Sildenafil Reduces the Risk of Thromboembolic Events in HeartMate II Patients with Low-Level Hemolysis and Significantly Improves the Pulmonary Circulation

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Summary

Low-level hemolysis (LLH) after left ventricular assist device implantation contributes to thromboembolic events (TE). Free plasma hemoglobin (fHb) scavenges nitric oxide (NO), which causes endothelial dysfunction and activates platelets. fHb also interacts with von Willebrand factor (vWF). We hypothesized that improved hemodynamic and enhanced NO signaling in HeartMate II (HMII) patients with LLH taking the phosphodiesterase-5 inhibitor sildenafil may reduce the risk of TE.

From 2011 to 2015, 83 patients underwent HMII implantation. Patients with LLH as defined by elevated lactate dehydrogenase ($400 < \text{LDH} \leq 700$ U/L) at hospital discharge were identified. Patients were categorized into 4 groups: 1) LLH + sildenafil, 2) LLH no sildenafil, 3) no LLH + sildenafil, and 4) no LLH no sildenafil. Adverse event-free survival was compared between the groups.

Thirty-four patients (40.9%) were discharged with LLH and 22 (64.7%) of them took sildenafil. LDH and fHb remained significantly elevated in both LLH groups compared to the no LLH patients ($P < 0.0001$). Overall incidence of pump thrombosis (PT) was 4.8% and of ischemic stroke (IS) was 8.4%. HMII patients with LLH not on sildenafil had higher risk of TE (hazard ratio (HR): 14.4, 95%-CI: 1.8-117.1, $P = 0.001$). vWF activity and bleeding incidence did not differ between the LLH and no LLH patients. Mean pulmonary artery pressure and pulmonary vascular resistance decreased significantly in HMII taking sildenafil ($P < 0.0001$) while cardiac index increased ($P < 0.0001$).

Sildenafil treatment among HMII patients with LLH reduced the risk of thromboembolic events and significantly improved and decompressed the pulmonary circulation during HMII support.

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Key words: Free plasma hemoglobin, Lactate dehydrogenase, Phosphodiesterase 5 inhibitors, Left ventricular assist devices, Pump thrombosis, Stroke, Nitric oxide

Continuous-flow (CF) left ventricular assist device (LVAD) support is a common form of treatment for end-stage heart failure with clear evidence of improving survival and quality of life.^{1,2} The number of implanted CF-LVADs as destination therapy is increasing.¹ Consequently, the prevalence of device-related complications is also increasing.³ Hemolysis represents one of the most common complications after CF-LVAD implantation with an estimated incidence of 15% to 30%,^{4,5} and it has been linked to pump thrombosis (PT), peripheral embolism, and higher morbidity and mortality.^{6,7} The Interagency Registry of Mechanically Assisted Circulatory Support (INTERMACS) defines hemolysis as elevated free plasma hemoglobin (fHb > 40 mg/dL) accompanied with clinical signs.⁸ Another accepted definition of hemo-

lysis after LVAD implantation is a rise in lactate dehydrogenase (LDH) levels to > 2.5 the upper limit of normal values (> 700 U/L).⁹ A study by Shah, *et al.* also demonstrated that LDH is a superior marker in detecting hemolysis in LVAD patients.¹⁰ The epidemiology of hemolysis in LVAD patients and the consequences of a hemolytic event are still incompletely understood. The released fHb due to erythrocyte destruction during hemolysis can directly activate platelets and induce a prothrombotic state.¹¹ Similar phenomena have been detected in patients with severe aortic stenosis.¹² Previous studies have demonstrated that fHb scavenges nitric oxide (NO) and reduces the bioavailability of NO during hemolysis, thus leading to endothelial dysfunction and platelet activation.^{13,14} At the same time, elevated fHb interacts with the A1 and 2

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domains of von Willebrand factor (vWF), which increases platelet adhesion to vWF.¹⁵⁾

Phosphodiesterase-5-inhibitors (PDE5-Is), such as sildenafil, enhance NO signaling and, therefore, the effect of NO.^{16,17)} PDE5-I have also been used during LVAD support to prevent right ventricular dysfunction or to improve it through decreases of pulmonary artery pressure and pulmonary vascular resistance (PVR) and therefore providing a higher cardiac output, and previous studies demonstrated the beneficial effect of PDE5-I use during LVAD support.¹⁸⁻²⁰⁾

We hypothesized that sildenafil might reduce the risk of thromboembolic events (TE) in HeartMate II (HMII) patients with low-level hemolysis (LLH), as defined by elevated LDH ($400 < \text{LDH} \leq 700$ U/L).

Methods

After obtaining informed consent from the patient and approval by the local ethics committee (Ethikkommission der RWTH Aachen, ethical vote no 151/09), blood samples were taken at predefined time points to perform additional analyses. Clinical data were retrospectively collected.

Study design and data collection: We conducted a single-center, retrospective, case-control study of patients who underwent HMII implantation during a 4-year period from April 2011 to April 2015. Patients were excluded from this analysis if they did not survive to discharge during the index hospitalization for LVAD implantation. Baseline demographic, baseline hemodynamic parameters from right heart catheterization and from echocardiography, and peri- and postoperative data were collected from our institution's database. Laboratory data (e.g., LDH, international normalized ratio (INR), fHb, hemoglobin (Hb), aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin and N-terminal pro b-type natriuretic peptide (NT-proBNP) were retrieved. fHb was not measured preoperatively. vWF antigen (vWF:Ag) as well as its collagen binding activity (vWF:CBA) and VWF ristocetin co-factor activity (vWF:RCO) were measured 3 months postoperatively. vWF multimer analysis was also performed 3 months postoperatively in a reference external laboratory. All patients were routinely followed-up in our outpatient clinic until July 2017. During follow-up visits patients had transthoracic echocardiography (TTE) studies to evaluate the right and left ventricular function (RV and LV). Additionally, almost all LVAD patients in our institution receive hemodynamic reevaluation 1 year after LVAD-implantation with right heart catheterization or a Swan-Ganz catheter to optimize the treatment and update the status if needed.

During the study period, our institutional anticoagulation protocol for HMII patients was to administer aspirin (100 mg) and phenprocoumon, a Vitamin-K-antagonist, with a goal INR of 1.8-2.2 after implant.

Adverse event monitoring: Each patient's history of adverse events (AE) history was evaluated during the inpatient treatment and at every outpatient visit. AEs were defined according to the 2013 INTERMACS Version 2.2 definitions for ischemic stroke (IS), hemorrhagic stroke

(HS), PT, bleeding and hemolysis.⁸⁾ IS or HS were defined by the presence of an acute cerebral infarction/bleeding noted on a cranial computed tomographic scan and accompanied by neurological symptoms. Signs and symptoms of suspected PT included at least 2 of the 3 following criteria: 1) Presence of hemolysis, 2) Presence of heart failure not explained by structural heart disease, 3) Abnormal pump parameters. PT was confirmed via direct visual inspection or by incontrovertible contrast radiographic evidence or by the absence of an appropriate Doppler flow signal that results in or could potentially induce circulatory failure caused by thromboembolism. Gastrointestinal bleeding (GIB) was defined by a drop in hemoglobin of > 1 g/dL, requiring a blood transfusion and endoscopic detection of bleeding source or angiodysplastic lesions of the gastrointestinal tract.

Patients groups: Patients with elevated LDH ($400 < \text{LDH} \leq 700$ UL) (normal range in our laboratory is < 280 U/L) at hospital discharge were identified. Patients were then categorized into 1 of 4 groups; group 1 taking sildenafil and had LLH (LLH + sildenafil), group 2 had LLH and were not taking sildenafil (LLH no sildenafil), group 3 did not have LLH and were on sildenafil (no LLH + sildenafil), and group 4 did not have LLH and were not on sildenafil (no LLH no sildenafil). If one of the following criteria was met, sildenafil was then prescribed after LVAD implantation on postoperative day 1: 1) mean pulmonary artery pressure (mPAP) > 25 mmHg; 2) pulmonary capillary wedge pressure (PCWP) > 15 mmHg; 3) PVR > 1.6 Wood units; 4) tricuspid annular plane systolic excursion (TAPSE) < 14 mm; or 5) right ventricular fractional area change (RV-FAC) < 30 %.

Statistical analysis: Continuous data were assessed for normality using the Kolmogorov-Smirnov test, and then presented as the mean $\pm$ standard deviation or median (minimum, maximum). Comparison of repeated continuous measurements between groups was performed by two-way ANOVA test or the Kruskal-Wallis test, as appropriate. For the comparison of continuous parameters without repeated measurements one-way ANOVA test, or multiple *t*-test were used, as appropriate.

Categorical variables are presented as frequencies, and differences were tested by Fisher's exact or the Chi-square test, as appropriate. Survival analysis was performed using Kaplan-Meier curves with the log-rank signed test, with time 0 being the date of implantation. Patients were censored for death, heart transplantation or device explant due to recovery. Cox regression modeling was used to calculate hazard ratios (HRs) and 95% confidence intervals [95% CIs] for events in groups. All data analyses were performed using SPSS 24 (IBM Corp., Armonk, NY, USA). A *P*-value < 0.05 was considered statistically significant.

Results

This study included 83 patients who underwent HMII implantation at our institution and survived to discharge from the index hospitalization. Mean age was 62.6 ± 8.5 years and 13 patients (15.7%) were female. The median (minimum, maximum) patient support with HMII among

Table I. Patient Characteristics and Preoperative Laboratory and Perioperative Data

	LLH + Sildenafil (n = 22)	LLH no Sildenafil (n = 12)	no LLH + Sildenafil (n = 34)	no LLH no Sildenafil (n = 15)	P value
Age, years	60.8 ± 10.7	61.2 ± 7.7	63.7 ± 8.2	63.8 ± 5.6	0.554
Female n (%)	5 (22.7)	3 (25)	3 (8.8)	2 (13.3)	0.368
BMI kg/m ²	28.1 ± 5.8	27.5 ± 3.5	26.5 ± 3.5	26.1 ± 8.3	0.427
HT n (%)	15 (68.2)	8 (66.7)	27 (79.4)	12 (80)	0.770
IDDM n (%)	2 (9.1)	1 (8.3)	7 (20.6)	0	0.202
PAD n (%)	4 (18.1)	3 (25)	10 (29.4)	4 (26.7)	0.811
ICM n (%)	17 (77.3)	9 (75)	25 (73.5)	13 (86.7)	0.764
DCM n (%)	5 (22.7)	3 (25)	9 (26.5)	2 (13.3)	0.764
PHT n (%)	18	0	26	0	0.0001
EF %	20.8 ± 7.6	20.5 ± 7.8	18 ± 4.3	20.7 ± 5.5	0.275
Prior dialysis n (%)	0	1 (8.3)	1 (2.9)	0	0.391
Prior cardiac surgery n (%)	6 (27.3)	1 (8.3)	3 (8.8)	5 (33.3)	0.106
INTERMACS level					
I	5 (22.8)	4 (33.3)	0	3 (20)	0.002
II	1 (4.5)	2 (16.7)	5 (14.7)	3 (20)	0.481
III	0	1 (8.3)	4 (11.8)	2 (13.3)	0.297
IV	16 (72.7)	5 (41.7)	25 (73.5)	7 (46.7)	0.314
BTT	4 (18.8)	3 (25)	8 (23.5)	4 (26.7)	0.708
DTT	18 (81.2)	9 (75)	26 (76.5)	11 (73.3)	0.708
Preop laboratory					
Creatinine mg/dL	1.1 ± 0.3	1.5 ± 2.2	1.2 ± 0.5	1.1 ± 0.4	0.484
NT-proBNP ng/L	3192 (614, 9059)	5236 (4780, 5692)	4884 (965, 9792)	1092 (820, 1365)	0.121
LDH U/L	226 (175, 939)	233 (148, 686)	213 (148, 686)	196 (124, 491)	0.349
Hb g/dL	13.1 (8.7, 16.7)	10.9 (8, 16)	13.1 (9, 15.9)	12.7 (7.8, 16)	0.880
Platelet count × 10 ³	199 (36, 345)	194.5 (63, 317)	221 (92, 468)	211.5 (92, 475)	0.351
AST U/L	40 (18, 213)	32 (20, 130)	26 (16, 92)	29.5 (13, 80)	0.011
ALT U/L	48 (16, 159)	39.5 (16, 91)	28.5 (11, 111)	24.5 (14, 71)	0.011
Total bilirubin	1.5 ± 1.4	0.9 ± 0.6	1.1 ± 1.1	1.1 ± 0.9	0.419
INR	1.2 ± 0.40	1.1 ± 0.08	1.2 ± 0.3	1.1 ± 0.2	0.268
MPAP mmHg	30.2 ± 8.9	22.6 ± 3.8	34.3 ± 9.7	21.4 ± 7.7	< 0.0001
PCWP mmHg	22.2 ± 6.3	16.1 ± 1.5	21.1 ± 9.3	14.3 ± 5.6	0.0001
PVR Wood units	2.5 ± 0.8	1.4 ± 0.4	3.0 ± 1.1	1.5 ± 0.9	< 0.0001
CI L/minute/m ²	2.0 ± 0.4	2.1 ± 0.3	1.9 ± 0.4	1.9 ± 0.3	0.905
TAPSE cm/second	15.3 ± 4.4	16.8 ± 2.1	14.3 ± 2.3	16.2 ± 2.8	0.066
RV-FAC %	36.9 ± 10.2	39.4 ± 9.4	35.4 ± 9.2	40.3 ± 9.6	0.348
Peri-op data					
HMII alone	10 (45.5)	6 (50.0)	13 (38.0)	7 (46.7)	0.797
HMII + CABG	9 (40.9)	4 (33.3)	11 (32.4)	5 (33.3)	0.928
HMII + TVR	1 (4.5)	1 (8.3)	5 (14.7)	2 (13.3)	0.117
HMII + CABG + Valve	2 (9.1)	1 (8.3)	5 (14.7)	1 (6.7)	0.884

AST indicates aspartate transaminase; ALT, alanine transaminase; BMI, body mass index; CABG, coronary artery bypass grafting; CBP, cardiopulmonary bypass; CI, cardiac index; Hb, hemoglobin; HMII, HeartMate II; HT, arterial hypertension; ICM, ischemic cardiomyopathy; IDDM, insulin-dependent diabetes mellitus; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; LDH, lactate dehydrogenase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAD, peripheral arterial disease; PHT, pulmonary hypertension; preop, preoperative; PCWP, pulmonary capillary wedge pressure; TVR, tricuspid valve replacement; TAPSE, tricuspid annular plane systolic excursion; EF, ejection fraction; DCM, dilated cardiomyopathy; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; and RV-FAC, right ventricular fractional area change.

all 83 HMII patients was 28 months (2, 83 months). The incidence of AE during follow-up in the study cohort (83 patients) was as follows: 16.9% hemolysis by INTERMACS definition with 0.10 events per patient-year (eppy); 4.8% PT (0.018 eppy); 8.4% IS (0.032 eppy); 6% TIA (0.023 eppy); 4.9% HS (0.018 eppy); and 45.1% GIB (0.59 eppy).

A total of 34 (40.9%) patients were discharged with LLH with a median LDH of 478 (410, 700) U/L. Among those patients, 22 (64.7%) had a median LDH of 483 (410, 700) U/L and were discharged with sildenafil treatment (LLH + sildenafil group) and 12 patients (35.3%)

had a median LDH of 457 (411, 700) U/L and were not taking sildenafil (LLH no sildenafil group). Baseline characteristics, preoperative laboratory, demographics, and operative data of all 4 groups are presented in Table I. Significantly more patients had INTERMACS level I in the LLH no sildenafil group than patients in the other 3 groups ($P = 0.002$). Regarding preoperative laboratory, only AST and ALT differed significantly between groups. AST was significantly higher in the LLH + sildenafil group compared to the no LLH + sildenafil and no LLH no sildenafil groups, 40 (18, 213) versus 26 (16, 92) U/L, $P < 0.001$ and 40 (18, 213) versus 29.5 (13, 80) U/L, $P =$

Table II. Time Course of Hemolysis and Liver Function Markers in the 4 Study Groups

	1. LLH + Sildenafil (n = 22)	2. LLH no Sildenafil (n = 12)	P-value 1 versus 2	P-value 1 versus 3	3. no LLH + Sildenafil (n = 34)	P-value 2 versus 3	4. no LLH no Sildenafil (n = 15)	P-value 3 versus 4
Lactate dehydrogenase U/L								
Pre-op U/L	226 (175, 939)	233 (148, 686)	0.703	0.004*	213 (148, 686)	0.314	196 (124, 491)	0.998
at discharge U/L	483 (410, 700)	457 (411, 700)	0.999	< 0.0001*	319 (160, 377)	< 0.001*	338 (15, 380)	0.996
3 months U/L	409 (301, 575)	571 (433, 840)	0.001*	0.511	312 (211, 1492)	< 0.001*	302 (217, 580)	0.763
6 months U/L	423 (313, 540)	474.5 (338, 908)	0.036*	0.301	324 (78, 893)	< 0.001*	285 (173, 602)	0.648
12 months U/L	374 (272, 580)	521 (330, 840)	0.006*	0.287	350 (225, 460)	< 0.001*	350 (225, 460)	0.837
Free plasma hemoglobin mg/dL								
at discharge mg/dL	45 (7, 71)	45 (33, 67)	0.999	< 0.0001*	20 (9.3, 77)	< 0.0001*	20 (10, 59)	0.975
3 months mg/dL	41.5 (17, 71)	48 (44, 107)	0.076	< 0.0001*	18 (5.3, 86)	< 0.0001*	27.9 (15.6, 69.2)	0.512
6 months mg/dL	26 (11, 66)	40.5 (25.4, 84)	0.017*	0.849	26.7 (11, 83)	0.0009*	25.2 (11, 76.2)	0.923
12 months mg/dL	31.5 (11, 55)	44.4 (12, 71)	0.041*	0.953	24 (12, 93)	0.006*	16 (6.2, 59.7)	0.839
Total bilirubin mg/dL								
Pre-op mg/dL	1.3 (0.2, 5.9)	0.8 (0.2, 2.5)	0.325	0.245	0.6 (0.3, 5.6)	0.961	0.8 (0.2, 3.6)	0.991
at discharge mg/dL	0.6 (0.2, 2.2)	0.6 (0.3, 2.2)	0.974	0.966	0.6 (0.2, 2.7)	0.865	0.5 (0.2, 2.2)	0.907
3 months mg/dL	0.6 (0.2, 3.9)	0.9 (0.2, 3.9)	0.833	0.969	0.5 (0.2, 3.8)	0.577	0.6 (0.3, 5.5)	0.972
6 months mg/dL	0.4 (0.2, 2.8)	1.3 (0.3, 2.1)	0.957	0.686	0.5 (0.2, 2.1)	0.469	1.2 (0.3, 2.1)	0.643
12 months mg/dL	0.5 (0.2, 3.9)	1.2 (0.4, 2.1)	0.485	0.662	1.1 (0.2, 3.3)	0.938	0.4 (0.2, 2.4)	0.757
Alanine aminotransferase U/L								
Pre-op U/L	47 (16, 159)	39.5 (16, 91)	0.073	0.006*	28 (11, 111)	0.903	24.5 (14, 71)	0.893
at discharge U/L	37 (14, 136)	30 (14, 84)	0.636	0.028*	22 (8, 75)	0.719	26.5 (14, 104)	0.755
3 months U/L	17 (9, 54)	17.5 (11, 37)	0.979	0.956	17 (8, 48)	0.999	21.5 (13, 53)	0.856
6 months U/L	25 (12, 83)	20.5 (14, 29)	0.652	0.594	21 (8, 48)	0.997	23 (8, 60)	0.911
12 months U/L	27 (15, 49)	20.5 (11, 45)	0.870	0.441	20 (8, 42)	0.973	19.5 (11, 43)	0.999
Aspartate aminotransferase U/L								
Pre-op U/L	40 (18, 213)	32 (20, 130)	0.491	< 0.001*	26 (16, 92)	0.045*	29.5 (13, 80)	0.659
at discharge U/L	26 (22, 54)	25 (14, 134)	0.663	0.353	25 (14, 134)	0.998	36 (15, 59)	0.607
3 months U/L	27 (19, 47)	26 (16, 88)	0.769	0.450	26 (16, 88)	0.997	27 (21, 38)	0.996
6 months U/L	29 (24, 55)	28 (18, 78)	0.963	0.405	28 (18, 78)	0.996	29 (12, 52)	0.969
12 months U/L	30.5 (19, 46)	29 (12, 81)	0.336	0.963	29 (12, 81)	0.999	27 (16, 40)	0.967

Data are presented as median (minimum, maximum), comparisons of repeated measurements between groups were conducted with 2-way ANOVA with Tukey's correction. * indicates significance.

0.045, respectively. ALT was also significantly higher in the LLH + sildenafil group compared to both no LLH groups (Table II). Total bilirubin did not differ between the groups at any measured time point (Table II).

Laboratory data: Detailed results of laboratory data at all measured time points are presented in Table II and an overview of LDH and fHb time courses are presented in Figure 1. LDH remained significantly elevated over time in the LLH no sildenafil group compared to the no LLH groups ($P < 0.0001$) (Figure 1). Within the LLH groups LDH was significantly lower over time in the LLH + sildenafil group compared to the LLH no sildenafil group ($P = 0.006$). LDH values in the LLH + sildenafil group were significantly higher at discharge compared to both no LLH groups (no LLH + sildenafil and no LLH no sildenafil) and then at 3, 6 and 12 months post HMII implantation LDH in the LLH + sildenafil did not differ significantly compared to no LLH patients either on sildenafil or not on sildenafil (Figure 1 and Table II).

fHb was also significantly higher over time in both LLH groups compared to the no LLH groups ($P < 0.0001$) and within the LLH groups, fHb was significantly lower in the LLH +sildenafil group compared to the LLH no sildenafil group ($P = 0.002$) (Figure 1 and Table II). INR did not differ between all groups at all measured time points (Figure 2).

vWF and LLH: All patients had an acquired von Willebrand disease (AvWD), as determined by the loss of high-molecular-weight multimers (HMWM) via Western blot and by reduced vWF:CBA/vWF:Ag and vWF:RCO/vWF:Ag ratios. The values of vWF:Ag, vWF:CBA and vWF:RCO in all groups 3 months postoperatively are presented in Table III. All vWF values did not differ significantly between groups.

Outcome by sildenafil status: The mean dose of sildenafil was 60 ± 15 mg/day. The distribution of AE in each group is presented in Table IV. Among the patients in the LLH no sildenafil group 25% had PT and 2.9% of the patients in the no LLH + sildenafil group had PT, whereas no patients in the LLH + sildenafil and no LLH no sildenafil groups had PT ($P = 0.005$). Among the LLH no sildenafil patients 33.3% had IS and 8.8% of the no LLH + sildenafil patients had IS and no IS occurred in the LLH + sildenafil and no LLH no sildenafil groups ($P = 0.004$). The incidence of TIA was 9.1% in the LLH + sildenafil group, 16.7% in the LLH no sildenafil group compared to 6.7% in the no LLH no sildenafil group, and no TIAs in the no LLH + sildenafil group were noted ($P = 0.764$). Comparing all TEIs including IS, PT and TIA between HMII patients with LLH ($n = 34$) and HMII patients without LLH ($n = 49$) regardless of sildenafil status, HMII patients with LLH had a significantly higher incidence of

TEs compared to HMII without LLH ($P = 0.025$).

The incidence of hemolysis according to the INTERMACS definition was 13.6% in the LLH + sildenafil group, 50% in the LLH no sildenafil group, 8.8% in the no LLH +sildenafil group, and 13.3% in the no LLH no sildenafil group ($P = 0.011$).

The incidences of HS and GIB did not differ significantly between the 4 groups (Table IV).

The 1 year estimated survival free from TE (IS or PT) in the LLH + sildenafil group was $95 \pm 4\%$ compared

to $32 \pm 15\%$ in the LLH no sildenafil and $92 \pm 4\%$ in the no LLH + sildenafil group ($P = 0.0001$, log-rank (Mantel-Cox) test) (Figure 3A). All groups had a similar INR during the follow-up (Figure 2).

Cox regression analysis revealed a significantly higher risk for TE among HMII patients with LLH not taking sildenafil compared to LLH + sildenafil patients (HR: 14.4, 95%-CI: 1.8 - 117.7, $P = 0.001$) and compared to no LLH no sildenafil patients (HR: 11.5, 95%-CI: 2.1 - 31.7, $P = 0.043$). The HR was reduced in LLH + sildenafil patients compared to LLH no sildenafil patients (HR: 1.8, 95%-CI: 1.8 - 117.7, $P = 0.013$). There was no difference in the survival free from gastrointestinal bleeding (GIB) in all 4 groups ($P = 0.369$) (Figure 3B). The overall survival regardless of AEs did not differ between the 4 groups ($P = 0.110$) (Figure 3C).

Hemodynamic parameters: Patients who received sildenafil (LLH + sildenafil and no LLH + sildenafil groups) had significantly higher mPAP, PVR and PCWP compared to HMII patients who did not receive sildenafil (Tables I and V). On the other hand, cardiac index (CI), TAPSE and RV-FAC did not differ between all 4 groups preoperatively.

mPAP, PCWP decreased significantly 1-year postoperative in all groups but not in the no LLH no sildenafil group (Table V).

The mean difference of mPAP comparing pre- and postoperative measurements in the LLH + sildenafil group was 7.2 ± 1.2 mmHg (30.2 ± 0.9 versus 22.3 ± 6.9 mmHg, $P < 0.0001$), and in the no LLH + sildenafil group, the mean difference in mPAP was 7.1 ± 1.1 mmHg (34.3 ± 9.7 versus 28.5 ± 7.2 , $P < 0.0001$) (Table V). In contrast, the mean difference in mPAP in the LLH no sildenafil group was only 4 ± 1.4 mmHg (Table V). PCWP also decreased significantly when comparing preoperative with 1 year post-implantation measurements, but

Table III. Von Willebrand Factor Parameter Measurements

	LLH + Sildenafil (n = 22)	LLH no Sildenafil (n = 12)	no LLH + Sildenafil (n = 34)	no LLH no Sildenafil (n = 15)	P value
vWF: Ag %	236 (116, 654)	148 (126, 278)	204 (94, 284)	233 (114, 720)	0.558
vWF: RCo	143 (75, 180)	102 (85, 147)	125 (71, 173)	115 (85, 302)	0.594
vWF: CBA	193 (85, 302)	115 (103, 166)	130 (58, 240)	166 (126, 278)	0.698
vWF: RCo/vWF: Ag	0.58 ± 0.12	0.59 ± 0.18	0.64 ± 0.14	0.53 ± 0.17	0.269
vWF: CBA/vWF: Ag	0.72 ± 0.15	0.67 ± 0.11	0.71 ± 0.19	0.69 ± 0.13	0.716

vWF indicates von Willebrand factor; Ag, antigen; CBA, collagen binding activity; RCo, ristocetin cofactor activity; and HMWM, high-molecular-weight multimers.

Table IV. Distribution of Adverse Events Between Groups

	LLH + Sildenafil (n = 22)	LLH no Sildenafil (n = 12)	no LLH + Sildenafil (n = 34)	no LLH no Sildenafil (n = 15)	P-value
PT n (%)	0	3 (25)	1 (2.9)	0	0.005
IS n (%)	0	4 (33.3)	3 (8.8)	0	0.004
TIA	2 (9.1)	2 (16.7)	0	1 (6.7)	0.764
HS	1 (4.5)	0	2 (5.9)	1 (6.7)	0.843
GIB	6 (27.3)	4 (33.3)	16 (17.6)	5 (33.3)	0.473
Hemolysis n (%)	3 (13.6)	6 (50)	3 (8.8)	2 (13.3)	0.011

PT indicates pump thrombosis; IS, ischemic stroke; HS, hemorrhagic stroke; GIB, gastrointestinal bleeding; and TIA, transitory ischemic attack.

again the mean decrease of PCWP values was higher in the sildenafil groups (7.2 mmHg) compared to the no sildenafil groups (4.3 mmHg) (Table V).

PVR did not decrease significantly when comparing pre- and postoperative measurements in HMII patients, who did not have sildenafil (LLH no sildenafil and no LLH no sildenafil groups) (Table V). On the other hand, PVR decreased significantly 1-year after implantation in both sildenafil groups; LLH + sildenafil (2.5 ± 0.8 versus 1.7 ± 0.5 Wood units, $P < 0.0001$) and no LLH + sildenafil (3.0 ± 1.1 versus 2.1 ± 0.9 Wood units, $P < 0.0001$) (Table V). CI increased in all groups (Table V) but the mean increase was higher in HMII patients, who had sildenafil (0.5 L/minute/m^2) (Table V). TAPSE increased significantly 1-year after HMII implantation only in the no LLH + sildenafil group. RV-FAC increased significantly 1-year post-implantation only in both sildenafil groups (Table V).

Discussion

Our study examined the clinical effect of LLH in patients on HMII support, as assessed by elevated LDH, and the association between sildenafil and TE. In our study, we found that patients on HMII support with LLH had a higher risk for TE and higher incidence of hemolysis and that the use of sildenafil in HMII with LLH reduced the risk of TE and sildenafil improved the pulmonary circulation significantly during HMII support regardless of LLH status. Similar results were described by Saeed, *et al*²¹⁾ who found that HMII patients with LLH not on sildenafil had a higher risk (HR 8.8) for TE compared to HMII with LLH on sildenafil. The difference in TE between the groups was not related to the variation in anticoagulation, as INR did not differ between all 4 groups. PDE5-Is intensify the effect of endogenous NO by stabilizing the

second-messengers cyclic adenylylate monophosphate (cAMP) and cyclic guanylate monophosphate (cGMP).²²⁾ cGMP activates the cGMP-dependent protein kinases (cGKs) and also inhibits phosphodiesterase 3, and thus inhibits the destruction of cyclic adenosine monophosphate (cAMP). cAMP also activates the cAMP-dependent protein kinases (PKA), which are known platelet inhibitors. The NO/cGMP-induced platelet inhibition can also be resolved by both cGK- and PKA-dependent mechanisms.²³⁻²⁵⁾ However, it is still unclear how cGKs mediate the platelet-inhibitory effect of the NO/cGMP. Many hypotheses have been presented and it has been shown that NO/cGMP inhibits intracellular calcium release,²⁶⁾ which is an important step to full activation of platelets.²⁷⁾ It was unclear how NO/cGMP resolve this effect but recently Antl, *et al*²⁸⁾ demonstrated that cGK phosphorylates inositol-1,4,5-trisphosphate receptor-associated cGMP kinase substrate, which leads to inhibition of intracellular calcium release.

Our hypothesis was that sildenafil, a PDE5-I, which is usually prescribed to CF-LVAD patients to treat pulmonary hypertension and right heart failure, might reduce the risk of TE in HMII patients with LLH, due to enhanced NO/cGMP signaling. In addition to a reduction of TE incidence in HMII patients on sildenafil, we detected that LDH and fHb decreased in LLH patients over time in comparison to LLH patients not on sildenafil. This effect might be a result of the hemodynamic effect of sildenafil since it lowers pulmonary vascular resistance and pulmonary capillary wedge pressure and increases LV preload without causing systemic hypotension²⁹⁻³¹⁾ and therefore leads to an increase in LVAD flow, which in turn may have contributed to lower TE due to better flow through the device.

In our study, we found decreases in mPAP and PCWP and an increase of CI in all HMII patients 1 year

Figure 3. Kaplan-Meier curves: Overall survival and survival free from thromboembolic events and GIB. **A:** Survival free from thromboembolic events. **B:** Survival free from GIB. **C:** Overall survival of all 4 groups. GIB indicates gastrointestinal bleeding.

Table V. Pre- and One-Year Postoperative Hemodynamic Parameters

	1. LLH + Sildenafil (n = 22)	2. LLH no Sildenafil (n = 12)	P-value 1 versus 2	P-value 1 versus 3	3. no LLH + Sildenafil (n = 34)	P-value 2 versus 3	4. no LLH no Sildenafil (n = 15)	P-value 3 versus 4
mPAP mmHg								
Pre	30.2 ± 8.9	22.6 ± 3.8	0.020*	0.145	34.3 ± 9.7	< 0.0001*	21.4 ± 7.7	< 0.001*
post	22.3 ± 6.9	18.9 ± 3.3	0.315	0.138	28.5 ± 7.2	< 0.0001*	18.9 ± 5.7	0.008*
P-value pre versus post	< 0.0001*	0.029*			< 0.0001*		0.222	
PCWP mmHg								
pre	22.2 ± 6.3	16.1 ± 1.5	0.027*	0.998	21.1 ± 9.3	0.032*	14.3 ± 5.6	< 0.001*
post	14.9 ± 5.8	11.3 ± 2.8	0.319	0.465	17.5 ± 5.8	0.018*	9.1 ± 2.8	0.009*
P-value pre versus post	< 0.0001*	< 0.01*			< 0.001*		< 0.01*	
PVR Wood units								
pre	2.5 ± 0.8	1.4 ± 0.4	0.005*	0.224	3.0 ± 1.1	< 0.001*	1.5 ± 0.9	< 0.001*
post	1.7 ± 0.5	1.2 ± 0.5	0.308	0.646	2.1 ± 0.9	0.034*	1.2 ± 0.6	0.031*
P-value pre versus post	< 0.0001*	0.776			< 0.0001*		0.680	
CI L/minute/m ²								
pre	2.0 ± 0.4	2.1 ± 0.3	0.999	0.974	1.9 ± 0.4	0.988	1.9 ± 0.3	0.999
post	2.5 ± 0.5	2.3 ± 0.2	0.326	0.146	2.4 ± 0.5	0.994	2.3 ± 0.2	0.369
P-value pre versus post	< 0.0001*	0.040*			< 0.0001*		0.010*	
TAPSE mm								
pre	15.3 ± 4.4	16.8 ± 2.1	0.566	0.634	14.3 ± 2.3	0.089	16.2 ± 2.8	0.204
post	16.4 ± 2.5	17.1 ± 1.4	0.931	0.285	15.3 ± 2.4	0.150	17.5 ± 2.3	0.034*
P-value pre versus post	0.219	0.994			0.011*		0.357	
RV-FAC %								
pre	36.9 ± 10.2	39.4 ± 9.4	0.897	0.941	35.4 ± 9.2	0.615	40.3 ± 9.6	0.361
post	41.5 ± 7.6	38.9 ± 8.2	0.764	0.610	38.6 ± 6.2	0.999	41.7 ± 7.7	0.667
P-value pre versus post	0.002*	0.983			0.009*		0.856	

mPAP indicates mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; CI, cardiac index; TAPSE, tricuspid annular plane systolic excursion; and RV-FAC, right ventricle fractional area change. Data are presented as the mean ± SD. Comparisons of repeated measurements between groups were conducted with 2-way ANOVA with Tukey's correction.

*indicates significance.

after implantation, as a result of the decompression of pulmonary circulation during HMII support, results that were similar to those noted by Alturi, *et al.*³²⁾ The mean increase of CI and decreases of mPAP and PCWP were higher in HMII, who had sildenafil.

In addition, PVR decreased significantly 1-year post-implantation only in HMII patients, who had sildenafil and also the RV-FAC increased significantly in these patients. According to our findings and in accordance with previous studies,¹⁸⁻²⁰⁾ we can conclude that the use of sildenafil during HMII supports add a further decompression effect of the pulmonary circulation during HMII support providing a better hemodynamic effect. These findings strengthen the above-mentioned hypothesis about the hemodynamic effect of sildenafil which might contribute to the lower TE incidence in HMII patients with LLH receiving sildenafil.

The etiology of hemolysis in patients with CF-LVAD is still not fully understood; red blood cell damage might be the direct result of shear stress induced by the device itself. However, many other factors, such as oxidative stress, medications, and infections contribute to the process of hemolysis.^{4,33,34)} NO is continuously synthesized in the endothelium and has many functions, such as the regulation of homeostatic vascular functions through vasodilation and inhibition of platelet activation and thrombosis.^{35,36)} Elevated fHb, as it appears in hemolysis conditions, increases the scavenging of NO and enhances its consumption, resulting in several cellular dysfunctions,

such as vasoconstriction, platelet activation and systemic and pulmonary hypertension, in patients with hemolytic disorders.^{13,35,37)} In our study, fHb remained significantly higher in LLH patients compared to no LLH patients, but fHb decreased significantly in LLH patients taking sildenafil compared to LLH patients not on sildenafil.

Elevated fHb, which occurs in pump induced hemolysis, can also contribute to the pro-coagulation pathway via its interaction with vWF. fHb interacts with the A1 and 2 domains of VWF, which in turn increase the adhesion of platelets to VWF.¹⁵⁾ In our study, we could not detect a significant difference in the measured vWF parameters in HMII patients with LLH or between patients taking sildenafil and not taking sildenafil. The incidence of GIB also did not differ between the groups.

Limitations of the study: Our study is limited by the usual shortcomings of a small-cohort single-center study. Our data suffer potential bias in patient selection and data acquisition; therefore, caution should be taken when interpreting the results. Due to the low number of AEs, adjustment in the regression model was not performed to covariates, which may have an impact on TE, and accordingly, the estimated hazard ratio is under powered.

Hemolysis in LVAD patients is multifactorial, and as a result, we cannot exclude the possibility that unmeasured confounders influenced our results. Another limitation was the small number and heterogeneity of the patients, which may limit the generalization of the results.

Conclusion

LLH in HMII patients as defined by elevated LDH is associated with higher thromboembolic rates. Sildenafil treatment among HMII patients with LLH reduced the risk of thromboembolic events. Sildenafil significantly improved the pulmonary circulation and added a further decompression effect during HMII support. Further investigation is needed to understand the pathophysiological role of PDE5-Is in the reduction of thromboembolic events among patients with CF-LVAD devices.

Disclosures

Conflicts of interest: The authors state that there are no conflicts of interest to declare.

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