Supplementary Information

Supplementary Methods

Study Population

We included consecutive patients from the Canadian HPS Program (Toronto, Ontario) with moderate to very severe HPS, defined as: liver disease; evidence of intrapulmonary vascular dilatation on contrast echocardiography (microbubbles appearing in the left heart ≥ 3 cardiac cycles after appearing in the right heart, following injection of 10 mL of agitated saline in a peripheral arm vein); and standing room air PaO_2 < 70 mmHg [with alveolar-arterial oxygen gradient ($AaDO_2$) ≥ 20 mmHg],[1] who had physiologic testing as per a pre-defined clinical protocol between November 2013 to June 2018. The study was approved by St. Michael's Hospital's Research Ethics Board (Toronto) prior to data collection (all patients consented to have their data included in a database for analysis).

Interventions

Each patient was electively admitted to the cardiac intensive care unit at St. Michael's Hospital (Toronto). We inserted an indwelling radial artery catheter (Becton Dickinson, Franklin Lakes, USA) to measure repeated arterial blood gases (ABGs) and mean systemic arterial pressure (MAP), and an internal jugular venous Cordis sheath through which a balloon-tipped pulmonary artery catheter (Edwards Lifesciences, Irvine, USA) was floated to measure haemodynamics. Placement of the pulmonary artery catheter was confirmed by chest radiograph.

We conducted the following series of interventions in order to measure the effects position change, inhaled nitric oxide (iNO), methylene blue (MB), and combinations thereof on oxygenation and haemodynamics (Figure 1):

1. Supine position (room air)
2. Trendelenburg position (-20°) (room air)
3. Seated position (room air)
4. Seated position (40% FiO_2)
5. Seated position [40% FiO_2 + 20 parts per million (ppm) iNO]
6. Seated position (room air)
7. Seated position (room air + MB) (at one, two, three, four, and five hours after MB infusion)*
8. Supine position (room air + MB)
9. Seated position (40% FiO_2 + MB)
10. Seated position (40% FiO_2 + MB + 20 ppm iNO)
11. Supine position (40% FiO_2 + MB + 20 ppm iNO)

* All patients (n=26) received iNO. Eight patients did not receive MB due to current use of SSRIs (n=4), TCAs (n=1), or both an SSRI and a TCA (n=1); and inability to tolerate full day testing (n=2), leaving 18 patients for MB administration.

After each intervention, we drew an ABG, measured MAP through the radial arterial catheter, and measured the following variables through the pulmonary artery catheter: central venous pressure (CVP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO) (measured by thermodilution, using triplicate values within ≤ 10% of one another). After each position change, we ensured that both pulmonary artery catheter and arterial line transducers were maintained at the phlebostatic axis. For all seated measurements, participants sat at the edge of

Gupta S, *et al. Thorax* 2021;0:1–4. doi: 10.1136/thoraxjnl-2020-216128

the bed with their legs over the side of the bed. We waited a minimum of ten minutes after each position change and twenty minutes after any change in FiO_2 and/or administration of iNO before starting measurements. Nitric oxide was delivered with oxygen (FiO_2 at 40%) through the INOmax DSIR (IKARIA Inc., Seattle, USA). We maintained FiO_2 at 40% with use of an oxygen blender, adjusted in accordance with readings from a continuous sampling line near the participant mask. To isolate the effect of iNO, we placed participants on oxygen at 40% FiO_2, with 0 ppm iNO through the INOmax DSIR, measured all variables, then added iNO at 20 ppm to the same circuit and re-measured. MB was infused at a dose of 3mg/kg over 15 minutes, and we measured MB effects hourly after completion of the infusion, up to five hours, which was previously reported to be the peak MB effect time in HPS (and thus set a priori as the main time point for MB effect reporting).[2] All other interventions after MB infusion (interventions eight to eleven above) were completed serially after the five-hour post-infusion mark. Given the risk of serotonin toxicity, participants using selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) or tricyclic antidepressants (TCAs) were not given MB, and completed interventions one to five only.

Outcomes

Our primary outcome was PaO_2 from ABG analysis. We also calculated the $AaDO_2$, using the following equation:

$$AaDO_2 = [F_iO_2(P_{atm} - P_{H2O}) - (PaCO_2/0.8)] - P_aO_2$$

Where $AaDO_2$ represents the alveolar-arterial oxygen gradient; FiO_2 represents the fraction of inspired oxygen; P_{atm} represents the atmospheric pressure (760 mmHg); P_{H2O} represents the partial pressure of water vapour (47 mmHg); $PaCO_2$ represents the arterial partial pressure of CO_2; and PaO_2 represents the arterial partial pressure of O_2.

We also assessed changes in CO, MAP, MPAP, PCWP, pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR), calculated as follows:

$$PVR = [80 \times (MPAP - PCWP)]/CO$$

$$SVR = [80 \times (MAP - CVP)]/CO$$

Where MPAP represents mean pulmonary artery pressure; PCWP represents pulmonary capillary wedge pressure; MAP represents mean arterial pressure; and CO represents cardiac output.

Statistical Analyses

We provide descriptive statistics (means with standard deviations and/or proportions) for baseline patient characteristics, and oxygenation (PaO_2, $AaDO_2$) and haemodynamic variables with each intervention. We used paired t-tests to compare the mean baseline value to the mean value following each intervention, for PaO_2 and key haemodynamic variables. In an exploratory analysis, we measured Pearson's correlation coefficient for change in PaO_2 with each intervention and the following baseline characteristics: age; time since HPS diagnosis; standing PaO_2; orthodeoxia value; macroaggregated albumin shunt fraction (MAA – a nuclear study used to estimate anatomic shunt, measured with Abrams' technique, which exclusively compares brain and lung technetium counts to estimate shunt as opposed to total body counts which can be influenced by decreases in renal blood flow caused by cirrhosis);[3] and non-invasive shunt fraction (calculated based on an ABG on 100% FiO_2). We used two-sample t-tests to compare change in PaO_2 with each intervention between the following clinically

relevant categories: male vs female; baseline (standing) PaO_2 > 50 mmHg vs ≤ 50 mmHg;[4] baseline orthodeoxia > 4 mmHg vs ≤ 4 mmHg;[1,5] and MAA shunt fraction ≥20% vs < 20%.[4,6] All analyses were performed using Stata/MP 15 (StataCorp LLC, College Station, USA); a p-value of < 0.05 was considered significant.

Gupta S, *et al. Thorax* 2021;0:1–4. doi: 10.1136/thoraxjnl-2020-216128

Supplementary Tables

Supplementary Table 1. Characteristics of Study Participants

Participant	Etiology of Liver Disease	Child-Pugh Score	MELD Score	Standing PaO_2 (mmHg)	Standing $AaDO_2$ (mmHg)	Orthodeoxia[a] (mmHg)	MAA Shunt[d] (%)	Oximetric Shunt[b] (%)	DLCO[c] (% predicted)
1	Alcohol	B7	11	48	66	1	13	18	72
2	Autoimmune hepatitis	B8	12	53	66	5	1	12	25
3	Alcohol	B9	10	53	60	12	15	7	62
4	NASH	C10	18	44	70	21	25	11	55
5	Hepatitis C	C10	12	64	44	9	8	6	50
6	NASH	B7	9	47	57	22	17	26	50
7	Hepatitis C	B7	15	40	73	17	27	25	50
8	Hepatitis C	B7	9	39	75	13	31	8	27
9	Cryptogenic cirrhosis	B8	17	33	88	30	30	27	29
10	NASH	A6	11	38	85	15	18	21	52
11	Hepatitis C/ alcohol	A6	8	52	58	2	18	21	52
12	NASH/ alcohol	B9	17	54	66	24	11	15	54
13	Hepatitis C/ alcohol	C10	20	56	58	15	15	13	54
14	NASH	B8	13	48	68	10	14	10	57
15	Hepatitis C	A6	9	59	25	4	19	15	53
16	Hepatitis C	A6	10	35	79	6	44	10	40
17	NASH	B7	10	62	47	7	19	5	61
18	NASH	B8	22	58	41	-2	NA	9	NA
19	Alcohol	A5	13	35	83	11	51	18	61
20	Sickle cell hepatopathy	B9	15	45	70	16	24	12	47
21	NASH	C10	15	38	70	11	35	30	63

22	Autoimmune hepatitis	B7	11	29		87	NA	54	NA	51	
23	Cryptogenic cirrhosis	A5	8	47		57	NA	25	NA	75	
24	Alcohol	B7	16	48		68	22	13	15	56	
25	NASH	B8	14	41		70	9	38	17	40	
26	Portal vein thrombosis	B7	9	34		77	2	77	19	NA	
Mean (SD) or Proportion	NASH 31% Hepatitis C 19% Alcohol 15% Autoimmune Hepatitis 8% Other 27%	A 23% B 62% C 15%	12.8 (3.8)	46.2	(9.6)	65.6 (14.9)	11.8 (8.1)	26 (17)	15 (7)	51	(13)

Age and sex were omitted from the table to protect patient identities

[a] Measured as the difference in PaO_2 between supine and standing position; 19/24 (79%) fulfilled published criteria for orthodeoxia (> 4 mmHg PaO_2 drop with supine to upright position change)[1]

[b] Calculated by comparing arterial blood gas results on room air versus 100% FiO_2 (normal value ≤ 6%)

[c] Values were adjusted for haemoglobin, where available

[d] Normal value ≤ 6%

CP denotes Child-Pugh; MELD denotes model of end-stage liver disease; PaO_2 denotes arterial partial pressure of O_2; MAA denotes macroaggregated albumin; FiO_2 denotes fraction of inspired O_2; DLCO denotes diffusing capacity of the lung for carbon monoxide; COPD denotes chronic obstructive pulmonary disease; NASH denotes non-alcoholic steatohepatitis; ILD denotes interstitial lung disease

Supplementary Table 2. Hourly Changes in Oxygenation Following Methylene Blue Administration (n=18)

Time after Methylene Blue Infusion	Change in PaO_2 (mmHg)	Change in PaO_2 (%)	p-value	N (%) with ≥10% improvement in PaO_2	N (%) with ≥20% improvement in PaO_2
1 hour (Seated)	0.2 (3.0)	0 (6)	0.76	0	0
2 hours (Seated)	0.4 (3.7)	1 (8)	0.66	2 (11)	0
3 hours (Seated)	1.4 (3.5)	3 (7)	0.11	4 (22)	0
4 hours (Seated)	1.2 (3.8)	3 (8)	0.19	5 (28)	0
5 hours (Seated)	1.1 (4.6)	2 (10)	0.34	3 (17)	1 (6)
5 hours (Supine)	2.3 (4.8)	5 (10)	0.06	6 (33)	1 (6)

Values reported as mean (SD)

All comparisons are to no intervention, in the same position, on the same FiO_2

PaO_2 denotes arterial partial pressure of oxygen

Supplementary Table 3. Changes in Key Haemodynamic Variables, by Intervention

Intervention	N	Systemic Vascular Resistance Change (dyn·sec·cm^{-5})	Change (%)	p-value	Pulmonary Vascular Resistance Change (dyn·sec·cm^{-5})	Change (%)	p-value	Mean Pulmonary Artery Pressure Change (mmHg)	Change (%)	p-value
Supine vs Seated (Orthodeoxia)	24	-192.0 (156.6)	-20 (15)	<0.001	-1.2 (40.3)	13 (57)	0.88	3.2 (6.2)	55 (102)	0.02
Trendelenburg vs Supine	21	-30.3 (86.8)	-3 (14)	0.13	8.1 (22.1)	21 (54)	0.12	2.7 (3.5)	15 (17)	0.002
Methylene Blue[a] (Seated)	18	-32.3 (131.0)	-3 (12)	0.31	5.1 (29.7)	21 (72)	0.49	3.9 (3.5)	28 (31)	<0.001
Methylene Blue[a] (Supine)	18	33.0 (236.2)	7 (33)	0.56	13.1 (47.4)	78 (299)	0.26	2.7 (4.9)	21 (34)	0.03
Inhaled NO (Seated)	24	41.3 (159.4)	9 (26)	0.22	-26.4 (79.8)	-8 (49)	0.12	-0.8 (3.3)	-4 (26)	0.23
Inhaled NO + Methylene Blue[a] (Seated)	18	31.4 (242.2)	8 (35)	0.59	-7.8 (39.3)	12 (67)	0.41	2.5 (2.4)	18 (24)	<0.001
Inhaled NO + Methylene Blue[a] vs Inhaled NO Alone (Seated)	18	-9.4 (245.1)	-1 (23)	0.87	15.9 (57.1)	53 (102)	0.25	3.6 (3.5)	29 (29)	<0.001

Values reported as mean (SD)

All comparisons are to no intervention, in the same position, on the same FiO_2, unless stated otherwise

[a] Measured five hrs after methylene blue infusion

NO denotes nitric oxide

Supplementary Table 4. Pearson's Correlations Between Baseline Patient Characteristics and Change in PaO_2, by Intervention

	Correlation (R) with Change in PaO_2, by Intervention (95% Confidence Interval)					
	Trendelenburg vs Supine	Supine vs Upright (Orthodeoxia)	iNO (Seated)	Methylene Blue (Seated)	Methylene Blue (Supine)	iNO + Methylene Blue (Seated)
Age (years)	-0.13 (-0.53, 0.32) p=0.56	-0.02 (-0.40, 0.37) p=0.91	-0.04 (-0.42, 0.35) p=0.85	-0.03 (-0.49, 0.44) p=0.91	-0.19 (-0.60, 0.30) p=0.45	0.14 (-0.35, 0.57) p=0.57
Time since HPS Diagnosis (years)	-0.21 (-0.59, 0.24) p=0.36	0.25 (-0.15, 0.58) p=0.24	-0.24 (-0.57, 0.16) p=0.23	0.19 (-0.30, 0.60) p=0.44	-0.14 (-0.67, 0.35) p=0.58	0.19 (-0.30, 0.60) p=0.46
PaO_2 (standing, mmHg)	0.07 (-0.37, 0.49) p=0.77	-0.29 (-0.61, 0.11) p=0.16	-0.06 (-0.44, 0.34) p=0.78	0.11 (-0.38, 0.55) p=0.68	-0.35 (-0.70, 0.14) p=0.15	-0.11 (-0.55, 0.38) p=0.68
Orthodeoxia (mmHg)	-0.31 (-0.67, 0.17) p=0.19	NA	-0.05 (-0.44, 0.36) p=0.80	0.21 (-0.30, 0.63) p=0.41	0.12 (-0.38, 0.57) p=0.64	-0.11 (-0.56, 0.39) p=0.68
^{99m}Tc-MAA (%)	0.25 (-0.22, 0.62) p=0.30	0.02 (-0.38, 0.41) p=0.92	-0.05 (-0.44, 0.35) p=0.82	-0.31 (-0.69, 0.20) p=0.23	0.36 (-0.15, 0.72) p=0.16	-0.00 (-0.48, 0.48) p=1.00
Non-invasive shunt fraction (%)	0.01 (-0.45, 0.46) p=0.96	0.13 (-0.29, 0.51) p=0.54	-0.27 (-0.61, 0.15) p=0.21	0.31 (-0.20, 0.69) p=0.23	0.10 (-0.40, 0.55) p=0.71	0.06 (-0.43, 0.53) p=0.81

All comparisons are to no intervention, in the same position, on the same fraction of inspired oxygen, unless stated otherwise

Effect of methylene blue interventions (both seated and supine) were measured at five hours after intravenous administration

PaO_2 denotes arterial partial pressure of oxygen, ^{99m}Tc-MAA denotes ^{99m}Tc macroaggregated albumin, iNO denotes inhaled nitric oxide

Supplementary Table 5. Differences in PaO_2 Response to Each Intervention, by Baseline Patient Characteristics

	Mean Change in PaO_2 by Category (mmHg), by Intervention (95% confidence Interval and p-value[a] for difference in change)					
	Trendelenburg vs Supine	Supine vs Seated (Orthodeoxia)	iNO Seated	Methylene Blue Seated	Methylene Blue Supine	iNO + Methylene Blue Seated
Sex M (n=11) F (n=15)	M 1.0, F 2.4 Difference: 1.4 (-3.0, 5.8) p=0.25	M 3.7, F 1.9 Difference: -1.8 (-7.8, 4.2) p=0.27	M 18.2, F 9.8 Difference: -8.4 (-28.2, 11.4) p=0.13	M 3.9, F 0.7 Difference: -3.2 (-22.6, 16.2) p=0.53	M 6.6, F 3.2 Difference: -3.4 (-21.1, 14.3) p=0.47	M 20.0, F 18.2 Difference: -1.8 (-42.4, 38.8) p=0.86
PaO_2 while standing >50mmHg[1] Yes (n= 9) No (n=17)	No 1.4, Yes 2.4 Difference: 1.0 (-3.98, 5.98) p=0.45	No 3.1, Yes 2.0 Difference: -0.9 (-6.64, 4.84) p=0.55	No 13.2, Yes 13.6 Difference: 0.4 (-21.0, 21.8) p=0.94	No 2.4, Yes 2.0 Difference: -0.4 (-21.8, 21.0) p=0.94	No 7.0, Yes -0.5 Difference: -7.5 (-25.6, 10.6) p=0.14	No 22.5, Yes 10.2 Difference: -12.3 (-50.4, 25.8) p=0.24
Known Orthodeoxia (>4mmHg)[1] Yes (n=19) No (n= 5)	No 3.1, Yes 1.5 Difference: -1.6 (-8.5, 5.3) p=0.39	NA	No 15.6, Yes 14.3 Difference: -1.3 (-30.6, 28.0) p=0.86	No -3.6, Yes 4.4 Difference: 8.0 (-16.2, 32.2) p=0.23	No -0.4, Yes 6.5 Difference: 6.9 (-16.5, 30.3) p=0.28	No 25.0, Yes 19.5 Difference: -5.5 (-55.5, 44.5) p=0.67
^{99m}Tc-MAA (≥20%)[4] Yes (n=12) No (n=13)	No 0.8, Yes 2.5 Difference: 1.7 (-2.9, 6.3) p=0.18	No 2.7, Yes 2.8 Difference: 0.1 (-5.2, 5.4) p=0.94	No 11.6, Yes 16.1 Difference: 4.5 (-17.4, 26.4) p=0.44	No 3.2, Yes 2.0 Difference: -0.8 (-14.7, 13.1) p=0.82	No 0.2, Yes 8.1 Difference: 7.9 (-8.5, 24.3) p=0.09	No 19.4, Yes 19.7 Difference: 0.3 (-32.0, 32.6) p=0.97

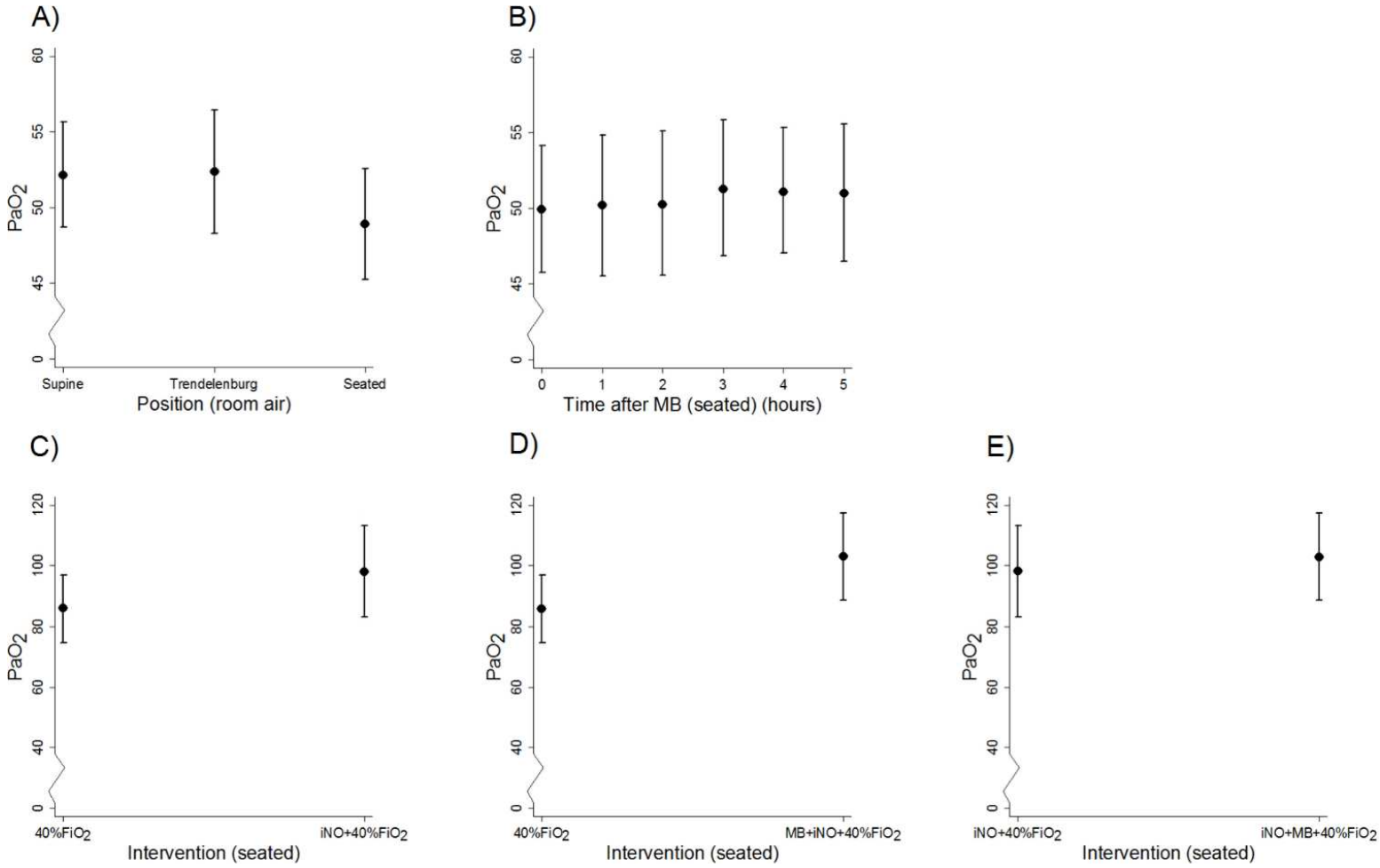
All comparisons are to no intervention, in the same position, on the same fraction of inspired oxygen, unless stated otherwise

Effect of methylene blue interventions (both seated and supine) were measured at five hours after intravenous administration

[a] Two-sample t-test

PaO_2 denotes arterial partial pressure of oxygen, ^{99m}Tc-MAA denotes ^{99m}Tc macroaggregated albumin, iNO denotes inhaled nitric oxide

Supplementary Figure 1. Effect of Various Interventions on Partial Pressure of Oxygen (PaO_2)



The mean PaO_2 with each intervention is represented by a data point, and whiskers represent the 95% confidence intervals. P-values for changes between states (panels A,C,D,E) are provided in Table 2 and p-values for changes at each time point after methylene blue infusion (panel B) are provided in Supplementary Table 3. A) Effect of position changes (n=21). B) Effect of methylene blue at five hours (n=18). C) Effect of inhaled nitric oxide (n=26). D) Effect of inhaled nitric oxide combined with methylene blue at five hours (n=18). E) Effect of inhaled nitric oxide combined with methylene blue at five hours compared to inhaled nitric oxide alone (n=18).

PaO_2 denotes the arterial partial pressure of oxygen, MB denotes methylene blue, iNO denotes inhaled nitric oxide, FiO_2 denotes fraction of inspired oxygen

Gupta S, *et al. Thorax* 2021;0:1–4. doi: 10.1136/thoraxjnl-2020-216128

References

1. Krowka, M.J., et al. International Liver Transplant Society Practice Guidelines: Diagnosis and Management of Hepatopulmonary Syndrome and Portopulmonary Hypertension. Transplantation **100**, 1440-1452 (2016).
2. Schenk, P., et al. Hepatopulmonary syndrome: prevalence and predictive value of various cut offs for arterial oxygenation and their clinical consequences. Gut **51**, 853-859 (2002).
3. Abrams, G.A., Jaffe, C.C., Hoffer, P.B., Binder, H.J., Fallon, M.B. Diagnostic utility of contrast echocardiography and lung perfusion scan in patients with hepatopulmonary syndrome. Gastroenterology **109**, 1283–1288 (1995).
4. Nayyar, D., Man, H.S., Granton, J. & Gupta, S. Defining and characterizing severe hypoxemia after liver transplantation in hepatopulmonary syndrome. Liver Transpl **20**, 182-190 (2014).
5. Krowka, M.J. & Cortese, D.A. Hepatopulmonary syndrome. Current concepts in diagnostic and therapeutic considerations. Chest **105**, 1528-1537 (1994).
6. Nayyar, D., Man, H.S., Granton, J., Lilly, L.B. & Gupta, S. Proposed management algorithm for severe hypoxemia after liver transplantation in the hepatopulmonary syndrome. Am J Transplant **15**, 903-913 (2015).