



Thromboembolic complications
after left ventricular assist
device implantation is
associated with platelet
activation as measured via the
Platelet Activity State assay

Background / Study Objective



- Shear-mediated platelet activation is the major driver of thromboembolic complications associated with LVADs.
- The possibility of detecting shear-mediated platelet activation with a specific methodology is still not readily available for clinical use.
- The Platelet Activity State (PAS) assay may serve as a reliable tool to quantitate shear-mediated platelet activation and correlate it with clinical VAD-related complications.

Aim of the study: To assess platelet activation in patients with LVAD using the PAS assay and to correlate results with thromboembolic complications associated with LVADs.

Patients



- 10 consecutive patients implanted with LVADs were involved in the study (5 Thoratec HMII, 5 Heartware HVAD, 76 ± 71 days after implant).
- 334 cumulative days of LVAD support.
- Patients received the following anti-thrombotic agents: Bivalirudin and subsequently coumadin + aspirin.
- LDH, fibrinogen and plasma free hemoglobin concentrations were measured contemporaneously with PAS determination. Physiological range values for LDH, fibrinogen and plasma free hemoglobin concentration in blood of healthy subjects were defined as 125-220 U/L, 150-400 mg/dl and 14-18 g/dl, respectively.

Methods



- Whole blood (20 ml) was drawn via venipuncture into 3 ml acid-citrate dextrose (ACD-A) from consenting LVAD patients.
- Blood was centrifuged at 500g for 15 min to obtain platelet-rich plasma (PRP).
- PRP was gel-filtered through a column of Sepharose 2B beads to collect gel-filtered platelets (GFP).
- GFP were diluted to a count of 20,000 platelet/ μ l.
- Platelet activation level was assessed via PAS assay (spectrophotometric analysis over 8 min, $\lambda = 405$ nM).
- Linear regression analysis was performed on the changes in absorbance/min. The values obtained were normalized against the thrombin generation rate of fully activated platelets, obtained by sonication (10 W for 10 s).
- All PAS values were expressed as a percentage of the maximum thrombin-generating capacity, with a maximum of 100%.

Results 1

29th EACTS
Annual Meeting

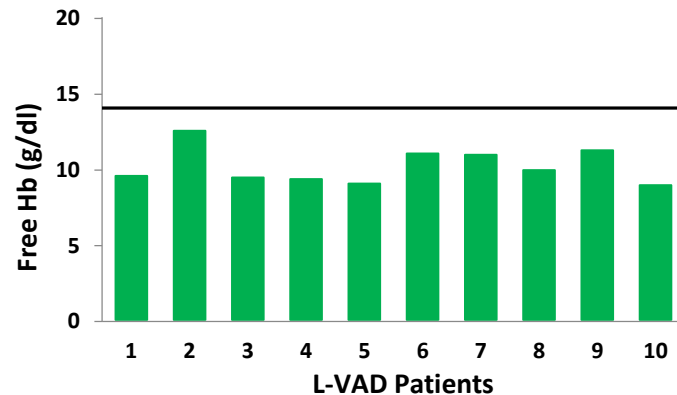
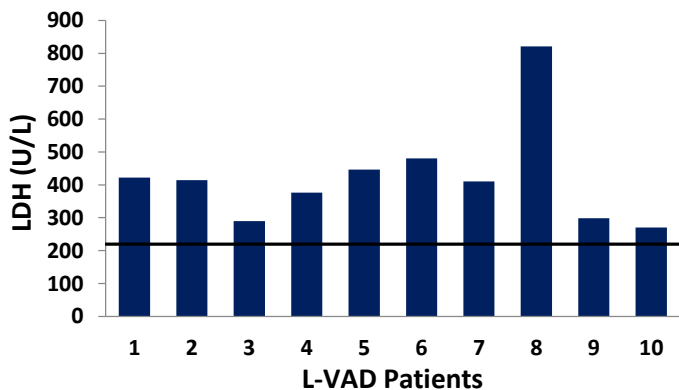
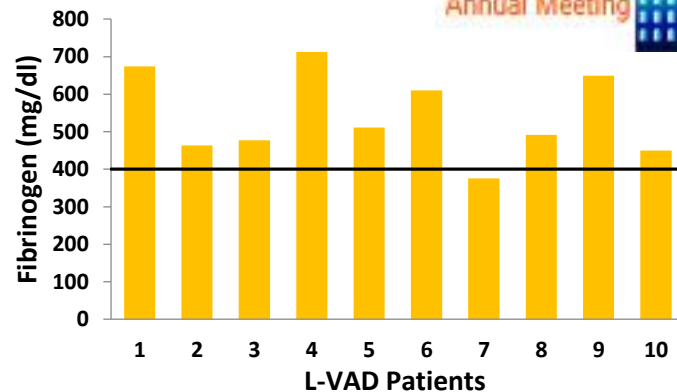
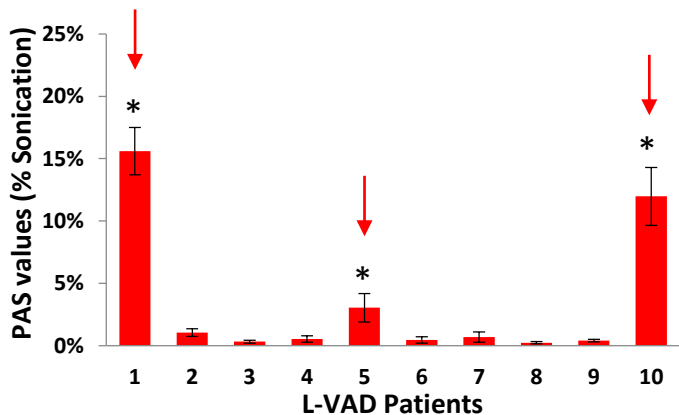


Figure 1. PAS assay, LDH, fibrinogen and free hemoglobin values obtained for ten LVAD patients (1-10). PAS values are reported as mean +/- standard deviation (number of replicates, n=10). Red arrows indicate patients who suffered clinically diagnosed thromboembolic events. * indicates statistically significant difference ($p < 0.05$). The Student–Newman–Keuls *post hoc* test was performed for comparisons between individual groups, after one-way ANOVA. The upper physiological limit (black lines in the graphs) of LDH and fibrinogen concentrations are considered 220 U/L and 400 mg/dl, respectively. The lower physiological limit of free hemoglobin concentrations is considered 14 g/dl.

Results 2



- Three patients had thromboembolic events following LVAD implantation (Pt.1 – VAD thrombosis, Pt.5 and Pt.10 – brain stroke).
- The level of platelet activation detected for patients with thromboembolic conditions (Pt.1 – $15,6 \pm 1,9\%$; Pt.5 – $3 \pm 1,1\%$; Pt.10 – $12 \pm 2,3\%$) was significantly higher compared to the other patients studied (mean value $0,54 \pm 0,27\%$).
- LDH, fibrinogen and free hemoglobin concentrations detected in all subjects were altered with respect to physiological values (except for fibrinogen level in Pt.7, see Fig. 1).
- LDH, fibrinogen and free hemoglobin concentrations were not able to discriminate between patients with thromboembolic events (Pt.1, 5 and 10) versus the other patients studied.

Conclusions



- Thromboembolic events are associated with higher level of platelet activation.
- The PAS assay appeared to be a sensitive tool for detecting abnormal levels of platelet activation in association with VAD-related thromboembolic events.
- Other clinical diagnostic measurements (LDH, fibrinogen, free hemoglobin concentration) may indicate the coagulation status of both patients and VADs but resulted to be a less sensitive methodology to determine shear-mediated platelet activation.
- Additional studies aimed at realizing a new-generation of diagnostic devices utilizing the PAS assay technique may help limit post-implantation complication rates and pharmacological therapy costs.