PVRI Chronicle

News, Discussions, Science and Medicine from the Pulmonary Vascular Research Institute

Today’s work, tomorrow’s possibility.
The Journal

PVRI Chronicle (ISSN 2057-5353) is a non-peer reviewed journal published on behalf of the Pulmonary Vascular Research Institute. The journal publishes articles, reviews and commentaries on the subject of pulmonary vascular diseases and actions within the PVRI. The journal is published bi-annually online and is available in print upon request.

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As the Olympic Games in Rio are coming to an end, I will stay with the theme and happily accept the torch Sachindra Joshi passed on to me. I will try to fill his shoes as new Editor in Chief for the PVRI Chronicle - though they seem a little large.

Who am I?

Well, I am still struggling with the same question myself, but here the easy answer: I live in the US in Pittsburgh Pennsylvania and try to uncover the mysteries underlying pulmonary hypertension from my perspective – focused on the innate immune system. While it may seem obvious to assume that I belong to the Department of Pulmonary Medicine (or at least cardiology), I am actually in the Department of Surgery, which became well known for their work in regards to understanding the innate immune system long before I ever learned to hold a pipette. I carry the official title of Assistant Professor and am lucky enough to have my own laboratory.

On a personal level I am a YOUNG 45 year old woman – and I guess thus making me still eligible to belong to the Committee of Young Clinicians and Scientists. I was born and raised in Germany and came to the US as young adult. I have three children, all teenagers, and just recently got divorced. And yes, as family, we are dealing with our own fair share of teenage related problems.

This current issue has all of the sections you have come to know previously and hopefully enjoy. Without listing all of the articles here I would like to thank all of the contributors who took time from their busy schedules to write and share their work here with us. I also like to make one point: If you look in detail at the contributors of this issues (and previous ones), you will notice that a large percentage of our articles come from the German group in Giessen. I cannot thank them all enough! However, where would we be if our friends in Giessen decided to stop writing articles for the chronicle? The PVRI is a global group creating connections between lung scientists and clinicians throughout the world. I urge you all to consider writing for us! While lung disease may be the same everywhere, every country has different approaches and experiences dealing with it and it would be lovely to have more international members share their work or comments with us. Some of my favorite talks at the meeting in Rome in January were the ones that presented on their approaches of treating patients with lung disease in countries struggling with obtaining all the “right and recommended” medicines. I was very impressed by their creativity and innovation. Wouldn't it be great if we had ten articles from ten different countries?

I will leave you with this quote as we struggle along to try and understand the underlying mechanisms of pulmonary vascular disease and trying to find a way to cure patients:

“Mistakes are, after all, the foundations of truth, and if a man does not know what a thing is, it is at least an increase in knowledge if he knows what it is not.”

- Carl Jung
Unexplained exertional dyspnea caused by low ventricular filling pressures: results from clinical invasive cardiopulmonary exercise testing

adapted from Oldham WM et al. Pulm Circ. Vol. 6, No. 1, pp. 55-62
http://www.journals.uchicago.edu/doi/full/10.1086/685054

Infographic Oldham

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ART CLUB
I recently had the pleasure of interviewing Dr. Stephen Chan for the PVRI Chronicle. Dr. Chan is an MD-PhD Clinician Scientist and Director of the University of Pittsburgh Center for Pulmonary Vascular Biology and Medicine. He is a practising cardiologist and a well funded, prolific scientist with numerous high impact publications. He also is a warm, welcoming and down-to-earth person who was happy to meet with me and answer all of my questions. The following conversation ensued:

Q: Tell me a little about your background and your career path leading to your current position.

A: I am a physician scientist and was originally interested in biomedical research that would directly impact the quality and service of patients in need. I had this desire since I was a young adult back in college and it is what drove me to pursue medical school and join the MD-PhD program at UCSF. I initially trained as a molecular biologist in virology and infectious disease. Though the training was very good, I ultimately decided to switch fields, when I became exposed to clinical cardiology and vascular disease in general. I believed I could make the most impact in this area. After graduating with both degrees, MD-PhD, I joined the internal medicine residency program in Boston at BWH followed by a fellowship in cardiology at Massachusetts General Hospital. It was during my fellowship that I became much more acquainted with the disease of pulmonary hypertension (PH). PH struck me from two perspectives: 1) our understanding of what this disease is, how it occurs and 2) how it is being treated. At the same time, I witnessed the devastating effects of this disease in our patients. This drove me, at least clinically, to delve deeper into exploration of the disease. Now, as a basic research scientist, PH became very appealing to me because I realized that we knew very little about the disease. It had been historically neglected and is also often considered an orphan disease. It was very exciting to me to feel I could make an impact in an area where there was still unmet need. That’s how I began to train as a physician scientist in pulmonary hypertension. I became a postdoctoral fellow with Joseph Loscalzo, who is the Chairman of Medicine in BWH. Dr. Loscalzo has been interested in the study of PH for decades. I also trained clinically at BWH with Aaron Waxman in the Center for Pulmonary Vascular Medicine. This was truly the start of my independent career both as a physician scientist as well as a clinician. I recently moved to the University of Pittsburgh where I now direct the Center for Pulmonary Vascular Biology and Medicine with the goal to combine both clinical and research operations to optimize patient care.

Q: Looking at the PH field right now, both from the perspective of a clinician and a physician scientist, would you consider that we have made a lot of headway in the past years and where do you see the field going in the next few years?

A: Over the past five to ten years it definitely seems that there has been an influx of new information, particularly from the molecular aspects of understanding this disease. I believe even from the clinical perspective we have now a better perspective of our patients, how they develop the disease and how it progresses. We seem to be on the upslope and I believe that we
are hitting an inflection point of gaining great traction into establishing new paradigms for this disease, both from a clinical and research perspective. It has been helpful not only in terms of the interests from our trainees who embraced the tasks of pushing the field to the next level, but also by increasing community awareness. The industry and pharma are also more aware and willing to invest into PH research, as is the federal government via the NIH. We are now beginning to see the payoff of this focus, at least in small form and still have a long way to go. Certainly other fields such as cancer and atherosclerosis for instance, are light-years ahead of us in terms of disease understanding and how to best approach the complexities of patient care and pathogenesis. I am convinced we are on the right track but we still have a long way to go.

Q: What do you think is the next big thing - or should be - in terms of PH research?

A: It depends on where you look. There are still a number of holes in our understanding of how we treat PH. I will highlight a few of the ones our program is interested in. The first is about early diagnosis and our understanding of early pathogenesis of PH. For years we have been fixated on trying to understand the end stages of this chronic disease. This has given us a limited understanding of where the disease is coming from. But we now know that probably what is going to drive the next era of therapeutics and management of PH is trying to understand how to identify those patients who are at risk or those who are just developing this disease. We would love to prevent PH rather than having to treat something that has developed for many years. We may want to understand the molecular processes initiating PH. We may want to try to design new diagnostics in order to understand the beginning stages of the disease. Investigating exercise physiology, for example, is one particular notion that may be addressed this way. Also, we may want to pursue novel imaging modalities of looking at early stage disease in the context of the pathways that we have yet to discover. I believe that early diagnostics will be an important area to focus on.

Secondly, it would be interesting to develop personalized medicine for PH. We are certainly behind the times compared to other areas in medicine, such as cancer or atherosclerosis, where “big data” are already in play in trying to allow for individualized management and patient care. I’m not just focused on genomics, but also other types of high throughput analyses, be it at the molecular level, such as metabolomix or expression data, or in the clinical realm where clinical analytics can be utilized to understand big population type data from electronic health patient. This is going to be a very important concept. I believe the integration of computational methods, bioinformatics, high throughput molecular understanding is going to allow us to drive that particular phase forward.

Third, we would be happy to usher in the next generation of therapeutics, be it small molecule inhibitors or otherwise, that would allow us to treat PH in a better fashion. This will also entail understanding the disease at a much finer level in order to target pathways other than the big three that we already have drugs for (prostacyclin, endothelin, and nitric oxide).

Q: Can you tell us a little about the PH program here at the University of Pittsburgh?

A: I arrived about 7 months ago as the Director for the Center for Pulmonary Vascular Biology and Medicine. It was an added bonus for me was that this program already had such phenomenal basic scientists and clinicians in place. This is an exciting time for us here at the...
University of Pittsburgh because we feel that we have a critical mass of scientists and clinicians that will allow us to integrate those disciplines to optimize patient care. We focus on innovation and research and we pride ourselves on research opportunities at the basic, translational and clinical levels. We believe we have the manpower as well as the commitment from the institution in order to pursue those types of endeavors. Several of our investigators are looking at many different pathways that we think are important in PH. At the molecular level, we are thinking not only of vasomotor tone, proliferation and survival mechanisms (all important in pulmonary vasculature and right ventricle), but also of other pathways that have not been previously studied. This includes metabolism, vascular stiffness, other phenotypes that we think are also playing an important role in this disease.

We also have a number of translational components that we are very proud of in terms of making sure that our basic science is really translated to the clinical realm not only in the therapeutic arm but also diagnostically. Accomplishing this doesn't happen just by accident and oftentimes in the field of PH it really relies upon the individual investigator to establish those types of collaborations. Because we have a critical mass of investigators who will be here long term, we have instituted a number of structural modalities to allow for that type of collaboration to occur. One of those, certainly, is the idea that we are very intent on building a very unique database of centralized information not only from our electronic health records system but also in combination with high throughput molecular data directly from bio-banked patient samples. This includes blood, plasma, tissue and cells. We believe that that will be a very prominent resource for what we think is going to be important in personalized medicine in pulmonary hypertension. We also have a very strong understanding that in order for our home-grown science to be translated to patient care we need to be on the forefront of that effort, rather than simply making the discovery and leaving it up to others who may or may not want to take it on. In that sense we've also put in a lot of infrastructure and resources in order to ensure that we have the ability to act as our own facility of developing drugs from the ground up, i.e. from the pre-clinical modeling, pharmacodynamics and pharmacokinetics all the way to first-in-human studies. We believe we have an opportunity to really take it from soup to nuts. So that's another very big push not only for our center but also for the institution in general to ensure that drug discovery remains a very high priority. We also would like to ensure that the integration of multiple different disciplines continues to be prioritized here. We pride ourselves on having a very integrated program at least between pulmonary and cardiology clinicians for the care of pulmonary hypertension. We also partner with a number of other disciplines such as rheumatology, sickle cell disease, interstitial lung disease, transplant, surgery and so on. That's a very big component of what we pride ourselves on and we want to train the next generation of investigators, both clinicians and researchers, in order to allow for them to have that type of training. Right now, we have both pulmonary and cardiology fellows training in PH in a number of different regards. We want to make sure that's a real dedicated program. That will take a little bit of institutional and/or philanthropic money to do so but we are intent on making sure that we prioritize the training and mentorship of our physician scientists going forward. Finally, one of the things that we also want to ensure is that we have a very meaningful relationship with our community of patients and advocacy groups in order to make sure that we offer the most holistic care to our patients. We
have a very large program of physician and patient outreach locally, nationally and internationally. We are interested in determining whether new innovative techniques such as telemedicine services may also be something that we could offer not only in the region but also to patients perhaps in resource-poor environments throughout the world. We think that our expertise may actually have an impact there as well. So those are some examples of where our program is going in addition to the individual efforts of our investigators to really advance the care of these patients.

Q: Being part of the PVRI you have probably seen the great contribution they make to the field. Where do you see the PVRI going in the future and what is an area that you would think this institute can have an instrumental role in pushing the field forward?

A: The PVRI is a great group and institution because I believe, at least from my work with them, two things are very apparent. One of which it is a very international program and I think that one of the key areas from what I’ve heard from the leadership of the PVRI is that they want to maintain true international collaboration and not just be dominated by the same groups in the developed regions such as the US, Europe and some of the countries in Asia but certainly everywhere else including resource poor environments. That is a huge unmet need for this disease in terms of making the appropriate diagnosis and treatment of patients. Again, as I mentioned, we would love to be able to partner with someone like the PVRI in order to institute a program of telemedicine of sorts in order for us to really allow for communication between experts in the field as well as patients throughout the world. That will allow for a much more connected group and I think it would optimize the care of those patients that really deserve better treatment then what they are getting right now. I think that is one very laudable goal of the PVRI that speaks to why we would love to partner with them. Secondly I noticed that the PVRI is very invested in research and innovation and that it is very apparent when going to their meetings. Their research component is a very big priority and we appreciate that here in our program because innovation is probably our top priority in terms of research and development. We would love to work in concert and conjunction with the PVRI as it goes forward. I think that the PVRI also has a great opportunity to really invest more resources for research in general. I think that that program is still getting off the ground in terms of it but I think that it really puts itself in a good position to do so and we’d love to partner in that realm as well.

Q: For the young junior faculty and post-docs, given the current environment of difficult funding and limited resources not just here in the US but worldwide, what advice do you have for them and what do you think they should focus on the most in securing a transition to the next step and having a successful future as researchers and physician scientists?

A: It certainly it is a very difficult time for everybody in terms of getting funding. Often times it is difficult for a person, especially at a young stage, to be optimistic if they hear all of the stories of difficulties in funding and how hard a road it really is. On the flip side, however, I actually feel quite a bit of optimism being in this field at this stage of the game. I think that there are two reasons for that. One of which, is that it is a phenomenal time to be a scientist and a research investigator in pulmonary hypertension. The technologies have gotten so
much better than they had been ten or fifteen years ago. There are so many more opportunities for a young investigator to really sink their teeth into and truly make an impact. Around fifteen years ago, there had been a very limited amount of research, so you’re going to have to start from scratch. At this stage, I feel there is so much technology and advancement, not just in the PH field, but also in other disciplines that we can learn from and leverage in order to make important discoveries in this field. I think there is great excitement and optimism to be had in terms of making profound discoveries at this stage in the next five to ten years. I like the direction in that regard. In terms of raising money I think that ironically, even though financial investment in research and research endeavors in general throughout the world has decreased, the PH field is actually on an upswing. If you were to look at the amount of money that is being invested and is available to the PH researcher and the young investigator included, I think that it is actually more than it was fifteen years ago. Fifteen years ago, there was less awareness and there was less interest from industry and federal partners to understand this disease better. Now I think we have critical mass and an inflection point of interest and persona in order to make this happen. I think that it is actually a really good time for a young investigator to be in this field. Even though we don’t get as much money on an absolute level as say cancer or other types of cardiovascular disease, I still feel the ratio of really good investigators to the amount of money that is being spent is very favorable for the person who is doing very strong science and making very strong discoveries. If you are to compare it to some of the other fields where there is a lot more saturation in terms of the competition, I feel perhaps even the competition is less in this regard and that, if you are well trained and you have really good ide-
A Colloquium on HIV and Pulmonary Diseases

Summary

Infection with human immunodeficiency virus (HIV) is now a chronic disease, thanks to the unquestioned success of antiretroviral therapies. Nevertheless, patients, clinicians and researchers are still facing challenges thirty years after the discovery of the virus. HIV has cleverly tricked both the host immune system and antiretroviral therapy (ART). As a first instance, the many HIV subtypes and recombinant forms have different susceptibilities to antiretroviral drugs, which may represent an issue in countries where ART is just being made available. Second, even under ART-induced viral suppression, HIV still promotes inflammation, deregulates bystander cell biology, and induces oxidative stress in the host. Third, the preference of HIV for CXCR4 as co-receptor may also have noxious outcomes including potential malignancies. Furthermore, HIV still replicates cryptically in anatomical reservoirs like the lung and impairs bronchoalveolar cell immune responses, rendering the lung susceptible to co-morbidities. Hence, it is becoming evident that HIV-infected individuals are significantly more susceptible to long-term HIV-associated complications, particularly now that HIV-infected individuals on ART live as long as the uninfected population. With this review, we will focus on chronic obstructive pulmonary disease (COPD), pulmonary arterial hypertension and lung cancer to hopefully braid concepts, give good starting points, and food for thought to pulmonologists, HIV specialists, cardiologists, and the new generations of scientists to jumpstart new efforts towards HIV-associated pulmonary diseases as common goal.

Introduction

Our respiratory tract is exposed to a myriad of everything from gases, dusts, pollens, oxidants, gastric contents, live pathogenic and non-pathogenic bacteria, fungi, and viruses. Together, these represent relentless challenges to the respiratory immune system, which relies in physical aerodynamic and immune barriers to maintain the lungs in good health; this would ensure an undisturbed gas exchange process, which is the ultimate physiologic goal of the lung. In addition, systemic diseases like infection with human immunodeficiency virus (HIV) may affect the lung. This colloquial review will focus on the impact of HIV in pulmonary immunology and the new challenges in both developed and developing countries, focusing on the non-infectious complications of HIV disease.

Human Immunodeficiency Virus: A Constant Challenge

HIV causes AIDS: that's not new; it has been the topic of intense research efforts all over the world for over 30 years now. HIV produces billions of virions per day and has a rapid turnover with new generations every 2.6 days [1]. Due to its extensive genetic variability, the main group of HIV type 1 is subtyped into nine genetic variants (A, B, C, D, F, G, H, J, and K), all of which recombine, which in turn introduce differences in mutation rates and fitness. Understanding HIV interactions with the host is
essential to learn about the viral strategies to induce pathogenicity and to identify potential additional therapeutic targets. Let’s start a discussion of key concepts on HIV entry, persistence and pathogenesis.

**HIV receptors.** HIV enters the cells via interactions with CD4 receptor in the host cell and C-C chemokine receptor-5 (CCR5) and C-X-C chemokine receptor-4 (CXCR4). The CCR5 is a receptor for RANTES/CCL5, MIP-α/CCL3, and MIP-β/CCL4 in primary macrophages [2]. The CCR5 receptor is expressed in microglia, T lymphocytes, macrophages and dendritic cells (DC). On the other hand, CXCR4 is a 7-transmembrane G protein-coupled receptor used by HIV as co-receptor for preferential entry to T cells lines [3]. Its natural ligand is stromal derived factor-1 (SDF-1/CXCL12) [4]. Conventionally, HIV virions that use CCR5 as portal of entry are designated as “R5”, while virions using CXCR4 are referred to as “X4”. The HIV preference for CCR5 co-receptor switches to a preference for CXCR4 over the course of HIV infection; this co-receptor switch predicts progression to AIDS in ~50% of HIV+ individuals [5].

**HIV-mediated evasion of immune surveillance.** HIV hides in cells by downregulation of key host receptors to evade immune surveillance [6]. For example, HIV-Nef is a key player in HIV pathogenesis by enhancing infectivity and downregulating critical molecules such as major histocompatibility complex-1 (MHC-1) and CD4 receptors [7]. It is known that Nef downregulates the CD4 receptor by targeting it to the endocytic degradation pathway in clathrin-coated vesicles. CD4 downregulation stimulates viral replication in primary T cells [8]. HIV Nef also downregulates MHC-1 by sequestering it in the trans-Golgi and hence, it prevents the recycling of this receptor from the Golgi to the membrane. Nef has highly conserved protein-protein interaction domains essential for these functions [9]. In addition, the Nef signature motifs used to downregulate MHC-1 are also used to downregulate CXCR4 and CCR5 which decreases the chances of HIV superinfection [10, 11] and the SOS call to the immune system.

**HIV Hiding Places: Reservoirs.** HIV-infected individuals who are compliant to antiretroviral therapy (ART) show an apparent clearance of the virus in the peripheral blood shortly after initiating therapy. Nevertheless, viral particles can be detected after interruption of antiretrovirals [12-14] and there is genetic HIV evolution over time in patients with undetectable viremia, suggesting a continuous low level replication of HIV even below the limits of clinical detection. This notion has been supported by the finding that in the presence of suppressive ART, the integrated HIV (AKA. archival, proviral HIV) and extrachromosomal HIV (episomal, surrogate for recent infection) belong to different viral populations [15].

**Where does the virus hide?** Resting T lymphocytes (memory cells) or long-lived myeloid cells (macrophages and DC) remain transcriptionally silent for long periods of time while having integrated copies of the HIV genome, especially in the presence of antiretroviral therapy [16-19]. The activation of these cells resumes the production of infectious particles and hence, the story repeats all over again by infection of new cells ⇔ reseeding of the reservoir ⇔ return to resting state and perpetuation of the persistence of HIV. At the organ/system level, anatomic compartments that may serve as reservoirs of HIV include the central nervous system [20, 21], the genitourinary tract [22, 23], and the gut-associated lymphoid tissue [24, 25].

The pulmonary microenvironment can also embrace high levels of HIV replication [26] [27] [28]. In the alveoli, lymphocytes are more susceptible to HIV infection than macrophages. HIV infects 1 in 100 of CD4+ alveolar lymphocytes [29] and 1 in 1,000 alveolar macrophages (AM) [30]. The alveolar space harbors small and large sub-populations of macrophages, which differ not only in morphology...
but also in cell surface markers [31, 32]. HIV preferentially infects the small macrophages, which exhibit more of highly active inflammatory phenotypes [33].

May the lungs act as anatomical reservoirs for HIV? Studies in the 90’s showed significantly complete phylogenetic separation of the HIV lineages in the lung, blood, brain and testis [34] [35]. A decade later, studies that compared HIV env sequences spanning the second constant and the fifth variable region (C2-V5) from matched blood and lung samples (either lung spuota or BALc) found lung-specific evolution in up to 56% of HIV-infected individuals [36]. The existence of HIV reservoirs demonstrates that ART does not eliminate from the host. Although evidence points to lung-specific viral evolution, the lung is not as anatomically enclosed as the brain and hence, viruses circulate freely - aided by the active blood flow through the cardiopulmonary system. In light of this, it is clear that HIV may contribute to immune disturbances leading to pulmonary complications.

HIV as an intrapulmonary pathogen: While some studies suggest that alveolar macrophages are resilient to HIV infection and remain competent to respond to Streptococcus pneumoniae [37, 38] and Cryptococcus neoformans [39], others suggest that HIV alters the pulmonary cell biology to the point that compared to HIV-uninfected counterparts, HIV-infected individuals have lower secretion of IFN-γ and tumor necrosis factor-alpha (TNF-α) in the lung, significantly increased RANTES and lysozyme in the BALf [40], marked cellular activation and accumulation of inflammatory mediators in the alveolar space, including increased HIV-specific CD8+ T cells. All these get complicated by smoking, which certainly complicates the immunologic landscape in the lungs [41], [42].

The presence of HIV in the lungs also impacts bystander pulmonary resident cells like endothelial cells. Although pulmonary endothelial cells are resistant to HIV infection [43], EC remain susceptible to apoptosis when exposed to HIV proteins [43-46] which suggests that while EC may not represent a cellular source of HIV in the lung, they certainly remain susceptible to the cytopathic effects of HIV that may eventually lead to HIV-associated pulmonary complications including chronic obstructive pulmonary disease (COPD), lung cancer, pulmonary arterial hypertension (PAH), fibrosis and infections [47, 48]. For instance, COPD is characterized by limited expiratory airflow, affecting individuals from 45 to 52 years of age [49], is the third leading cause of mortality in the world [50], and a significant risk factor for hospitalizations in the HIV-infected population [51-53] regardless of smoking status and antiretroviral therapy [51, 54], and is associated with inflammatory markers [48] [55, 56], increased oxidative stress [57]. Of note, contrasting studies reported that there is not a significant risk for COPD (odds ratio (OR)= 1.61) or lung cancer (OR= 2.65) in HIV-infected individuals, particularly in the era post-antiretrovirals [58]. However, it is very likely that the reported findings were masked by the presence of unrecognized COPD because that study relied on self-reported pulmonary diagnoses, which were not clinically confirmed. Together, this suggests that the presence of infectious pathogens like HIV, coupled with abnormal inflammatory responses and oxidative stress all contribute mechanistically to HIV-COPD.

Emphysema is a form of COPD characterized by apoptosis of epithelial and alveolar cells, with various degrees of inflammation. While cigarette smoking is a major cause of COPD/emphysema [59], HIV is another risk factor for COPD, regardless of smoking status. Histologically, HIV is mostly found in the emphysematous regions of the lung, while very rare HIV+ cells are present in normal lung areas [60], suggesting a direct role of HIV and/or HIV proteins in emphysema. One of the mechanistic insights offered for the lung endothelial cell apoptosis is the upregulation of the inflammatory cytokine...
endothelial monocyte activating polypeptide II (EMAP II) [61] and that such upregulation is induced by gp120 signaling through the CXCR4 receptor and activation of p38 MAPK [45].

Relevant to chronic bronchitis, CXCR4 and HIV-X4 viruses are implicated in the overproduction of mucus and mucous cell metaplasia in human bronchial epithelial cells in vitro, via the CXCR4/α7-nicotinic acetylcholine receptor/γ-aminobutyric acid (GABA)-A receptor axis [62]. These results further support a potential role of HIV in higher incidence of COPD.

2) Pulmonary arterial hypertension is a rare disease in the general population, affecting 1-2 persons per million individuals but is significantly more frequent in the HIV-infected population, regardless of gender, age, socio-demographic characteristics, duration of HIV diagnosis, and interventions with ART.

Listed in the Group 1 of clinical classification of the 5th World Symposium of Pulmonary Hypertension [63] HIV-associated PAH is characterized by increased inflammatory cytokines, atypical pulmonary vascular remodeling featured quasi-malignant phenotype of pulmonary endothelial cells [64, 65], and highly glycolytic pulmonary artery vascular cells [66, 67]. In addition, a signature of PAH is the presence of cells that obliterate the lumina of pulmonary arteries (plexiform lesions) [68]; therefore, the mean pulmonary artery pressures increase (mPAP >25 mmHg), ending fatally due to right heart failure. PAH can be screened by echocardiography, which measures PASP and diagnosed by right heart catheterization (RHC), which measures mPAP; final diagnosis is made based on mPAP > 25 mm Hg, pulmonary arteriolar wedge pressure <15 mmHg and pulmonary vascular resistance > 3 Wood units. Clinically, HIV-PAH presents as any idiopathic PAH. Symptoms are often nonspecific and insidious, so they are attributed to other complications of HIV or HIV itself. The time of presentation to the diagnosis is often long, from 6 to 2 years[69], or many times it is just overlooked. The prevalence of PAH in HIV-infected population is usually reported to be 1 in 200 (0.5%) individuals. Nonetheless, the awareness of HIV-PAH has increased in medical communities worldwide, as evidenced by the coordination of taskforces aimed to screen patients who are asymptomatic for pulmonary arterial hypertension. PAH has been recently reported to affect 0.2-12.7% of HIV-infected individuals in several countries, based on either echocardiographic PASP or RHC. Based on these results, and the fact that PAH screening and diagnostic tools are not part of the routine clinical care to HIV-infected individuals, there are two questions on the table: should all patients with PAH should undergo HIV testing [70]? or should all patients with HIV undergo screening for PAH?

A study comparing pressures measured by Doppler-based echocardiography vs right heart catheterization showed that 19.7% of the Doppler-based measurements were inaccurate, missing the PAH phenotype in 1/3 patients [71] Despite this, the reality is that many patients may just decline the RHC procedure, are ineligible or it may just not be available, especially in low-resource settings. Hence, the best scenario in many instances is to retrieve echocardiography data and use PASP 30-35 mm Hg as a cutoff for echocardiographic abnormalities associated with PAH, with the caveat that some of these data may still be underestimated but at least, accounted for.

The increased prevalence of HIV-PAH (whether accurate or underestimated) has been documented by several studies worldwide but, do we have new ideas about new mechanisms and targets for therapy? Do we really know what in HIV increases the chances of PAH? There is no definitive proof that HIV causes PAH and no evidence that HIV infects lung endothelial cells [57]. However, viral proteins and their interactions with molecular partners in the infected cells may damage endothelial cells, induce inflammation and deregulate apoptosis and proliferation of vascular endothelial cells in the lung, resulting in pulmonary vascular remodeling featured in PAH patients [44, 45, 72-74]. Primate
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models recapitulate intimal and medial hyperplasia along with elevated pulmonary pressures associated with human PAH, as reported after infection of the animals with chimeric SHIVenv virions [75]. Additional HIV proteins like Nef co-localizes with EC in PAH-like plexiform lesions and promotes severe dysfunction of the Golgi tethers at the subcellular level [76-78]. Additional studies found Nef signature sequences associated with the PAH phenotype in humans [79]. Together, these studies suggest that HIV proteins play key roles in the pathogenesis of HIV-PAH.

The combination of HIV –and/or its proteins- and recreational drugs like cocaine exacerbates pulmonary arteriopathies. For example, HIV Tat and cocaine disrupts tight junction proteins, increases the expression of platelet-derived growth factor and increases the proliferation of pulmonary smooth muscle cells particularly when Tat and cocaine are combined[80]. Moreover, macaques exposed to the simian immunodeficiency virus (simian homologue of HIV) and morphine exhibit significant pulmonary vascular remodeling and oxidative-stress mediated apoptosis of endothelial cells followed by proliferation of apoptosis-resistant cells followed by proliferation of apoptosis-resistant cells [81].

3) HIV-associated malignancies that define AIDS (e.g. Kaposi's sarcoma, non-Hodgkin lymphoma, and cervical cancer) have decreased in the post-ART era; nonetheless, the incidence of non-AIDS – defining cancers (NADC) have tripled: lung cancer is now the leading cause of NADC. Up to 52% of deaths in the post-ART era have been ascribed to NADC, including liver, gastric, colorectal and lung malignancies, which occurred in patients with fairly well-controlled HIV disease [82-84]. The most common type of lung cancer in the HIV-infected population is the adenocarcinoma [85], although non–small cell lung cancer (NSCLC) was found in 88% of cases with HIV-associated lung cancer [86]. Compared to the HIV-uninfected population, patients with HIV present with a younger age (mean 50 years) at the time of diagnosis with lung cancer [87]. In addition, most of the affected patients are smokers and unfortunately, present with symptoms of advanced cancer [88]. HIV itself is an independent risk factor for lung cancer, regardless of smoking, COPD and bacterial pneumonia [85]. One of the mechanisms proposed for HIV-associated lung cancer is immunosuppression [83, 84, 89] [90]. Separate studies found a 2.2 relative risk of lung cancer in HIV-infected immunosuppressed patients (with CD4 counts <200 cells/mL), compared to uninfected [83]. Contrasting data from several groups show that lung cancer in HIV-infected individuals is not associated with CD4 counts [91-93], despite the inverse relationship with HIV viral load [94]. Additional mechanistic views into lung malignancies associated with HIV infection are provided by respiratory infections and genomic instability. The HIV-infected population is particularly prone to bacterial pneumonia, in addition to mycobacterial Pneumocystis, and viral respiratory infections. Pulmonary infections, in turn, may increase the risk of lung malignancies in the HIV-infected population [91, 95, 96]. In addition, genomic instability, reflected by microsatellite alterations, has been hypothesized to increase the risk of lung cancer in HIV. Microsatellite alterations, but not the loss of heterozygosity, were significantly increased (6-fold higher) in HV-associated lung carcinomas [97]. What produces this HIV-related genomic instability? A previously unrecognized interaction between HIV and endogenous retrotransposable elements has been uncovered with the finding that HIV infection results in accumulation of Type 1 long-interspersed nuclear elements (L1s) DNA in primary CD4+ lymphocytes [98].

Inflammation is also an ingredient in the recipe for HIV-associated cancers. A study that evaluated activated inflammatory pathways (IL-6 and C-reactive protein) and coagulation pathways (D-dimer) in HIV-infected patients found that individuals with higher levels of IL-6 had significantly higher risk for cancer [99].
Despite the potential underestimations due to calculations based on self-reported disorders, the use of screening tools for diagnosis or even undocumented patient cohorts, the higher susceptibility of HIV+ patients to serious lung complications including COPD, PAH and lung cancers is evident. Inflammation, oxidative stress, de-regulated apoptosis and proliferation, and malignant phenotypes are common denominators in the quest for mechanistic hints. Many of the specifics regarding the direct and indirect role of the virus in these diseases remain undetermined.

**Food For Thought: THE CARDS ON THE TABLE**
We have won many battles against HIV/ADS aided by antiretrovirals but not the war. We are still learning about the HIV molecular tricks and facing challenges thirty years after its discovery. This review presents key concepts of HIV persistence and focused on how the lung resents HIV, echoing to HIV-associated pulmonary complications like COPD, pulmonary arterial hypertension and lung malignancies. We still need systematic epidemiological surveillance to document HIV-associated pulmonary complications globally; therefore, we insist that the crosstalk between pulmonologists, cardiologists, and HIV specialists is essential to document the true prevalence of these diseases, which otherwise would go unnoticed and untreated before the patient's quality of life is seriously deteriorated. Antiretroviral drug toxicity, resistance and drug-drug interactions are issues affecting both the developed and the developing world, which certainly require extensive research, pharmacological formulations, and implementation of revised therapeutic strategies. New research enterprises are certainly warranted at the basic science level. For instance, the role of HIV and HIV-proteins in PAH, particularly at the sub-cellular level and the impact in cellular crosstalk (e.g. EC, macrophages, T cells, SMC) remain as opportunities to carve deeper niches to eventually identify novel therapeutic targets. In addition, it is necessary learn more about the modulation of cellular sources of viruses within reservoirs in order to strategize for a functional eradication of HIV reservoirs. Finally, the role of chronic inflammation in HIV-associated pulmonary diseases is certainly a unifying, hypothesis-generating topic that can take us to the next level.

AUTHOR DISCLOSURE STATEMENT
No competing financial interests exist.

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A Colloquium on HIV and Pulmonary Diseases


COPD/emphysema: cause or consequence of aging/senescence?

Prelude

Chronic obstructive pulmonary disease (COPD) is described as treatable and preventable, but also incurable disease, manifested as persistent airflow limitation with alveolar wall destruction resulting in emphysema which is not fully reversible (1). It is proven to involve inflammation, for instance induced by cigarette smoke, including oxidant-antioxidant and protease-antiprotease imbalances (2, 3). COPD is an increasing global health problem, which is predicted to become the third most common cause of death by 2030. On the one hand, tobacco smoke and air pollution in industrial countries, on the other hand, in house cooking with wood in developing countries are the major causes for COPD. However, in the time of increasing life expectancy, age may contribute to the development of COPD as well (Fig. 1). This is supported by observations that incidence of COPD positively correlates with advanced age (1, 4).

Aging is without doubt an important factor in many diseases (5,6), but there is also an intensive discussion about characterization of premature aging (7) and senescence, cellular aging and its involvement in diseases (8). This article aims to discuss if aging and senescence are critical events for the development of COPD/emphysema. Based on our previous article “Pulmonary arterial hypertension and aging: Is there a connection?” (Vol. 3, Issue 2) describing an involvement of aging in the context of pulmonary hypertension (PH), we now discuss its role in the context of COPD, a disease often associated with PH.

Main article

Aging is a natural and inevitable process represented as progressive loss of physiological integrity, leading to impaired function and lower adaptive capacity to stress (5, 9). Even though aging is one of the most obvious processes, clearly visible at all levels from the cell to organism, it still remains poorly understood. Despite intensive research, there is no unique strong, well-accepted hypothesis providing comprehensive explanation for the mechanism of aging (5, 6), in particular in the context of diseases. However, there are hypotheses which survived constant scientific
challenging. Somatic mutation theory is one of them, which suggests mutations to be accumulated in somatic cells resulting in senescent phenotype (10). Mitochondrial theory suggests similar mechanism, but in mitochondrial DNA causing leaky electron transport chain and increased ROS release (11). Replicative senescence is supported by telomere loss theory, placing telomere shortening as hallmark of senescence (12). Indeed, stress and in particular oxidative stress (shown to be increased in COPD patients) are proven to cause telomere shortening (13). Altered protein and accumulated waste theories have also been postulated associated with decline in activities of proteasomes (14, 15) and chaperones (16), and cellular waste accumulation (17). Kirkwood’s network theory (disposable soma theory) indicates a connection of the mentioned hypotheses. He believes that they occur simultaneously, causing, or at least supporting, each other (5, 18).

Aging is not linked to the chronological age in all cases. For that reason the term premature aging and senescence are used (5, 8). Major efforts are made in distinguishing senescence and premature aging from the actual, chronological, aging of an organism (7). This gives a solid ground to continue discovering triggers (e.g. ROS, cigarette smoke) (4, 9, 19) and markers (e.g. telomerase activity, cell cycle arrest) (12, 16) of senescence.

The lung, like any other organ, ages with progressive functional impairment and reduced capacity to respond to environmental stresses and injury (20, 21). Lung function declines with increasing age and that decline is even greater in smoking individuals (3, 22). Physiologic aging of the lung is associated with dilatation of alveoli with an enlargement of airspaces, a decrease in gas exchange surface area and elasticity (24). This age-dependent loss of elastin fibers is similar to the loss of skin elasticity and wrinkling of the skin that occurs in elderly (25). Age-related changes in lung, also called ‘senile emphysema’, do not include alveolar wall destruction or bronchial inflammation, the usual pathological changes seen in COPD patients (22). Also COPD in non-smokers is not just due to accelerated aging, but aging could contribute to the development of COPD in both, smokers and nonsmokers (22, 26).

Many studies show that telomeres are shorter in COPD patients, but it is unknown whether telomere shortening is a cause or a consequence of COPD (13). Telomere shortening occurs in normal aging and in smokers and patients with COPD. It provides a biological marker for aging and has a critical role in cellular senescence (7, 13, 19). Studies suggest that telomere shortening could make individuals more susceptible to the development of emphysema and lowers the threshold for damage induced by cigarettes (13). Aging cells accumulate damaged proteins due to decreased autophagy (degradation and removal of damaged protein and organelles in lysosomes), and exposure to cigarette smoke can lead to additional cellular damage pushing cells towards senescence (17).

As mentioned above, the most known hallmarks of senescence include telomere attrition (11), cumulative DNA damage, impaired DNA repair (5, 10), protein damage (14, 16, 29) and accumulation of waste products (17). It is believed that these hallmarks lead to terminal cell cycle arrest as an evolutionary preserved defense mechanism preventing malignant transformation (27, 28). In lung alveoli, cells replicate to replace damaged cells but eventually cannot keep up with replacement because of replicative senescence. The consequences of the lack of alveolar cell replacement, which is the case in senescent lung, can be an increase in alveoli size and a decrease of the surface area (13). In aged tissue, this repair system may become inefficient due to the impaired regenerative capacity of stem cells, leading to the accumulation of senescent cells and lowering threshold towards damage which cannot be repaired (6, 26).
COPD pathology and aging are both complex processes involving many mechanisms on cellular level as well as on the level of whole organism. Despite the obvious connection, it still remains unclear which players are really important and in which conditions. Our brief discussion is aimed to stimulate thinking about a core riddle behind the COPD pathology and senescence/aging.

**The question for interactive discussion**

Based on the above described facts, ideas and suggestions, additionally we would like to raise few questions: Is there a strong connection between COPD/emphysema and aging? Is COPD/emphysema a cause or consequence of aging? Senescence and aging are shown to severely impair the regenerative potential of cells and tissues. Thus, does COPD/emphysema develop due to accumulated stress or inadequate repair? Is this disease age-related, or just the lung cannot recover anymore that efficiently? Should the focus of ongoing research be directed to the mechanisms of insult or repair? These questions are directed to all the scientists, clinicians and others interested in this topic across the world to try to answer and expose their own views, perspectives and visions in the next volume/issue of the PVRI Chronicle.

**References:**


Figure 1: Correlation of lung function and age. Lung function increases with growth until maturity and drops with age. Environmental factors such as smoking (amongst others producing reactive oxygen species (ROS)) can negatively influence lung function. Modified from Ito and Barnes (21)
**Targeting PPARγ in Lung fibroblasts: Prospects of therapeutic treatment for IPF**

**Prelude**

Idiopathic pulmonary fibrosis (IPF) is a devastating disease and the most common form of idiopathic interstitial pneumonias. Former assumptions described IPF as a chronic inflammatory disease resulting from a response to an unknown stimulus which later progresses to lung injury and fibrosis. Median survival of patients diagnosed with IPF is reported to be from 2.5 to 3.5 years, and most available treatment compounds have not proved effective, including the antioxidant N-acetylcysteine which is only known to slow the decline in lung functions though has no impact on mortality. Another approach to therapeutically target IPF was by using agonists of peroxisome proliferator-activated receptor γ (PPARγ), as they were reported to possess anti-inflammatory and anti-fibrotic potentials. However, first trials of PPARγ agonists in pulmonary fibrosis did not yield conclusive results, possibly due to vastly diverse experimental approaches used to assess the efficacy of the treatment. Thus, the role of PPARγ in the pathomechanisms of IPF remains elusive. This interactive discussion therefore summarizes the therapeutic role of PPARγ in IPF model systems and proposes new experimental strategies for treatment of IPF with PPARγ agonists. Our aim is also to challenge all researchers interested in this field to express their opinions on the potential of using PPARγ-associated treatment for IPF patients.

IPF is a progressive disease that can result in respiratory failure. IPF is characterized by scarring of lung tissues due to uncontrolled deposition of extracellular matrix (ECM) proteins and further disruption of the lung architecture and normal function. Clinical staging of IPF has been well delineated to inform management practices...
and clinical trials26. Clinical development of IPF starts with a slow progression over time followed by acute exacerbation of symptoms, loss of lung function and early death11, 27, 28. Devastating effects of IPF and high mortality rates therefore demand biological studies to experimentally model the disease, find the underlying molecular mechanisms, and target the pathways involved in IPF progression as means of potential treatment. Despite the clear clinical staging of the disease, pathogenesis of IPF has not yet been fully elucidated. Current hypothesis suggests that an initial lung injury disrupts the alveolar-capillary basement membrane leading to a deterioration of re-epithelialisation and re-endothelialisation and a further cytokine-mediated fibroblast proliferation13. One of the major cytokines involved is the transforming growth factor β1 (TGFβ1)14. TGFβ1 is a ubiquitously expressed cytokine that functions in numerous biological pathways and was shown to be both important for normal physiology, and implicated in playing a role in diverse diseases15, 16. This cytokine is specifically known to mediate the recruitment and activation of fibroblasts during injury17, stimulate the regeneration of connective tissue, and inhibit its degradation18. Additionally, TGFβ1 stimulation leads to transdifferentiation of fibroblasts into aggressive myofibroblasts which are responsible for the production and secretion of components of the ECM19, 20, 21, 22. Interestingly, interfering with the pathways that lead to myofibroblast expansion have been proposed as potential means of treatment for IPF patients 23 since regimens targeting inflammation have been ineffective 24, 25.

**Anti-fibrotic potential of PPARγ agonists in IPF**

In previous studies PPARs were shown to control multiple physiological activities29 and disease conditions. PPARs are part of a superfamily of nuclear receptors activated by lipid-derived substrates30 and regulate gene expression by binding to specific nucleotide sequences called PPAR response elements (PPREs) within promoters31 together with their heterodimeric partners, retinoid X receptors (RXRs)32. Three isotypes of PPARs characterized in lower vertebrates and mammals are PPARα, PPARβ and PPARγ33 of which the latter is subdivided into γ1, γ234, γ335, and γ436 isoforms. PPARs are ubiquitously and specifically expressed in various tissues and cell types37.

Recently, natural and synthetic activators of PPARγ were reported to elicit anti-fibrotic properties and inhibit myofibroblast differentiation and collagen secretion in cultured human lung fibroblasts10, 38, 39, 40. Burgess et al. (2005) suggested that endogenous and synthetic activators of PPARγ can control TGFβ1-mediated profibrotic effects, reverse primary human pulmonary myofibroblast differentiation and collagen production10. In this study, primary human fibroblasts were treated with different PPARγ agonists concomitant with recombinant human TGFβ1. We suggest that the therapeutic effect of PPARγ agonists on myofibroblast after exogenous addition of TGFβ1 to fibroblasts in culture would possibly model the pathogenic mechanism in patients though this may not be the focus of the authors. Furthermore, in this study, PPARγ antagonist could not reverse the inhibition of differentiation of fibroblasts into myofibroblasts by PPARγ agonists, indicating the existence of a possible PPARγ-independent mechanism by the agonists.

Another study by Milam and colleagues (2008) demonstrated that treatment of lung fibroblasts in culture with PPARγ agonists, Troglitazone and ciglitazone, and in vivo administration of Troglitazone to bleomycin-treated lung
mice inhibited TGFβ1-induced myofibroblast differentiation and collagen secretion, showing the potentials of PPARγ agonists as novel therapeutic agents for the treatment of fibrotic lung diseases. Noteworthy is the fact that the mechanism of the inhibition of profibrotic phenotype caused by TGFβ1 is addressed in this study. In the models used, PPARγ agonists were administered in a pre-treatment setting: before TGFβ1 stimulation in vitro, and before induction of lung injury in vivo. Despite the important findings of the study, a drawback in the in vivo model used has to be mentioned: It is known that inflammation caused by lung injury may lead to many disease outcomes and not IPF alone. In addition, pre-treatment with PPARγ ligands may interfere with the inflammatory process before a possible lung remodeling could occur. Thus, the experimental approach could only confirm the anti-inflammatory potential of PPARγ agonists which was reported earlier. In attempts to assess the effectiveness of agonist treatment in an established IPF model, troglitazone was given to another group of mice on day 11 following bleomycin instillation. However, authors provided no evidence of the extent of tissue remodeling and fibrosis on day 11 after bleomycin instillation alone. In all, the authors investigated only the preventive role of PPARγ agonists and not the proposed therapeutic effects on IPF. This experimental model however presents further questions regarding the therapeutic potential of PPARγ ligands.

Ferguson et al. (2009) also reported that PPARγ ligands and 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) block critical TGFβ1-mediated profibrogenic activities through pathways independent of PPARγ after co-treating human lung fibroblasts with TGFβ1 and PPARγ ligands. The study did not investigate the exogenously secreted collagens in the ECM. Moreover, it is well known that IPF progression is characterized by aberrant cellular release of collagens, proteoglycans, tenascin, laminin, and fibronectin into the ECM. The study also proposed possible roles of PPARγ agonists and CDDO in the inhibition of myofibroblast differentiation in IPF, though it would be interesting to know the regulation of fibrosis markers by PPARγ agonists after TGFβ1 stimulation.

Lin et al. (2010) defined the role of rosiglitazone, a PPARγ agonist, in suppressing myofibroblast transdifferentiation. The study suggested a potential preventive role of PPARγ agonist in pulmonary fibrosis since human fetal lung fibroblasts were co-treated with TGFβ1 and rosiglitazone. Rosiglitazone downregulated αSMA, however IPF markers specifically the abundance of ECM proteins such as collagen was not investigated.

**Question for interactive discussion**

Translation of in vitro and in vivo studies to clinical applications may be very challenging from the viewpoint of predicting the efficacy of the developed experimental approaches in alleviating clinical symptoms of patients. Though much progress has been made towards the understanding of the pathogenesis of IPF with regards to the PPARγ pathway, studies are often limited to focusing on the inhibition of fibroblast to myofibroblast conversion using PPARγ agonists (figure 1A). Cells accumulated at the fibroblastic foci of IPF patients present a mixed population of fibroblasts and myofibroblasts. We therefore query the molecular effect of PPARγ agonists on the already existing myofibroblasts or TGF-β1 stimulated fibroblasts. Additionally, we propose that experimental models should be developed...
to investigate the role of PPARγ ligands on ECM deposition and reversal of the progressive fibrotic phenotype after TGFβ1 stimulation in vitro (figure 1B) and in vivo using alternate lung fibrosis mouse models. Studies in this direction might complement the already existing knowledge about the anti-fibrotic potential of PPARγ agonists.

Fig. 1 Models for studying anti-fibrotic action of PPARγ agonists. (A) Experimental models previously described: co-treatment of fibroblast with TGFβ1 and PPARγ agonist (I) and treatment of PPARγ agonist followed by TGFβ1 stimulation (II). TGFβ1 stimulates fibroblast conversion to myofibroblast and PPARγ agonist inhibits the process. (B) Proposed model for future studies: TGFβ1 transdifferentiation of fibroblast to myofibroblast followed by PPARγ agonist treatment. Are PPARγ agonists capable of reversing myofibroblasts to fibroblasts or could they prevent/promote synthesis of excess collagen and ECM deposition by myofibroblasts? Fig. 1 Models for studying anti-fibrotic action of PPARγ agonists. (A) Experimental models previously described: co-treatment of fibroblast with TGFβ1 and PPARγ agonist (I) and treatment of PPARγ agonist followed by TGFβ1 stimulation (II). TGFβ1 stimulates fibroblast conversion to myofibroblast and PPARγ agonist inhibits the process. (B) Proposed model for future studies: TGFβ1 transdifferentiation of fibroblast to myofibroblast followed by PPARγ agonist treatment. Are PPARγ agonists capable of reversing myofibroblasts to fibroblasts or could they prevent/promote synthesis of excess collagen and ECM deposition by myofibroblasts?
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Diagnosis and markers for the assessment of COPD; can a consensus be reached?

Prelude

Chronic obstructive pulmonary disease (COPD) is a progressive disorder which is characterized by airflow limitation that is not fully reversible [1]. It affects central airways causing chronic bronchitis, peripheral airways leading to small airway disease, and the lung parenchyma giving rise to emphysema [2]. Further, COPD has already become the third leading cause of death worldwide [3]. The major therapeutic goal is prevention of further exacerbations, yet the currently available treatment options are not considered to be very effective. Hence, implementing the onset biomarkers for the early diagnosis of the disease could improve the clinical assessment of COPD. Here we summarize the currently available markers and methods for COPD evaluation and diagnosis.

Generally, the assessment of COPD is based on physiological, symptomatic and nowadays – on newly emerging biological markers. Physiological markers are not straightforward to identify and validate, further requiring a standardized approach. These markers include:

Spirometry – this is used to measure the volume of forcibly exhaled air in 1 second (FEV1)/forced vital capacity FVC [4], which is a marker for determination of staging, diagnosis, and treatment of the disease [5]. A fixed ratio of (FEV1/FVC)<70% defines airflow limitation. COPD patients lose lung function over a period of time, thus, a decline in FEV1 is used as an indicator of disease progression [6]. However, spirometry has its limitations. Since lung volumes are also affected during the normal process of aging, this method tends to be biased towards over diagnosis of the disease in some elderly patients [4]. Moreover, COPD is a complex disease which is accompanied by numerous pathological complications that are often ignored due to the poor understanding of
the relationship between spirometry and the symptoms [6].

**Lung volume or lung hyperinflation** characterizes an increase in the gas volume in the lungs in comparison to the predicted value [7]. It is another marker for the evaluation of COPD and determination of disease severity. Absolute lung volume is evaluated by measuring the increase in total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV) and the decrease in inspiratory capacity (IC) [8]. Conventionally, lung hyperinflation is said to exist when the values of TLC, FRC and RV exceed 120–130% of the predicted volume [7]. These values are considered to be clinically relevant, however, cut-offs remain invalidated [7]. Moreover, high variability among COPD patients is a major problem in reproducing the cut-off values, and remains to be addressed in future studies [7].

**Gas exchange** \( \text{DL}_{\text{CO}} \) relies on the diffusing capacity of the lungs and is measured via the transfer of carbon monoxide from the airspace to the pulmonary capillary blood [9]. This method is used in the diagnosis of patients with emphysema [10]. A major drawback in assessing \( \text{DL}_{\text{CO}} \) is the high price of the necessary equipment making it less available in the primary care units. Moreover, it requires specific training for the technical staff and its reproducibility depends on the expertise of the staff [9]. Furthermore, Salzman and colleagues argue that \( \text{DL}_{\text{CO}} \) cannot be definitive for either confirming or excluding the diagnosis of emphysema because a reduction in \( \text{DL}_{\text{CO}} \) accompanied by normal lung mechanics values (FEV1, FVC, FEV1/FVC, and lung volumes) might be suggestive of an emphysema, whereas normal \( \text{DL}_{\text{CO}} \) does not rule it out [11].

**Exercise capacity or a 6-Minute Walk Test (6MWT)** is the estimation of the distance covered during a 6-min walk where oxygen saturation, cardiac frequency, and respiratory intensity are recorded [12]. There are many sources of variability for this test, such as patients’ age, sex, weight, height, etc., making this test highly unreliable in diagnosing COPD [8, 13].

**The body mass index (BMI) and the BODE index.** The BMI is calculated as weight/height squared in kg/m² in the journal the superscript is written wrongly. Body mass in this case is assumed to be comprised of fat mass and fat free mass (FFM). After accounting for BMI, FFM, airflow limitation (expressed by the FEV1), Dyspnea (expressed with the modified MRC scale), and exercise capacity (expressed with the 6-minute walking distance) an integral value is calculated – the so called “BODE index”, which provides information for COPD staging. In addition, the BODE index can serve as a prognostic factor of COPD where the decrease in BMI and FFM are associated with the impairment of the muscle function, health status, exercise capacity, and decreased survival of COPD patients [14, 15].

**Imaging** includes computed tomography (CT), functional positron emission tomography (PET) and magnetic resonance imaging (MRI). CT reflects the morphologic changes in the lung parenchyma, pulmonary vasculature, central and peripheral airways [16]. In addition, CT is the best way to assess the severity of emphysema [17]. A disadvantage of this technique is associated with radiation risk, limiting the multiple longitudinal scans in clinical trials. In contrast, MRI does not involve radiation and can be used as a substitute in the clinics [18]. However, MRI is more time-consuming and expensive [19]. Finally, functional imaging through (MRI) and (PET) using hyperpolarized helium and xenon can be done [20, 21]. However, further standardization is necessary to achieve optimal results.
Exacerbations are short periods of worsening of the patients’ symptoms and characterized by as sputum hyper-production, increased cough, and dyspnoea [22]. Exacerbations represent the disease progression and clinical instability, and are associated with an increased risk of mortality [23]. Exacerbations are recorded by the patients by using a questionnaire or a diary card where they provide the information on the frequency, time of onset, severity, and duration of the periods of worsening symptoms. However, lack of standardized criteria makes the evaluation and comparison of clinical studies difficult [4], resulting in a poor correlation between manifestation of exacerbations and pathological changes occurring in the lung [19].

Other markers used for the assessment of COPD belong to the symptomatic group. These markers are assessed by medical doctors by recording frequency and nature of coughing, colouring of sputum, shortness of breath, and wheeze [6]. However, there is no agreed form for quantifying symptomatic data, response options and the equivalence between different questionnaires [6]. The symptomatic measures of COPD comprise the following:

Baseline/Transition Dyspnea Index (BDI/ TDI), Borg-Scale, and MRC (Medical Research Council) scale are the most frequently used scales for the identification of dyspnoea in clinical practice. The BDI/TDI is based on an interview conducted by the staff which converts the patients’ experience of dyspnoea into numerical parameters [8]. Such studies are limited, biased, and lack standardization when translating patient’s answers into parameters [24]. The Borg-scale (or CR-10), is a 10 point category scale that incorporates the description of the severity corresponding numbers [25]. CR-10 requires a detailed instruction for the patients on how to use this scaling system [26]. The MRC scale is a five-point scale that describes breathlessness and the severity of dyspnoea [27]. This method is also limited by bias [28].

St. George’s Respiratory Questionnaire (SGRQ) covers three domains: frequency and severity of symptoms, activities that cause or are limited by breathlessness, and the impact these disturbances have on patients’ life, which is reflected in the end as an integral score. However, these scores are influenced by patient’s age, sex, education, and comorbidities [29].

Moreover, pulmonary biomarkers can also be used to assess the COPD disease status. These group of markers comprises an estimate of the presence and involvement of inflammatory cells (macrophages, neutrophils, and lymphocytes), levels of cytokines (IL-6, IL-8, TNF-α) myeloperoxidases (MPO), neutrophil elastases (NE), expired nitric oxide (NO), and carbon monoxide (CO), which can be sampled from either sputum, blood, bronchoalveolar lavage (BAL), exhaled breath, or bronchial biopsies [12]. Bronchial biopsies provide the information on the structural changes in the epithelium, muscles, and glands where structural components can be dissected and studied separately [30]. The interaction between inflammatory and resident cells can be investigated by immunostaining [12]. However, there are several drawbacks to bronchial biopsies. First, this procedure is invasive and unsafe to patients with a severe disease stage [31]. Second, the airway wall examined during biopsy might not reflect the pathological alterations in the lung parenchyma and peripheral airways.

Bronchoalveolar lavage (BAL) allows the study of chemical and cellular components in the epithelial lining fluid of the peripheral airways [32]. Unfortunately, the low sample numbers
obtained from BAL are not sufficient for statistical analysis.

Sputum analysis provides the information of the mediators in the central airways [33]. However, this analysis is not representing the environment in the distal airways that might be important for the diagnosis of COPD. Moreover, sputum contains thick mucus that has to be removed before processing the sample, and dithiothreitol (DTT) used to solubilize the sputum alters the proteins making them unrecognizable by the antibodies [34, 35].

Exhaled gas analysis is a non-invasive method for monitoring COPD airways inflammation [12]. It measures exhaled nitric oxide (eNO) and exhaled carbon monoxide (CO) in breath. Unfortunately, reproducibility and sensitivity issues are not yet been clarified [12].

Blood samples; there are several studies suggesting that CRP, TNF-α, IL-6 and IL-8 play a role in COPD and in its exacerbations [36, 37]. However, blood sampling requires more randomized studies with larger sample sizes in order to confirm the sensitivity and specificity of this method [38].

**Summary and questions for interactive discussion**

We summarized the available COPD assessment methods and their limitations. Currently, there exists no consensus concerning the best criteria to be used for the assessment of COPD despite the variety of markers and methods available for diagnosing this disease. However, reproducibility and effectiveness of the methods were shown to be limited. Moreover, by the time COPD patients seek medical help, they already manifest significant (and often severe) symptoms of the disease. Therefore, we would like to forward the following questions to the scientific community worldwide: how reliable are the biological markers for COPD? What are the best and most specific markers to be used for assessment of COPD? What are the markers that will allow the early diagnosis of COPD? We would like to invite all those interested in this field to reply and express their views on this topic in the next volume of PVRI Chronicle.

**Current available methods and markers of COPD assessment**

Abbreviations: IL interleukin, MPO myeloperoxidase, NE neutrophil elastase, TNFα tumor necrosis factor alpha, CRP C-reactive protein, BAL bronchoalveolar lavage, NO nitric oxide, CO carbon monoxide, SGRQ St. George’s Respiratory Questionnaire, MRC medical research council, CT computed tomography, MRI magnetic resonance imaging, DLCO diffusion capacity of the lung for carbon monoxide, PET functional positron emission tomography.
Figure 1: Current available methods and markers of COPD assessment
References


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The ENTELLIGENCE Young Investigator Program is supported through an educational grant from Actelion Pharmaceuticals US, Inc.
Stress on Lungs: A Search Through 8-ISO Prostaglandin-F2α

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High Altitude Pulmonary Edema (HAPE) is a form of altitude illness that develops in travellers on rapid ascent to or physical exertion at altitudes (> 2500 m; 8200 ft) [1,2]. The altitude, speed and mode of ascent and, above all, individual susceptibility are the most important determinants for the occurrence of HAPE. It commonly strikes the second night at a new altitude and rarely occurs after more than 4 days at a given altitude, owing to adaptive cellular and biochemical changes in pulmonary vessels [1,2]. Briefly, HAPE is characterized by hypoxia-induced pulmonary hypertension (PH), oxygen sensing redox switches and intravascular over perfusion [1-4].

Prolonged exposure to HA increases oxidative damage, which could be the consequence of increased reactive oxygen species (ROS), decreased antioxidants and free radical-mediated reduction in pulmonary NO bioavailability [4,5]. Ladakhi highlanders, living for several thousand years at such a great height have adapted mechanisms different from lowlanders for combating oxidative stress, which is the most severe repercussion of HA [5,6]. Hence, the relevant pathways and its constituents that maintain redox balance become an important focus of attention. In this regard 8-iso prostaglandin F2α (8-isoPGF2α) is believed to be one of the most accurate markers to estimate oxidative stress, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease such as HAPE [3,5].

8-ISO Prostaglandin-F2α

Isoprostanes are prostaglandin (PG)-like substances that are produced in vivo independently of cyclooxygenase (COX) enzymes, mainly by free radical-induced peroxidation of arachidonic acid [7]. The formation of PG-like compounds during auto-oxidation of polyunsaturated fatty acids was first reported in the mid-1970s, but isoprostanes were not discovered to be formed in vivo in humans until 1990 [8]. F2-isoprostanes are a group of 64 compounds isomeric in structure to cyclooxygenase-derived PGF2 and one of the most important isoforms is 8-isoPGF2α [7,8]. They are released in response to cellular activation; circulate in plasma and are excreted in the urine. Morrow et al. first described the non-enzymatic production of a series of prostaglandin-like compounds during peroxidation of membrane phospholipids by free radicals and reactive oxygen species. Further, isoprostanes, appear to act through tyrosine kinase, Rho, and Rho kinase (ROCK), leading to decreased activity of myosin light chain phosphatase (MLCP) [8]. The measurement of 8-iso-PGF2α in tissues and/or biological fluids provides a valuable approach to the quantification of oxidative stress as well as a biochemical basis for assessing therapeutic intervention, as there is tremendous increase in reactive oxygen species at hypobaric hypoxia thereby predisposing the healthy sojourners towards HAPE [3]. Increased formation of 8-isoPGF2α has been detected in human cardiovascular diseases; it associates with enhanced plasma levels of noradrenaline and angiotensin II contributing to elevated vasoconstrictor effects and induces mitogenesis in vascular smooth muscle cells [3,5]. Furthermore,
8-Isoprostaglandin concentration was found to be elevated in asthma, COPD [9], cystic fibrosis (CF) [10] and pulmonary sarcoidosis [11]. A considerable body of literature exists documenting an increased production of indicators of oxidative stress in breath, blood, urine and tissue of laboratory rats in response to hypoxia [3, 12-13]. In conclusion, increased 8-iso-PGF2α level has been associated with oxidative stress and pulmonary stress that, in turn, contributes to endothelial dysfunction, which is vital in the development of HAPE. Thus, this molecule holds immense potential for providing a major improvement in the field of high altitude.

References


Therapeutic options for pulmonary hypertension (PH) have increased over the past years and include pharmacological approaches as well as surgical procedures. Pharmacological therapies approved for pulmonary arterial hypertension (PAH, group 2 according Nice classification, Table 1) include drugs addressing the signaling pathways of nitric oxide (NO), prostacyclin and endothelin (Figure 1), well known to be phosphodiesterase-5- (PDE5) inhibitors (sildenafil, tadalafil), prostacyclins/prostacyclin analoga (iloprost, epoprostenol, treprostinil) or endothelin receptor antagonists (ERAs: bosentan, ambrisentan). In the last years the spectrum of drugs was extended by a stimulator of the soluble guanylate cyclase (sGC) “riociguat” (Adempas, FDA approval: 2013), the prostacyclin receptor agonist “selexipag” (FDA approval: 2015) and the ERA “macitentan” (Opsumit, FDA approval: 2013). Most importantly, for the first time pharmacological therapy for another form of PH besides PAH became available, as riociguat was approved for the treatment of chronic thromboembolic PH (CTEPH). For continuous intravenous treatment with treprostinil the application of an implantable pump has been developed. This article provides an overview about currently approved new therapies for PH.

**Macitentan for treatment of PAH**

Macitentan (Opsumit, 10mg, once per day, oral) was approved by FDA in 2013 and acts as dual ERA with better tissue penetration and less interaction with other drugs [3]. In contrast to other ERAs liver toxicity seems to be negligible, as the respective clinical trial showed liver toxicity on the level of placebo. The placebo controlled study “SERAPHIN” (Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome) enrolled 742 patients with a mean follow up of 3.5 years. For the first time a composite endpoint of morbidity and mortality (e.g. lung transplantation, initiation of treatment with i.v. or subcutaneous prostanoioids or worsening of PAH) was chosen for an approval-relevant clinical phase III trial. The trial showed that application of 3 or 10 mg Macitentan reduced the relative risk to reach the combined primary endpoint by 30 or 45%, respectively, compared to the placebo group. The combined endpoint was mainly reduced by decreasing PAH related hospitalizations and worsening of PAH, but not mortality. Moreover, Macitentan was the first ERA, which showed positive effects in combination with PDE5 inhibitors. Main side effect at a dose of 10 mg was a decreased a hemoglobin value [10]. In contrast to bosentan, Macitentan neither interacts with Warfarin [11] nor with oral contraceptive [7].

**Riociguat for treatment of PAH**

Riociguat (Adempas, standard dose 2.5 mg, three times per day, oral) was approved for the therapy of PAH in 2013 and acts as sGC stimulator. Thus, Riociguat addresses sim-
monophosphate (cGMP) signaling pathway. In contrast to sildenafil, which increases the concentration of cGMP by inhibition of its degradation, Riociguat enhances the sensitivity of the sGC for NO and stimulates production of cGMP independently of NO [14]. In the study conducted for approval of Riociguat for PAH (“PATENT-1” Pulmonary Arterial Hypertension Soluble Guanylate Cyclase–Stimulator Trial 1) Riociguat improved the primary endpoint of 6-minute-walk-distance as well as secondary endpoints such as exercise capacity, pulmonary vascular resistance and time to clinical worsening. Adverse effects included particularly systemic arterial hypotony [5]. Therefore, in clinical practice Riociguat is up-titrated during a time period of approximately 6 weeks according to a titration scheme supervised by monitoring of blood pressure (starting dose 1 mg, three times per day) until reaching the standard dose, if tolerated. Combination of Riociguat and sildenafil is contraindicated due to arterial hypotony [2].

Riociguat for treatment of CTEPH
CTEPH is the only form of PH for which a potential curative approach is available, which is the removal of thromboembolic material by the surgical procedure of pulmonary endarterectomy (PEA). PEA is performed during circulatory arrest in deep hypothermia [9]. Furthermore, life-long sufficient anticoagulation is considered standard basic therapy. Since approval of Riociguat in April 2014, the first pharmacological therapy for CTEPH was approved. However, Riociguat is only approved for technical inoperable CTEPH, or if the benefit-risk ratio limits PEA, as well as in persistent or recurrent PH after PEA. First line therapy for CTEPH is still PEA. Thus every patient should be evaluated for PEA after diagnosis of CTEPH, before considering pharmacological therapy. Moreover, pharmacological therapy with Riociguat for the purpose of bridging the time until PEA in conditions of severe CTEPH has not yet been evaluated in clinical studies. In the randomized controlled CHEST trial (Chronic Thromboembolic Pulmonary Hypertension Soluble Guanylate Cyclase–Stimulator Trial) application of Riociguat resulted in improved exercise capacity determined by the six minute walk distance, and decreased pulmonary vascular resistance [4]. However, time-to-clinical worsening (defined as combined end point of e.g. mortality, transplantation) was unchanged. In a prior study in CTEPH, Bosentan improved hemodynamics, but not exercise capacity, allowing this drug to only be used as off-label medication [8].

Selexipag for treatment of PAH
Selexipag is an oral, selective prostacyclin receptor agonist with long half-life (6.2-13.5 h) and was approved by FDA at the end of 2015. In the clinical phase III trial GRIPHON PGI2 (Receptor agonist In Pulmonary arterial Hypertension) Selexipag -as mono- or combination-therapy with ERAs and/or PDE5-inhibitors- reduced the mixed endpoint of morbidity and mortality [12].

New application forms in PAH

Treprostinil: implantable pump
Intravenous application of prostacyclins can be cumbersome due to the persistent indwelling i.v. line which predisposes for infections and dislocations. A more preferable way of application was achieved by development of a fully implantable pump system with a titanium reservoir which can be re-filled from outside every 2-4 weeks percutaneously by an injection through a membrane with a special needle by trained personnel. The pump is implanted in the subcutaneous tissue of the abdominal wall and delivers the treprostinil in the superior
vena cava via a tunneled catheter. The pump is driven by a purely mechanical system and does not need any battery. The pump speed is constant, so that the delivered dose is adjusted by varying the concentration of treprostinil filled into the reservoir. The pump can be implanted during local anesthesia and is thus suitable for PAH patients presenting with severe disease [1].

Outlook:
In the past year several promising drugs for treatment of PAH have been evaluated in clinical trials. Unfortunately some of them did not fulfill the requirements for approval (e.g. the serotonin receptor agonist, inhaled vasoactive peptide). Particularly the tyrosine kinase inhibitor Imatinib showed significant improvements in the primary endpoint, but development was stopped due to serious side effects [6]. In a current study efficacy and safety of inhaled NO for treatment of PAH is tested (NCT01457781). Soon results of a trial with dichloroacetate which reverses metabolic alterations of PAH will be published (NCT01083524). Another study with Tacrolimus was recently finished. Case reports show beneficial effects, but final results have to be waited for [13].

Figure 1: Signaling pathways and pharmacological therapy (red: recently approved therapeutics)

Abbreviations: IP receptor: Prostacyclin receptor, ETA/B: Endothelin A/B, GMP: guanosine monophosphate, cGMP: cyclic guanosine monophosphate, sGC: soluble guanylate cyclase, PDE5: phosphodiesterase 5, cAMP: cyclic adenosine monophosphate, ERA: endothelin receptor antagonist.
**References:**


### Table 1: Clinical Classification of PH and therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Definition</th>
<th>Approved therapy</th>
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<tbody>
<tr>
<td><strong>Group 1 (PAH)</strong></td>
<td></td>
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<tr>
<td>1.1 Idiopathic pulmonary arterial hypertension (IPAH)</td>
<td>Precapillary: (\text{mPAP} \geq 25 \text{ mmHg} ), (\text{PAWP} \leq 15 \text{ mmHg})</td>
<td>PAH, Therapy of underlying disease, basic therapy (diuretics, oxygen supplementation...), specific therapy (PDE5-inhibitors, sGC stimulator, ERAs, prostacyclins/analog, prostacyclin receptor agonist)</td>
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<tr>
<td>1.2 Hereditary pulmonary arterial hypertension (HPAH)</td>
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<td>1.2.1 BMPR2</td>
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<tr>
<td>1.2.1 others (ALK1, Endoglin)</td>
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<tr>
<td>1.3 Drug and toxin induced (DPAH)</td>
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<td>1.4 Associated pulmonary arterial hypertension (APA):</td>
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<td>1.4.1 Connective tissue diseases</td>
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<td>1.4.2 HIV-Infection</td>
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<td>1.4.3 Portal hypertension</td>
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<td>1.4.4 Congenital heart disease</td>
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<td>1.4.5 Schistosomiasis</td>
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<td>1.5 Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis</td>
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<td>1.6 Persistent pulmonary arterial hypertension of the newborn</td>
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</table>

| **Group 2 (PH due to left heart disease)** | | |
| 2.1 Systolic dysfunction | Postcapillary: \(\text{mPAP} \geq 25 \text{ mmHg} \), \(\text{PAWP} > 15 \text{ mmHg}\) | Therapy of underlying disease |
| 2.2 Diastolic dysfunction | | |
| 2.3 Valvular diseases | Combined post-/ precapillary | |
| 2.4 Congenital/ acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies | | |
| 2.5 Congenital/ acquired pulmonary veins stenosis | | |

| **Group 3 (PH due to lung disease and/or hypoxia)** | | |
| 3.1 Chronic obstructive disease | Precapillary: \(\text{mPAP} \geq 25 \text{ mmHg} \), \(\text{PAWP} > 15 \text{ mmHg}\) | Therapy of underlying disease, oxygen supplementation |
| 3.2 Interstitial lung disease | | |
| 3.3 Other pulmonary diseases with mixed restrictive or obstructive pattern | | |
| 3.4 Sleep-disordered breathing | | |
| 3.5 Alveolar hypoventilation disorders | | |
| 3.6 Chronic exposure to high altitude | | |
| 3.7 Developmental lung diseases | | |

| **Group 4 (Chronic thromboembolic PH and other pulmonary artery obstructions)** | | |
| 4.1 CTEPH | Precapillary: \(\text{mPAP} \geq 25 \text{ mmHg} \), \(\text{PAWP} \leq 15 \text{ mmHg}\) | Anticoagulation, PEA, specific pharmacological therapy (sGC stimulator) |
| 4.2 Other pulmonary artery obstructions | | |
| 4.2.1 Angiosarcoma | | |
| 4.2.2 Other intavascular tumors | | |
| 4.2.3 Arteritis | | |
| 4.2.4 Congenital pulmonary arteries stenoses | | |
| 4.2.5 Paracites (hydatidosis) | | |

| **Group 5 (PH with unclear and/or multifactorial mechanisms)** | | |
| 5.1 Hematological disorders | Precapillary: \(\text{mPAP} \geq 25 \text{ mmHg} \), \(\text{PAWP} \leq 15 \text{ mmHg}\) | Therapy of underlying disease |
| 5.2 Systemic disorders | | |
| 5.3 Metabolic disorders | Postcapillary: \(\text{mPAP} \geq 25 \text{ mmHg} \), \(\text{PAWP} > 15 \text{ mmHg}\) | |
| 5.4 Others | Combined pre-/ postcapillary | |

BMPR: bone morphogenic protein receptor, ALK: activin-like kinase, CI: cardiac index
References:

8. Jais X, D’armini AM, Jansa P et al. (2008) Bosentan for treatment of inoperable chronic thromboembolic pulmonary hypertension: BENEFIT (Bosentan Effects in iNopErable Forms of chronIc Thromboembolic pulmonary hypertension), a randomized, placebo-con-
trolled trial. Journal of the American College of Cardiology 52:2127-2134
Pulmonary Arterial Hypertension (PAH) is a progressive disease, characterized by pulmonary vascular remodeling and right ventricular remodeling in hypertrophy, but eventually dilatation and right heart failure [1]. Today, there are several drug classes registered to hamper the disease progression [2]. However, no curative medication for PAH is available. Due to dedicated research, several factors playing an important role in the pathobiology of PAH are identified [3]. Many of these factors also play an important role for the compensatory cardiac adaptation, which poses a treatment paradox – what is beneficial to the lungs, might be harmful for the heart - when it comes to medication targeting these factors.

From the PH center at the VU University medical center, Michiel Alexander de Raaf defended his PhD entitled: “targeting the cause, affecting the course”. The thesis discussed the treatment paradox using experimental models resembling important aspects of PAH.

Important conclusions which could be made from the PhD program were that the different experimental models, like (chronic) hypoxia, monocrotaline and Sugen Hypoxia for example, supply different answers to the treatment paradox [4]. This is due to the fact that every animal model resembles only a ‘set’ of aspects seen in the human disease. With better understanding of endothelial dysfunction, cellular signaling and ‘quasi-malignant phenotypes’ of the disease, the interpretation of what we understand about PAH is still evolving. The scientific progress that has been made also translates to the evolution of animal models; there is a continuous need to characterize and improve animal models by including the representation of aspects which has become more appreciated to have an important role in PAH [5]. This also means, that using older animal models such as monocrotaline or chronic hypoxia to study the efficacy of medication targeting endothelial proliferation – an aspect not represented in these animal models -, may lead to unpredictable translation to the clinic [4, 6, 7]. The best solution to solve the treatment paradox pre-clinically, making the translation gap smaller, is to use multiple animal models; pulmonary vascular remodeling models representing the drug target for ‘targeting the cause’, and right ventricular pressure overload models to understand if the drug is not worsening cardiac function by ‘affecting the course’. To answer the latter, Pulmonary Artery Banding could be used. With Pulmonary Artery Banding, right ventricular adaptation under exposure of the medication can be measured and evaluated. A good example of the understanding gained from using multiple animal models is the evaluation of histone deacetylases (HDACs) for PAH. In animal models not representing the hyperproliferative endothelium, several HDACs showed a beneficial treatment effect. The thesis shows that HDAC activity in these experimental models is already low in the lungs and elevated in the right heart. Testing these HDAC’s in the Pulmonary Artery Banding model resulted in cardiac worsening and within the Sugen Hypoxia model, no treatment effect was visible [6, 8]. This would conclude that using multiple animal models, predictability of the contemplated beneficial treatment potential.
can be made. Bridges were made to the field of the developmental biology to understand the treatment paradox in PAH [9]. During the prenatal phase, the increased pulmonary vascular resistance is needed for the development of the right heart. Medication which lowers the pulmonary vascular resistance results in cardiovascular malformations. Indeed, with the reactivation of fetal aspects in PAH in both pulmonary vascular remodeling as cardiac adaptation, is shows many parallels with cardiopulmonary development.

References


This walking tour through Rome was made as social event at the PVRI Annual Meeting in Rome 2016. The tour was guided by PVRI member and medical biologist Michiel Alexander de Raaf, who lived in Rome for one year during an internship.

Romans consider their squares as living rooms, full with memories of nice and romantic stories of the city. Historically, not all stories can be exactly true as they are told nowadays. However, with a city full of history, the Romans don’t care or even know about this and these stories became treasures of the Roman ambiance and culture. The stories are often not known by tourists and provide a nice glimpse in the history of Rome.

Piazza Mattei
The tour started at “Piazza Mattei”, the square called after the Mattei family who had several palaces close to this square. The Duke of the Mattei family was desperately in love with a young woman. The father of the young woman did not approve the proposed marriage, as the Duke was ‘famous’ about his gambling addiction and lost all his money. On a market day, the father visited the Duke to tell him the news that he is not willing to marry his daughter to someone without money. Of course, the Duke was desperately in love and told the father to return the next day. The next day, there was no market and the father saw for the first time the fountain of the turtles (“Fontane Felle Tartarughe”). The Duke lied and told him that he ordered to build the fountain in one night to convince the father about his richness. The father was convinced and the couple married. As the father did not want other people to see the beauty of the fountain as he did for the first time, the window facing the fountain is immured.

Area Sacrale Largo Torre Argentina
At “Area Sacrale Largo Torre Argentina” we saw the four oldest temples of the Roman Empire. They were probably already built by the Etruskians, who lived in this region before recorded Roman history. It is said that the temples were forgotten during the Roman reign, to whose Gods the temples were built for. To overcome the problem of dishonoring the gods, the complete area was called ‘sacred’. During the reign of Julius Gaius Caesar, this area was the playground of world history. As Julius Caesar was renovating the curia (senate house) at the “Forum Romanum”, the Senate of Rome used the area “sacrale” for their daily meetings. It was here, in the temple on the right side, where Caesar was murdered. Due to the age of these temples, we know that this area is also one of the oldest building sites of Rome. The area of the Roman forum and the Colosseum were still swamps in that time. It was known that a monster was living in that swampy area which could ‘grasp’ people by its bad breath; dying a horrible death. Therefore, the area was said to have ‘sick air’, which is in Latin ‘male aria’ and the base for the disease malaria.

Piazza Navona
The “Piazza Navona” square is built on the foundations of the hippodrome built by Dominitianus. Horse races were very popular in ancient Rome. There were four teams (blue,
Rome Tour

white, red, green) and racing with chariots they
needed to make 7 turns around the “spina”, which
is a structure in the middle of the hippodrome. In
the centre of the “spina”, an Egyptian obelisk was
placed and later moved to “Piazza del Popolo”. The
obelisk to be found nowadays at “Piazza Navona”
is a replica made by the students of the architect,
Bernini (they were not familiar with the Egyptian
hieroglyphs, and placed them upside down). Bernini
was not invited to the competition to
make this fountain in the center, but he submitted
his proposal and was chosen to construct it. The
other architect, Borromini, was very angry, as
he wanted the assignment. To ease the situation,
he was ordered to build the church next to the
fountain. The fountain consists of four men who
represent the four ‘biggest’ rivers at that time;
Donau, Ganges, Nile and Rio de la Plata. As the
beginning of the Nile was not known at that time,
the head of the ‘river god’ is covered by a cloth. The
river god of “Rio de la Plata” is also special as the
face is not of a human adult. One story tells that
this face is a monkey as the ‘New World’ was not
Christian yet. Another story tells the face is a baby
because the ‘New World’ was new and immature.
Borromini found a mathematic mistake in the
drawings of Bernini and the fountain would
collapse by the weight of the obelisk. Bernini
went through his calculations several times but
was not able to find his mistake. The only way
to find the error and solve it, was to rely on one
of his students. The daughter of Borromini was
desperately in love with one of the students of
Bernini. This student was sent to declare his love
to Borromini’s daughter who let him in the house.
At night he copied the calculations and the next
day they were given to his master Bernini. Bernini
was able to fix the mathematical error and the
fountain was saved from collapsing. To annoy
Borromini, who was still not aware that Bernini
knew about the mistake and fixed it, he ‘saved’
the fountain from collapsing by stabilizing the
obelisk with silk wires. As “Piazza Navona” is one
of lowest areas of the city, there is the possibility
to make an artificial lake of 60 cm deep, which
was used in summertime during the 18th-19th
century.

San Luigi dei Francesi

We passed this church and in the back and on
the left, we were able to see original Caravaggio
paintings. Please note that the paintings depict
stories from the Bible, while the ‘setting’ is
not from Ancient Rome era, but Medieval, so
‘contemporary’ in the time of Caravaggio himself.
This is probably done, as in many outside Italy,
to narrow the ‘translational’ gap for the Medieval
people to understand better the stories told in the
Bible.

Piazza della Rotonda – Pantheon

The Pantheon is built on the fields of Mars. This
field was sacred in Ancient Rome and it was
permitted to build buildings here. The fields were
used for elections; Roman people walked to the
area dedicated to a specific consul to vote for him.
Also the two Roman legions in order to keep
ambitious Roman generals with their legions out
of the governmental center of the Empire, had
their camps here. However, Rome was growing
and held at its maximum approximately 1.3
million inhabitants. So, the field of Mars needed
to be used for residential buildings. To keep all
gods happy with this need, the first building built
was a temple for all the gods; the Pantheon. And
there were a lot of Gods to please, partly due to the
following aspect. To stabilize the power of Rome
and to destabilize Roman generals to become too
powerful (the divide and rule idea by Caesar),
Roman legions needed to move every 6 months
from their garrison to another. This was good for
stabilizing the political and military power but by
this strategy the legionnaires were exposed to a
lot of new cultures and religions (keep in mind
1 out of 10 Romans were in the army). Many of these religions were spread through the empire. For example, the Persian Mithras became very popular among soldiers and altars were found throughout the Empire (England, Spain, Rome, etc.). With the Pantheon, the Romans kept the ‘promise’ that all gods were represented in the city of Rome.

**Sant’Ignazio di Loyola in Campo Marzio**
This is one of the most beautiful churches in Rome and not so famous nor touristic. Every catholic province (with a cardinal) was allowed to build a church to represent themselves in Rome. However, most popes did not want that the church became more beautiful than their own churches as the Saint Peter in the Vatican. Therefore, the pope stopped the budget of the construction. Major problem was that without the money, the dome on top of the church could not be completed. Giovanni Tristano, the architect, ‘constructed’ the dome by painting the dome (which interior is the observatory of Galilei). When opening the church, the pope walked to the ‘yellow’ stone and was astonished by seeing the dome, not realizing that it was fake, and walked -very angry - out. Also the other breathtaking fresco which makes the church optical much higher is painted by Andrea Pozzo.

**Fontana di Trevi**
The Trevi fountain is probably the most well known tourist hotspot in Rome. It is said that it is called “Trevi” as this means ‘three’ and the square is the connection point of three main streets. Another story tells of the Trevi Fountain getting its water from a river with 3 different springs. When Ancient Rome was flowering, the city had numerous aqueducts feeding the city with fresh water. After the ‘Sacco di Roma’, the Gauls destroyed all aqueducts except one. This same one still functions today and gives its water to the Trevi Fountain. From there, all other fountains in the city are fed by the Trevi Fountain. Therefore, the water from the Trevi Fountain is the freshest water of the city. Drinking from the water together with your partner assures an eternal relationship. Two anecdotes were told at the Trevi Fountain. The first was about a little girl who was living next to the fountain when it was built. As she was sick, she was afraid that she was not able to see the final result. Indeed, she died before the construction was finished and therefore the architect, Nicola Salvi (but the fountain was drawn by Bernini 50 years earlier), made a little statue of her head and placed it on the façade of the church next to the fountain, as she could now ‘see’ the Trevi fountain for centuries.

Another very nice story is revolves around the construction of the fountain. At the time, there was a barbershop on the right side of the square. Every day, the barber came to talk with Nicola Salvi. He was full of ideas -all of which had one goal. He wanted to make his barbershop more appealing; when you were looking to the Trevi fountain, your eyes were directed to his barbershop. The architect Salvi was increasingly frustrated by these recommendations, and as a result, on the very last day of the construction, he placed a big rock on the right side of the square, including a big vase on top, so that when you were looking at the Trevi fountain, you could not see anything of the barbershop.
From left to right:
Piazza Mattei, Area Sacramento Largo Torre Argentina, Fontana di Trevi, Piazza Della Rotorida - Pantheon,
Piazza Navona, San Luigi dei Francesci,
When Djuro Kosanovic sent me an e-mail saying that he had rented a castle for the weekend and asking me to join him, I had mixed feelings of surprise and excitement. Would this finally the moment to push our scientific collaborative friendship further? Perhaps I had misjudged the situation a bit, I thought. Especially, when I noticed that I was not the only one being invited. And then I understood. It was the PVRI retreat for the Committee of Young Clinicians and Scientists. It was work. Science is a tough game.

The first -and therefore historic - PVRI CYCS Retreat was located at the castle of Waldeck in Germany. Waldeck castle is known to have had a substantial influence on world history. In 1858 for example, Emma of Waldeck-Pyrmont was born there. She married the Dutch king William the Third who needed to stabilize his legacy after his unsuccessful previous marriage with his niece Sophia. Emma gave birth to Queen Wilhelmina and after the death of the king, she was regent in the period when Wilhelmina was still a child. As we feel the influence of the historic Waldeck castle around us, we hope that this can be reflected in our efforts to support and evolve the PVRI.

The program started on Friday afternoon by welcoming everybody. We had a lovely dinner and the CYCS board discussed the agenda. Later that evening, we had a wonderful tour of the castle, seeing the dungeons, prisons and the well. The well was dug by two prisoners who were released when they found water. After 27 years, and 140 meters of well, they lost their eyesight but finally found water. Upon release, one of the prisoners was so excited that he had a heart attack and unfortunately died!

On Saturday, we had a CYCS plenary council meeting in the morning, addressing all ongoing topics of our committee. Some of these topics were discussed immediately and others were postponed to Sunday. In the afternoon we had a splendid social teambuilding event by leaving the castle using a cable track and went sailing on the lake. In the evening, after dinner, we started our scientific session which was full of interactive discussions. In total we had 14 presentations (from the 11 attendees) and covered a broad spectrum of today’s cutting-edge science in the field of PVD. On Sunday, the day started with the second half of the CYCS plenary council meeting, where we discussed the remaining topics. My Waldeck Retreat finished a bit earlier; as my wife is also a scientist and was on her way to a congress herself, I headed back home to continue my duties as father. The others remained and continued in the same spirit and ambiance as the days before, having the second part of the scientific sessions closed by a dinner at the castle.

Aside from the scientific success, we had also other successes. Regarding the PVRI CYCS tasks, the timing of this retreat was superb, as we were able to discuss our ongoing projects and start preparations for the Annual Meeting in Miami. We had two ‘plenary council meetings’ (and one board meeting) to discuss and evaluate all ongoing projects. We have never had so many topics discussed and never had so many ideas to explore and improve the council. I sincerely believe that we took a vast number of steps forward and that the things discussed at Waldeck will echo in a more effective CYCS for a long time.

Regarding friendship, this retreat had a marvelous atmosphere; giving ample possibility to start and
strengthen friendships. This is already reflecting in the increased dedication and activity we experience now among our members. All those who took part have been very enthusiastic about the retreat and look forward to the possibility of making such an event in the future an even bigger success.

All attendees of the PVRI CYCS Retreat at Waldeck, would like to acknowledge Mariola Bednorz for the wonderful organization of this historic meeting. This retreat showed the strength of our CYCS, and personally I feel very fortunate to have experienced this.
Obituary of Professor Almaz Aldashev (1953-2016)
Vice-President of National Academy of Sciences of Kyrgyz Republic
Director of the Institute of Molecular Biology and Medicine
Bishkek, Kyrgyz Republic

Prof Martin Wilkins
MD FRCP FBPhS FMedSci
Head of Department of Medicine, Imperial College London

We are saddened to announce the death of Almaz Aldashev, a leading scientist in the world of pulmonary vascular research.

Almaz was born and much of spent his life in Kyrgyzstan where he devoted his academic life to pulmonary disease. He is best known to us for his work in pulmonary hypertension but he had many other interests, both within and outside of science. While Kyrgyzstan was part of the Soviet Union his travels were confined to Russia and Central Asia, but with the independence of Kyrgyzstan in 1991 he was able to reach out more freely to western scientists. He developed a network of collaborators in Europe and America and linked them to physicians and scientists within the newly independent Central Asian states. A man of integrity and politically astute, it was natural that he should rise to high office in his country’s leading science community. He was steeped in the history of Central Asia and a warm and engaging guide to Kyrgyzstan, now the Kyrgyz Republic. He was proud of his country and was happy and at home traveling the highlands, meeting local villagers and sharing stories and songs. His loss will be felt at home and abroad.
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... that enables us to challenge what’s possible.