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Fibrin clot permeability (Ks) in patients on left ventricular assist device

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Patients on left ventricular assist devices (LVAD) are prone to excessive hemostasis disturbances due to permanent contact of artificial pump surfaces with blood components. We aimed to investigate if fibrin clot permeability is altered in patients on long-term continuous-flow LVAD therapy and if the clot permeability is associated with clinical characteristics and adverse events. We investigated 85 end-stage heart failure patients (90.6% men, age 48.6–63.8 years) scheduled for continuous flow long-term LVAD support according to current clinical indications. The patients were assessed periodically: prior to LVAD implantation (T1), 3–6 months (T2) after LVAD implantation, 6–12 months after (T3) and then every 6 months. We tested the first three blood samples (T1-T3) and the last available blood sample (T4), but no longer than 5 years after LVAD implantation. We assessed hemostasis parameters (Activated Partial Thromboplastin Time (APTT) Prothrombin Time, Activated Partial Thromboplastin Time, Fibrinogen, D-dimer, Antithrombin, Thrombin Time, Factor VIII, and von Willebrand Factor, aspirin-induced platelet inhibition, adenosine-diphosphate test) changes during the study period. Fibrin Clot Permeability was evaluated using a pressure system and Permeability Coefficient (Ks) was calculated. We observed a decrease in fibrin clot permeability (Ks) between T1, T2, T3 and T4 time periods; $P < 0.01$ for each comparison. Fibrin clot permeability was negatively correlated with fibrinogen concentration: $r = -0.51$, $P < 0.001$, factor VIII activity $r = -0.42$, $P < 0.001$. There was no association of Ks with age, Left Ventricular Ejection Fraction (LVEF) and medications $P > 0.001$, however cumulative measurements in patients on aspirin showed shortening of Ks in this group $P = 0.0123$. Major adverse cardiac and cerebrovascular events (MACCE) occurred in 36.5% patients, bleeding events in 25.9%, Net Adverse Clinical Events (NACE) in 62.4%; 31.7% patients died, and 17.6% underwent transplantation. The transplantation was considered as the endpoint. Discrepancies in Ks were observed between patients with MACCE, bleeding, and NACE, and patients without adverse events. Ks showed a constant trend towards normalization ($P < 0.01$) only in patients without adverse events. Patients with advanced heart failure have disturbed clot structure. A trend towards normalization of the Ks values is associated with fewer thromboembolic and bleeding complications in this group of patients.

Keywords Heart failure, Left ventricular assist device, Fibrin clot permeability, Ks

The management of advanced heart failure patients (HF) includes, among long-term therapeutic options, continuous-flow left ventricular assist devices (LVADs)[1]. With organ shortages and a growing number of heart failure cases, there's an increasing demand for durable circulatory support systems. Long-term mechanical circulatory support raises major concerns regarding driveline issues, comprising primarily of driveline infections at the exit site, right ventricular heart failure, and thromboembolic and bleeding complications[2].

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In contrast to heart transplantation (HTx), where the most challenging period occurs within the first year following HTx by a relative stabilization thereafter[3], LVAD patients seem to accumulate complications over time.

The most detrimental complications in continuous flow LVAD therapy are bleeding and thromboembolic events, with ischemic strokes and intracranial bleeding being particularly disabling. Demographic trends in Europe indicate an increasing predicted lifespan coupled with ageing population[4]. Heart failure prevalence rises with age, and older patients with HF often have a higher rate of comorbidities[5]. Efforts are being made not only to extend life expectancy but also optimize the quality of life in HF patients. Complications such as ischemic and hemorrhagic strokes significantly impact both life expectancy and quality of life in LVAD patients. Complications associated with comorbidities and advanced age[6] substantially increase healthcare and caregiver burden. Consequently, efforts are underway to identify significant risk factors to prevent these complications.

Optimal medication, including heart failure treatment, appropriate anticoagulation, antiplatelet therapy, and blood pressure control, can impact the risk of bleeding and thromboembolic complications[7]. Despite optimal management, complications remain high at 14% in the first year and 20% within the first two years of the long-term continuous-flow LVAD support. The incidence of hemorrhagic and ischemic strokes is nearly equal, and both contribute to increased mortality[8]. Efforts are ongoing to mitigate their effects and prevent their occurrence. We hypothesize that prothrombotic conditions may precede these adverse events.

Fibrin clot permeability is a parameter describing functional fibrin clot properties, defined by the Darcy constant (Ks). The parameter characterizes the density of the fibrin net and its porosity. Denser and more compact fibrin clots appear to be more resistant to lysis and are correlated with thromboembolic events.

Factors affecting fibrin networks are both genetic and environmental. Briefly, denser fibrin nets that are more resistant to lysis promote prothrombotic states. These nets are present in patients with diabetes[9], atherosclerosis, coronary artery disease, and acute coronary syndromes[10,11].

Denser fibrin clot structures have been reported in anticoagulated patients with ischemic stroke. Lower permeability values (Ks) are observed in patients with cardiovascular disease and thromboembolism. Altered fibrin clot permeability is also noticed in patients with dysfibrinogenemia[9,12,13]. There were observations connecting the presence of prothrombotic fibrin clot properties in patients with congestive heart failure. Significantly faster formation of dense fibrin clots in heart failure patients, predisposing them to thromboembolic complications compared to healthy controls, was observed. There was no correlation with left ventricular ejection fraction, as the left ventricular ejection fraction in advanced heart failure is only one of the numerous factors describing the overall clinical state[14] Furthermore, prothrombotic fibrin clot properties may occur during acute viral infections such as SARS-CoV-2 infection[15].

An international study on standardizing fibrin clot permeability measurements suggests its potential in characterizing thromboembolic diseases[16]. The properties of fibrin clots seem to be reversible with pharmacologic interventions such as anticoagulants, antihypertensive drugs, antiplatelet drugs, folic acid, omega-3 fatty acids[17], and intense low-density lipoprotein lowering treatment[18]. Impaired fibrin clot formation may result among others causes, from dilution coagulopathy[19].

Aligned with these findings, our study aimed to investigate the role of changes in fibrin clot permeability (Ks) in patients on long-term LVAD support.

Materials/patients and methods

Patient selection

We recruited 85 consecutive adult patients (over 18 years old) diagnosed with advanced heart failure, meeting at least one of the following inclusion criteria: a history of at least 3 hospitalizations due to HF decompensations in the preceding 12 months, dependence on inotropes or temporary mechanical circulatory support, progressive end-organ dysfunction attributed to the low cardiac output, very limited exercise capacity (VO2 peak < 12 ml/kg/min), or an inability to exercise. Exclusion criteria encompassed current pregnancy, ongoing infections, recurrent malignant arrhythmias, severe right ventricular failure, phenotypes of hypertrophic or restrictive cardiomyopathy hindering safe LVAD implantation, known contraindications to antiplatelet or anticoagulation therapies, life-limiting severe comorbidities or cancer (with an expected survival of less than two years), severe renal or liver failure, absence of social support or unstable psychosocial background.

Implantation was performed either as a bridge to transplantation or bridge to candidacy. The study was conducted at a single high-volume heart transplantation center between May 10, 2017 and September 10, 2021. All patients provided informed consent before participation in and underwent assessment adhering to the inclusion and exclusion criteria preimplantation.

Bioethics committee approval

The study adheres to the principles of the Declaration of Helsinki and received approval from the Medical University of Silesia Bioethics Committee (PCN/CBN/0022/KB1/144/21/22).

Pump implantation and management

Two available continuous-flow LVAD systems (HeartWare, Medtronic or HeartMate 3, Abbot) were used. The choice between two types and surgical approach (full sternotomy vs. mini- sternotomy) was at the operator's discretion. Both devices had national health insurance approval at the time of implantation. Both pumps consisted of an inflow cannula inserted into the left ventricle connected to a continuous-flow centrifugal pump placed in the chest. The outflow cannula transported blood from the pump to the ascending aorta. Both pumps were externally powered, connected to an external energy source via the driveline tunneled in abdominal tissues and exiting on the skin surface. Pump flow was regulated by altering the rotation speed in the external controller[20].

Pump speed adjustment was performed by an experienced transplant physician based on echocardiography and patient' clinical condition[21].

The vitamin K antagonists were caseated prior to the procedure in order to obtain appropriate hemostasis during the operation.

Medication

The anticoagulation regimen comprised titrated warfarin (vitamin K antagonist) to achieve a target international normalized ratio (INR) of 2–3. Patients underwent INR home tests for all-day self-monitoring. In instances of warfarin cessation, patients were bridged with therapeutic doses of low molecular weight or unfractionated heparin.

Acetylsalicylic acid at a daily dose of 75 mg was the preferred an antiplatelet therapy. We monitored aspirin-induced platelet inhibition (ASPI) to assess aspirin response. If the ASPI test fell below 745AU × min, we maintained the dose; if it exceeded 745AU × min, we increased the dosage to 150 mg daily. Should ASPI test levels remain elevated, we switched antiplatelet medication to clopidogrel 75 mg daily and monitored the response using adenosine-diphosphate (ADP) tests. If the ADP test resulted below 534 AU × min, we maintained the dosage; otherwise, we doubled the dosage. Details of the anticoagulation and antiplatelet regimen are described elsewhere[22].

Heart failure guideline-directed medical therapy included angiotensin-convertingenzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), angiotensin receptor- neprilysin inhibitor (ARNI), mineralocorticoid receptor antagonists (MRA), sodium-glucose contransporter-2 (SGLT-2), beta-blockers, diuretics, and optionally, depending on etiology, statins. Comorbidities were managed based on individual indications.

Follow-up

Subsequent to acquiring informed consent for participation in the study, patients were regularly followed up at scheduled visits: prior to LVAD implantation, 3–4 months post-implantation, 6–12 months post-implantation, and thereafter every 6 months post the first year (for a maximum follow-up of 5 years). The last available blood sample was analyzed (median 21 months; IQR 10–29 months).

Major adverse cardiac and cerebrovascular events (MACCE) encompassed all-cause mortality, ischemic stroke, transient ischemic attack, peripheral embolism, pulmonary embolism, and pump thrombosis. Ischemic stroke, transient ischemic attack, peripheral embolism and pulmonary embolism were defined based on separate criteria[23–26]. We documented all symptomatic events during follow-ups and outpatient visits. Bleeding complications were classified based on criteria established by the International Society on Thrombosis and Hemostasis[27]. Net adverse clinical events (NACE) comprised a composite of MACCE and bleeding complications. Transplantations were calculated separately and were not included in MACCE or NACE assessments.

Laboratory investigations

Assessment timeline

Basic clinical and laboratory parameters were analyzed at four time points: directly before to LVAD implantation (T1), 3–4 months post-implantation (T2), 6–12 months post-implantation (T3), and the last available blood sample (T4). Fasting venous blood samples were collected between 7:30 and 09:00 AM. Routine laboratory tests were used to evaluate basic biochemical parameters and complete blood count.

Coagulation profile

Citrated venous blood samples were collected using S—Monovettes: Citrate 9NC/2.9 mL (9NC:0.106 mol/L) to assess Prothrombin Time, Activated Partial Thromboplastin Time, Fibrinogen, D-dimer, Antithrombin, Thrombin Time, Factor VIII, and von Willebrand Factor.

Assay details

Prothrombin time (PT) and INR we evaluated using STA Neoptimal reagent, Diagnostica Stago, Canada, with a reference range of 70–120%.

Activated Partial Thromboplastin Time (APTT) was assessed with STA Cephascreen reagent, Diagnostica Stago, Canada, with a normal range of 24–35 s.

Thrombin Time (TT) was evaluated with STA Thrombin, Diagnostica Stago, Canada with a reference range of 14–21 s.

Fibrinogen levels were determined using STA Liquid Fib reagent, Diagnostica Stago, Canada, with a normal range of 200–400 mg/dL.

D-dimer assessment employed an immunoturbidimetric method (TA Liatest D-DI Plus reagent, Diagnostica Stago, Canada) with a reference range of 0–0.5 ug/mL FEU.

Antithrombin time (AT) utilized a colorimetric method with the STA Stachrom AT III reagent, Diagnostica Stago, Canada, and reference range of 80–120%.

Factor VIII assessment involved STA Immunodef VIII, Diagnostica Stago, Canada, with a reference range of 60–150%.

Von Willebrand factor assessment used an immunoturbidimetric method (STA Liatest VWF: Ag reagent, Diagnostica Stago, Canada) with normal range was 50–160%.

Platelet function assessment

ASPI test

Platelet function in response to acetylsalicylic acid was evaluated using impedance aggregation in a Multiplate analyzer (Roche Diagnostics, Mannheim, Germany), with a reference range from 745 to 1361 AU × min.

ADP test

Platelet function in response to clopidogrel was assessed via impedance aggregation in a Multiplate analyzer (Roche, Diagnostics, Mannheim, Germany), with a reference range of 534 to 1220 AU × min.

Fibrin clot permeability assessment

Fibrin clot permeability was evaluated using a pressure system[28]. Clots were obtained from 120uL citrated plasma by applying 1 IU/mL of human thrombin and 20 mM $CaCl_2$ according to established standards[16]. The volume and mass of the buffer flowing through the clot was measured over a specified time period. All analyses were performed in duplicate. The interassay variability coefficient was less than 8%

Permeability coefficient (Ks) calculation

The Ks ($\times 10^{-9}$ cm^2) was calculated using the following formula:

$$\mathrm{Ks}\ \left(\times 10^{-9}\ \mathrm{cm}^2\right) = \mathrm{Q} \times \mathrm{L} \times \eta/\mathrm{t} \times \mathrm{A} \times \Delta\mathrm{P},$$

where: Q (cm^3) represents the flow rate at time t (s), L (cm) signifies the length of the fibrin gel, η (dyne × s/cm^2) denotes the viscosity of the liquid, A (cm^2) signifies the cross-sectional area, and ΔP (dyne/cm^2) represents the pressure gradient.

There are no reference range for this measurement available. According to an international study on the standardization of fibrin clot permeability measurement: methodological considerations and implications for healthy control values[16] average Ks for healthy controls is: 9.91×10^{-9} cm^2.

Statistical analysis

Continuous variables were presented as means and standard deviations for normally distributed data or medians with lower and upper quartiles (IQR: 25th to 75th) for data with non-normal distribution. Categorical variables were presented as percentages. Normal distribution was verified using the Shapiro–Wilk test. Statistical tests included the chi-squared test for categorical variables and the Student's t-test or Mann–Whitney U test for continuous variables. Friedman rank sum test and exact all-pairs comparisons tests of Friedman-type ranked data[29] and Mann–Whitney U test with Holm-Bonferroni correction for multiple comparisons were used. To assess a monotonic trend the Page's ordered aligned rank sum test was used. Repeated measures correlation was also calculated[30]. A P value < 0.05 was considered statistically significant. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 4.3.1 (R Core Team, 2023).

Results

Clinical characteristics of investigated patients

As depicted in Table 1, the study involved 85 patients, with a mean age of 54.9 ± 11.9 years (median age: 58.2 years, range 48.6–63.8); 77 patients (90.6%) were male. The most frequent cause of heart failure among these patients was ischemic etiology. At baseline, the medication was as follows: 38 patients (44.7%) received vitamin K antagonists, 24 patients (28.2%) were prescribed acetylsalicylic acid, 5 patients (5.9%) received clopidogrel, 44 patients (51.8%) were on ACE inhibitors, 12 patients (14.1%) used ARB or combinations, 74 patients (87.1%) were administered aldosterone antagonists, 65 patients (76.5%) were on beta blockers, and 51 patients (60%) were prescribed statins.

NACE encompassed MACCE and bleeding complications. Throughout the entire follow-up, MACCE occurred in 31 (36.5%) patients, bleeding events in 22 (25.9%) patients, and NACE in 53 (62.4%) patients. The cumulative incidence of adverse events and transplantations is presented in Table 2.

The Ks values in the studied group

Baseline Ks values did not differ significantly between patients receiving vitamin K antagonists (n = 38) and without VKA antagonists (n = 47): Ks 3.86 (1.17) for patients receiving warfarin prior to the procedure and Ks 4.1 (1.11) for the rest of population; P = 0.3899.

At baseline 24 patients (28.2%) were prescribed acetylsalicylic acid and 5 patients (5.9%) received clopidogrel. In the subgroup with aspirin the Ks was 3.90 (0.90) vs. 4.03 (1.23) in patients without aspirin; P = 0.69. In the whole subgroup of patients with antiplatelet drugs the baseline differences were nonsignificant too: Ks 3.999(0.84) for patients with antiplatelet drugs vs. 3.936(1.2) for patients without antiplatelet drugs; P = 0.836.

We observed a trend toward normalization of the Ks values in the entire patient cohort (two-sided probability: P < 0.001, Page's test). Figure 1 illustrates the timeline of Ks values in the entirety of LVAD patients.

Patients with ischemic etiology of heart failure exhibited significantly lower Ks values (P = 0.037) compared to patients with other HF etiologies. Nevertheless, these values changed over time in a similar manner (Fig. 2). In patients with diabetes the baseline value was nonsignificant shortened vs. patients without diabetes 3.75 (1) vs 4.15(1.22), P = 0.38, however the total Ks measurements in diabetes patients were significantly shortened P = 0.017.

History of stroke prior to implantation and other analyzed comorbidities did not affect the Ks values.

Variable	
Age at the time of implantation, years	58 [49–64]
NYHA class, median [IQR]	4[3.5–4]
NYHA III	21 (24.7)
NYHA III advanced	5 (5.9)
NYHA IV	59 (69.4)
INTERMACS median [IQR]	3 [2–4]
INTERMACS 1	0
INTERMACS 2	27 (31.8)
INTERMACS 3	33 (38.8)
INTERMACS 4	24 (28.2)
INTERMACS 5	1 (1.2)
Echocardiographic parameters	
LVEF, %	15 [11–17]
LVEDD, mm	75[70–82]
NTproBNP [pg/mL]	7222 [4537–14054]
HF etiology	
Ischemic HF	49 (57.6)
Dilated CM	29 (34)
Inflammatory	6 (7.1)
Non-compaction	1 (1.2)
Anthracycline	1 (1.2)
Toxic	1 (1.2)
Comorbidities	
Prior stroke	13 (17.6)
Hypertension	38 (44.7)
Diabetes mellitus	35 (41.2)
CAD	49 (57.6)
Impaired glucose tolerance	3 (3.5)
CKD stage ≥ 3	37 (45.1)
Type of LVAD support	
HM3	72 (84.7)
Implanted HW	13 (15.3)

Table 1. Baseline characteristics of LVAD patients (prior to LVAD implantation). Data are shown as numbers (%), mean ± standard deviation or median [interquartile range]. *ACEI* angiotensin-converting enzyme inhibitors, *ARB* angiotensin II receptor blockers, *CAD* coronary artery disease, *CKD* chronic kidney disease, *CM* cardiomyopathy, *INTERMACS* Interagency Registry for Mechanically Assisted Circulatory Support [The INTERMACS score assesses the clinical status of patients with heart failure, classifying them into seven levels from the most stable (level 7) to the most critical cases requiring immediate intervention (level 1)], *HM3* HeartMate 3, *HW* HeartWare, *IQR* interquartile range, *NYHA* New York Heart Association class, *LVEDD* left ventricle end diastolic dimension, *LVEF* left ventricular ejection fraction.

Events	Cumulative incidence
MACCE, no of events	34
Bleeding events, no. of events	23
NACE, no. of events	55
Heart transplantation, no. (%)	15 (17.6)
Death no. (%)	27 (31.8)

Table 2. Major adverse cardiac and cerebrovascular events (MACCE), bleeding events, Net Adverse Clinical Events (NACE), deaths and transplantations. Cumulative incidence.

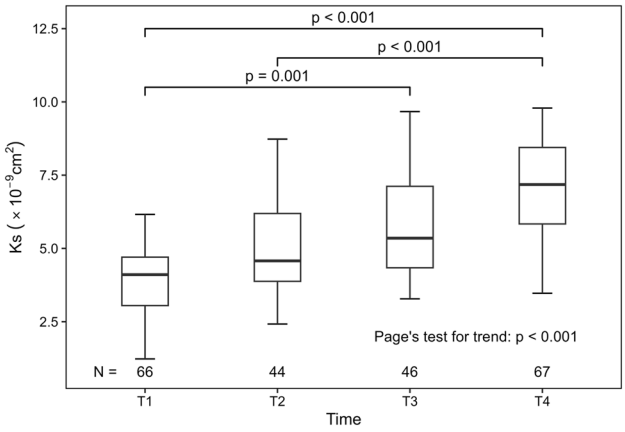

Figure 1. Clot permeability values (Ks) in the whole group of patients during LVAD support. Significant differences (P < 0.001) were observed between T1 vs. T3, T1 vs. T4 and T2 vs. T4 timepoints. In the box-whisker plot, the box represents the interquartile range (lover and upper quartiles), with the horizontal line inside the box indicating the median value. Whiskers illustrate the minimum and maximum values.

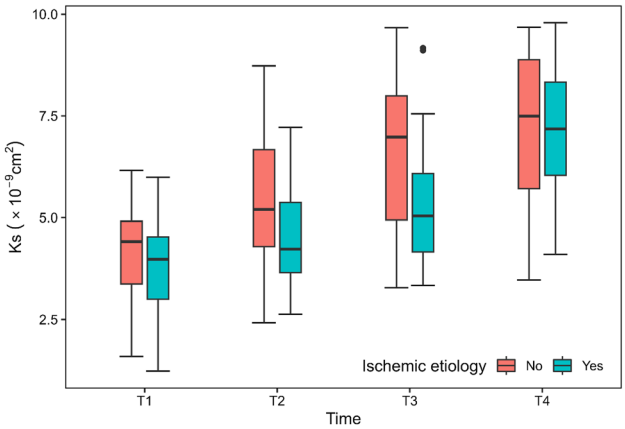

Figure 2. Ks changes over time in regard to HF etiology. In patients with ischemic etiology significant differences in Ks values were observed between the all analyzed timepoints (T1–T2, T1–T3, T1–T4, T2–T3, T2–T4 and T3–T4); P < 0.05. In regard other etiologies the differences were significant between: T1–T2, T1–T3, T1–T4, T2–T4 and T3–T4 timepoints; P < 0.05. P value with Holm-Bonferroni correction.

However, the favorable trend in Ks improvement was exclusively observed in patients with no prior ischemic stroke (significant differences observed in: T1 vs. T2, T1 vs. T3, T3 vs. T4, T2 vs. T3, T2 vs. T4 and T3 vs. T4; P < 0.001).

We did not identify significant differences in Ks concerning medication (antiplatelet therapy, statins, angiotensin receptor blocker, ARB/ angiotensin converting enzyme inhibitors, ACEI, mineraloid receptor antagonists, MRA). However, the cumulative measurements of patients receiving aspirin at a different time points showed shortening in patients receiving aspirin; P = 0.0123.

While no significant correlations were found with ASPI and ADP, correlations were observed with fibrinogen, D-dimer, antithrombin, hemoglobin, platelet count, INR and factor VIII activity. Further details are presented in Table 3.

The Ks values in relation to outcomes

Ks values did not differ with respect to survival status during follow-up. Regarding to survival within the observation period, differences in Ks significantly improved only in the group of survivors (P < 0.001) at each timepoint. In contrast, among deceased patients, a significant difference was observed only between T1 and T4 time points.

No differences were found in Ks values concerning bleedings, MACCE, and NACE (Table 4).

Patients without MACCE, NACE, and bleedings exhibited significant differences (P < 0.001) between all-time points. In the NACE group, differences (P < 0.05) were only observed between T1 vs. T3, T1 vs. T4, T2 vs. T4 and T3 vs. T4. Among patients with MACCE, differences were significant (P < 0.05) only at T1 vs. T4 and T2 vs. T4. Conversely, in patients experiencing bleedings, no differences were observed among these time points.

Laboratory parameter	Mean/median value, SD/IQR	Repeated measures correlation, r, [95% CI]	P value
ASPI [AU × min]	T1: 556.5 [352.8–850] T2: 462 [273–766] T3: 440 [232–766] T4: 464.5 [271.5–870]	− 0.177; 95% CI: [− 0.32 to − 0.031]	0.039
ADP [AU × min]	T1: 401 [285.8–630.8] T2: 355 [229.8–591.2] T3: 445 [264–601] T4: 460 [307–671]	− 0.0034; 95% CI: [− 0.166 to 0.138]	0.97
Fibrinogen [mg/dL]	T1:431 [334.8–496.8] T2: 432 [374–474.5] T3: 394 [333.5–458.5] T4: 379 [329–444]	− 0.51; 95% CI [− 0.62 to − 0.398]	P < 0.001
D-dimer [µg/mL]	T1: 1.48 [0.56–3.57] T2: 1.94 [1.42–2.55] T3: 1.295 [0.95–2.02] T4: 1.23 [0.71–1.94]	− 0.18; 95% CI [− 0.29 to − 0.065]	0.038
Antithrombin [%]	T1:79 [67–90] T2: 91 [85–102] T3: 94.5 [85–103.75] T4: 90.5 [80.5–104]	0.22; 95% CI: [0.12 0.41]	0.012
Factor VIII activity [%]	T1:266.5 [221–341.8] T2: 202.5 [170–240.5] T3: 166 [128–214] T4: 182 [135.5–236]	− 0.42; 95% CI [− 0.58 to − 0.31]	P < 0.001
vWF [%]	T1: 252 [165–341] T2: 168 [126–223] T3: 173.5 [129.2–215.8] T4: 197.5 [146.8–306.8]	− 0.22; 95% CI [− 0.38 to − 0.074]	0.015
CRP [mg/dL]	T1: 11.5 [4.4–26.4] T2: 9.2 [4–27.2] T3: 5.3 [2.8–10.5] T4: 9.2 [3.2–30.4]	− 0.058; 95% CI: [0.15–0.44]	0.5
Hemoglobin, mmol/L	T1: 7.6 [6.7–8.9] T2: 7.75 [6.98–8.33] T3: 8.3 [7.55–9.3] T4: 8.4 [7.1–8.2]	0.26; 95% CI: [− 0.25 to 0.09]	0.002
Platelet count, 103/µL	T1: 195 [149.8–243] T2: 240.5 [186.8–273] T3: 195 [156.5–242.5] T4: 183 [156–232]	− 0.28; 95% CI [− 0.39 to − 0.17]	0.0007
International normalized ratio, %	T1: 1.36 [1.14–1.76] T2: 2.29 [2.03–2.4] T3: 2.24 [2–2.56] T4: 2.23 [1.75–2.55]	0.27; 95% CI [0.11–0.42]	0.0016
Thrombin time, s	T1: 16.9 [15.7–18.3] T2: 16.2 [15.3–17.2] T3: 16 [15.2–16.7] T4: 16.8 [15.7–17.6]	− 0.15; 95% CI [− 0.23 to 0.09]	0.095
Activated partial thromboplastin time, s	T1: 41.8 [33.9–50.1] T2: 44.35 [40.6–49] T3: 42.9 [38.25–49.55] T4: 41.7 [37.2–47.5]	0.047; 95% CI [− 0.23 to 0.17]	0.58

Table 3. Correlation between repeated measurements of Ks with selected clotting parameters.

Discussion

Fibrin clot permeability is a measure of pore size in a fibrin clot. Dense fibers are more resistant to lysis, and some studies suggest that they are associated with adverse cardiovascular events. Our study is the first to demonstrate that in the group of patients with left ventricular assist devices (LVAD) implanted due to advanced heart failure, fibrin clot permeability changes over time.

Studies and available standards present, that in healthy volunteers the Ks value is higher than in patients with adverse cardiovascular events. The Ks is a measure of average pore size, and the lower the Ks, the more compact the clots, which are typical of CAD and most prothrombotic conditions[16]. According to this fact it may be supposed, that the LVAD support may have beneficial effect on clotting hemostasis in patients with end stage heart failure.

It seems surprising, considering the fact that current generation LVAD devices is still connected with high incidence of ischemic and hemorrhagic strokes[31]. However according to results presented in healthy volunteers in available studies, our patients with end stage HF scheduled for LVAD therapy had strongly disturbed clotting hemostasis[16]. The clotting hemostasis and fibrin clot permeability is strongly influenced by inflammatory states as showed by other authors[14]. On the other hand, advanced heart failure is considered as an proinflammatory condition and in patients with reduced left ventricular ejection fraction elevated levels of pro-inflammatory cytokines are present[32]. Based on our results, the LVAD seems to reduce this condition rather than aggravate it.

Bleedings	Patients with bleedings	Patients without bleedings	P value
T1	4.09 [3.07–4.57]	4.12 [3.05–4.71]	0.81
T2	3.88 [3.11–6.41]	4.63 [3.98–6.12]	0.77
T3	4.49 [4.05–6.05]	5.97 [4.73–7.3]	0.75
T4	6.91 [5.6–7.9]	7.55 [5.86–8.57]	0.75
MACCE	**Patients with MACCE**	**patients without MACCE**	**P value**
T1	4.13 [3.05–5.01]	3.98 [3.05–4.55]	1
T2	4.54 [3.75–7.1]	4.68 [3.93–6.08]	1
T3	4.36 [3.55–6.51]	5.46 [4.89–7.3]	0.37
T4	7.51 [5.96–7.93]	7.15 [5.68–8.85]	1
NACE	**Patients with NACE**	**Patients without NACE**	**P value**
T1	4.11 [3.05–4.85]	4.13 [3.15–4.64]	1
T2	4.54 [3.71–6.97]	4.68 [3.94–6.03]	1
T3	4.49 [4.05–6.05]	6.12 [4.96–7.3]	0.33
T4	7.14 [5.77–7.99]	7.63 [5.81–8.89]	0.62

Table 4. The Ks values ($\times 10^{-9}$ cm^2) concerning bleedings, MACCE and NACE, shown median [IQR].

The possible mechanism could be the alleviation of pulmonary congestion and improvement of systemic perfusion. Adverse consequences such as blood contact with artificial surfaces and continuous flow do not seem to outweigh the benefits in terms of proinflammatory state and clot structure.

We demonstrated, based on repeated measures correlation, that in this group of patients, the Ks was connected with known risk factors of thromboembolic complications such as fibrinogen concentration, factor VIII activity, vWF activity, D-dimer, and antithrombin. In our study, we confirmed a moderate negative correlation of Ks with fibrinogen and factor VIII activity and a weak positive correlation with antithrombin. The negative correlation with vVF factor activity can be assessed as a weak. Our results are concordant with patients with type 1 diabetes, where fibrin clot structure was correlated with fibrin concentration. Fibrin clot permeability in this group of patients was correlated with clinical microangiopathy, microalbuminuria and glycemic control defined by HbA1c[9].

In previous studies involving patients on vitamin K antagonists with atrial fibrillation, it was shown that patients with lower Ks are more prone to transient ischemic attacks or ischemic strokes[33]. Ks was also found to be valuable in predicting recurrent venous thromboembolic complications[9]. Similarly, reduced fibrin clot permeability on admission in acute pulmonary embolism patients seemed to predict future residual obstruction of pulmonary arteries[34]. The Ks value was significantly lower at baseline and at 6–7 days after the incident in patients who developed residual pulmonary arteries obstruction. The authors created predictive model based on Ks showing that the baseline Ks level $\leq 6.55 \times 10^{-9}$ had area under the ROC curve of 0.91 in predicting the future residual pulmonary vascular obstruction.

Patients with Fontan circulation had lower Ks values than controls, and as in the group of LVAD patients the Ks correlated negatively with fibrinogen concentration and factor VIII activity[35].

The etiology of heart failure seems to have an important influence on clot structure. In our study, we observed that patients with ischemic etiology and patients with diabetes had shortened Ks. This is concordant with other studies where Ks was negatively correlated with hypo- or hyperglycemia in type 2 diabetes patients[36]. Duration of diabetes exceeding 5 years and HbA1c level over 6.5% in a study comprising 156 consecutive patients with type 2 diabetes was also associated with shortened Ks values[37].

In patients diagnosed with atrial fibrillation factors such as age 65–74 years, presence of heart failure and hypertension was independently associated with lower Ks[38].

Cross-sectional studies showed that atherosclerotic diseases have an impact on altered fibrin clot structure. Factors such as oxidative stress, chronic inflammation, endothelial dysfunction and increased platelet activation are common pathway for cardiovascular risk factors and clotting disturbances[17,39].

Despite observations from previous studies indicating that interventions influencing cardiovascular risk factors such as administration of statins or ACE inhibitors administration increased the clot permeability[18,40] we did not observe differences based on the heart failure medication. However, our finding might stem from the relatively limited sample size.

Of important value is not only the Ks value itself, which is strongly disturbed at baseline in these HF patients, but also changes in fibrin clot structure may be of significant value for future prognosis. The beneficial changes were clearly visible only in patients without adverse events such as bleeding, MACCE and NACE. Both types of complications, as shown in the literature, are often connected, and bleedings in this group of patients may precede ischemic complications[41].

Limitations of the study

The number of patients is low, as we investigated a highly specified group of patients with advanced HF scheduled for LVAD implantation. We analyzed two types of LVADs (HVAD and HM3) together, as separating them could negatively impact the study's power. All patients received warfarin after implantation, which influences fibrin clot permeability. Furthermore, we had no control group; the patients were compared with themselves. The lack

of statistical significance in Ks values in the group of patients with MACCE, NACE, and/or bleeding may be a consequence of a type 2 error due to the small number of patients and thus low study power.

Summary

In our study, we observed that patients with advanced heart failure have disturbed clot structure.

The Ks values increased in patients without end-points compared to those who suffered from a MACCE, NACE, or bleeding, thereby suggesting a role of fibrin structure characteristics in the outcomes of patients on LVAD.

Data availability

The data that support the findings of the study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Author contributions
AK: study design, data analysis, writing of manuscript, MS: statistical analysis, writing of manuscript, BH: writing of manuscript, JK: writing of manuscript, TH writing of manuscript, SP: writing of manuscript, PP acquisition of funding.

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Competing interests
The authors declare no competing interests.

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