricted in order to participate in a trial. Unfortunately, evidence-based literature is scarce and thus expert opinion will continue to predominate in these areas. It is a need to strive for factorially designed protocols that answer the following questions: when, how, or should combination therapy be utilized? Should combination treatment always include a parenteral prostacyclin? Should upfront combination therapy be used given the high attrition rates early in the disease? If upfront therapy is used, should we randomize based on estimates of risk or apply broadly to all? If a step-wise approach is used, when should it be added and which parameters should be used to justify combination treatment? Should ‘failed’ therapies ever be stopped, and when should patients be referred to transplant or palliative therapies? To answer these questions and keeping in mind the limited number of patients, the global PH community must be dedicated and act as a cohesive unit promoting strategy trials rather than drug approval trials.

**Table 2** Independent predictors of survival of the three modern registries of patients with pulmonary arterial hypertension

<table>
<thead>
<tr>
<th>Parameter</th>
<th>REVEAL⁵²</th>
<th>French Registry⁵⁹</th>
<th>PHC Registry⁵⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>Age–gender interaction</td>
<td>Gender</td>
<td>Language</td>
</tr>
<tr>
<td>PAH subtype</td>
<td>Connective tissue disease, familial PAH, portopulmonary PH</td>
<td>Connective tissue disease</td>
<td>Language</td>
</tr>
<tr>
<td>Functional Class</td>
<td>I, III, IV</td>
<td>Per class increase</td>
<td>Cardiac index</td>
</tr>
<tr>
<td>Haemodynamics</td>
<td>Right atrial pressure, PVR</td>
<td>Cardiac index</td>
<td>Language</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>BNP, NT-proBNP</td>
<td>Continuous</td>
<td>Language</td>
</tr>
<tr>
<td>Walking distance</td>
<td>&lt;165 m, &gt;440 m</td>
<td>Continuous</td>
<td>Language</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td>Creatinine</td>
<td>Language</td>
<td>Language</td>
</tr>
<tr>
<td>Imaging and diagnostic studies</td>
<td>Pericardial effusion</td>
<td>Language</td>
<td>Language</td>
</tr>
<tr>
<td></td>
<td>Diffusion capacity for carbon monoxide</td>
<td>Language</td>
<td>Language</td>
</tr>
</tbody>
</table>

**Figure 5** c-indices for the different prognostic equations.

**Figure 6** The cliff effect illustrating the abrupt decline in the 12-month Kaplan–Meier survival curve beyond a calculated REVEAL Risk Score of 10.

**Figure 7** Seven-year survival estimates of patients in the REVEAL registry.
• A significant barrier to conducting clinical drug trials in PAH is accurate clinical phenotyping.
• Disease-specific efficacy endpoints using novel markers, which are validated and accurately reflect ‘disease modification,’ need development.
• ‘Hard’ endpoints such as those used in two large ongoing trials in PAH (‘SERAPHIN’ and ‘GRIPHON’) including death, transplant, or time to clinical worsening should be used in future clinical trials instead of older ‘soft’ endpoints such as 6 min walking distance or functional class.
• Phase IV trials, which clearly define and solidify the use of present-day therapeutics and interventions, need to be carried out, similar to the ongoing ‘AMBITION’ trial, which is analysing upfront drug combination vs. monotherapy.
• It will be important to (i) define haemodynamic criteria for the diagnosis of ‘out-of-proportion’ PH due to left heart disease and (ii) to standardize haemodynamic challenges, i.e. exercise protocols, agents, fluids, and volumes.
• New trials and registries in WHO groups 2–4 and in patients with ‘out of proportion PH’ need to be designed to define both the use and safety of PH-specific drugs in these groups.

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