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RESEARCH ARTICLE

Red Blood Cell Mechanical Fragility as a Potential Predictor of Long-Term Hemolysis from Ventricular Assist Devices

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ABSTRACT

Introduction: Recent improvement in design and performance of Left Ventricular Assist Devices facilitate their use for destination therapy. The shear stresses in such devices can damage patients' red blood cells (RBC), leading to increased hemolysis, which has been associated with pump thrombosis and patient mortality. Here we report an investigation assessing RBC mechanical fragility as a potential metric of blood damage and its potential utility in monitoring LVAD performance.

Methods: Twenty subjects were recruited from the Center for Circulatory Support at the University of Michigan. Thirteen were implanted with the HeartWare HVAD (Medtronic, Inc.) and 7 with HeartMate 3® LVAD (HM3; Abbott Labs). Blood samples were obtained before surgery and at 1 hour, 24 hours, 1 week and 4 weeks after. Hemolysis biomarkers, total lactate dehydrogenase (LDH) and LDH isoenzymes, bilirubin, haptoglobin, and serum hemoglobin were determined through standard clinical tests. Mechanical fragility, as a metric of sub-hemolytic RBC damage, was determined using electromagnetically driven bead milling in a tube with a cylindrical bead, combined with non-invasive spectrophotometric analysis of induced hemolysis upon selected durations of the stress application. Certain variations of the stressing regime were employed for comparison.

Results: RBC mechanical fragility, as assessed through some of the stress application regimes, was correlated with certain hemolysis metrics like bilirubin, LDH, and LDH1. Specifically, a subset of pre-surgery RBC mechanical fragility markers were strongly correlated with bilirubin levels measured 1 day, 1 week, and 1 month (though not immediately) after the surgery. While such correlation with unconjugated bilirubin declined in significance over time, the correlation to conjugated bilirubin reached significance at 1 month. Mechanical fragility values determined in albumin-supplemented medium at 1-day post-surgery, showed strong correlation to total LDH and LDH1 at 1-month post-surgery ($p < 0.01$, R^2 up to 0.45), with the correlation with LDH1 stronger than with total LDH.

Conclusions: These data demonstrate the potential for some RBC mechanical fragility metrics as predictive prognostic biomarkers for hemolysis induced by implantable circulatory support systems. With appropriate tailoring of testing parameters to best suit the application, mechanical fragility assays could help facilitate the transition to greater utilization of ventricular assist devices.

Keywords: red blood cells, hemolysis, mechanical fragility, circulatory support, LVAD, HeartMate 3

Introduction

Heart Failure (HF) is a common and often fatal disease, with over 6 million adults diagnosed with HF in the US alone [1]. The prevalence of HF is increasing world-wide due to improvement in treatment options and due to aging population. Over time, patients may become unresponsive to pharmacotherapy solutions, which may not be able to control potentially life-threatening symptoms, leading to deterioration of a patients' condition [2]. Heart transplant was long considered the only option for HF patients; however, not every patient was able to receive a heart transplant due to organ availability, age, or medical co-morbidities. Left Ventricular Assist Devices (LVADs) were initially developed to provide a bridge to transplant, by temporally supporting heart function [3]. Such support, if offered by a durable implanted pump, with the power source being outside the body, supports the heart in maintaining circulation. Part of the blood flow is diverted by the implanted pump into an external circuit, connecting the left atrium or ventricle to the aorta, thus reducing the load on the left ventricles, and helping to maintain healthy circulation.

Recent technological advances have allowed rapid improvement in the design and performance of durable LVADs, making them a treatment option for patients with end-stage heart failure or cardiogenic shock refractory to medical therapy. Their use has been shown to significantly increase survival, functional status, and quality of life of patients [4]. More and more frequently, durable LVADs are used not just as a bridge to heart transplantation but as permanent therapy for patients that may be ineligible for or unable to secure heart transplantation. Currently, 78.1% of patients receive durable LVAD implants as destination therapy. Moreover, despite the patient cohort receiving durable LVADs being both older and sicker, survival rates from 2016 to 2020 at 1 and 2 years past implantation have continued to increase, reaching 82.8% and 74.1%, respectively [5]. Significant reductions in adverse events including stroke, gastrointestinal bleeding, infection, and device malfunction / pump thrombosis have also been reported – despite an increasing proportion of patients, up to 50% in 2019, receiving durable LVAD implants in the highest acuity of patient illness, i.e., INTERMACS patient profiles 1 and 2 [6].

Long-term therapy applications place ever-increasing demands on reliability and safety of LVAD systems. Until recently, continuous flow pumps with axial design, like the HeartMate II (HM2; Abbott Labs, Chicago, IL), were the most frequently implanted durable LVAD in the United States. Long-term circulatory support with durable LVADs involves prolonged contact and collision between blood cells and foreign surfaces, and high mechanical shear stresses caused by the pump's action. Strong turbulent flows present in these devices can damage patients' RBC, causing reduced cell lifespan due to stress-induced hemolysis [7-9]. Hemolysis is commonly recognized as a major complication of mechanical circulatory support and has been strongly associated with pump thrombosis [10,11] and with patient morbidity and mortality [12].

RBC lysis results in the release of hemoglobin (Hb), and Hb degradation products like heme, into the bloodstream. This induces a cascade of effects resulting in decreased NO availability, impaired microcirculation and diminished tissue oxygen supply [13,14]. It was reported that even low levels of cell-free hemoglobin can significantly increase RBC aggregation, leading to thrombosis, especially at low shear conditions [15]. Patients on long-term circulatory support often exhibit significant alterations of blood rheology, including prevalent RBC damage short of immediate hemolysis (i.e., "sub-lethal" cell trauma), and hence early RBC removal from circulation. Sub-lethal RBC damage was also cited as a cause of decreased microperfusion and hypoxic RBCs, leading to end organ dysfunction caused by cellular ischemia [16]. It has also been implicated in shortened cell life span [17] and general deterioration in RBC mechanical properties [18]. In cases involving prolonged mechanical circulatory support, adverse outcomes have been linked to chronic anemia [19]. Such was observed to persist even without detectable extraordinary-induced hemolysis [20], possibly due to a decreased lifespan of circulating RBC due to sub-lethal cell trauma [21].

In LVADs, shear-induced hemolysis is driven by both the amount of stress that the blood is exposed to and by the duration of such exposure within the pump. In general, among the design elements affecting pump-induced blood damage, the biggest impact was shown to be from the potentially hemolytic impact of clearance gaps and associated blood flow disturbance and turbulence [22,23]. The newer systems were designed for lower speed / longer residence time compared to previous generation devices like HM2 with higher flow speed but shorter blood residence time [24]. Such systems included the HeartWare HVAD (Medtronic, Inc., Minneapolis, MN) and the HeartMate 3® LVAD (HM3; Abbott Labs, Chicago, IL), however, as of June 2021, FDA no longer recommends Medtronic's HVAD for use in patients with end-stage heart failure, due to possible pump delay or failure to

restart and higher frequency of neurological adverse events and mortality compared to other commercially available devices (like HM3).

The newer centrifugal flow pumps are designed with non-contact levitation of the internal impellor but differ in levitation mechanisms that influence the size of the gaps between the internal impellor and housing. The centrifugal pump designs aim to balance the relative contributions of stress magnitude and the duration of blood exposure to stress to improve device hemocompatibility (minimize hemolysis and thrombosis). Flow pulsation, through sequential changes in pump speed, also aims at reducing possible thrombosis, which was linked to better long-term clinical outcomes [25]. Benefits of pulsation may, however, be offset if fast changes in rotor speed would induce additional fluid shear stress and turbulence throughout the volume of the pump [26,27].

Measurements of hemolysis – either directly through serum (cell-free) hemoglobin, or indirectly through metrics like bilirubin, haptoglobin, or lactate dehydrogenase (LDH) – remain the main ways to assess blood damage induced by an implanted device. However, standard hemolysis-related biomarkers, being reflective of RBC hemolysis, do not account for possible ongoing sub-lethal cell damage to RBC – which can lead to gradual decline in cell lifespan, early removal from circulation, and/or impaired cell function [28]. Progressively accumulating sub-hemolytic damage was suggested as an underlying cause of chronic anemia reported for many LVAD patients [19,20].

RBC mechanical fragility (MF) is a property that reflects how well RBC can withstand mechanical stress [29,30], and tracking a sample's induced hemolysis at progressively increasing stress applications can yield a fragility "profile." Different methods or configurations of the stressing can generate significantly different stresses, not just in magnitude (of stress duration and/or intensity level), but also in stress type – depending on the flow parameters (laminar vs turbulent, turbulent flow regime, size of eddies), cell environment, interaction with foreign surfaces, etc. [31-34], leading to potentially very different results. This contributes to the challenge of determining the optimal type(s) and other parameters of the stress application for MF assays so as to best reflect RBC membrane changes and/or defects that may exist in each particular situation (e.g., during LVAD operation). In the present work, we report the results of an investigation aimed at assessing the use of RBC MF testing implemented via several different regimes as a potential metric of blood damage caused by two recent models of implantable LVADs (HVAD and HM3), and MF's potential utility in monitoring post-op LVAD performance. This follows a previous article on this study, which was focused more on the differences in performance between the two devices. [35]

Materials and Methods

Patients and sample collection: Twenty subjects were recruited from the Center for Circulatory Support at the Frankel Cardiovascular Center, Michigan Medicine (University of Michigan). Twelve (60%) of the subjects were males and eight (40%) were females. Thirteen of the subjects were implanted with the HVAD, and 7 with the HM3. All implants for both devices were performed through a median sternotomy incision with cardiopulmonary bypass. (Additional details on the operating procedure, as well as a comparison of performance between the two device types in this study, was reported previously [35].) Mean age was 54±15 years, with 11 subjects having blood type A (55%), and 8 blood type O. Blood samples were collected preoperatively and shortly after the surgery (1 hr., ± 1 hr.), and then at approximately 1 day (1 ± 0.2 days), 1 week (6 ± 1 days), and 4 weeks (29 ± 5 days) following LVAD implantation. Preoperative total patient blood volume was estimated using the Nadler method [36], and was 5.5 ± 1 L. There was no known history of pre-existing hemolytic or bleeding disorder in any of the subjects. Study aliquots of blood were collected from blood samples for clinical indications. Informed consent was obtained for each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in approval of the study by the University of Michigan IRB (HUM00106930).

Standard laboratory analysis: Established laboratory tests were performed by the clinical laboratory of the University of Michigan Center for Circulatory Support, and for each blood sample collection included hemoglobin, lactase dehydrogenase total (LDH-T) and isoensymes, with special attention to type 1 (LDH1), haptoglobin, serum (cell-free) hemoglobin, and bilirubin (total conjugated and unconjugated).

RBC Mechanical Fragility (MF) testing: Bead milling with different sizes of cylindrical beads, as used in this study, had been shown to generate different types/combinations of mechanical stress in liquid medium, thus allowing testing of RBC MF samples under substantially different conditions and with potentially different results [37]. This is in addition to other (quantitative) variable stressing parameters, such as stressing duration/intervals, and bead oscillation frequency and amplitude (contributing

to stress intensity). In this study, a proprietary electromagnetic bead mill was used to oscillate a cylindrical magnetic bead within an enclosed tube to induce mechanical shear stress in the sample. This was combined with a proprietary spectral analysis method which allows noninvasive measurement of hemolysis level in the sample within the tube/cuvette, at desired cumulative durations of stress, for data points constituting a MF profile. These testing elements were described previously [38]. Three different stressing regimes were employed: Regime 1 used a tube/cuvette 34 mm in length and 4.1 mm in diameter, with a 9 mm x 3.7 mm (L x ∅) magnetic bead with a biocompatible coating, oscillated within the cuvette at 10 Hz at 0.2V power level. Diameters of the chamber and the bead remained the same for other regimes; however, Regime 2 employed a cuvette 44 mm in length with a 18 mm long bead, with 3 Hz oscillations induced at 0.6V. Regime 3 was the same as regime 2, except with the sample medium supplemented with 4 g/dL albumin. Physiological concentrations of albumin had been shown previously to significantly reduce RBC propensity to hemolyze [34]. Under some stress application regimes, its presence was suggested to alter bead end and annulus flows, resulting in quantitatively and qualitatively altered mechanical stress, with consequently altered RBC response [39]. Thus, while such supplementation was impactful for stress generated with the parameters of regime 2, it had minimal to no impact on stress when generated with the parameters of regime 1.
MF was characterized via MF "profiling", with hemolysis being determined non-invasively (per noted approach) at 1 min intervals, up to 10 min of cumulative stress duration. Area under the curve (AUC) values at 3 and 10 min of applied stress durations were selected to represent each profile. The values measured at 3 and 10 minutes of stress application, for each of the stressing regimes, are naturally significantly correlated – as the 3-minute AUC represents the most labile fraction of total RBC population, as further assessed by the cumulative 10-minute profile. (Each sample was tested in triplicate; typical standard deviation (SD) of these results was within 5 percent.)
Citrate-stabilized blood samples were diluted with Additive Solution 3 (AS3) to hemoglobin (Hb) concentration of 0.5 g/dl, effectively reducing possible cell-cell interactions. Dilution was verified by a Hemoglobin 201 system from HemoCue (Angelholm, Sweden), with final concentrations verified using NanoDrop1000 (ThermoFisher; USA). Induced hemolysis in a sample at respective points in time was calculated based on the difference in absorbance at 576 nm, a wavelength of oxygenated Hb maximum, and at 685 nm, the local minimum for the oxygenated Hb form. It was expressed as a fraction of free hemoglobin (HB^F) relative to total hemoglobin concentration (Hb^T) according to Formula 1, which included the correction for sample hematocrit as detailed by Sowemino-Coker [40].

$$Hem = \frac{Hb^F_{576} - Hb^F_{685}}{Hb^T_{576} - Hb^T_{685}} * (1 - Hematocrit)$$

(Formula 1)
Total hemoglobin concentration for each diluted RBC sample was determined spectrophotometrically by first subjecting a small (50-100 µL) aliquot to ultrasound for 15 seconds (0.1 second pulses, with 0.2 intervals between pulses, on ice), delivered by a Branson Digital Sonifier 450 (Danbury, CT), at 15% intensity (from the manufacturer-specified 400 Watt). In control experiments, such treatment was shown to fully lyse RBC without inducing hemoglobin oxidation.
Materials: Bovine serum albumin (BSA) was purchased from RPI Corp (Mt. Prospect, IL); all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and Fluka (Honeywell, Morristown, NJ).
Statistical Methods: Values are presented as mean ± standard deviation. Data for this analysis was separated into single time points, thus avoiding the need for repeated-measurement corrections, with correlations between such single time point values analyzed using linear regression models. A *p-value* < 0.05 was considered significant.

Results

Evolution of hemolysis-related biomarkers up to one month from LVAD implantation is presented in Figure 1. An increase in serum Hb, bilirubin and total LDH and LDH1 at 1 day after surgery, with the concomitant decrease in haptoglobin and total hemoglobin, is consistent with the presence of perioperative hemolysis. No extraordinary hemolysis seems to be apparent at 7 and 29 days after surgery. At one-month of follow up, total hemoglobin is partially recovered (albeit still remaining somewhat lower than it was before surgery), haptoglobin increases to about its pre-surgery level, serum hemoglobin levels remain steady, and bilirubin and LDH/LDH1 are gradually decreasing from the maximum level reached at 1 day after the operation (Figure 1A-F). Interestingly, there was a difference in the timing of changes in LDH and in LDH1 fraction, with the increase in LDH1 lagging the increase in total LDH (Figure 1E-F).

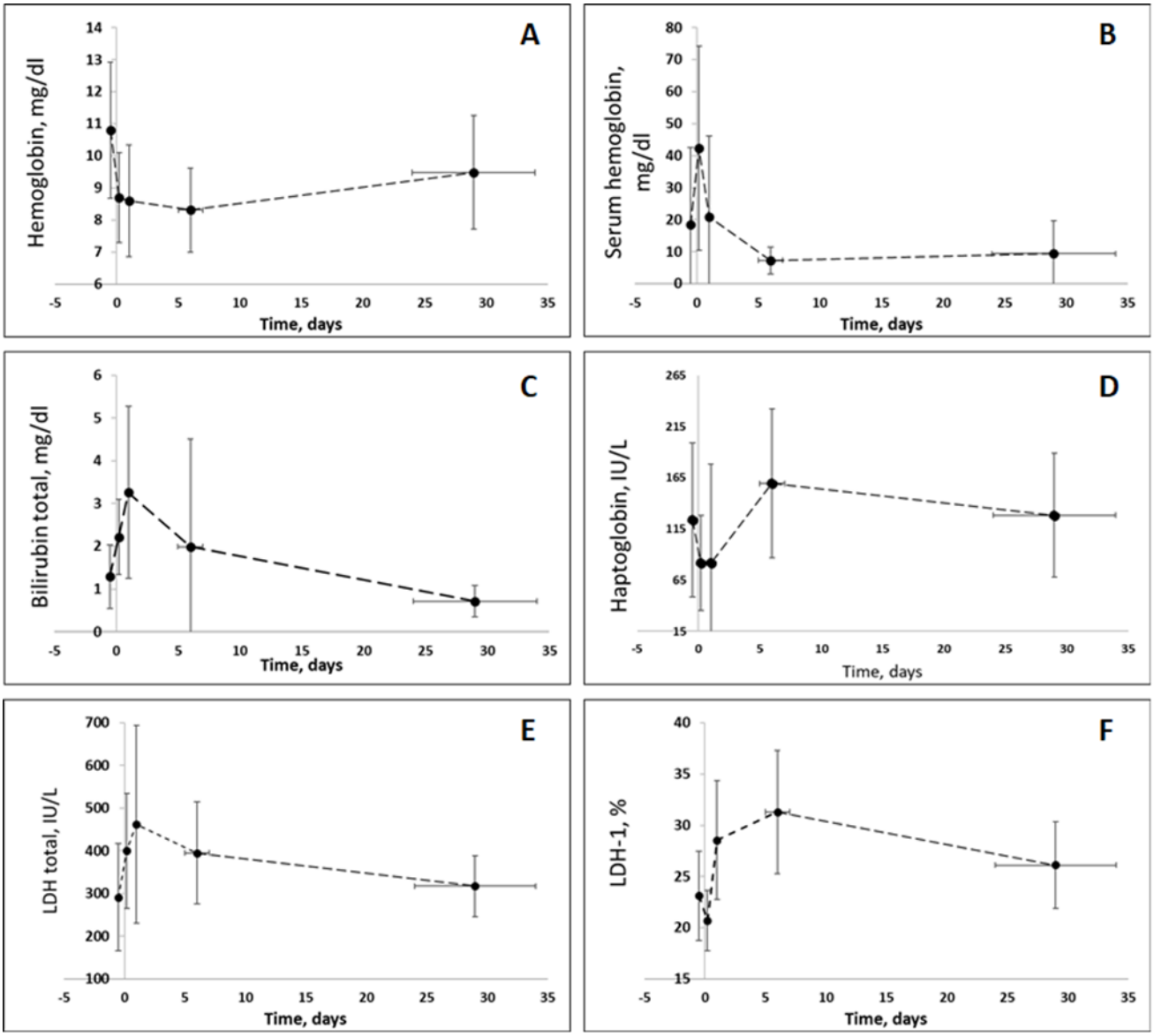

Figure 1. Changes in select clinical chemistry biomarker values measured before (marked as time = -1 days), and after the surgery, for A) Total hemoglobin; B) Serum (cell-free) hemoglobin; C) Total bilirubin; D) Haptoglobin; E) Total LDH; and F) LDH-1.

Evolution of MF-related biomarkers (Figure 2) shows overall similarity in response among the three MF regimes used in this study. RBC MF assessed at all three regimes decrease at 1-day post-surgery, possibly due to the impact of packed RBC (pRBC) transfusion. While no correlation was observed between the decline in each individual patient's RBC MF from its post-surgery level, such analysis did not account for the actual quality of pRBC units – which can vary significantly due to multiple factors, including donor-to-donor variability [41-43]. This decrease was followed by an increase in MF at 6 days and then at a month after the surgery, with either a new decrease (for testing performed in BSA-supplemented medium) or essentially no change (for non-supplemented medium). Despite the overall similarity in the direction of the changes, the correlation between the three regime MF metrics lacked significance.

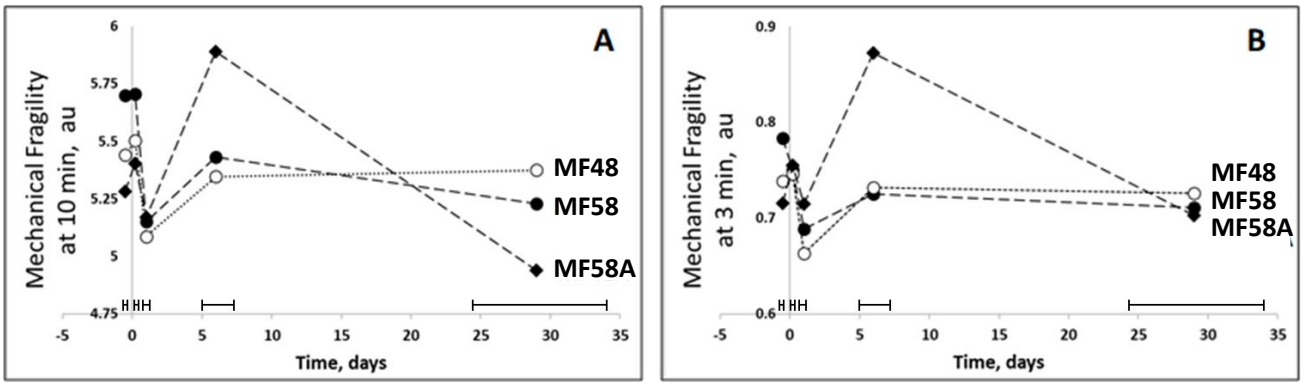

Figure 2. Changes in select Mechanical Fragility (MF) biomarker values measured before (marked as time = -1 days), and after the surgery, for A) 10 minutes stress application (MF58-10, MF48-10, and MF48A-10), and B) 3 minutes of stress application (MF58-3, MF48-3, and MF48A-3). Error bars over the time axis indicate variability in sample collection times. Coefficients of variability for Mechanical Fragility were 15% for MF48-10 and MF58-10, 20% for MF48-3, 25% for MF58-3 and MF48A-10, and 35% for MF48A-3.

Assessing correlation between RBC MF and hemolysis markers at specific sample collection time points allows to evaluate patient-specific changes while avoiding the need for repeated-measure corrections. There was no correlation between the MF metrics measured at any time point with any of the 3 regimes and serum (cell-free) hemoglobin or haptoglobin. MF metrics measured before and immediately after the surgery showed significant ($p<0.05$; R^2 in the range of 0.24 to 0.45) correlation with patient bilirubin measured at a later time. Specifically, pre-surgery MF58-10 and MF58A-10 markers were strongly correlated with bilirubin levels measured 1 day, 1 week, and 1 month, but not immediately (1 hour) after the surgery. Strong correlation with unconjugated bilirubin declined in significance over time, losing significance at one-month post-surgery then being replaced with the correlation to conjugated bilirubin (Figure 3; data for MF58-10 is shown with similar dependence observed in an albumin-supplemented stress application regime). Analogous, but weaker correlations were observed for samples collected at about 1 hour after the surgery, with, e.g., MF5810 being a significant ($p < 0.05$, $R^2 = 0.28$) predictor of total bilirubin at 1 day and of total and conjugated bilirubin at 1 month after the surgery (R^2 values of 0.25 and 0.33 correspondingly).

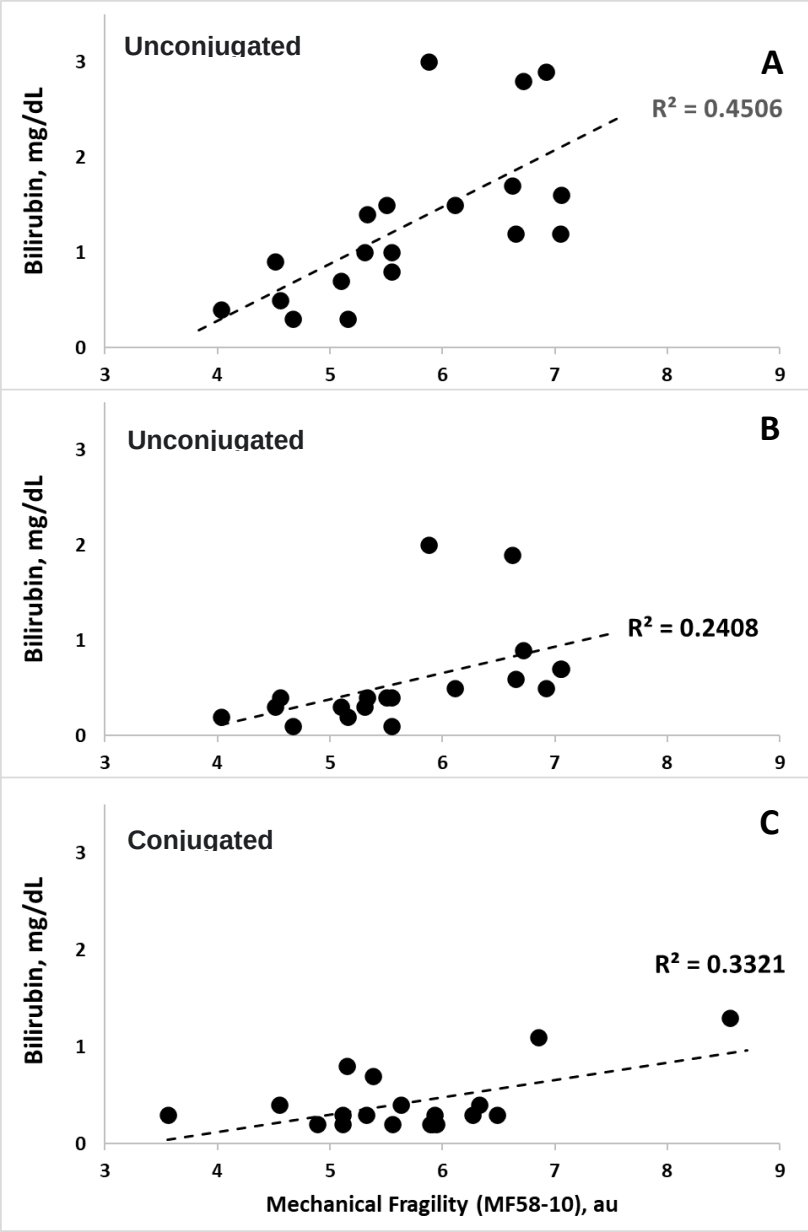

Figure 3. Correlation between Mechanical Fragility (MF5810) measured pre-surgery with unconjugated bilirubin measured at one day (A) and one week (B), and conjugated bilirubin measured at one month after the surgery (C).

No MF biomarkers showed correlation to either total LDH or LDH1, except for those measured in albumin-supplemented medium (MF58A-3 and MF58A-10) at one day after the surgery. Those were found to be significantly ($p < 0.01$, R^2 up to 0.45) correlated with LDH1 values at one-month post-surgery (Figure 4). Both metrics showed much lower correlation coefficients towards total LDH, with only MF58A-3 retaining statistical significance at $p < 0.05$ and $R^2 = 0.23$.

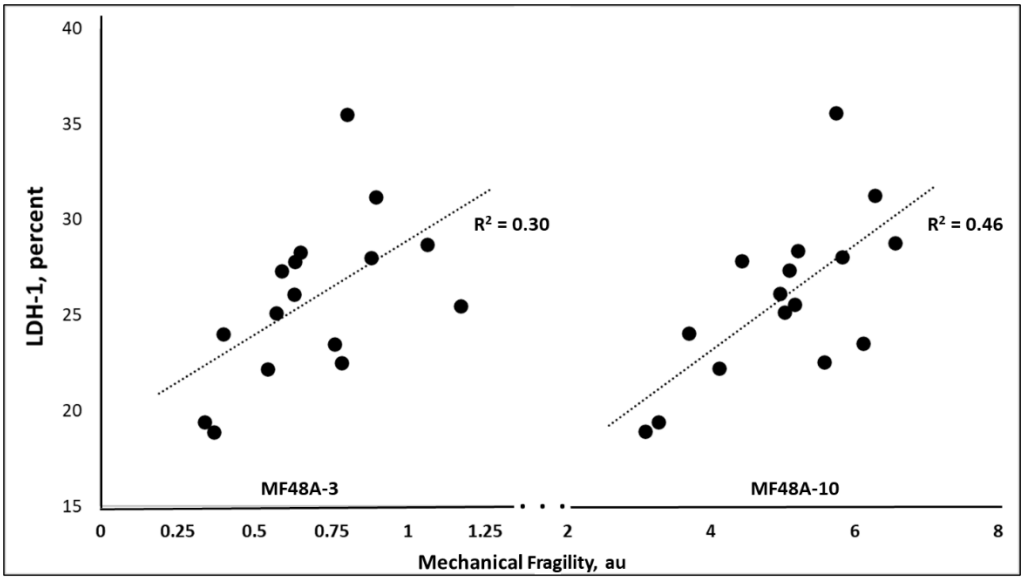

Figure 4. Correlation between Mechanical Fragility (MF58A after 3 and 10 minutes of stress application) measured at day 1 after the surgery with LDH-1 (percent of total) measured 30 days after the surgery.

Discussion

The HVAD system utilizes a spinning, wide-blade impeller suspended with a hybrid levitation system combining a hydrodynamic bearing (a layer of blood is needed to lift the rotor inside the pump) and passive magnetic levitation with a rare-earth magnet. Its short inlet cannula and bearing-less impeller with primary, secondary, and tertiary blood flow paths is thought to reduce the blood transit time through the device to enhance blood flow and reduce device-induced blood trauma [44,45]. Typical operating speed is between 2400 and 3200 rpm, with the average speed in this study (2700 ± 200 rpm) falling within that range. The HM3 system is designed with a fully magnetically levitated rotor, wide flow gaps, and textured surfaces [46]. Use of full magnetic levitation means that no blood bearing is needed to levitate the rotor, eliminating that possible source of blood damage. Typical operating speed is between 4000 and 6000 rpm (5300 ± 200 rpm in this study). Of the two systems, the HM3 is the newer design and has larger gaps along the side, top and bottom of the impeller and the pump housing and impeller. These features are designed to further reduce blood damage with associated hemolysis and sub-hemolytic cell damage. Resultant improved performance drove the replacement of axial-flow systems like HM2 with first hybrid levitation and then with full magnetic levitation systems, which in 2019 represented 77.7% of all the implants. While hybrid levitation devices are still utilized, axial-flow system use dropped to just 1.8%, from 50% in 2017 when full magnetically levitated HVAD and HM3 were introduced. Recently in June of 2021, the HVAD has been removed from commercial distribution due to a device malfunction related to failure to re-start leaving the HM3 device as the only commercially available durable LVAD.

For many years the HeartMate 2 system was considered a gold-standard in LVAD therapy, often used as a hemocompatibility benchmark for newer magnetically levitated devices. Preliminary evaluation that compared HM2 with the newer HM3 reported significant reduction in shear stress exposure for HM3, associated with nearly 2-fold decrease in hemolysis-associated cell-free hemoglobin as well as a markedly lower modified index of hemolysis [46]. Overall, hemolysis levels are reported to be exceptionally low in HM3, with no hemolysis events reported for a 50-patient study [47]. The pivotal MOMENTUM 3 study reported a significantly improved net hemocompatibility score for HM3 as compared to HM2, with such improvement linked to the absence of observed hemolysis [48]. The HVAD system, which was reported to induce hemolysis compared to static sample [49], also showed less induced hemolysis when compared to HM2 [50]. Other work, however, reported elevated shear stress and shear rate in the aortic arch with the HVAD when compared to both HMII and HM3 [51]. Overall, data indicate higher hemolysis in axial VADs as compared to centrifugal VADs, likely due to axial systems having longer blood residence time along with higher shear stresses [52]. Notably, not all hemolysis-related complications are associated with the implanted device. A case of hemolysis, as evidenced by an elevation of LDH, plasma hemoglobin and new coronary thrombus after HM3 implantation, has been attributed to underling

polycythemia as the likely proximal cause of the complication [53]. In another case, elevated hemolytic biomarkers were associated with an unrelated clinical condition, in that case an atypical hemolytic uremic syndrome [54]. We also previously reported higher post-surgery hemolysis with HM3 as compared to HVAD; however, a strong correlation of such hemolysis with cardiopulmonary bypass time suggested that it is likely the procedure and not the device itself that is the proximal cause of the difference [35].

In this work we report potential correlation of RBC mechanical fragility, as assessed by certain stress application regimes, with hemolysis metrics like bilirubin, LDH, and LDH1. Better correlation was achieved between RBC MF and LDH1, as compared with total LDH biomarker. Notably, correlations between hemolysis and mechanical fragility biomarkers depended on the stress application regimes. For example, correlation with LHH/LDH1 biomarker was observed only with medium supplementation with BSA (MF58A regime), and the MF48 stress application regime, unlike MF58 and MF58A regimes, was not a significant predictor of bilirubin at one week and one month after surgery. Here, changes in hemolysis biomarkers at 1-day post-surgery are more likely to be associated with the surgery itself and potentially with blood transfusion, while the longer-term values at one week and one month could be closer associated with the device's impact on circulating RBC. As the causes of blood damage are likely to be different in these two cases, the outcome of such damage is likely to manifest in different hemolytic and sub-hemolytic RBC. Similar to the observations made previously [35], these results show the importance of selecting and optimizing in vitro applied stress configurations to the nature of the blood damage and outcomes being predicted. This highlights the need for the results based on any single stress application regime or assay method to be interpreted with caution, as they would be unlikely to represent the whole picture. For example, the conclusion made by Madden et al. that "Shear forces across existing LVADs create similar levels of biochemical hemolysis, as assessed by LDH, despite baseline differences in a patient's red cell fragility" should be understood strictly in terms of the particular stress application method used for the assay [55]. In that case, what was assayed was pre-surgery RBC osmotic fragility (OF) – a property dependent on ion and water transport across the membrane in addition to membrane structural integrity. In that regard, depending on the structural membrane elements being damaged, even mechanical/flow-based stresses can induce significantly different changes in RBC membrane properties, as was demonstrated previously [34,39].

Increasing use of LVADs as a destination therapy, as opposed to being a bridge to transplant, puts higher priority on understanding the long-term effects of device performance. Probably the longest – 13 years – time of ongoing support on LVAD was reported for a 65 year-old patient who received a magnetically levitated Incor LVAD (Berlin Heart; Berlin, Germany) [56]. Published analysis of real-world outcomes for HVAD system report 51 percent Kaplan–Meier survival through 7 years after the implant [57]. Despite the increased focus on long-term destination therapy applications, most of the available data focuses on shorter time frames (typically 2-years of system performance). Notably, while hemolysis is considered one of the critical concerns affecting clinical outcomes for LVAD patients, it is not being consistently assessed. There is no agreed-upon definition for even what constitutes "hemolysis", with studies using different diagnostic criteria and cut-off values for each hemolysis-related biomarker. Inconsistency of hemolysis definition is also reflected in Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) guidelines, which reflect the focus on short-term device performance (within 72 hours post-implant) and heavily rely on total LHD values (Table 1). Even if supplemented with cell-free hemoglobin, as recommended by the Academic Research Consortium (ARC), results can be ambiguous without further investigation [58,59]. Moreover, even low-level extraordinary hemolysis can have significant detrimental impact on circulation [15,60], especially if such effects extend beyond perioperative time.

Table 1. INTERMAC definition of hemolysis [61].

Severity	Definition
Minor Hemolysis	A plasma-free hemoglobin value greater than 20 mg/dl or a serum lactate dehydrogenase (LDH) level greater than two and one-half time (2.5x) the upper limits of the normal range at the implanting center occurring after the first 72 hours post-implant **in the absence** of clinical symptoms or findings of hemolysis or abnormal pump function.
Major Hemolysis	A plasma-free hemoglobin value greater than 20 mg/dl or a serum lactate dehydrogenase (LDH) level greater than two and one-half times (2.5x) the upper limits of the normal range at the implanting center occurring after the first 72 hours post-implant **and associated with** clinical symptoms or findings of hemolysis or abnormal pump function. Major Hemolysis requires the presence of one or more of the following conditions: ○ Hemoglobinuria ("tea-colored urine") ○ Anemia (decrease in hematocrit or hemoglobin level that is out of proportion to levels explainable by chronic illness or usual post-VAD state) ○ Hyperbilirubinemia (total bilirubin above 2 mg%, with predominately indirect component) ○ Pump malfunction and/or abnormal pump parameters

Conclusions

Modifications of biomarker thresholds, definitions, and reporting requirements would likely be needed with the development of new and improved ventricular assist devices and with further transition to even longer, multi-year support. New approaches and biomarkers for evaluation and prediction of such longer-term effects would be highly desirable. Red cell mechanical fragility, or cell stability under mechanical stress, could be one class of such biomarker, being (as shown in this work) potentially predictive of future hemolytic state. As also evident from this work, establishing such MF-based prognostic biomarkers would likely require optimization of stress application regimes and configurations targeted to best probe the specific blood damage induced by the particular implant devices over prolonged periods of time.

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Disclosure

Authors Tarasev, Chakraborty, and Alfano were employees of, and hold equity in Blaze Medical Devices, a company that developed the RBC MF testing technology used in this work. Author Alfano contributed to the work solely in his capacity with Blaze. Author Tarasev is presently employed with Functional Fluidics, Inc., a company that develops assays and tests for assessment of cell function, and which now holds exclusive rights in the technology developed by Blaze.

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