IMPROVING OUR UNDERSTANDING OF PORTOPULMONARY

HYPERTENSION

A Thesis

by

Sandeep Sahay, MD

Submitted to the Graduate and Professional School of
Texas A&M University
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	David P Huston
Committee Members,	Burton F Dickey
	Edward A Graviss
Head of Department,	Carol Vargas

May 2022

Major Subject: Medical Sciences

Abstract

Pulmonary arterial hypertension (PAH) is characterized by elevated pulmonary artery pressure and pulmonary vascular resistance, right heart failure, exercise limitation, and an increased risk of death. Histopathologic examination reveals intimal proliferation, medial hypertrophy, and adventitial fibrosis in the small muscular pulmonary arteries. Plexiform lesions and *in situ* thrombosis are also seen. Most commonly idiopathic, PAH may also be associated with portal hypertension, termed portopulmonary hypertension (PoPH). Previous studies have shown a prevalence of histopathologic changes of PAH of 0.61% in autopsies of patients with cirrhosis, and PoPH was the third most common form of PAH in a population-based epidemiologic study in France. Recent cohort studies showed that the prevalence of POPH in patients presenting for liver transplant evaluation is between 5 and 6%. Patients with POPH have an increased risk of death, even with specific PAH treatment. In many cases, POPH greatly complicates or precludes liver transplantation, significantly affecting the course of hepatic failure in these patients. The etiology of PAH in patients with portal hypertension (characterized by systemic vasodilatation) is unclear. We have shown that female sex and autoimmune etiology of liver disease are associated with the risk of POPH. Although germline mutations in the gene that codes for bone morphogenetic protein receptor type II(BMPR2) have been associated with idiopathic and familial forms of PAH, they have not been found in patients with POPH.

In this thesis, a step-by-step approach is proposed to advance our understanding of POPH, and the outcome of this project is to provide a comprehensive systemic review of POPH, diagnosis, evaluation and management. In addition, we want to understand the leading cause of mortality in patients with POPH and why liver transplant is so underutilized.

Acknowledgements

This project would not have accomplished without the help and support of many people, and I would like to express my deepest thanks particularly to:

Professor David Huston, who is my advisor for this master's program. My Mentor, Professor Adaani Frost, who taught me immensely through-out the time I worked with her. She encouraged me routinely. Support from Royanne Holy, research manager, Jordin Rice, RN along with research team at HMH, was tremendously important for me to successfully complete this master's. Sacrifices and support from my family, my wife, Dr Jaya Kala and my lovely kids, Sajal, Jaanvi and my parents cannot go unnoticed for any academic success I achieve in my career. Thank you for helping to make this project a success. Drs. Dickey and Graviss for all their guidance.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

Funding Sources

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

Pulmonary Arterial Hypertension (PAH)

Portopulmonary Hypertension (PoPH)

Bone Morphogenetic Protein Receptor type II(BMPR2)

Pulmonary Artery Pressure (PAP)

Pulmonary Vascular Resistance (PVR)

Idiopathic/hereditary PAH (IPAH/HPAH)

The French Pulmonary Hypertension Registry (FHPR)

New York Heart Association Functional Class [NYHA FC]

Transjugular Intrahepatic Portosystemic Shunt (TIPS)

Mean Pulmonary Artery Pressure (mPAP)

Bone Morphogenic Protein 9 (BMP9)

Toll-like receptors (TLRs) lipopolysaccharides (LPS)

16α-hydroxyestrone (16α-OHE1)

Single nucleotide polymorphisms (SNPs)

2-hydroxyestrogen/16-α-hydroxyestrone (2-OHE/16α-OHE1)

16-α-hydroxyestradiol (16α-OHE2)

Immunohistochemical (IHC)

Second heart sound (P2)

Computerized tomography (CT)

Ventilation/perfusion (V/Q)

Chronic Thromboembolic Pulmonary Hypertension (CTEPH)

Transthoracic Echocardiography (TTE)

Right Ventricular Systolic Pressure (RVSP)

Right Heart Catheterization (RHC)

American Association for the Study of Liver Diseases (AASLD)

The International Liver Transplant Society (ILTS)

Right ventricular (RV)

Hepatic Venous Pressure Gradient (HVPG)

Wedge Hepatic Vein Pressure [WHVP]

Free Hepatic Vein Pressure [FHVP]

Pulmonary Artery Wedge Pressure (PAWP)

Liver Transplantation (LT)

World Health Organization (WHO)

6-minute walk distance (6MWD)

Model for End-Stage Liver Disease (MELD)

Organ Procurement and Transplant Network (OPTN)

CHAPTER 1

Background and Demographics of POPH

Pulmonary arterial hypertension (PAH) is a chronic progressive disease characterized by elevated pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) eventually leading to right heart failure and premature death. PAH associated with portal hypertension is called portopulmonary hypertension (PoPH) and is a subset of Group 1 PH (1). Similar to other causes of PAH, PoPH is characterized by the presence of intimal proliferation, medial hypertrophy, adventitial fibrosis of the muscular arteries and plexiform arteriopathy, and is thought to be histopathologically indistinguishable from other PAH phenotypes (2–4). Importantly, there does not seem to be a link between the presence or severity of PoPH with the degree of underlying liver dysfunction or hepatic venous pressure gradient (5, 6).

Patients with PoPH may be treated with PAH specific therapies, which may decrease the severity of the disease, improve functional parameters and hemodynamics, and allow for liver transplant (LT) (7). Despite a rapidly evolving understanding of PoPH, challenges remain in the diagnosis, treatment and transplantation of patients with PoPH. In this review, we will discuss the latest expert opinion in diagnosis and management of PoPH, as well as identify key areas of future research focus.

The exact prevalence of PoPH is difficult to determine. The annual incidence of all types of PAH is <10 patients per million population, and PoPH patients are a subset of this group (8). PoPH was the third most common form of PAH in a population-based epidemiologic study in France (9). Historically, PoPH is thought to be 5–10% of all patients with PAH (10, 11). More recently, French authors have reported that the proportion of newly diagnosed (incident) patients with PoPH is as high as 15% of all patients with PAH and is continuing to rise as wider screening practices are adopted (12). PoPH is thought to be present in anywhere between 2 and 10% of patients with portal hypertension (6, 13). Furthermore, while the vast majority of cases of PoPH are in patients with portal hypertension related to cirrhosis, non-cirrhotic causes of portal hypertension (including portal vein thrombosis, granulomatous disease, auto-immune disorders, drug reactions, infections, and congenital abnormalities) are also important contributors (14, 15). McDonnell et al. showed a prevalence of histopathologic changes of PAH of 0.61% in autopsies of patients with cirrhosis (16), and small cohort studies have shown a PoPH prevalence in patients with cirrhosis undergoing transplant evaluation to be between 5 and 6% (17–19). Although the hemodynamic profile of patients with PoPH is better than in patients with idiopathic/hereditary

PAH (IPAH/HPAH), their overall mortality is similar or worse (12, 20–22). The French Pulmonary Hypertension Registry (FHPR) shows a 1, 3, and 5-years survival of 84, 69, and 51%, respectively, for patients with PoPH (12), which is similar to the survival data for patients with IPAH, HPAH and anorexigen associated PAH (23). In contrast, analysis of the US based REVEAL registry showed that patients with PoPH had significantly poorer survival when compared to patients with IPAH/FPAH: 67 vs. 85% at 2 years, and 40 vs. 64% at 5 years (Figure 1) (20). These findings were similar in the Spanish REHAP registry, where the 5-year mortality was 49 and 69%, for PoPH and IPAH/HPAH, respectively (Figure 2) (24, 25). Furthermore, severe PoPH is associated with significantly decreased survival in patients undergoing LT (7, 26), and severe PoPH precludes liver transplantation and significantly affects the course of hepatic failure in these patients (26, 27).

The lack of better outcomes in patients with PoPH as compared to their IPAH/FPAH counterparts is not entirely clear, especially in light of their relatively better hemodynamic profile and functional status (20, 25). One possible explanation is the increased deaths from liver related events, which account for 25–33% of deaths in patients with PoPH, as compared to only 5% in patients with IPAH (22, 25). In patients with liver disease, higher Child-Pugh Scores are associated with poorer outcomes (14, 22), which makes it clear that the severity of liver disease (rather than PoPH) is the main factor in poor outcomes in this population. Another possibility is a relative delay in PAH-specific treatments within the PoPH cohort. PoPH patients started on PAH-specific therapy live longer, despite poorer hemodynamics, when compared to PoPH patients not on therapy (25). However, the percentage of PoPH patients on pulmonary vasodilators at both time of enrollment and at 90-days post enrollment into the REVEAL registry was significantly lower than their IPAH/FPAH counterparts (20). The cause of this delay is likely multifactorial, and includes milder symptoms, better hemodynamics, and lack of definitive data suggesting the ideal agent in patients with PoPH.

Likewise, the difference in survival seen between different PoPH cohorts are also likely due to a variety of factors, which include differences in regional screening practices for the presence of PoPH. Patients in the US and Spain with cirrhosis are typically screened at the time of liver transplant evaluation, which likely skews the diagnosis of PoPH to patients with more severe liver disease. In contrast, all patients with cirrhosis are screened for PoPH in France, which likely

leads to increased rates of diagnosis of PoPH in patients with milder liver disease, which in turn increases the overall survival rates. Rates of referrals of patients to PH specific centers may differ by region (the REVEAL registry included PAH patients treated in the community in addition to academic PH-centers). There may also be differences in baseline characteristics of patients enrolled in each respective registry (including New York Heart Association Functional Class [NYHA FC] and severity of underlying liver disease at the time of enrollment). Additional prospective studies are needed as PoPH-specific management guidelines are established and standardized, to see if this gap can be improved.

Figure 1 Two-year Survival from Enrollment
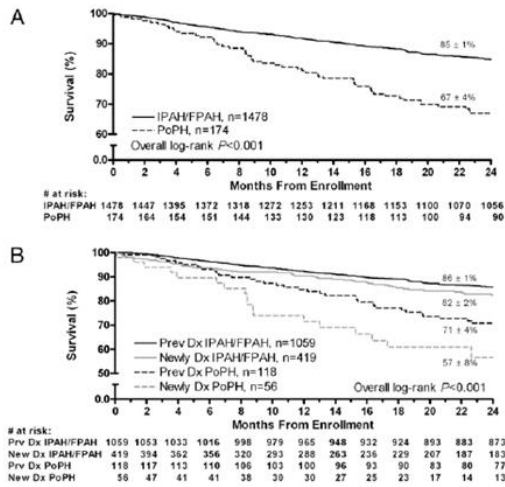
Figure 1. Two-year survival from enrollment. **A,** Patients with PoPH and IPAH/FPAH. **B,** Stratified by duration of disease from enrollment. **Dx** = diagnosis, **FPAH** = familial pulmonary arterial hypertension, **IPAH** = idiopathic pulmonary arterial hypertension, **PoPH** = portopulmonary hypertension. Reproduced with permission of Elsevier: Chest 141 (4) 906-915; DOI: 10.1378/chest.11-0160 Epub 21 July 2011.

Figure 2 Kaplan-Meier Estimates of 5-yr Survival from Time of Diagnosis in Different Pulmonary Hypertension Subtypes
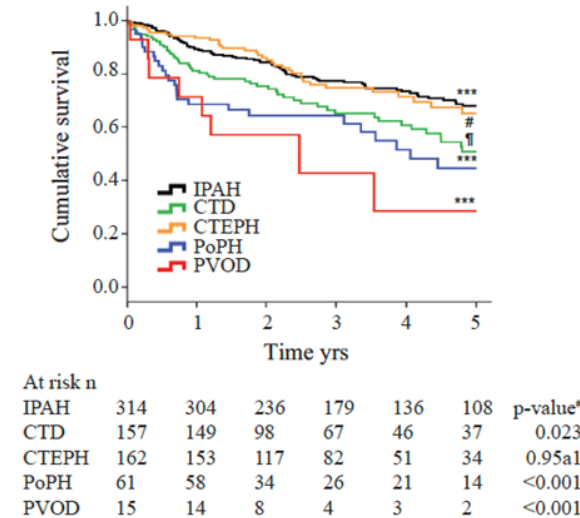
Figure 2. Kaplan-Meier estimates of 5-yr survival from time of diagnosis in different pulmonary hypertension subtypes. **IPAH** = Idiopathic Pulmonary arterial hypertension, **CTD** = connective tissue disease, **CTEPH** = chronic thromboembolic pulmonary hypertension, **PoPH** = portopulmonary hypertension, **PVOD** = pulmonary veno-occlusive disease. The p-value for the overall comparison is <0.001. #: compared with IPAH. Reproduced with permission of the © ERS 2020: European Respiratory Journal 40 (3) 596-603; DOI: 10.1183/09031936.00101211 Published 31 August 2012

References

1. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. (2019) 53:1801913. doi: 10.1183/13993003.01913-2018

2. Jonigk D, Golpon H, Bockmeyer CL, Maegel L, Hoeper MM, Gottlieb J, et al. Plexiform lesions in pulmonary arterial hypertension composition, architecture, and microenvironment. Am J Pathol. (2011) 179:167–79. doi: 10.1016/j.ajpath.2011.03.040

3. Edwards BS, Weir EK, Edwards WD, Ludwig J, Dykoski RK, Edwards JE. Coexistent pulmonary and portal hypertension: morphologic and clinical features. J Am Coll Cardiol. (1987) 10:1233–8. doi: 10.1016/S0735-1097(87)80123-7

4. Humbert M, Guignabert C, Bonnet S, Dorfmuller P, Klinger JR, Nicolls MR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J. (2019) 53:1801887. doi: 10.1183/13993003.01887-2018

5. Krowka MJ. Portopulmonary hypertension. Semin Respir Crit Care Med. (2012) 33:17–25. doi: 10.1055/s-0032-1301731

6. Hadengue A, Benhayoun MK, Lebrec D, Benhamou JP. Pulmonary hypertension complicating portal hypertension: prevalence and relation to splanchnic hemodynamics. Gastroenterology. (1991) 100:520–8. doi: 10.1016/0016-5085(91)90225-A

7. Krowka MJ, Plevak DJ, Findlay JY, Rosen CB, Wiesner RH, Krom RA. Pulmonary hemodynamics and perioperative cardiopulmonary-related mortality in patients with portopulmonary hypertension undergoing liver transplantation. Liver Transpl. (2000) 6:443–50. doi: 10.1053/jlts. 2000.6356

8. Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. Eur Respir J. (2007) 30:104–9. doi: 10.1183/09031936.00092306

9. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med. (2006) 173:1023–30. doi: 10.1164/rccm.200510-1668OC

10. Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, et al. Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. Chest. (2010) 137:376–87. doi: 10.1378/chest. 09-1140

11. Zelniker TA, Huscher D, Vonk-Noordegraaf A, Ewert R, Lange TJ, Klose H, et al. The 6MWT as a prognostic tool in pulmonary arterial hypertension: results from the COMPERA registry. Clin Res Cardiol. (2018) 107:460– 70. doi: 10.1007/s00392-018-1207-5

12. Savale L, Guimas M, Ebstein N, Fertin M, Jevnikar M, Renard S, et al. Portopulmonary hypertension in the current era of pulmonary hypertension management. J Hepatol. (2020) 73:130–9. doi: 10.1016/j.jhep.2020. 02.021

13. Chen HS, Xing SR, Xu WG, Yang F, Qi XL, Wang LM, et al. Portopulmonary hypertension in cirrhotic patients: Prevalence, clinical features and risk factors. Exp Ther Med. (2013) 5:819–24. doi: 10.3892/etm. 2013.918

14. Sithamparanathan S, Nair A, Thirugnanasothy L, Coghlan JG, Condliffe R, Dimopoulos K, et al. National Pulmonary Hypertension Service Research Collaboration of the United, and Ireland, Survival in portopulmonary hypertension: Outcomes of the United Kingdom National Pulmonary Arterial Hypertension Registry. J Heart Lung Transplant. (2017) 36:770– 9. doi: 10.1016/j.healun.2016.12.014

15. Schouten JN, Garcia-Pagan JC, Valla DC, Janssen HL. Idiopathic noncirrhotic portal hypertension. Hepatology. (2011) 54:1071–81. doi: 10.1002/hep.24422

16. McDonnell PJ, Toye PA, Hutchins GM. Primary pulmonary hypertension and cirrhosis: are they related? Am Rev Respir Dis. (1983) 127:437–41. doi: 10.1164/arrd.1983.127.4.437

17. Colle IO, Moreau R, Godinho E, Belghiti J, Ettori F, Cohen-Solal A, et al. Diagnosis of portopulmonary hypertension in candidates for liver transplantation: a prospective study. Hepatology. (2003) 37:401– 9. doi: 10.1053/jhep.2003.50060

18. Kawut SM, Taichman DB, Ahya VN, Kaplan S, Archer-Chicko CL, Kimmel SE, et al. Hemodynamics and survival of patients with portopulmonary hypertension. Liver Transpl. (2005) 11:1107–11. doi: 10.1002/ lt.20459

19. Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. Portopulmonary hypertension: Results from a 10-year screening algorithm. Hepatology. (2006) 44:1502– 10. doi: 10.1002/hep. 21431

20. Krowka MJ, Miller DP, Barst RJ, Taichman D, Dweik RA, Badesch DB, et al. Portopulmonary hypertension: a report fromthe US-based REVEAL Registry.Chest. (2012) 141:906–15. doi: 10.1378/chest.11-0160

21. K.L. Swanson, R.H. Wiesner, S.L. Nyberg, C.B. Rosen, and M.J. Krowka, Survival in portopulmonary hypertension: Mayo Clinic experience categorized by treatment subgroups. Am J Transplant 8 (2008) 2445-53.

22. J. Le Pavec, R. Souza, P. Herve, D. Lebrec, L. Savale, C. Tcherakian, X. Jais, A. Yaici, M. Humbert, G. Simonneau, and O. Sitbon, Portopulmonary hypertension: survival and prognostic factors. Am J Respir Crit Care Med 178 (2008) 637-43.

23. M. Humbert, O. Sitbon, A. Chaouat, M. Bertocchi, G. Habib, V. Gressin, A. Yaici, E. Weitzenblum, J.F. Cordier, F. Chabot, C. Dromer, C. Pison, M. Reynaud-Gaubert, A. Haloun, M. Laurent, E. Hachulla, V. Cottin, B. Degano, X. Jais, D. Montani, R. Souza, and G. Simonneau, Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation 122 (2010) 156-63.

24. P. Escribano-Subias, I. Blanco, M. Lopez-Meseguer, C.J. Lopez-Guarch, A. Roman, P. Morales, M.J. Castillo-Palma, J. Segovia, M.A. Gomez-Sanchez, J.A. Barbera, and R. investigators, Survival in pulmonary hypertension in Spain: insights from the Spanish registry. Eur Respir J 40 (2012) 596-603.

25. M. Lazaro Salvador, C.A. Quezada Loaiza, L. Rodriguez Padial, J.A. Barbera, M. Lopez-Meseguer, R. Lopez-Reyes, E. Sala Llinas, S. Alcolea, I. Blanco, P. Escribano Subias, and R. investigators, Portopulmonary hypertension: prognosis and management in the current treatment era. Results from the REHAP Registry. Intern Med J (2020).

26. M.J. Krowka, M.S. Mandell, M.A. Ramsay, S.M. Kawut, M.B. Fallon, C. Manzarbeitia, M. Pardo, Jr., P. Marotta, S. Uemoto, M.P. Stoffel, and J.T. Benson, Hepatopulmonary syndrome and portopulmonary hypertension: a report of the multicenter liver transplant database. Liver Transpl 10 (2004) 174-82.

27. N. Sussman, V. Kaza, N. Barshes, R. Stribling, J. Goss, C. O'Mahony, E. Zhang, J. Vierling, and A. Frost, Successful liver transplantation following medical management of portopulmonary hypertension: a single-center series. Am J Transplant 6 (2006) 2177-82.

CHAPTER 2

Pathogenesis of PoPH

The underlying mechanisms for the development of PoPH are poorly understood and continue to be an area of active research. Liver cirrhosis and portal hypertension lead to splanchnic vasodilatation and formation of portosystemic shunts, which are thought to contribute to PoPH pathogenesis in multiple ways (**Figure 3**). Mechanistically, increased intrahepatic resistance to blood flow due to cirrhosis results in an increased portal pressure gradient and portosystemic collateralization through the reperfusion/dilation of preexisting blood vessels and through the generation of new vessels[28]. On a molecular level, portosystemic shunting enables blood to bypass the liver, and thus evade hepatic metabolism of vasoactive substances. This direct reduction in the peripheral vascular resistance, combined with indirect vasodilation via intestinal vasoactive substances enable blood to bypass the liver and reach systemic circulation, ultimately resulting in a hyperdynamic state. This phenomenon has been seen in patients immediately after undergoing a transjugular intrahepatic portosystemic shunt (TIPS) placement, and persist for at least 1 month[29]. Likewise, levels of vasoactive substances acting in the pulmonary circulation (including pulmonary vasodilators nitric oxide, and prostacyclin, as well as pulmonary vasoconstrictors endothelin-1, thromboxane A2, and serotonin), are similarly dysregulated in cirrhosis[30; 31; 32]. The imbalance of these vasoactive substances in the pulmonary vasculature results in net vasoconstriction and pulmonary vascular resistance elevation.

Shear stress from persistently high blood flow leads to endothelial cell injury and the activation and repression of genes that participate in the vascular remodeling process, and is believed to play a role in various forms of PAH[33; 34; 35]. Exposure of and damage to the underlying arterial smooth muscle results in smooth muscle proliferation and thickening of the tunica intima, media, and adventitia within the pulmonary vasculature[4]. The arterial wall thickening, in turn, can result in more sluggish pulmonary blood flow, platelet aggregation, and thrombus formation, which may become recanalized over time[3]. Autopsy data of patients with PoPH has confirmed that the end stage of this process is medial and intimal thickening, plexiform lesions and fibrotic venular obstruction[36]. Together, these vascular lesions result in permanently elevated PVR that distinguishes PoPH from other causes of elevated mean pulmonary artery pressure (mPAP) in patients with portal hypertension.

Downregulation of bone morphogenic protein receptor 2 (BMPR2), a member of the TGF-β superfamily, has been shown to play a key role in development familial pulmonary hypertension,

and mutations in the BMPR2 gene encoding this receptor has also been found in 15-40% of idiopathic cases of PAH[37; 38]. Bone Morphogenic Protein 9 (BMP9) is a circulating factor produced by hepatic stellate cells[39]. Recent studies have shown that patients with PoPH have significantly lower circulating levels of BMP9 as compared to control patients with advanced liver disease and no evidence of pulmonary hypertension[40]. BMP9 levels were likewise significantly lower in patients with PoPH as compared to patients with other etiologies of group 1 PAH[41]. Moreover, selective enhancement of endothelial BMPR2 with exogenous BMP9 has been shown to reverse the presence of PAH in multiple mouse models of PAH[42]. Together, these findings suggest that BMP9 and BMPR2 are associated with downstream signaling pathways and also likely play a role in the pathogenesis of PoPH.

More recently, activation of toll-like receptors (TLRs) by bacterial lipopolysaccharides (LPS) from bacterial translocation has been implicated in the pathogenesis of PAH. Mouse models deficient in BMPR2 and exposed to LPS were shown to develop evidence of pulmonary hypertension, which did not occur in their wild-type counterparts[43]. The underlying mechanism for this is thought to be rooted in increased IL-6 production and activation of the STAT3 transcription factor, which is associated with increased expression of TLR4[43]. TLR4 is the main receptor for LPS and has been previously show to promote the development of PAH[44; 45; 46]. Together, this data suggests that exposure to bacterial endotoxins, which occur more frequently in cirrhotic patients due to recurrent gut bacterial translocations, may provide a 'second hit' towards the development of PoPH in an otherwise predisposed host.

Figure 3: Highlighting the pathogenesis of PoPH

FIGURE 3 | Pathogenesis of Portopulmonary Hypertension (PoPH). Hepatic fibrosis results in portal venous hypertension, increased resistance to hepatic blood flow, and splanchnic vasodilation. Splanchnic vasodilation results in increase in overall circulating volume. and the diversion of blood flow from the liver to the heart via porto-systemic shunting resulting in an overall hyperdynamic state. Dysregulation of key regulator of pulmonary vascular tone associated with cirrhosis results in pulmonary vasoconstriction. At the same time, damage to the pulmonary endothelium and the underlying smooth muscle results in permanent vascular remodeling, which ultimately leads to the development of pulmonary hypertension. CO, cardiac output; NO, nitric oxide; ET-1, endothelin-1; TXA2, thromboxane A2; 5-HT, serotonin; E2, estrogen; BMP9, Bone Morphogenic Protein 9; BMPR2, Bone Morphogenic Receptor 2. Please refer to the text for additional details.

References

28. M. Bolognesi, M. Di Pascoli, A. Verardo, and A. Gatta, Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. World J Gastroenterol 20 (2014) 2555-63.

29. P. Van der Linden, O. Le Moine, M. Ghysels, M. Ortinez, and J. Deviere, Pulmonary hypertension after transjugular intrahepatic portosystemic shunt: effects on right ventricular function. Hepatology 23 (1996) 982-7.

30. A. Huertas, C. Guignabert, J.A. Barbera, P. Bartsch, J. Bhattacharya, S. Bhattacharya, M.R. Bonsignore, L. Dewachter, A.T. Dinh-Xuan, P. Dorfmuller, M.T. Gladwin, M. Humbert, T. Kotsimbos, T. Vassilakopoulos, O. Sanchez, L. Savale, U. Testa, and M.R. Wilkins, Pulmonary vascular endothelium: the orchestra conductor in respiratory diseases: Highlights from basic research to therapy. Eur Respir J 51 (2018).

31. B. Shenoda, and J. Boselli, Vascular syndromes in liver cirrhosis. Clin J Gastroenterol 12 (2019) 387-397.

32. M. Porres-Aguilar, J.T. Altamirano, A. Torre-Delgadillo, M.R. Charlton, and A. Duarte-Rojo, Portopulmonary hypertension and hepatopulmonary syndrome: a clinician-oriented overview. Eur Respir Rev 21 (2012) 223-33.

33. R. Rodriguez-Roisin, M.J. Krowka, P. Herve, M.B. Fallon, and E.R.S.T.F.P.-H.V.D.S. Committee, Pulmonary-Hepatic vascular Disorders (PHD). Eur Respir J 24 (2004) 861-80.

34. N. Baeyens, C. Bandyopadhyay, B.G. Coon, S. Yun, and M.A. Schwartz, Endothelial fluid shear stress sensing in vascular health and disease. J Clin Invest 126 (2016) 821-8.

35. E. Pascall, and R.M. Tulloh, Pulmonary hypertension in congenital heart disease. Future Cardiol 14 (2018) 343-353.

36. M. Furuta, T. Sato, I. Tsujino, M. Tanino, T. Watanabe, and M. Nishimura, An autopsy case of portopulmonary hypertension associated with idiopathic portal hypertension. European Respiratory Journal 42 (2013) P2649.

37. P.P.H.C. International, K.B. Lane, R.D. Machado, M.W. Pauciulo, J.R. Thomson, J.A. Phillips, 3rd, J.E. Loyd, W.C. Nichols, and R.C. Trembath, Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet 26 (2000) 81-4.

38. J.R. Thomson, R.D. Machado, M.W. Pauciulo, N.V. Morgan, M. Humbert, G.C. Elliott, K. Ward, M. Yacoub, G. Mikhail, P. Rogers, J. Newman, L. Wheeler, T. Higenbottam, J.S. Gibbs, J. Egan, A. Crozier, A. Peacock, R. Allcock, P. Corris, J.E. Loyd, R.C. Trembath, and W.C. Nichols, Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. J Med Genet 37 (2000) 741-5.

39. A. Desroches-Castan, E. Tillet, N. Ricard, M. Ouarne, C. Mallet, L. Belmudes, Y. Coute, O. Boillot, J.Y. Scoazec, S. Bailly, and J.J. Feige, Bone Morphogenetic Protein 9 Is a Paracrine Factor Controlling Liver Sinusoidal Endothelial Cell Fenestration and Protecting Against Hepatic Fibrosis. Hepatology 70 (2019) 1392-1408.

40. E.R. Rochon, M.J. Krowka, S. Bartolome, G.A. Heresi, T. Bull, K. Roberts, A. Hemnes, K.A. Forde, K.L. Krok, M. Patel, G. Lin, M. McNeil, N. Al-Naamani, B.L. Roman, P.B. Yu, M.B. Fallon, M.T. Gladwin, S.M. Kawut, and G. Pulmonary Vascular Complications

of Liver Disease 2 Study, BMP 9/10 in Pulmonary Vascular Complications of Liver Disease. Am J Respir Crit Care Med (2020).

41. I. Nikolic, L.M. Yung, P. Yang, R. Malhotra, S.D. Paskin-Flerlage, T. Dinter, G.A. Bocobo, K.E. Tumelty, A.J. Faugno, L. Troncone, M.E. McNeil, X. Huang, K.R. Coser, C.S.C. Lai, P.D. Upton, M.J. Goumans, R.T. Zamanian, C.G. Elliott, A. Lee, W. Zheng, S.P. Berasi, C. Huard, N.W. Morrell, R.T. Chung, R.W. Channick, K.E. Roberts, and P.B. Yu, Bone Morphogenetic Protein 9 Is a Mechanistic Biomarker of Portopulmonary Hypertension. Am J Respir Crit Care Med 199 (2019) 891-902.

42. L. Long, M.L. Ormiston, X. Yang, M. Southwood, S. Graf, R.D. Machado, M. Mueller, B. Kinzel, L.M. Yung, J.M. Wilkinson, S.D. Moore, K.M. Drake, M.A. Aldred, P.B. Yu, P.D. Upton, and N.W. Morrell, Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. Nat Med 21 (2015) 777-85.

43. E. Soon, A. Crosby, M. Southwood, P. Yang, T. Tajsic, M. Toshner, S. Appleby, C.M. Shanahan, K.D. Bloch, J. Pepke-Zaba, P. Upton, and N.W. Morrell, Bone morphogenetic protein receptor type II deficiency and increased inflammatory cytokine production. A gateway to pulmonary arterial hypertension. Am J Respir Crit Care Med 192 (2015) 859-72.

44. E.M. Bauer, R.S. Chanthaphavong, C.P. Sodhi, D.J. Hackam, T.R. Billiar, and P.M. Bauer, Genetic deletion of toll-like receptor 4 on platelets attenuates experimental pulmonary hypertension. Circ Res 114 (2014) 1596-600.

45. E.M. Bauer, R. Shapiro, H. Zheng, F. Ahmad, D. Ishizawar, S.A. Comhair, S.C. Erzurum, T.R. Billiar, and P.M. Bauer, High mobility group box 1 contributes to the pathogenesis of experimental pulmonary hypertension via activation of Toll-like receptor 4. Mol Med 18 (2013) 1509-18.

46. K.C. Young, S.M. Hussein, R. Dadiz, D. deMello, C. Devia, D. Hehre, and C. Suguihara, Toll-like receptor 4-deficient mice are resistant to chronic hypoxia-induced pulmonary hypertension. Exp Lung Res 36 (2010) 111-9.

CHAPTER 3

Role of Estrogen in Pathogenesis of PoPH

Recently, altered estrogen metabolism has been implicated as a culprit pathway in development of other forms of WHO group I PAH. Elevated serum estrogen has been reported in males with idiopathic PAH. Additionally, urine metabolites of estrogen have been shown to be elevated in familial PAH. However, there is no such study ever conducted in patients with PoPH. Aromatase plays a critical role in the metabolism of estrogen, and aromatase expression in the liver is induced in some liver conditions. Under normal circumstances estrogen is metabolized by oxidation in extrahepatic tissues by CYP450 by hydroxylation at the C-16 and C-2 position, resulting in 16α-hydroxyestrone (16α-OHE1) in extrahepatic tissues (Figure 4). While 2-OHE is a weak mitogen, 16α-OHE1 stimulates cellular proliferation by activating the estrogen receptor, making it both more mitogenic and potentially more "genotoxic". Disturbances that alter the relative proportion of these downstream byproducts of estrogen metabolism may therefore have an impact on disease expression in the pulmonary vascular bed. Thus, individuals who metabolize a larger proportion of estrogen to 16α-OHE1 may be at increased risk of developing PH that result from both the mitogenic and genotoxic effects of estrogens. Higher urinary 16 α-OHE1/2-OHE ratio are both risk factors for heritable PAH. There is growing evidence that an altered estrogen hormone also contributes to the pathogenesis of PoPH. Estrogen binds to the promoter region of the BMPR2 gene and regulates its expression[47]. Polymorphisms in CYP1B1 which metabolizes estrogen has been shown to be associated with penetrance of PAH in women with BMPR2 mutations, but not men[48]. Expression of CYP1B1 has also been shown to be increased in experimental models and human PAH[49]. A candidate gene study to identify genetic risk factors in PoPH found single nucleotide polymorphisms (SNPs) in the genes coding for estrogen receptor 1 and aromatase [50]. Recently, Al-Naamani et al showed that in PoPH patients, the risk allele rs7175922 in CYP19A1 was associated with significantly higher levels of estradiol, urinary 2-hydroxyestrogen/16-α-hydroxyestrone (2-OHE/16α-OHE1), plasma levels of dehydroepiandrosterone-sulfate and plasma levels of 16-α-hydroxyestradiol (16α-OHE2) compared to the patients with liver disease but with no PoPH [51]. 16α-OHE1 leads to the development of PAH in animals via upregulation of micro-RNA (miR-29) and has been associated with abnormal markers of insulin resistance[52]. 16α-OHE1 has also been shown to increase oxidative-stress related proliferation in pulmonary arterial smooth muscle cells from PAH patients[53]. Similarly, Sahay et al performed qualitative immunohistochemical (IHC) staining of the explanted livers of PoPH patients and compared them with normal and cirrhotic livers (Figure 5). However, limited by the small sample size, the authors

did not find any significant difference between the PoPH and cirrhotic livers, but aromatase expression was increased compared to the normal liver[54]. Additional research is needed to explore the links between altered estrogen metabolism and the development of portopulmonary hypertension (PoPH) in patients with liver disease.

Figure 4 : Estrogen biosynthesis and metabolism
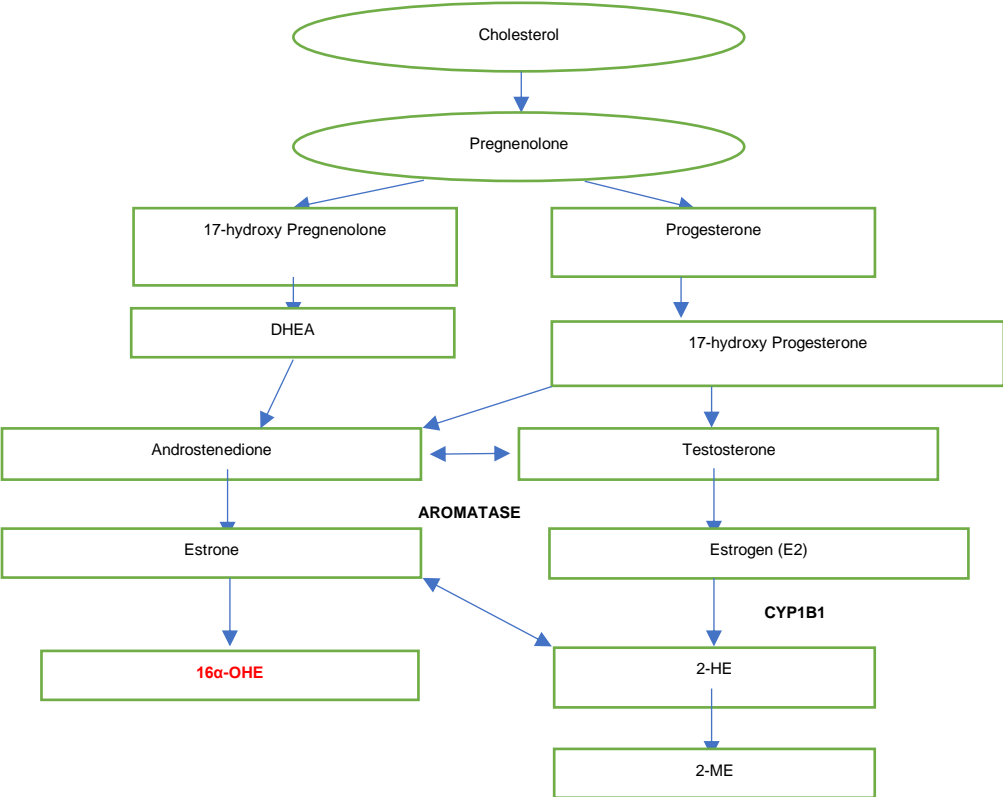
Figure 5 : Immunohistochemical (IHC) staining for aromatase of the explanted liver from patients with liver cirrhosis and with POPH with positive control of placental tissue (Panel D). Panel A shows IHC staining of normal hepatocytes without increased expression of aromatase. Panel B and C are photomicrographs of cirrhotic patients without and with PAH respectively. As shown in Panel B and C, hepatocytes show increased expression of aromatase which is abnormal but not distinguishable between the two.
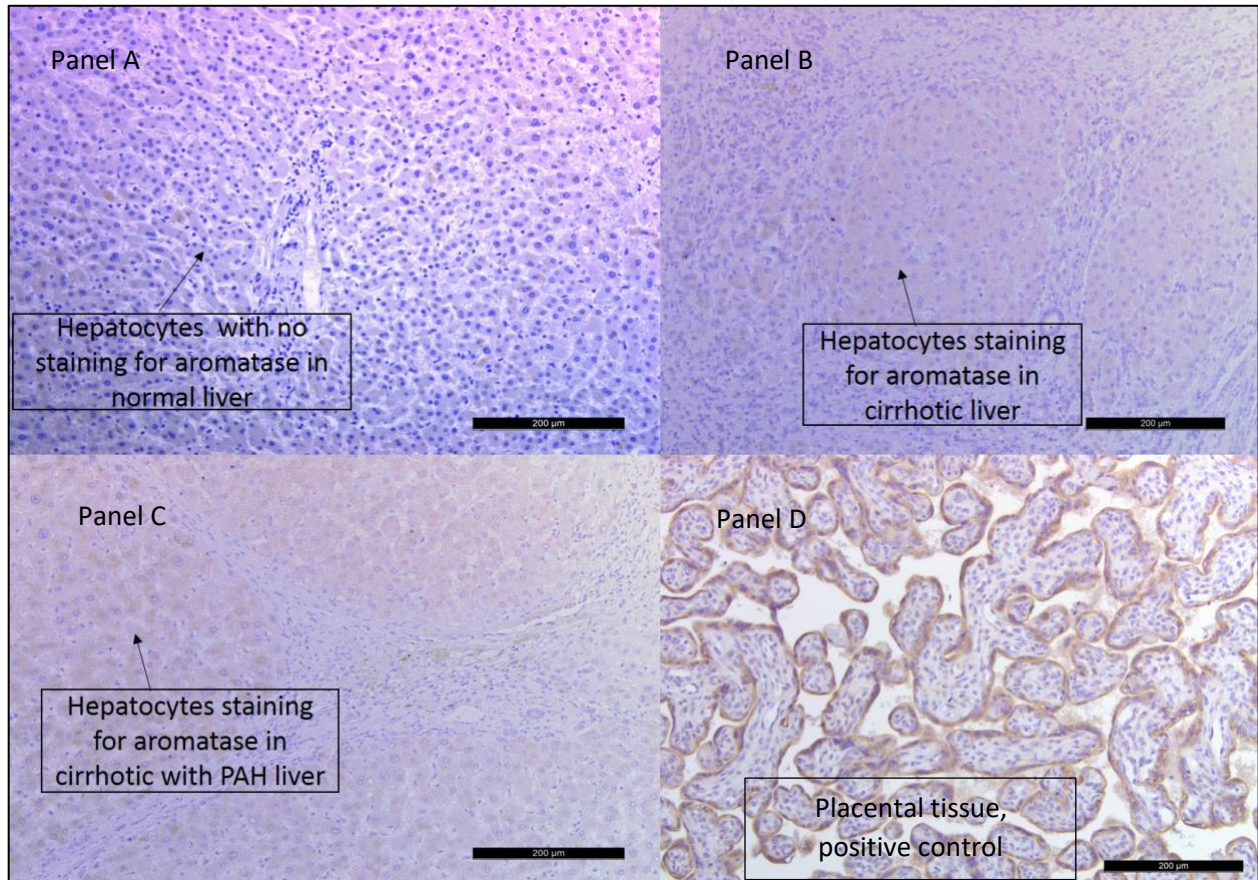
References

47 E.D. Austin, R. Hamid, A.R. Hemnes, J.E. Loyd, T. Blackwell, C. Yu, J.A. Phillips Iii, R. Gaddipati, S. Gladson, E. Gu, J. West, and K.B. Lane, BMPR2 expression is suppressed by signaling through the estrogen receptor. Biol Sex Differ 3 (2012) 6.

48 E.D. Austin, J.D. Cogan, J.D. West, L.K. Hedges, R. Hamid, E.P. Dawson, L.A. Wheeler, F.F. Parl, J.E. Loyd, and J.A. Phillips, 3rd, Alterations in oestrogen metabolism: implications for higher penetrance of familial pulmonary arterial hypertension in females. Eur Respir J 34 (2009) 1093-9.

49 K. White, A.K. Johansen, M. Nilsen, L. Ciuclan, E. Wallace, L. Paton, A. Campbell, I. Morecroft, L. Loughlin, J.D. McClure, M. Thomas, K.M. Mair, and M.R. MacLean,

Activity of the estrogen-metabolizing enzyme cytochrome P450 1B1 influences the development of pulmonary arterial hypertension. Circulation 126 (2012) 1087-98.

50 K.E. Roberts, M.B. Fallon, M.J. Krowka, R.S. Brown, J.F. Trotter, I. Peter, H. Tighiouart, J.A. Knowles, D. Rabinowitz, R.L. Benza, D.B. Badesch, D.B. Taichman, E.M. Horn, S. Zacks, N. Kaplowitz, S.M. Kawut, and G. Pulmonary Vascular Complications of Liver Disease Study, Genetic risk factors for portopulmonary hypertension in patients with advanced liver disease. Am J Respir Crit Care Med 179 (2009) 835-42.

51 N. Al-Naamani, M.J. Krowka, K.A. Forde, K.L. Krok, R. Feng, G.A. Heresi, R.A. Dweik, S. Bartolome, T.M. Bull, K.E. Roberts, E.D. Austin, A.R. Hemnes, M.J. Patel, J.K. Oh, G. Lin, M.F. Doyle, N. Denver, R. Andrew, M.R. MacLean, M.B. Fallon, S.M. Kawut, and G. Pulmonary Vascular Complications of Liver Disease Study, Estrogen Signaling and Portopulmonary Hypertension: The Pulmonary Vascular Complications of Liver Disease Study (PVCLD2). Hepatology (2020).

52 X. Chen, M. Talati, J.P. Fessel, A.R. Hemnes, S. Gladson, J. French, S. Shay, A. Trammell, J.A. Phillips, R. Hamid, J.D. Cogan, E.P. Dawson, K.E. Womble, L.K. Hedges, E.G. Martinez, L.A. Wheeler, J.E. Loyd, S.J. Majka, J. West, and E.D. Austin, Estrogen Metabolite 16alpha-Hydroxyestrone Exacerbates Bone Morphogenetic Protein Receptor Type II-Associated Pulmonary Arterial Hypertension Through MicroRNA-29-Mediated Modulation of Cellular Metabolism. Circulation 133 (2016) 82-97.

53 K.Y. Hood, A.C. Montezano, A.P. Harvey, M. Nilsen, M.R. MacLean, and R.M. Touyz, Nicotinamide Adenine Dinucleotide Phosphate Oxidase-Mediated Redox Signaling and Vascular Remodeling by 16alpha-Hydroxyestrone in Human Pulmonary Artery Cells: Implications in Pulmonary Arterial Hypertension. Hypertension 68 (2016) 796-808.

54 S. Sahay, R. Barrios, M. Deavers, Y. Ren, and A.E. Frost, Aromatase Expression of Liver in Portopulmonary Hypertension, A63. RIDE THE LIGHTNING: BASIC AND TRANSLATIONAL STUDIES IN PULMONARY HYPERTENSION, pp. A2102-A2102.

CHAPTER 4

Screening and Diagnosis of PoPH

Screening for portalpulmonary hypertension (PoPH) should begin with a detailed history and physical exam (**Figure 5**). Common symptoms of PoPH include unexplained dyspnea on exertion, fatigue, weakness, orthopnea, near-syncope, syncope, palpitations, or chest pain, with dyspnea being by far the most common. On physical exam, these patients often have an accentuated pulmonic component of the second heart sound (P2), a right-sided S3, and a right-sided S4. Additionally, the presence of a tricuspid regurgitant murmur or a pulmonic insufficiency murmur can be heard in 40% and 13% of patients, respectively[55]. Other signs of right heart failure, including jugular venous distention, ascites, and bilateral lower extremity edema, may also be seen[56]. Additionally, a patient history screening for other known WHO group 1 PAH risk factors should be taken. This includes use of drugs such as methamphetamines, use of dietary supplements, prior history of chemotherapy and history of autoimmune diseases. Clinicians should also review the medical history for any other conditions known to be associated with PH, such as left heart disease, chronic lung disease, obstructive sleep apnea, venous thromboembolism, and hematologic disorders. If the history and physical arouses any suspicion for PoPH, the patient should undergo an echocardiogram.

Laboratory workup should include serological screening tests for connective tissue diseases, hepatitis and HIV. Additional evaluation with pulmonary function tests (including spirometry, lung volumes, diffusion capacity, and a six minute walk test) and a computerized tomographu (CT) scan of the chest should be obtained to screen for the presence of significant lung disease, and to establish a baseline which can then be used to track disease progression[11; 57]. A ventilation/perfusion (V/Q) scan should also be performed to screen for the presence of chronic thromboembolic pulmonary hypertension (CTEPH) (especially in the setting of portal vein thrombosis). See **Table 1** for additonal symptoms, physical exam and imaging findings.

Transthoracic echocardiography (TTE) is the best screening test for PoPH, as it is for other forms of PAH. All patients with portal hypertension should also be screened for PoPH with a TTE. A list of the most common echocardiographic findings that are seen in patients with pulmonary hypertension can be seen in Table 1. Although traditionally used throughout the literature to screen for the presence of pulmonary hypertension, right ventricular systolic pressure (RVSP) calculations using the simplified Bernoulli equation are prone to error, stemming from the difficulty in accurately assessing a patient's right atrial pressure. In their analysis of

echocardiographic screening of patients undergoing liver transplant evaluation, Krowka et al. demonstrated only moderate correlation between the RVSP and the PA systolic pressures that were measured on right heart catheterization (RHC)[19]. Moreover, this discrepancy increased with increasing values of RVSP. Likewise, the cutoff values of RVSP used for ruling out the presence of PoPH have differed between studies, and the ideal threshold value for a positive screen has been a matter of debate. As such, it has been suggested that the use of RVSP should be replaced by continuous wave Doppler measurements of peak tricuspid regurgitant velocity, with cutoff values of 2.8 m·s^{-1} for intermediate and 3.4 m·s^{-1} for high probability for the presence of PH[58]. Issues with calculating RVSP notwithstanding, current guidelines from the American Association for the Study of Liver Diseases (AASLD) recommend evaluation with right heart catheterization in all patients being considered for LT with an RVSP $\geq$ 45 mmHg on screening echocardiogram[59]. The International Liver Transplant Society (ILTS) guidelines recommend a RHC in patients with RVSP > 50 mmHg and/or evidence of right ventricular (RV) hypertrophy or dysfunction on TTE[60]. Clinicians should understand that when lower RVSP cutoffs are used, the sensitivity will approach 100%, but the specificity will decrease, resulting in a significant number of false positives[17]. However, given the high mortality associated with severe PoPH and LT, performing an unnecessary RHC is preferable to "missing" PoPH. In practice, all patients in whom there are TTE findings suspicious for PoPH (elevated RVSP or signs of RV dysfunction) should undergo RHC.

Right heart catheterization hemodynamics interpretation in PoPH

The diagnosis of PoPH includes hemodynamic evaluation of both the pulmonary and hepatic circulation systems. Portal hypertension can be challenging to diagnose noninvasively. Esophageal varices and skin collaterals are suggestive of portal hypertension. On ultrasound, decreased portal vein flow velocity, and portal vein biphasic or flow reversal are suggestive of portal hypertension[61]. A hepatic venous pressure gradient (HVPG) (wedge hepatic vein pressure [WHVP] – free hepatic vein pressure [FHVP]) of > 5 mmHg is diagnostic of sinusoidal portal hypertension[62].

On RHC, the patient must have both an elevated mPAP and PVR and a normal pulmonary artery wedge pressure (PAWP) for a positive diagnosis. During the most recent World Symposium on Pulmonary Hypertension, the hemodynamic criteria for the diagnosis of pre-capillary

pulmonary hypertension (which includes WHO Group 1) were revised. With this change, patients with a mPAP > 20 mmHg (previously > 25 mmHg), a PVR of ≥ 3 Wood units (WU) (≥240 dynes), and a PAWP < 15 mmHg meet the diagnostic criteria for the presence of PAH[1].

Careful evaluation of a patient's hemodynamic profile on RHC is crucial in order to distinguish PoPH from other clinical conditions that are common in patients with liver disease that can also result in elevated mPAPs. These conditions can be classified into three broad categories: hyperdynamic state, volume overload and true PoPH[63]. See **Figure 6** for comparisons between these hemodynamic profiles.

Patients with decompensated liver disease may present with an elevated mPAP, PAWP and PVR. This may be due to a combination of volume overload, hyperdynamic state, and PoPH (perhaps all three). Volume overload is managed with careful diuresis, as patients with liver disease are prone to kidney injury[64]. A hyperdynamic state can be intrinsic to liver disease, but may also be due to other treatable medical comorbidities (such as anemia, arteriovenous shunts, and hyperthyroidism)[65; 66; 67]. This common clinical scenario speaks to the challenge of diagnosing and treating PoPH, which is best done at expert centers which are most familiar with these complicated patients.

Figure 6 : Algorithm for diagnosis of PoPH

(TTE: transthoracic echocardiography; CTD: Connective tissue disease; CT: computed tomography; PFT: Pulmonary function tests; V/Q: Ventilation perfusion test)
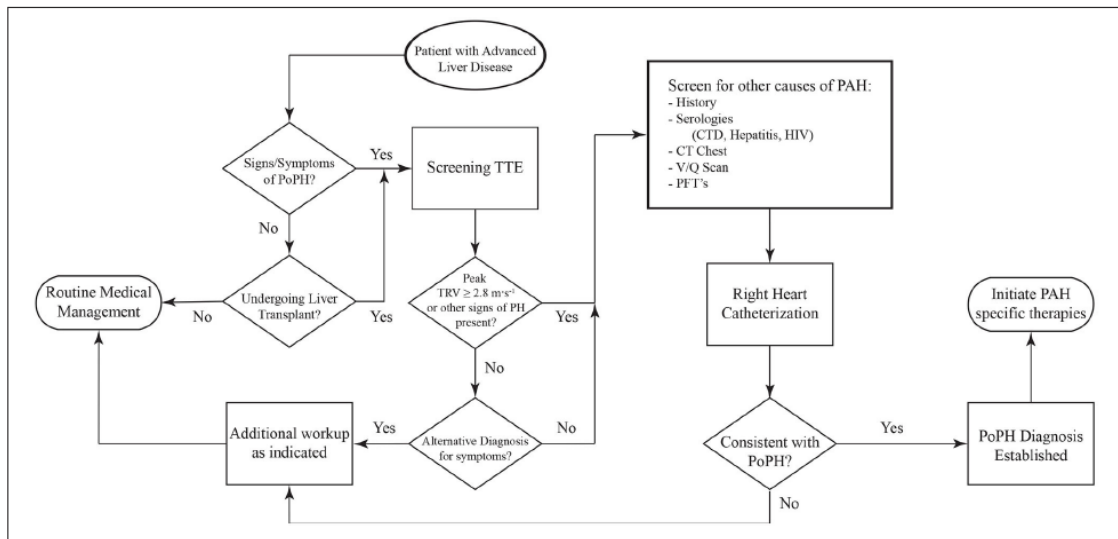
Table 1 Clinical symptoms and examination findings in PAH

TABLE 1 | Comon clinical and diagnostic findings in patients with Pulmonary Hypertension.

Symptoms	Physical exam	CT findings	TTE findings (57)
Dyspnea on exertion	Accentuated P2	PA diameter ≥29 mm	Peak TR velocity > 2.8 m/s
Fatigue	Right sided S3	PA to Ascending Aorta	RV/LV basal diameter > 1.0
Weakness	Right sided S4	diameter ratio of ≥1	Flattening of interventricular septum
Orthopnea	TR Murmur	RA Dilation	RV outflow Doppler acceleration
Near-syncope	Pulmonic Insufficiency Murmur	RV Dilation	<105 msec
Syncope	Jugular Venous Distension		Early diastolic pulmonary regurgitation
Palpitations	Ascites		velocity >2.2 m/s
Chest Pain	Lower Extremity Edema		PA diameter > 25 mm
	Cyanosis		IVC diameter >21 mm
			RA end-systole area >18 cm^2

TR, Tricuspid Regurgitation; PA, Pulmonary Artery; RA, Right Atrium; RV, Right Ventricle; LV, Left Ventricle; IVC, inferior vena cava.

Figure 7: Highlighting the hemodynamic changes seen in patients with liver disease
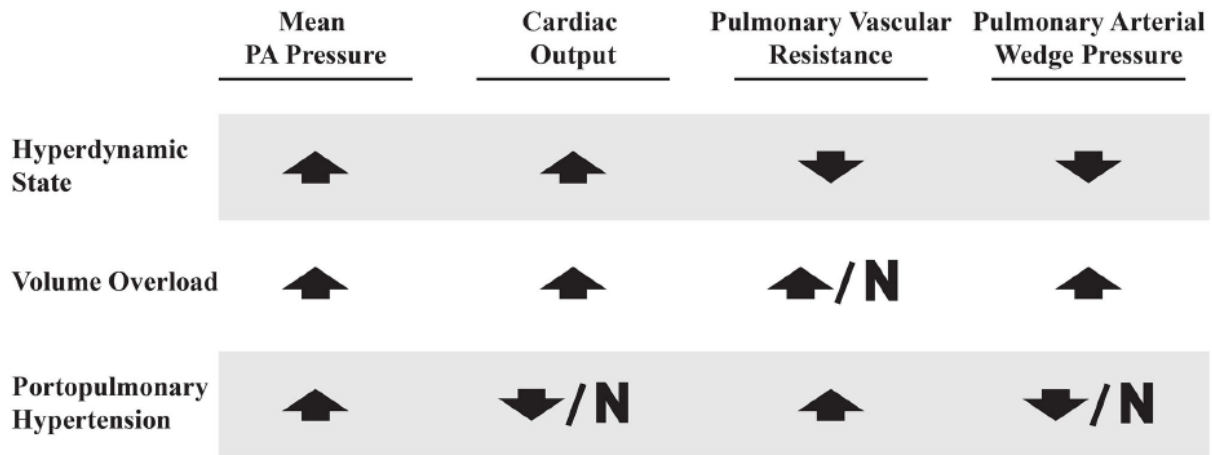
References

55 S. Rich, D.R. Dantzker, S.M. Ayres, E.H. Bergofsky, B.H. Brundage, K.M. Detre, A.P. Fishman, R.M. Goldring, B.M. Groves, S.K. Koerner, and et al., Primary pulmonary hypertension. A national prospective study. Ann Intern Med 107 (1987) 216-23.

56 B.D. Robalino, and D.S. Moodie, Association between primary pulmonary hypertension and portal hypertension: Analysis of its pathophysiology and clinical, laboratory and

hemodynamic manifestations. Journal of the American College of Cardiology 17 (1991) 492-498.

57 A. Frost, D. Badesch, J.S.R. Gibbs, D. Gopalan, D. Khanna, A. Manes, R. Oudiz, T. Satoh, F. Torres, and A. Torbicki, Diagnosis of pulmonary hypertension. Eur Respir J 53 (2019).

58 E.M. Lau, Y. Tamura, M.D. McGoon, and O. Sitbon, The 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: a practical chronicle of progress. Eur Respir J 46 (2015) 879-82.

59 P. Martin, A. DiMartini, S. Feng, R. Brown, Jr., and M. Fallon, Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. Hepatology 59 (2014) 1144-65.

60 M.J. Krowka, M.B. Fallon, S.M. Kawut, V. Fuhrmann, J.K. Heimbach, M.A. Ramsay, O. Sitbon, and R.J. Sokol, International Liver Transplant Society Practice Guidelines: Diagnosis and Management of Hepatopulmonary Syndrome and Portopulmonary Hypertension. Transplantation 100 (2016) 1440-52.

61 D.B. Hernández, M.A. Serrano, J.A. Gabutti, I.E. Casanova, and M. Guerrero, Portal hypertension. What, when and how of portal gradients, TIPS and Neo-porta. European Congress of Radiolog (2018).

62 R. de Franchis, and V.I.F. Baveno, Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol 63 (2015) 743-52.

63 R. Cartin-Ceba, and M.J. Krowka, Portopulmonary Hypertension. Clinics in Liver Disease 18 (2014) 421-438.

64 G. Garcia-Tsao, C.R. Parikh, and A. Viola, Acute kidney injury in cirrhosis. Hepatology 48 (2008) 2064-77.

65 S.C. Park, L.B. Beerman, J.C. Gartner, B.J. Zitelli, J.J. Malatack, F.J. Fricker, D.R. Fischer, R.A. Mathews, W.H. Neches, and J.R. Zuberbuhler, Echocardiographic findings before and after liver transplantation. Am J Cardiol 55 (1985) 1373-8.

66 Y.N.V. Reddy, V. Melenovsky, M.M. Redfield, R.A. Nishimura, and B.A. Borlaug, High-Output Heart Failure: A 15-Year Experience. J Am Coll Cardiol 68 (2016) 473-482.

67 B. AbuHalimeh, M.J. Krowka, and A.R. Tonelli, Treatment Barriers in Portopulmonary Hypertension. Hepatology 69 (2019) 431-443.

CHAPTER 5

Treatment of PoPH and Liver Transplantation

Treatment of Portopulmonary Hypertension (PoPH)

The importance of screening for PoPH was established by Krowka et al. in a retrospective review of case reports and case series, which looked at the relationship between the presence of untreated PoPH and cardiopulmonary related mortality in patients undergoing liver transplantation (LT)[7]. In this seminal work, Krowka et al. examined 43 patients with PoPH (confirmed by RHC) who were not on medical treatment and underwent LT. The patients were grouped based on severity of pulmonary hypertension. Patients with a mPAP between 25 and 35 mmHg were considered to have mild disease, while those with a mPAP between 35 and 50 mmHg were considered moderate, and those with a mPAP greater than 50 mmHg were considered severe. In these groups, 100% of the patients with severe and 50% of the patients with moderate PoPH died due to cardiopulmonary related events (the majority died during the transplantation hospitalization). There were no mortalities reported among the patients with mild PoPH (mPAP < 35 mmHg). As a result of this study, routine screening for the presence of pulmonary hypertension is now recommended in all patients undergoing evaluation for LT.

After this landmark study [7] established that PoPH patients with higher mPAP are at high risk of mortality with LT, the findings prompted the question of whether medical therapy could mitigate some of those identified risks. Treatment of PoPH prior to or instead of liver transplantation remains challenging and is fraught with a paucity of data and large knowledge gaps. Randomized controlled trials of pulmonary hypertension specific medical therapies for patients with portopulmonary hypertension have been limited by concerns regarding adverse hepatic effects of PAH specific medications[68].

Retrospective data on treatment of PoPH is quite heterogenous, with some studies showing improvement in hemodynamics and outcomes, and others showing no treatment effect. There is higher quality data from prospective non-randomized studies, from which a few themes emerge. A recently published prospective cohort study from the French Pulmonary Hypertension Registry (FPHR) examined data on 637 patients with PoPH, 90% of whom were started on PAH-specific therapies[12]. These patients experienced significant improved World Health Organization (WHO) functional class, 6-minute walk distance (6MWD) and hemodynamic parameters. The ANGEL study is a recent single arm prospective trial of ambrisentan in 31 patients with PoPH[69]. This trial showed improved hemodynamics (PVR, mPAP, cardiac index [CI]) and functional class, but no change in 6MWD. From these studies, it is reasonable to conclude that PAH directed therapy has favorable effects on hemodynamics and functional outcomes, but the effects on mortality and candidacy for LT are unclear.

As previously stated, the vast majority of randomized controlled trials of PAH-specific therapies in PAH patients have excluded patients with PoPH. One notable exception is the PATENT-1 trial by

Ghofrani et. al. (phase 3, double-blind, randomized controlled trial), which randomized 443 patients with various etiologies of WHO group 1 PAH to receive either placebo or two different doses of riociguat[70]. Riociguat is a soluble guyanyly cyclase stimulator which causes vasodilatation by increasing production of nitric oxide and also making endothelium more sensitized to nitric oxide. This trial is significant in that it included 13 patients with PoPH, 11 of whom received the highest dose of study medication (2.5 mg daily). The primary end point of the PATENT-1 trial was 6MWD, which was significantly improved in the riociguat 2.5mg group. Secondary hemodynamic (PVR, mPAP and CO) and functional (WHO functional class, Borg dyspnea scale and time to clinical worsening) endpoints were also significantly improved in the riociguat 2.5mg daily group. The PATENT-2 trial was an extension of the PATENT-1 study, in which all eligible patients from the first study were given riociguat and followed for 2 years. PATENT-2 showed sustained improvements in functional class and 6MWD[71]. A recently published subgroup analysis of the 13 patients with PoPH who were enrolled in PATENT-1 and 2 showed similar improvements in WHO functional class, 6MWD and PVR[72]. Importantly, riociguat was well tolerated by the PoPH patients.

The first ever randomized controlled trial of PAH specific therapy in patients with PoPH was published in 2019. Macitentan is a dual endothelin receptor antagonist which mediates pulmonary vasodilatation by blocking endothelin receptors. The PORTICO study, which randomized 85 patients with PoPH to receive either macitentan or placebo for 12 weeks, found that the macitentan group had a 35% reduction in PVR, the primary endpoint[73]. While there were significant improvements in some secondary endpoints (mPAP and CI), there were no significant improvements in WHO functional class and 6MWD. Importantly, macitentan was well tolerated in this population, with no hepatic side effects. A notable limitation of PORTICO is that it excluded patients with Child-Pugh class C liver disease and patients with a Model for End-Stage Liver Disease (MELD) score of $\geq$19, which are groups with known poor survival[12].

It is still unclear whether this significant reduction in PVR could translate into more meaningful outcomes for patients (improved functional capacity, decreased mortality) or alter candidacy for liver transplant. However, a recently published post-hoc analysis of the PORTICO study found that patients in the macitentan treatment group had significantly decreased waitlist and perioperative mortality risk categories as compared with the placebo arm[74].

Taken together, these studies show that PAH specific therapies can improve the hemodynamics and functional outcomes in patients with PoPH. Importantly, these medications seem to be well tolerated in patients with liver disease and PoPH. By extension, they can help patients become candidates for LT and potentially live longer. In the absence of PoPH specific treatment guidelines, clinicians should follow the general treatment principles of PAH, with special attention to the unique considerations for patients with PoPH. More research is needed to elucidate the nuances of PAH specific therapies in the PoPH population.

Liver Transplantation

As previously discussed, the severity of PoPH is an established risk factor for liver transplant waitlist mortality[75] and poor perioperative outcomes[7]. As such, decisions about candidacy for liver transplantation in patients with PoPH are complex and best done by experienced centers.

The following general principles are recommended by the American Association for the Study of Liver Disease (AASLD)[59] for management of patients with liver disease who are being considered for LT. All patients who are undergoing evaluation for LT should be screened for PoPH with transthoracic echocardiography, and patients with RVSP $\geq$ 45 mmHg should be further evaluated by RHC. Patients with mPAP $\leq$ 35 mmHg do not require treatment with PAH specific therapies and can proceed with further evaluation for LT. Patients with mPAP > 35 mmHg should be evaluated by an experienced PH specialist and be considered for PAH specific therapy. If the mPAP can be lowered to $\leq$ 35 mmHg and the PVR can be lowered to < 5 WU with medical therapy, LT can be pursued.

For some patients with mild liver disease and PoPH, their higher risk for waitlist mortality is not accurately captured by the MELD score[76]. LT candidates with PoPH are eligible for MELD exception points if the following criteria are met: initial mPAP > 35 and PVR > 3 WU; patients are treated with PAH specific therapy; and post-treatment mPAP is lowered to < 35 mmHg[76].

Giving MELD exception points to patients with PoPH is controversial for a number of reasons. The U.S. based Organ Procurement and Transplant Network (OPTN) requires a RHC and resubmission of full hemodynamic data every three months[76] to maintain the MELD exception points, which is burdensome and often impractical for both patients and transplant centers. Despite having to submit full hemodynamic data every 3 months, only mPAP < 35 mmHg is used to maintain eligibility MELD exception points. Some patients may achieve a lower PVR with PAH directed therapy but continue to have an elevated mPAP from volume overload and/or a high flow state[60]. In an analysis of patients with PoPH on the LT waitlist, initial PVR was an independent predictor of waitlist mortality, while pre- and post-treatment mPAP were not[75]. This suggests that a broader hemodynamic picture should be used to maintain MELD exception points eligibility.

In one analysis of 155 patients on the LT waitlist with approved PoPH MELD exception points, less than half of the patients actually met the standardized OPTN MELD exception criteria. Furthermore, almost one third of patients in this study lacked sufficient hemodynamic data to diagnose PoPH or had hemodynamic data that was inconsistent with PoPH[77]. These researchers found that overall mortality was higher for patients given PoPH MELD exception points (as compared to patients listed without exception

points), which shows the need for more research into the methods of risk stratification for patients with PoPH.

In the era of the AASLD/International Liver Transplantation Society (ILTS) guidelines and MELD exception points, data on the natural course of PoPH after LT is limited. In one retrospective cohort study, hemodynamics and survival data for 35 patients with PoPH were examined[78]. After LT, 6 months, 1-year and 3-year survival rates were 80%, 77% and 77%, respectively. Of the 27/35 patients who survived more than six months after LT, all were able to be weaned from intravenous epoprostenol. Furthermore, most of the patients had improvement in their hemodynamics after LT, with 30% having a mPAP < 25 mmHg at last follow up. This data suggests that carefully selected patients with PoPH who undergo LT will have significant improvement in their hemodynamics.

References

68 O. Sitbon, D.S. O'Callaghan, and L. Savale, Portopulmonary hypertension: light at the end of the tunnel? Chest 141 (2012) 840-842.

69 I.R. Preston, C.D. Burger, S. Bartolome, Z. Safdar, M. Krowka, N. Sood, H.J. Ford, W.F. Battarjee, M.M. Chakinala, M. Gomberg-Maitland, and N.S. Hill, Ambrisentan in portopulmonary hypertension: A multicenter, open-label trial. J Heart Lung Transplant (2020).

70 H.A. Ghofrani, N. Galie, F. Grimminger, E. Grunig, M. Humbert, Z.C. Jing, A.M. Keogh, D. Langleben, M.O. Kilama, A. Fritsch, D. Neuser, L.J. Rubin, and P.-S. Group, Riociguat for the treatment of pulmonary arterial hypertension. N Engl J Med 369 (2013) 330-40.

71 L.J. Rubin, N. Galie, F. Grimminger, E. Grunig, M. Humbert, Z.C. Jing, A. Keogh, D. Langleben, A. Fritsch, F. Menezes, N. Davie, and H.A. Ghofrani, Riociguat for the treatment of pulmonary arterial hypertension: a long-term extension study (PATENT-2). Eur Respir J 45 (2015) 1303-13.

72 R. Cartin-Ceba, M. Halank, H.A. Ghofrani, M. Humbert, J. Mattson, A. Fritsch, and M. Krowka, Riociguat treatment for portopulmonary hypertension: a subgroup analysis from the PATENT-1/-2 studies. Pulm Circ 8 (2018) 2045894018769305.

73 O. Sitbon, J. Bosch, E. Cottreel, D. Csonka, P. de Groote, M.M. Hoeper, N.H. Kim, N. Martin, L. Savale, and M. Krowka, Macitentan for the treatment of portopulmonary

hypertension (PORTICO): a multicentre, randomised, double-blind, placebo-controlled, phase 4 trial. Lancet Respir Med 7 (2019) 594-604.

74 M. Krowka, E. Cottreel, M.M. Hoeper, N.H. Kim, N. Martin, O. Sitbon, and J. Bosch, Macitentan Improves Risk Categorization for Liver Transplant Mortality in Patients With Portopulmonary Hypertension: A PORTICO Study Post Hoc Analysis. Liver Transpl 26 (2020) 935-940.

75 H.M. DuBrock, D.S. Goldberg, N.L. Sussman, S.D. Bartolome, Z. Kadry, R.J. Salgia, D.C. Mulligan, W.K. Kremers, S.M. Kawut, M.J. Krowka, and R.N. Channick, Predictors of Waitlist Mortality in Portopulmonary Hypertension. Transplantation 101 (2017) 1609-1615.

76 Guidance to Liver Transplant Programs and the National Liver Review Board for: Adult MELD Exception Review.

77 D.S. Goldberg, S. Batra, S. Sahay, S.M. Kawut, and M.B. Fallon, MELD exceptions for portopulmonary hypertension: current policy and future implementation. Am J Transplant 14 (2014) 2081-7.

78 L. Savale, C. Sattler, A. Coilly, F. Conti, S. Renard, C. Francoz, H. Bouvaist, C. Feray, P. Borentain, X. Jais, D. Montani, F. Parent, C. O'Connell, P. Herve, M. Humbert, G. Simonneau, D. Samuel, Y. Calmus, C. Duvoux, F. Durand, J.C. Duclos-Vallee, and O. Sitbon, Long-term outcome in liver transplantation candidates with portopulmonary hypertension. Hepatology 65 (2017) 1683-1692.

CHAPTER 6

Causes of Death and Challenges in Management of POPH

The causes and circumstances surrounding death are poorly understood in patients with portopulmonary hypertension (PoPH). Only a few patients are able to successfully receive liver transplantation and it is still not well known if patients die of their liver disease or complications of their PAH. To answer this, we performed a retrospective study to find specific reasons for dying and characteristics surrounding this process in patients with PoPH [79]. A total of 69 patients with PoPH (age 56.0 ± 8.9 years), with 49 % females were included. Causes of death were available in 52 (75%) patients, of these PoPH either directly or indirectly contributed to death in 13/52 (25%) of patients, meanwhile 39/52 (75%) of the patients died due to progressive liver disease and its related complications. Decompensated liver disease was the leading cause of death in this cohort 20/52 (38%) while 19/52 (37%) died due to conditions associated with liver disease. About half, 36/69 (52%) of patients died in a healthcare environment and 23/36 (64%) during a hospitalization at Cleveland Clinic. A total of 59/69 (74%) of patients received PAH-specific therapies. Six patients died after liver transplantation (in three death was related to PAH related complications). Most of the patients in this cohort of PoPH patients were considered unsuitable for liver transplantation for a variety of reasons. Advanced health care directives were available in only 28% of patients. Most patients with PoPH died due to complications of their liver disease. PAH directly or indirectly contributed to death in a third of them. A quarter of them did not receive PAH-specific therapy prior to their death.

Challenges in the diagnosis and management of PoPH

There are a number of barriers to the effective diagnosis and treatment of patients with PoPH. As previously discussed, accurate diagnosis of PoPH is challenging, and the differential for an elevated mPAP in the setting of liver disease is broad. There is only one randomized controlled trial for PAH specific therapy in patients with PoPH. Advanced liver disease symptoms have significant overlap with the side effects of PAH specific therapies (e.g. nausea, fatigue, and fluid overload), which can make the initiation and maintenance of treatment challenging[67].

Many of the drugs used to treat PoPH are metabolized in the liver, and drug metabolism is altered by liver disease and other medications that patients may be taking[67]. Additionally, patients may have hepatic encephalopathy or lack the social support needed to safely administer parenteral therapy or take medications multiple times per day[67]. More generally, many of the treatment barriers that apply to all patients with PAH apply to patients with PoPH: late referrals,

lack of an experienced center in close proximity to where the patient lives, and insurance issues. There is significant variability in the screening and management practices of PoPH, discordance between published guidelines and actual practice patterns, and disagreements about the role of LT in PoPH [80].

References

79 Sahay S, Al Abdi S, Melillo C, Newman J, Dweik RA, Heresi GA, Tonelli AR. Causes and Circumstances of Death in Portopulmonary Hypertension. Transplant Direct. 2021 Jun 8;7(7):e710. doi: 10.1097/TXD.0000000000001162. PMID: 34124346; PMCID: PMC8191691

80 H.M. DuBrock, R.J. Salgia, N.L. Sussman, S.D. Bartolome, Z. Kadry, D.C. Mulligan, S. Jenkins, K. Lackore, R.N. Channick, S.M. Kawut, and M.J. Krowka, Portopulmonary Hypertension: A Survey of Practice Patterns and Provider Attitudes. Transplant Direct 5 (2019) e456.

CHAPTER 7

Conclusions

PoPH remains a challenging disease entity with many facets in need of further research. More research is needed into which types of PAH specific therapies will benefit patients with varying PoPH severities. The first randomized control trial in PoPH was recently published, which showed that macitentan significantly lowered PVR in patients with PoPH. It unfortunately remains unclear if hemodynamic improvements translate into successful liver transplantation or substantial survival benefit. While it is clear that patients with severe disease have high mortality with or without LT, their long-term response to treatment is unknown. It is unknown if mild PoPH needs to be treated or given MELD exception points, and the risk for progression to more severe disease is unknown.

Pre-operative risk stratification of patients prior to LT remains a challenge, as the traditional cut off of a mPAP of 35 mmHg is somewhat arbitrary and is based on a small retrospective review of case reports and series. There are no published guidelines on initiation of PAH specific therapies in PoPH, or whether to continue PoPH treatment post-LT. Finally, there is no centralized national registry of PoPH patients who undergo LT; this would serve as a foundation for focusing on the aforementioned areas of need.

While there are indeed many barriers and challenges in caring for patients with PoPH, it remains a field with many areas of active investigation. As the mechanisms and treatment continue to be explored, we are confident that care for these complex patients will continue to improve.

Lastly, during my masters, I researched etiopathogenesis of PoPH and circumstances around which mortality of these patients occur (papers cited above). My research projects during this period helped us advance our understanding of the causes of death in patients with PoPH. Additionally, it also provided insight into the role of estrogen in disease development. Large studies are further needed to confirm these findings to confirm the role of altered estrogen metabolism in PoPH.

CHAPTER-8

Summary of Scholarly Activity

During three years of my master's program, I continued to participate in different scholarly activities. Apart from working on this thesis, I provide a summary of scientific publications in the last three years and also a summary of my other academic activities.

- Research participation: 18 studies as PI (7 RCT's, 5 multicenter international/national registries, 2 grants,4 retrospective studies)

- 2020 ILD research grant from ACCP Chest for $50K (mentored fellow)

- 2021 Proteomics & Genomics study in POPH (IIS $210K)

List of pubmed indexed scientific publications in the last three years

1: Beshay S, Guha A, Sahay S. Evaluation, Diagnosis, and Classification of Pulmonary Hypertension. Methodist Debakey Cardiovasc J. 2021 Jul 1;17(2):86-91. doi: 10.14797/OCDF4453.

2: Guha A, Sahay S. Insights and Advances in the Diagnosis, Treatment, and Management of Pulmonary Hypertension. Methodist Debakey Cardiovasc J. 2021 Jul 1;17(2):84-85. doi: 10.14797/IAME5992.

3: Sahay S, Guha A. Drs. Sandeep Sahay and Ashrith Guha Lead Issue on Pulmonary Hypertension. Methodist Debakey Cardiovasc J. 2021 Jul 1;17(2):83. doi: 10.14797/MULQ7820.

4: Nathan SD, Waxman A, Rajagopal S, Case A, Johri S, DuBrock H, De La Zerda DJ, Sahay S, King C, Melendres-Groves L, Smith P, Shen E, Edwards LD, Nelsen A, Tapson VF. Inhaled treprostinil and forced vital capacity in patients with interstitial lung disease and associated pulmonary hypertension: a post-hoc analysis of the INCREASE study. Lancet Respir Med. 2021 Jun 29:S2213-2600(21)00165-X. doi: 10.1016/S2213-2600(21)00165-X. Epub 5:

5. Sahay S, Al Abdi S, Melillo C, Newman J, Dweik RA, Heresi GA, Tonelli AR. Causes and Circumstances of Death in Portopulmonary Hypertension. Transplant Direct. 2021 Jun 8; 7(7):e710. doi: 10.1097/TXD.0000000000001162.

6: Piccari L, Bernardo RJ, Rodríguez-Chiaradía D, Vitulo P, Wort SJ, Sahay S. Pulmonary vasodilator treatment in pulmonary hypertension due to left heart or lung disease: time for a high-definition picture? Pulm Circ. 2021 May 29;11(2):20458940211018074

7: Sahay S, Farber HW. Pregnancy in PAH: Emotions versus reality. J Heart Lung Transplant. 2021 May;40(5):394-395. doi: 10.1016/j.healun.2021.01.2138. Epub 2021 Feb 13. PMID: 33663895.

8: Sahay S, Holy R, Lyons S, Parsley E, Maurer M, Weers J. Impact of human behavior on inspiratory flow profiles in patients with pulmonary arterial hypertension using AOS™ dry powder inhaler device. Pulm Circ. 2021 Jan 20;11(1):2045894020985345. doi: 10.1177/2045894020985345. PMID: 33532060; PMCID:

9: Sahay S, Holy R, Lyons S, Parsley E, Maurer M, Weers J. Assessment of pinch force strength in patients with pulmonary arterial hypertension in the era of AOS® dry powder inhaler based therapies. Respir Med. 2021 Jan 14; 177:106308. doi: 10.1016/j.rmed.2021.106308.

10: Youssef JG, Zahiruddin F, Youssef G, Padmanabhan S, Ensor J, Pingali SR, Zu Y, Sahay S, Iyer SP. G6PD deficiency and severity of COVID19 pneumonia and acute respiratory distress syndrome: tip of the iceberg? Ann Hematol. 2021 Mar;100(3):667-673. doi: 10.1007/s00277-021-04395-1.

11: Wesley Milks M, Sahay S, Benza RL, Farber HW. Risk assessment in patients with pulmonary arterial hypertension in the era of COVID 19 pandemic and the telehealth revolution: State of the art review. J Heart Lung Transplant. 2021 Mar;40(3):172-182. doi: 10.1016/j.healun.2020.12.005. Epub 2020 Dec 19.

12: Tonelli AR, Sahay S, Gordon KW, Edwards LD, Allmon AG, Broderick M, Nelsen AC. Impact of inhaled treprostinil on risk stratification with noninvasive parameters: a post hoc analysis of the TRIUMPH and BEAT studies. Pulm Circ. 2020 Dec 14;10(4):2045894020977025.

13: Beshay S, Sahay S. Risk stratification in pulmonary hypertension associated with interstitial lung disease: The holy grail? J Heart Lung Transplant. 2021 Mar;40(3):234-235. doi: 10.1016/j.healun.2020.11.010. Epub 2020 Nov 27. PMID: 33349520.

14: Sahay S, Tsang Y, Flynn M, Agron P, Dufour R. Burden of pulmonary hypertension in patients with portal hypertension in the United States: a retrospective database study. Pulm Circ. 2020 Nov 23; 10(4):2045894020962917. doi: 10.1177/2045894020962917.

15: Thomas C, Glinskii V, de Jesus Perez V, Sahay S. Portopulmonary Hypertension: From Bench to Bedside. Front Med (Lausanne). 2020 Nov 3; 7:569413. doi: 10.3389/fmed.2020.569413. PMID: 33224960; PMCID: PMC7670077.

16: Sahay S, Tonelli AR, Selej M, Watson Z, Benza RL. Risk assessment in patients with functional class II pulmonary arterial hypertension: Comparison of physician gestalt with ESC/ERS and the REVEAL 2.0 risk score. PLoS One. 2020 Nov 11;15(11):e0241504. doi: 10.1371/journal.pone.0241504.

17: Karmouty-Quintana H, Thandavarayan RA, Keller SP, Sahay S, Pandit LM, Akkanti B. Emerging Mechanisms of Pulmonary Vasoconstriction in SARS- CoV-2-Induced Acute Respiratory Distress Syndrome (ARDS) and Potential Therapeutic Targets. Int J Mol Sci. 2020 Oct 29;21(21):8081. doi: 10.3390/ijms21218081.

18: Ramjug S, Weatherald J, Sahay S, Khoury J, Foris V, Chandran N, Bokan A, Godinas L, Delcroix M. ERS International Congress, Madrid, 2019: highlights from the Pulmonary Vascular Diseases Assembly. ERJ Open Res. 2020 Oct 13;6(4):00304-2020. doi: 10.1183/23120541.00304-2020.

19: Beshay S, Sahay S, Humbert M. Evaluation and management of pulmonary arterial hypertension. Respir Med. 2020 Sep; 171:106099. doi: 10.1016/j.rmed.2020.106099. Epub 2020 Aug 19. PMID: 32829182.

20: Umoru G, Taitano M, Beshay S, Niravath P, Sahay S. Pulmonary arterial hypertension in breast cancer patients on HER2-targeted therapy: a review of FDA Adverse Events Reporting System data. ERJ Open Res. 2020 Jul 20;6(2):00199-2020. doi: 10.1183/23120541.00199-2020.

21: Sahay S, Farber HW. Management of hospitalized patients with pulmonary arterial hypertension and COVID-19 infection. Pulm Circ. 2020 Jun 15;10(3):2045894020933480. doi: 10.1177/2045894020933480. PMID: 32595934; PMCID: PMC7297206.

22: Clapham KR, Rao Y, Sahay S, Sauler M, Lee PJ, Psotka MA, Fares WH, Ahmad T. PECAM-1 is Associated WithOutcomes and Response to Treatment in Pulmonary Arterial Hypertension. Am J Cardiol. 2020 Jul 15; 127:198-199. doi: 10.1016/j.amjcard.2020.04.031.

23: Sahay S, Rahaghi F. Real-world experience using combination therapy with riociguat and risk assessment using REVEAL Lite 2.0 in patients with pulmonary arterial hypertension. Pulm Circ. 2020 Feb 28;10(1):2045894020910098. doi: 0.1177/2045894020910098

24: Rajagopal K, Bryant AJ, Sahay S, Wareing N, Zhou Y, Pandit LM, Karmouty-Quintana H. Idiopathic pulmonary fibrosis and pulmonary hypertension: Heracles meets the Hydra. Br J Pharmacol. 2021 Jan;178(1):172-186. doi: 10.1111/bph.15036.

25: Medrek S, Sahay S, Zhao C, Selej M, Frost A. Impact of race on survival in pulmonary arterial hypertension: Results from the REVEAL registry. J Heart Lung Transplant. 2020 Apr;39(4):321-330. doi: 10.1016/j.healun.2019.11.024. Epub 2020 Jan 24.

26: Menon AA, Sahay S, Braverman LE, Farber HW. Thyroid Dysfunction in Patients with Pulmonary Artery Hypertension (PAH): The Effect of Therapies Affecting the Prostanoid Pathway. Lung. 2019 Dec;197(6):761-768. doi: 10.1007/s00408-019-00283-8. 27: Sahay S. Evaluation and classification of pulmonary arterial hypertension. J Thorac Dis. 2019 Sep;11(Suppl 14):S1789-S1799. doi: 10.21037/jtd.2019.08.54.

28: Khirfan G, Tonelli AR, Ramsey J, Sahay S. Palliative care in pulmonary arterial hypertension: an underutilised treatment. Eur Respir Rev. 2018 Dec 19;27(150):180069. doi: 10.1183/16000617.0069-2018. PMID: 30567933.