Conference Abstracts

A record number of abstracts were submitted during the poster sessions at the Conference. The winning abstracts in Basic Science and Clinical Science were presented as oral abstracts during the scientific sessions and are included in this issue of *Advances*.

CLINICAL/TRANSLATIONAL SCIENCE:

β-2 Adrenergic Receptor Polymorphism and Gene Expression are Associated with Risk of Development of and Disease Severity in Pulmonary Arterial Hypertension Associated with Scleroderma

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Background: Little is known about the role of neurohormonal dysfunction in the pathophysiology of pulmonary arterial hypertension (PAH), particularly with respect to PAH-related to scleroderma (SSc-PAH). Single nucleotide polymorphisms (SNPs) in the β-2 adrenergic receptor (ADBR2) gene have been associated with cardiovascular disease and specifically, risk of development of left heart failure. Similarly, expression of related genes in peripheral blood mononuclear cells (PBMC) has been associated with disease severity in left heart failure. Therefore, we sought to 1) determine whether previously validated SNPs in ADBR2 were associated with the risk of development of PAH in SSc and 2) characterize the gene expression of ADBR2 in treatment-naïve SSc-PAH patients.

Methods: 308 SSc patients without PAH and 140 SSc-PAH patients provided blood for genetic analyses. Several SNPs of the ADBR2 gene (n=13), previously demonstrated to have functional significance in cardiovascular disease, were examined for their association with the risk of development of PAH in SSc at the single locus level using PLINK. Fifteen of the SSc-PAH patients, who were treatment-naïve at enrollment, also provided PBMCs for gene expression analyses using Illumina high-density BeadArrays. Pearson correlations between ADBR2 and clinical variables were calculated along with p-values and false discovery rates (FDR).

Results: Genetic analyses showed a significant association between the ADBR2 SNP Arg16Gly in the promoter region (rs17778257) and development of PAH in SSc ($p=0.03$). Gene expression profiles showed a strong positive correlation with ADBR2 expression and cardiac output ($r=0.81$, $p<0.0001$, $FDR<0.01$).

Conclusions: Preliminary results in this cohort suggest that functional SNPs in ADBR2 may be associated with the risk of development of PAH in SSc and that gene expression of ADBR2 is associated with disease severity. Given the known associations between ADBR2 and left heart failure, further study is warranted.

CLINICAL SCIENCE:

Living with Pulmonary Hypertension (PH): Unique Insights from an International Ethnographic Study

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Background: Despite the widespread use of quality of life (QoL) assessments in PH research, large gaps remain in our awareness of how patients perceive their disease. This study utilized ethnography, a unique qualitative research methodology based on participant-led observations, to acquire a better understanding of the patient's perspective of QoL, disease management, and the patient–healthcare professional (HCP) relationship in PH.

Methods: Patients were recruited through HCPs and patient associations. They were included if they were aged ≥18 years, had been diagnosed with pulmonary arterial hypertension (PAH) or chronic thromboembolic PH (CTEPH) for ≥6 months, and were receiving PAH-specific medication. The study used interviews and observations to assess real-life patterns of behavior. Patients completed diaries before being interviewed to gauge the emotional impact of living with PH. The researcher then observed the patient in their home for up to 6 hours, capturing the environment, interactions, and activities of everyday life. A total of 140 hours of footage were independently reviewed and analyzed by several ethnographers within the research team. The data were cut thematically and analyzed for behavioral themes including: daily routines, impact on lifestyle, future outlook, information and support sources, medication practicalities, compliance, and emotional attachment to therapy. These observations were then compared with stated patient responses.

Results: In total, 39 patients with PH (PAH=34, CTEPH=5; NYHA FC I=2, II=14, III=17, IV=6; 29 females; age range 19–91 years) were enrolled from 7 countries across 4 continents. In addition to the severe limitations imposed by the disease on patients' lifestyles, a striking outcome of the study was the reported secrecy surrounding PH. Many patients had a poor understanding of PH and found their 'invisible' disease difficult to explain to others. Feelings of insecurity, isolation, and depression were regularly reported, with many patients admitting to hiding their symptoms. The majority recalled the pre-diagnosis phase of PH as being defined by feelings of anxiety and a fear of being judged as lazy or unfit. Following diagnosis, access to medication played an integral role in their lives, providing symptomatic relief and improving QoL.

Thus, compliance with, and emotional attachment to, medication were observed to be high. The marked improvement in symptoms after initiation of therapy made assessment of disease progression more difficult as patients tended to compare their QoL against a pre-treatment level and were less aware of ongoing subtle changes. In terms of disease coping strategies, patients fell into 2 categories: solution-seekers who developed tactics to cope with PH on a daily basis; or disease-dominated who had a greater dependency on caregivers and a more passive attitude toward PH. Regardless of the strategy adopted, patients stated that extensive planning and adherence to daily routines were required in everyday life. Many patients were unaware of, and reluctant to discuss, their prognosis. The enforced dependencyon caregivers that followed diagnosis was an aspect of PH that patients were unprepared for, and the majority appreciated personal contact with expert PH HCPs.

Conclusions: This study provides a unique real-life insight into PH from the patient's perspective, uncovering a number of findings that would not typically be revealed by other qualitative approaches. It highlights the secrecy surrounding PH, the difficulties in describing the disease, and the challenges in assessing disease progression. A more tailored dissemination of information and a simple and understandable definition of PH may prove beneficial to patients. A greater appreciation by HCPs and caregivers of how patients perceive their disease and their QoL has the potential to improve PH management.

BASIC SCIENCE:

Pulmonary Arterial Hypertension Induces Gene Expression Changes in the Right Ventricle in Advance of Right Ventricular Failure that Are More Severe in Female Rats

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Background: Right ventricular (RV) failure is the leading cause of morbidity and mortality in patients with pulmonary arterial hypertension (PAH), and females represent up to 75% of patients with PAH. However, most animal models of PAH focus on male rats precluding an analysis of sex-specific changes in RV adaptation or dysfunction. We analyzed genome-wide mRNA expression patterns in the RV of both female and male rat models of severe PAH to determine whether changes occur prior to the onset of RV failure, whether these changes resemble those characteristic of left ventricular (LV) failure, and whether there are sex-specific biological differences in RV failure.

Methods: 6 week-old female and male rats underwent left pneumonectomy or sham surgery followed by 50 mg/kg MCT 7 days later to induce severe, neointimal PAH. Rats underwent transthoracic echocardiography and continuous ambulatory invasive right heart hemodynamic monitoring. Cardiac tissue was harvested and RNA expression profiles were generated by microarrays from female ($n=3$) and male ($n=4$) rats with PAH 10 days following MCT, and from female ($n=4$) and male ($n=4$) control rats.

Results: Experimental rats exhibited significantly elevated pulmonary pressures but grossly normal RV size and function prior to sacrifice. 195 genes were differentially expressed in the RV of rats with PAH relative to normal control rats. These genes were involved in calcium signaling, myocyte contraction, mitochondrial function, extracellular matrix remodeling, cell proliferation, and cell membrane and cytoskeleton structure. Expression changes in *Emp3*, *Fn1*, *Hspb1*, *Mgp*, *S100a4* and *Timp1* were confirmed by real-time quantitative PCR in RV. Expression of these genes was unchanged in the LV. In general, female PAH rats exhibited more extreme gene expression changes than male PAH rats.

Conclusions: We have documented gene expression changes in RV of rats with PAH prior to the appearance of significant RV enlargement. These changes resemble those occurring in LV failure but appear to be more severe in female relative to male rats.

BASIC SCIENCE:

Mechanobiological Feedback Amplification of Vascular Remodeling in Pulmonary Arterial Hypertension is Modulated by COX-2-Derived Prostanoids

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Background: Recent studies suggest that increased pulmonary arterial stiffness contributes significantly to increased right ventricular afterload and is associated with increased mortality in pulmonary arterial hypertension (PAH) patients, however the role of PA stiffening in the pathogenesis of PAH has not yet been fully elucidated.

Methods: Male Sprague-Dawley rats were treated with SU5416 (20 mg/kg) or vehicle subcutaneously and exposed to hypoxia (10% FiO_2) for three weeks followed by re-exposure to normoxia for 2, 5, or 10 weeks (for a total of 5, 8, and 13 weeks). Lungs were harvested and pulmonary arterioles were mechanically characterized using atomic force microscopy (AFM) microindentation. Human PASMC were cultured on synthetic polyacrylamide substrates of defined stiffness spanning a shear modulus range of 0.1 to 25.6 kPa.

Results: SU5416/hypoxia-exposed rats developed dramatic increases in right ventricular systolic pressure (58 ± 2.5 vs. 22 ± 1 mm Hg) and Fulton's index (0.63 ± 0.04 vs. 0.14 ± 0.04) compared with controls, as well as marked pulmonary vascular remodeling. SU5416/hypoxia-exposed rats developed significant increases in stiffness in pulmonary arterioles <100 μm (1.26 ± 0.8 kPa) compared with controls at 5 weeks, with progressive and sustained increases in PA stiffness at 8 and 13 weeks. Interestingly, pulmonary arterioles >100 μm demonstrated no increase in stiffness early following SU5416/hypoxia, however subsequently developed significant increases in

shear modulus at 8 weeks (2.8 ± 2.5 kPa) and 13 weeks (3.1 ± 1.1 kPa) compared with controls. PASMC grown on substrates that span this stiffness range demonstrated exaggerated contractility and enhanced matrix deposition with increasing substrate stiffness, as well as increased proliferation, decreased apoptosis, and reduced cyclooxygenase-2 (COX-2)-derived prostanoid expression. Treatment with iloprost, a PGI_2 analog, significantly attenuated stiffness-dependent increases in PASMC proliferation, matrix deposition, and contractility. Furthermore, increased matrix stiffness led to a significant reduction in COX-2 promoter activity in transiently transfected PASMC grown on substrates of pathologic stiffness.

Conclusions: Our results demonstrate that matrix remodeling in the pulmonary arterial wall fundamentally biases cellular behavior towards progressive vascular remodeling via previously unrecognized effects of matrix stiffening and suggest a central role for COX-2 in orchestrating stiffness-driven amplification of vascular remodeling.