Received: 9 August 2023 | Revised: 5 October 2023 | Accepted: 6 December 2023

DOI: 10.1111/aor.14693

Artificial Organs WILEY

MAIN TEXT

Increased phagocytosis capacity of circulating neutrophils in patients on continuous flow ventricular assist device support

Morcos A. Awad[1] | **Wenji Sun**[1] | **Dong Han**[1] | **Bartley P. Griffith**[1] | **Zhongjun J. Wu**[1,2]

[1]Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland, USA

[2]Fischell Department of Bioengineering, A. James Clark School of Engineering, University of Maryland, College Park, Maryland, USA

Correspondence
Zhongjun J. Wu, Department of Surgery, University of Maryland School of Medicine, 10 South Pine Street, MSTF 436, Baltimore, MD 21201, USA.
Email: zwu@som.umaryland.edu

Funding information
National Institutes of Health

Abstract

Background: Neutrophils take part in the innate immune response, phagocytosis, and pro-inflammatory cytokine release. The phagocytic capacity of circulating neutrophils in patients on continuous flow (CF) ventricular assist device (VAD) has not been well studied.

Methods: Blood samples from 14 patients undergoing CF-VAD implantation were collected and analyzed preoperatively (at baseline) and on postoperative days (POD) 3, 7, 14, and 28. Flow cytometry was used to assess the surface expression levels of CD62L, CD162, and macrophage antigen-1 (MAC-1) and neutrophil phagocytic capacity. Interleukin 1 (IL1), IL6, IL8, TNF-α, neutrophil elastase, and myeloperoxidase in plasma were measured using enzyme-linked immunosorbent assays.

Results: Among the 14 patients, seven patients had preoperative bridge device support. Relative to baseline, patients with no bridge device had elevated leukocyte count and neutrophil elastase by POD3 which normalized by POD7. Neutrophil activation level, IL6, IL8, and TNF-α increased by POD3 and sustained elevated levels for 7–14 days postoperatively. Elevated neutrophil phagocytic capacity persisted even until POD28. Similar patterns were observed in patients on a preoperative bridge device.

Conclusions: Neutrophil activation and phagocytic capacity increased in response to VAD support, while inflammatory cytokines remain elevated for up to 2 weeks postoperatively. These findings may indicate that VAD implantation elicits circulating neutrophils to an abnormal preemptive phagocytotic phenotype.

KEYWORDS

continuous flow ventricular assist device, inflammatory cytokines, neutrophils, phagocytosis

1 | INTRODUCTION

Neutrophils are polymorphonuclear (PMN) leukocytes that take part in the innate immune response, phagocytosis, and pro-inflammatory cytokine release. Neutrophil activation causes significant upregulation of the granulocyte surface integrin macrophage antigen-1 (MAC-1; also known as CD11b/CD18), which is considered a marker of systemic inflammation.[1,2] Neutrophil activation also augments their ligand affinity especially across the selectin and mucin families of intercellular adhesion molecules. An increase in affinity between the L-selectin CD62L on neutrophil surface and the mucin CD34 on resting endothelial cells mediates neutrophil rolling on the endothelium.[1,3,4] Adhesion and transmigration into the site of injury or foreign body ensues, thereby mounting an immune response and increasing phagocytic capacity. This process is also enhanced by an increase in binding affinity between CD162, a mucin-like P-selectin glycoprotein ligand-1 located on neutrophil surface, and CD62E/CD62P located on the surface of activated endothelial cells and/or platelets.[1,3]

Following neutrophil adhesion and transmigration, CD62L and CD162 shedding, as a mechanism of downregulating both surface receptors, is observed and used as a marker of neutrophil activation. On activation, neutrophils release molecules known as neutrophil extracellular traps which include myeloperoxidases (MPOs) and neutrophil elastases. MPOs mediate tissue damage during inflammatory responses, while elastases have been incorporated in pro-inflammation, thrombus formation, and activation of coagulation.[5,6]

Ventricular assist devices (VADs) have been reported to cause defects in immunologic response to stimuli and increase reactive oxygen species levels[2]; thereby leading to a pro-inflammatory environment. This in turn leads to increased granulocyte activation and leukocyte-derived microparticles.[7] On the one hand, a VAD acts as a foreign body in a human host, causing generalized neutrophil activation. It is also known that the high-speed rotation of the pump propeller in a continuous flow (CF) VAD introduces high mechanical shear stress to the circulation which activates immune cells.

There is limited literature on the changes that occur to neutrophils under in vivo conditions in patients with implanted CF-VADs. Previous studies on VAD patients reported a marked increase in neutrophil-to-lymphocyte ratio (NLR) by postoperative day (POD) one.[8,9] Although NLR normalized to preoperative levels by 1 month and plateaued by 3 months in some patients,[8,10] elevated levels at 3–6 months post-implantation were associated with higher rates of stroke and mortality.[9,10] Another study of 20 patients showed an increase in MAC-1 expression (i.e., activated neutrophils) postoperatively with sustained elevation even up to 30–60 days depending on VAD type.[11] A more recent study of 13 patients reported elevated levels of platelet–neutrophil aggregates compared to control patients who underwent other cardiac procedures.[12]

Given the limited literature on neutrophils in vivo in patients on CF-VAD support, this study aims to analyze markers of neutrophil activation and phagocytic capacity, as well as the levels of pro-inflammatory cytokines in patients with HVAD (HeartWare, Medtronic, Minneapolis, MN).

2 | METHODS

2.1 | Patients

From September 2018 to January 2020, 14 patients who underwent HVAD implantation consented for participation in the study. Informed consent was obtained based on an approved protocol by the institutional review board at the University of Maryland Baltimore. Patients were divided into two groups depending on the use of bridge device immediately prior to HVAD implantation: bridge device ($n = 7$) or no bridge device ($n = 7$). All patients were followed for 28 days.

Preoperative hemodynamics were collected from the last documented right heart catheterization prior to any mechanical circulatory support (MCS). Intraoperative and postoperative blood product utilization and rotational speed of implanted HVADs were also documented. Blood for routine clinical laboratory tests was collected in the preoperative and postoperative period. Additional blood was collected for the assays to assess alterations in circulating neutrophils at baseline (preoperative) and on POD 3, 7, 14, and 28.

2.2 | Reagents for assays

The antibodies used for flow cytometry assays to assess neutrophil surface markers included V450-labeled anti-human CD66b (neutrophil marker, clone G10F5, mIgMκ) (BD Biosciences, San Jose, CA, USA), FITC-labeled anti-human CD62L (neutrophil activation marker, clone DREG56, mIgG1κ) (BD Biosciences), PerCP-labeled anti-human CD45 (leukocyte marker, clone 2D1, mIgG κ) (BioLegend, San Diego, CA, USA), and APC-labeled anti-human CD162 (neutrophil activation marker, clone KPL-1, mIgG1κ) (BioLegend), and PE-labeled anti-human CD11b (neutrophil activation epitope, clone CBRM1/5, mIgG1κ, MAC-1 detection) (ThermoFisher Scientific, Waltham, MA, USA).

Corresponding isotypes for these antibodies were used for preparing negative controls. Neutrophil phagocytosis was detected by phagocytosis assay kit (IgG-DyLight 633, Cayman Chemical, Ann Arbor, MI, USA). Flow cytometry phosphate-buffered saline (FC-PBS) buffer was made from PBS with 0.1% bovine serum albumin (BSA) and 0.1% Sodium Azide (Sigma-Aldrich, St. Louis, MO, USA). Four percent paraformaldehyde (PFA) (Affymetrix Inc., Cleveland, Ohio, USA) was used to make 1% PFA. Red blood cell (RBC) was lysed by RBC lysing buffer (BD Biosciences). Commercial enzyme-linked immunosorbent assay (ELISA) kits for human PMN-Elastase (ThermoFisher Scientific), human myeloperoxidase, human IL-1β/IL-1F2, human IL-6, human IL-8/CXCL8, and human TNF-α (Quantikine, R&D Systems, Minneapolis, MN, USA) were used to quantify levels of human inflammatory cytokines.

2.3 | Flow cytometry assay

Blood samples were collected into BD vacutainer citrate tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Markers for neutrophil activation and receptor shedding were measured by flow cytometry. For neutrophil staining, 50 μL patient blood was added to a tube and stained with specific monoclonal antibodies: 2 μL of CD66b-V450, 5 μL of CD62L-FITC, 2.5 μL of MAC-1-PE, 0.2 μL of CD45-PerCP, and 1 μL of CD162-APC. Samples were incubated in the dark for 30 min and then fixed with 1% PFA for 30 min, followed by centrifugation at 400 × g at 4°C for 5 min. Blood samples were also stained with corresponding isotypes and incubated. RBCs were lysed with BD lysis buffer, and cells were pelleted by centrifugation. Samples were resuspended in FC-PBS buffer, followed by centrifugation at the same speed for 5 min. Samples were resuspended in 300 μL PBS. Flow cytometry data were acquired on BD LSRII (BD Biosciences, San Jose, CA, USA). An unstained cell sample was run first to determine FSC (forward scatter) and SSC (side scatter), then adjusted each detector settings to ensure the autofluorescence background was within the first decade of the log scale of fluorescence intensity. BD CompBeads Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (BD Biosciences) was used to optimize fluorescence compensation. This set provided distinct positive and negative population when both particles mixed with a fluorochrome-conjugated mouse Ig, κ antibody and the compensation level was manually set. Figure 1 shows the gating strategy for the flow cytometry data analysis. Granulocytes were gated based on CD45

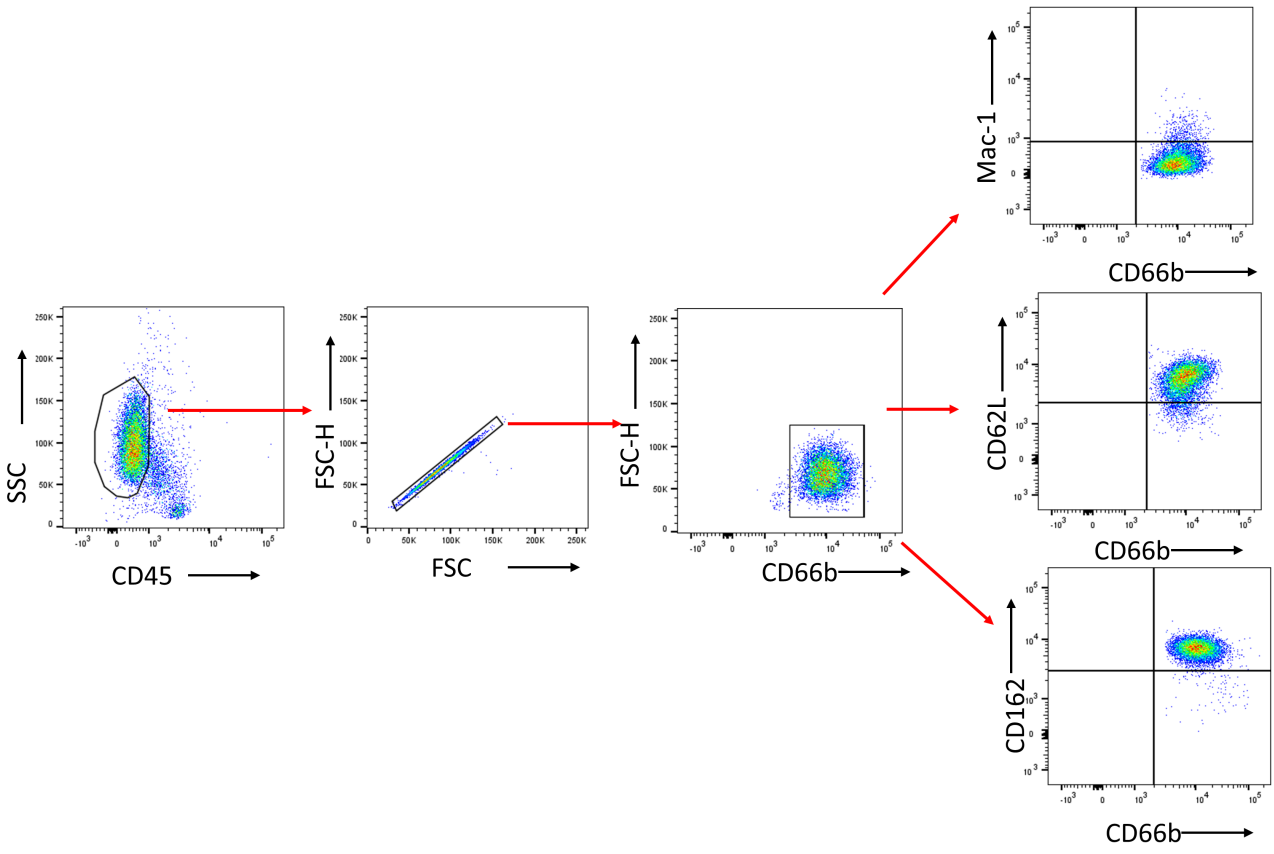

FIGURE 1 The gating strategy used to identify the neutrophil population and to determine the expression levels of Mac-1, CD62L, and CD162 on neutrophils. [Color figure can be viewed at wileyonlinelibrary.com]

expression level versus SSC, and doublets were removed by plotting the forward scatter height against area (FSC-H vs. FSC-A). Neutrophils were identified as $CD66b^+$ events. Levels of MAC-1, CD62L, and CD162 on neutrophil surface were expressed as mean fluorescence intensity (MFI) among $CD66b^+$ events. A total of 10 000 $CD66b^+$ events were collected for analysis. All flow cytometry standard data files were analyzed using FlowJo Software (BD bioscience, Ashland, OR, USA).

2.4 | Neutrophil phagocytosis assay

The neutrophil phagocytosis assay was performed following manufacturer instructions. Briefly, 50 µL whole blood was mixed with 0.5 µL DyLight 633 IgG-coated beads and incubated at 37°C for 60 min. Negative control was prepared by incubating the same mixture at 4°C. After an hour of incubation, phagocytosis was discontinued by placing samples on ice for 5 min. PE-labeled anti-CD66b antibody (5 µL) was added, and samples were incubated for another 30 min. RBC lysing buffer was added, and samples were washed with FC-PBS. After centrifugation at 1500 rpm for 5 min, the cell pellet was resuspended in 300 µL PBS and assessed with BD Calibur cytometry. $CD66b^+$ events were identified as neutrophils and 10 000 $CD66b^+$ cells were collected. Neutrophil phagocytosis was determined by the percentage of DyLight 633 IgG^+ events among $CD66b^+$ events.

2.5 | ELISA assay

Blood samples were collected into BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were centrifuged at 3000 rpm for 15 min at room temperature. Plasma was aliquoted and stored at −80°C until assayed. Samples and standards were run in duplicates per manufacturer instructions. Samples for PMN elastase and MPO were diluted to concentrations of 1:100 and 1:20, respectively. Absorbance of plasma in 96-well ELISA plates for each cytokine was measured using the SpectraMax M3 Spectrophotometer (Molecular Devices, San Jose, CA) at 450 nm, except for IL8 plates (at 490 nm).

2.6 | Statistical analysis

Data are presented as mean ± standard error or median [interquartile range (IQR)]. Fisher exact test was used for comparisons of categorical variables. Student's *t*-test or Mann–Whitney *U*-test was used for comparisons of continuous variables. For comparison with baseline data, mixed linear models were used with patient number as the subject variable and time of data collection as a fixed repeated-measure variable. Best-fit model estimated least squares means on post hoc analysis at each timepoint to compare between data points at baseline versus subsequent timepoints. Statistical analysis was performed using SAS® Studio [SAS Institute Inc., Cary, NC, USA]. A $p < 0.05$ was considered statistically significant.

3 | RESULTS

There was no difference in age, body mass index (BMI), gender, and disease etiology between both two groups (Table 1). Of the seven patients with bridge device, four patients were on intra-aortic balloon pump (IABP), two patients were on veno-arterial extracorporeal membrane oxygenation (VA-ECMO), and one patient had an IABP that was exchanged for VA-ECMO due to clinical deterioration. Median duration on bridge device was 10 days [IQR: 5.5–15 days].

Of the 14 patients in the study, 13 patients underwent right heart catheterization at a median of 13 days [IQR: 7–21 days] prior to HVAD implantation. Six of seven patients with bridge device underwent catheterization at a median of 3 days [IQR: 3–3.75 days] prior to initiation of any MCS. Preoperative central venous pressure (CVP) was higher in patients requiring a bridge device; however, other hemodynamics were similar between both groups (Table 1).

Intraoperatively, patients with a bridge device required more packed red blood cells (PRBC) and fresh frozen plasma (FFP) compared to patients with no bridge device (Table 2). However, postoperative blood product utilization was similar between both groups. No patient required platelet transfusion postoperatively in either group.

Preoperative hemoglobin was lower in patients with a bridge device; however, lactate dehydrogenase (LDH) was similar between both groups (Table 3). On POD28, LDH was lower in patients with a bridge device, while hemoglobin was similar between both groups. LDH did not change from baseline at any subsequent timepoint in either group. Blood urea nitrogen (BUN), creatinine, and HVAD rotational speeds were similar between both groups throughout the study period.

In patients with no bridge device, leukocyte count increased from baseline by POD3 then declined by POD7 thereafter (Figure 2A). This elevation was accompanied by a drop in CD62L surface expression (Figure 2B), suggesting receptor shedding. However, markers of neutrophil endothelial adhesion and general activation, CD162 (Figure 2C) and MAC-1 (Figure 2D), respectively, did not statistically change from baseline over time.

TABLE 1 Preoperative demographics of patients undergoing ventricular assist device implantation.

	No bridge device ($n=7$)	Bridge device ($n=7$)	p-value
Age (years)	60.5 ± 2.0	51.7 ± 1.3	0.19*
BMI (kg/m^2)	26.9 ± 0.6	28.8 ± 0.8	0.50*
Male	6 (85.7)	5 (71.4)	1.00†
Disease etiology			1.00†
Ischemic	4 (57.1)	3 (42.9)	
Non-ischemic	3 (42.9)	4 (57.1)	
Right heart catheterization hemodynamics			
CO (L/min)	3.9 ± 0.2	4.6 ± 0.2, $n=5$	0.40†
CI (L/min/m^2)	1.8 ± 0.1	2.1 ± 0.1, $n=6$	0.40†
CVP (mm Hg)	7.7 ± 1.1	15.5 ± 0.6, $n=6$	0.04†
Mean PCWP (mm Hg)	21.2 ± 0.8	24.6 ± 1.3, $n=5$	0.37†
Mean PAP (mm Hg)	33.7 ± 1.3	39.8 ± 1.4, $n=6$	0.23†

Note: Mean ± standard error; n (%). *Student t test; †Fisher exact test.

Abbreviations: BMI, body mass index; CI, cardiac index; CO, cardiac output; CVP, central venous pressure; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure.

TABLE 2 Blood product utilization for patients undergoing ventricular assist device implantation.

	No bridge device ($n=7$)	Bridge device ($n=7$)	p value‡
Intraoperative			
PRBC	1 [0–1]	3 [2–5]	0.03
Platelet	0 [0–0]	0 [0–1]	0.73
FFP	0 [0–0]	3 [0–4]	0.02
Cryoprecipitate	0 [0–0]	0 [0–5]	0.46
Postoperative			
PRBC	3 [0–4]	2 [0–9]	0.91
Platelet	0 [0–0]	0 [0–0]	1.00
FFP	1 [0–2]	0 [0–0]	0.27
Cryoprecipitate	0 [0–7]	0 [0–0]	0.73

Note: Median [interquartile range]. ‡Mann–Whitney U-test.

Abbreviations: FFP, fresh frozen plasma; PRBC, packed red blood cells.

In patients with a bridge device, leukocyte count increased from baseline by POD3 and remained elevated until returning to baseline by POD28 (Figure 2A). Surface expression of CD62L, CD162, and MAC-1 did not statistically change from baseline. However, there was an elevation in CD62L (Figure 2B) by POD28 relative to baseline. Comparing between both groups, there was no difference in leukocyte count, MAC-1, and CD162 surface expression throughout all timepoints. However, CD62L surface expression was higher at baseline in patients with no bridge device compared to their counterparts (Figure 2B).

With leukocyte count elevation, there was a significant increase in neutrophil phagocytic capacity at all subsequent timepoints compared to baseline in patients with and without bridge device (Figure 2E). This trend was similar between both groups at all timepoints.

There was no statistical difference in IL1 levels across time or between both groups at all timepoints (Figure 3A). In patients with no bridge device, there was an elevation by POD3 in IL6 (Figure 3B), IL8 (Figure 3C), TNF-α (Figure 3D), and PMN elastase per leukocyte count (Figure 3E). This elevation was followed by a drop in their respective levels by POD14, POD28, POD28, and POD7.

In patients with a bridge device, there was an elevation from baseline in IL6 (Figure 3B) by POD3, and in IL8 (Figures 3C) and TNF-α (Figure 3D) by POD7. This was followed by a drop to baseline in their respective levels by POD7, POD14, and POD28. MPO levels were similar across time in either group, except for a drop from baseline by POD28 in patients with bridge device (Figure 3F). Comparing between both groups, only IL6 (Figure 3B) and PMN elastase per leukocyte count (Figure 3E) were

TABLE 3 Laboratory values and ventricular assist device rotational speed at different timepoints.

	No bridge device ($n=7$)		Bridge device ($n=7$)		p-value *
	n	Value	n	Value	
Hemoglobin (g/dL)					
Baseline	7	11.4 ± 0.3	7	8.2 ± 0.1	0.002
Day 3	7	8.7 ± 0.2	7	8.4 ± 0.1	0.60
Day 7	7	8.7 ± 0.2	6	8.3 ± 0.1	0.46
Day 14	6	8.9 ± 0.2	7	8.7 ± 0.1	0.74
Day 28	7	9.9 ± 0.1	7	8.9 ± 0.2	0.10
LDH (units/L)					
Baseline	6	616.2 ± 62.2	6	820.3 ± 61.5	0.36
Day 3	6	842.8 ± 45.2	7	800.7 ± 18.4	0.72
Day 7	6	873.5 ± 48.8	7	659.6 ± 22.1	0.12
Day 14	6	787.0 ± 34.5	6	720.7 ± 40.7	0.62
Day 28	7	681.6 ± 12.9	7	536.3 ± 14.4	0.02
BUN (mg/dL)					
Baseline	7	28.9 ± 1.4	7	38.7 ± 2.3	0.20
Day 3	7	26.4 ± 1.5	7	38.7 ± 1.9	0.07
Day 7	7	21.4 ± 1.4	7	40.3 ± 2.7	0.04
Day 14	6	19.5 ± 0.8	7	36.6 ± 3.2	0.09
Day 28	7	18.7 ± 0.8	7	28.4 ± 1.5	0.05
Creatinine (mg/dL)					
Baseline	7	1.2 ± 0.0	7	1.3 ± 0.1	0.59
Day 3	7	1.0 ± 0.0	7	1.4 ± 0.1	0.08
Day 7	7	0.9 ± 0.0	7	1.3 ± 0.1	0.14
Day 14	6	0.9 ± 0.0	7	1.2 ± 0.1	0.16
Day 28	7	0.9 ± 0.0	7	1.0 ± 0.0	0.32
VAD rotational speed (rpm)					
Day 0	6	2710.0 ± 23.6	5	2520.0 ± 22.6	0.06
Day 3	6	2740.0 ± 26.2	7	2660.0 ± 35.4	0.53
Day 7	7	2725.7 ± 21.1	7	2654.3 ± 31.1	0.49
Day 14	6	2736.7 ± 21.2	7	2662.9 ± 29.3	0.48
Day 28	7	2685.7 ± 20.5	7	2625.7 ± 22.7	0.47

Note: Mean ± standard error. *Student t test. Baseline is a preoperative measure.

Abbreviations: BUN, blood urea nitrogen; LDH; lactate dehydrogenase; rpm, rotations per minute; VAD, ventricular assist device.

higher at baseline in patients with a bridge device relative to their counterparts.

4 | DISCUSSION

We examined several key receptors for endothelial interaction and phagocytic capacity of circulating neutrophils as well as inflammatory cytokines release in response to HVAD placement for up to 28 days in 14 patients with and without a bridge device. Patients with a bridge device had lower hemoglobin at baseline which is likely related to being on IABP or VA-ECMO. Although they required higher intraoperative PRBC use, postoperative hemoglobin and PRBC utilization were similar between both groups.

LDH as a measure of hemolysis did not change from baseline throughout the study. In an in vitro model with human blood, we have previously reported overall low levels of normalized index of hemolysis (NIH) associated with the HVAD (0.00525 ± 0.00183 g/100 L) and the HeartMate 2 (HMII, 0.00583 ± 0.00182 g/100 L).[13] Similar low hemolysis level was also reported for HVAD (NIH: 0.0068 g/100 L) in another in vitro study using bovine

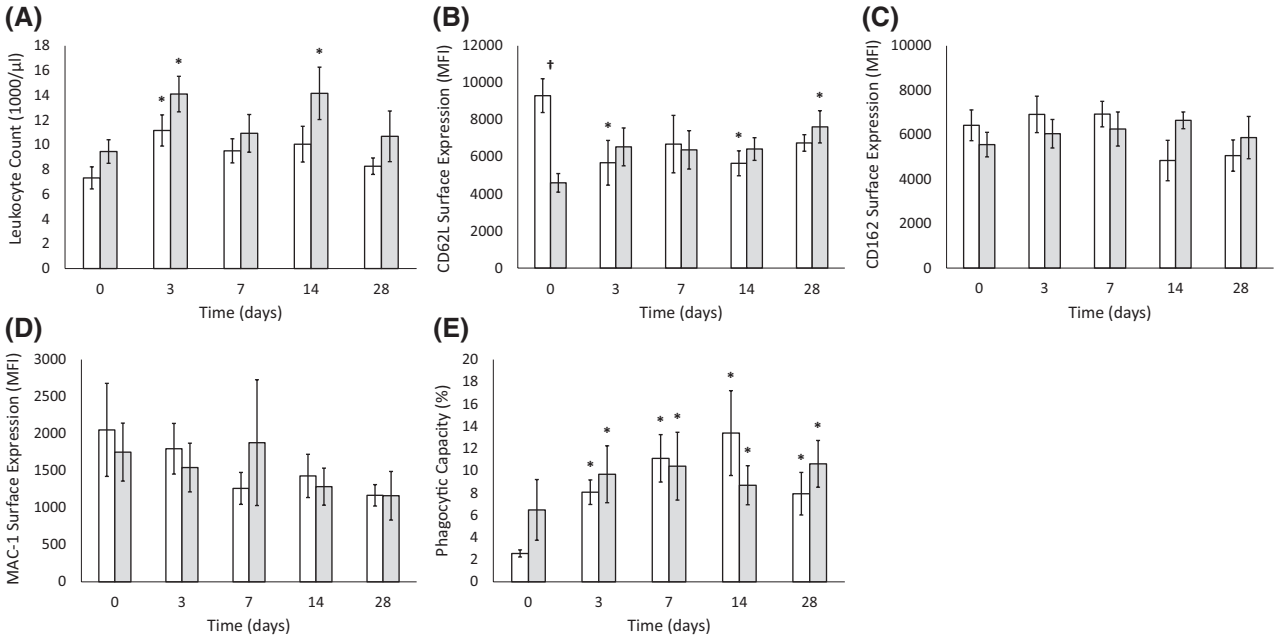

FIGURE 2 (A) Leukocyte count; (B) $CD62L^+$ neutrophils; (C) $CD162^+$ neutrophils; (D) $MAC\text{-}1^+$ neutrophils; (E) percentage of neutrophil phagocytic capacity. White bars represent patients with no bridge device; gray bars represent patients with bridge device (i.e., intra-aortic balloon pump or veno-arterial extracorporeal membrane oxygenation). Comparisons were performed for both groups relative to their respective preoperative ($t=0$) baseline value (*$p<0.05$), and between both groups at each timepoint (†$p<0.05$). MAC-1, macrophage antigen-1 (i.e., CD11b/CD18); MFI, mean fluorescence intensity.

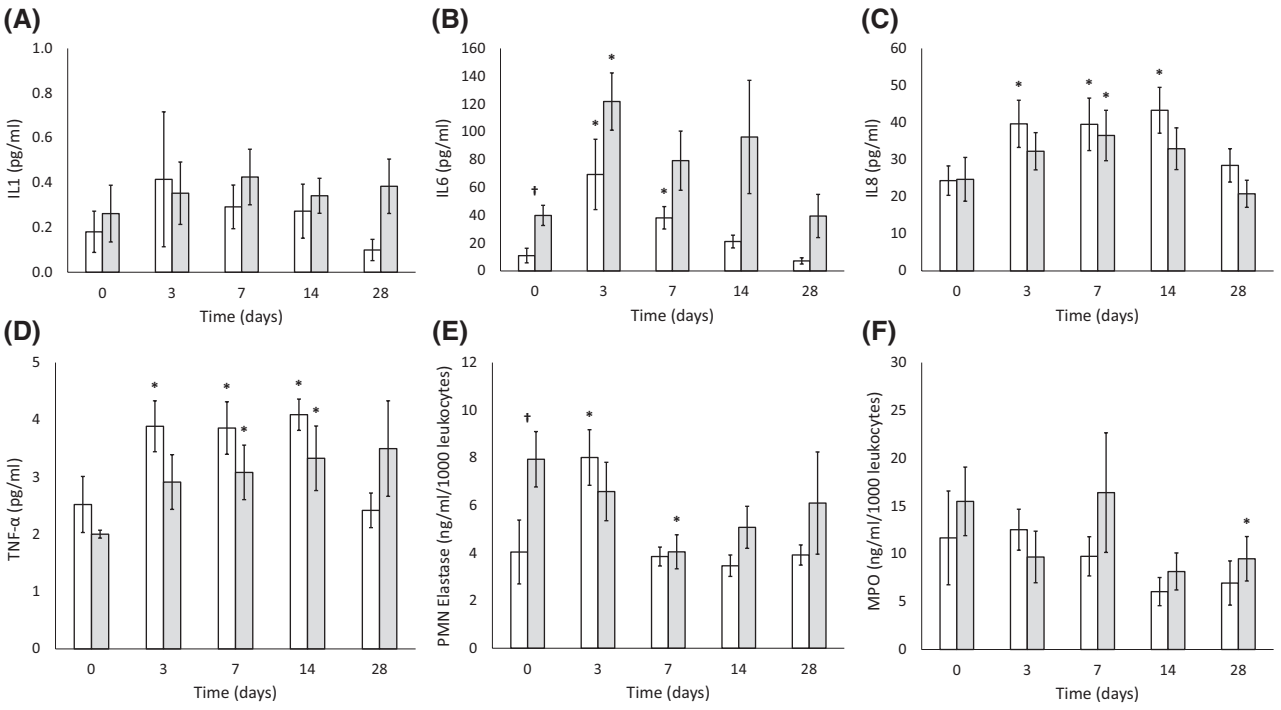

FIGURE 3 (A) IL1 levels; (B) IL6 levels; (C) IL8 levels; (D) TNF-α levels; (E) neutrophil elastase levels; (F) myeloperoxidase levels. White bars represent patients with no bridge device; gray bars represent patients with bridge device (i.e., intra-aortic balloon pump or veno-arterial extracorporeal membrane oxygenation). Comparisons were performed for both groups relative to their respective preoperative ($t=0$) baseline value (*$p<0.05$), and between both groups at each timepoint (†$p<0.05$). MPO, myeloperoxidase; PMN, polymorphonuclear.

blood to assess device-induced blood cell damage by three currently clinical used VADs.[14] But the reported NIH value for the HMII was significantly higher (NIH: 0.0224 g/100 L) in the same study. Both studies suggested that HVAD produced relatively low level of hemolysis. The low hemolysis level from the in vitro studies is consistent

with our observation that there was no significant increase in the LDH level in the patients. Although some previous in vivo work demonstrated high levels of RBC microparticles, which have previously been linked to cell hemolysis, in patients with VAD even at 3 months postoperatively.[15,16] Hemolysis is expected in patients on long-term VAD support secondary to pump propeller; however, for an overall clinical benefit, levels of hemolysis did not increase over time, as we show in this report.

We observed an early elevation in leukocyte count after HVAD implantation in both groups, with levels returning to baseline earlier in patients without a bridge device. Such inflammatory changes are expected early after surgical intervention, especially with the invasive thoracotomy and coring the left ventricular apex plus a foreign body (i.e., VAD) remaining in vivo. The immune response reaction after cardiac surgery is expected to include leukocytosis and systemic inflammatory response. Previous reports showed that lymphocyte count peaked on POD14 after VAD placement with levels not returning to baseline even until POD30, while granulocyte percentage peaked on POD14 and plateaued at lower levels afterward.[11] Patients on VAD also showed an elevation in NLR relative to baseline within the first 24 h postoperatively followed by normalization or plateau at lower levels around 3–6 months, with higher NLRs affiliated with increased mortality and adverse events.[9,10] Such early rise of leukocyte count was also observed after HVAD implantation in an animal model. Tuzun et al. reported that the leukocyte count increased from a baseline value of $7.2 \pm 3.1 \times 10^3/mm^3$ to $15.3 \pm 22.3 \times 10^3/mm^3$ at postoperative day one in six sheep implanted with the HVAD.[17] The leukocyte count returned to the baseline level at postoperative day 30.

In patients with no bridge device, we observed lower CD62L surface expression by POD3, possibly due to shedding, which subsequently normalized to baseline by as late as POD14. A similar pattern was observed in PMN elastase release and in levels of IL6, IL8, and TNF-α, but not IL1. However, we observed that neutrophil phagocytic capacity increased from baseline by POD3 but remained elevated even until POD28. We did not observe similar patterns in MPO release and neutrophil activation (i.e., elevation in MAC-1 and decline in CD162) over time. This may be secondary to neutrophil priming to enhance their phagocytic potential, but not yet to a degree of full activation as would be indicated by MAC-1 expression or MPO release. It may also be that fully activated neutrophils were cleared through phagocytosis by resident macrophages and dendritic cells.

Our group previously reported increased levels of neutrophil activation (i.e., elevation in MAC-1 and decline in CD62L and CD162) in an in vitro circulation loop using human blood with a CentriMag pump (Abbott Inc, Chicago, IL) over time.[18] Using a Couette-type blood-shearing device, we also showed an augmentation in neutrophil activation (i.e., elevation in MAC-1 and decline in CD62L but not CD162) with increasing levels of shear stress after only 0.5 s of exposure.[19] Pieper et al. found a similar trend of neutrophil activation when they used ovine blood in an in vitro loop with the CentriMag VAD to evaluate the leukocyte activation and damage caused by the pump over a 6-h circulation.[20] Their results showed the three pumping conditions (low flow, standard, and high speed) all caused a significant increase in $CD45^+$ leukocyte microparticles (LMPs) compared to the static control, with the most increase seen under the high-speed condition. They further confirmed that these LMPs with high CD11b expression were driven from activated neutrophils. Device-induced neutrophil activation indicated by increased LMPs in bovine blood was also reported by Radley et al. in an in vitro loop benchmarking study of the CentriMag, HMII, and HVAD.[14] In these studies, the changes in CD62L and CD162 expression were not assessed. A decline in CD62L, but not an elevation in CD11b (part of the MAC-1 complex) on neutrophils after exposure to $1000\,s^{-1}$ shear in a rheometer confirmed that neutrophils can be activated not only by biomaterials but also shear stress.[21] These in vitro studies indicated that contemporary VADs could induce leukocyte activation and it is possible to observe differences between different pump designs during in vitro testing that might translate to clinical performance.

A previous in vivo study of patients with HVAD showed an elevated MAC-1 expression on granulocytes (i.e., granulocyte activation) even until POD30.[11] Interestingly, MAC-1 expression was not higher than baseline during infection episodes. This is consistent with in vitro reports of neutrophil pseudopodia retraction and impaired phagocytic capacity in response to higher shear stress.[22,23] Neutrophil activation and generalized phagocytic capacity seem to increase in response to MCS for two primary reasons: foreign body presence and the resulting shear stress due to HVAD propeller rotation. However, this elevation could often be accompanied by a decline in neutrophil potential to fight off infection. This paradoxical effect may be related to neutrophil overload and exhaustion, which may explain the decline in neutrophil phagocytic capacity in vitro after exposure to $1000\,s^{-1}$ rheometer-based shear stress in some but not all experimental conditions.[21] Previously, our in vitro study showed that the CentriMag pump caused an initial increase in neutrophil phagocytic capacity in the device-assisted circulation at 1 h followed by a subsequent decline for up to 4 h in a rotational speed-dependent manner.[18]

We show that in patients without a bridge device, inflammatory cytokines (IL6, IL8, and TNF-α, but not IL1) increased by POD3 and normalize after 2 weeks. Similarly,

a previous study reported an elevation in TNF-α, IL6, and PMN elastase throughout the entire 12-week follow-up period.[24] A previous in vitro study showed no change in IL1α relative to baseline,[21] similar to what we currently show in vivo.

Limitations to this study include the small sample size and the lack of some blood samples for laboratory assays at specific timepoints for certain patients. All patients underwent HVAD placement which is no longer used in clinical practice. Nevertheless, this study still serves as an in vivo model which can be used to study leukocyte biological changes in response to MCS.

5 | CONCLUSION

In patients with no bridge device, we report an elevation in leukocyte count and neutrophil activation (i.e., reduced CD62L surface expression) in response to HVAD placement by POD3 which normalizes as late as POD14. This change is associated with a rise in PMN elastase, IL6, IL8, and TNF-α, but not MPO or IL1, which may still be elevated 7–14 days postoperatively. This is also accompanied by a consistent elevation in neutrophil phagocytic capacity from POD3 onward. Similar general patterns were observed in patients on IABP or VA-ECMO prior to HVAD placement, with higher PMN elastase release and IL6 levels in this patient population compared to those with no bridge device, which is likely related to MCS use prior to HVAD implantation.

AUTHOR CONTRIBUTIONS

Morcos A. Awad was involved in drafting the article, statistics, data analysis/interpretation. Wenji Sun was involved in data collection and laboratory assays. Dong Han was involved in critical review of the article and data analysis. Bartley P. Griffith was involved in critical revision of article. Zhongjun J. Wu was involved in concept/design, funding secure by, critical revision of article.

ACKNOWLEDGMENTS

The study was partially supported by the National Institutes of Health (Award Numbers: R01HL118372, R01HL124170, R01HL162940, and R01HL171120).

CONFLICT OF INTEREST STATEMENT

Z.J.W and B.P.G dislcose financial interest in Abiomed/Breethe, Inc., EConnect 1, Inc., and intellectual property for ECMO systems and blood pumps. Z.J.W dislcoses serving as a consultant with Daiichi Sanko, Inc. and CH Biomedical USA, Inc. All other authors declare no conflict of interest in the subject matter and materials discussed in this study.

ORCID

Morcos A. Awad https://orcid.org/0000-0003-2568-3330
Zhongjun J. Wu https://orcid.org/0000-0003-0807-7195

REFERENCES

1. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. Nat Rev Immunol. 2004;4(6):432–44.
2. Radley G, Ali S, Pieper IL, Thornton CA. Mechanical shear stress and leukocyte phenotype and function: implications for ventricular assist device development and use. Int J Artif Organs. 2019;42(3):133–42.
3. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. Lancet. 2006 Jul 8;368(9530):157–69.
4. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76(2):301–14.
5. Zhou Y, Xu Z, Liu Z. Impact of neutrophil extracellular traps on thrombosis formation: new findings and future perspective. Front Cell Infect Microbiol. 2022;12:910908.
6. Aratani Y. Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 2018;640:47–52.
7. Diehl P, Aleker M, Helbing T, Sossong V, Beyersdorf F, Olschewski M, et al. Enhanced microparticles in ventricular assist device patients predict platelet, leukocyte and endothelial cell activation. Interact Cardiovasc Thorac Surg. 2010;11(2):133–7.
8. Bhat G, Yost GL, Ibrahim K, Pappas P, Tatooles A. Risk stratification with longitudinal neutrophil to lymphocyte ratio assessment after left ventricular assist device implantation. Int J Artif Organs. 2018;41(8):445–51.
9. Ferrera C, Gonzalez Fernandez O, Bouzas N, Castrodeza J, Green T, Woods A, et al. Neutrophil to lymphocyte ratio is related to thrombotic complications and survival in continuous flow left ventricular assist devices. ASAIO J. 2020;66(2):199–204.
10. Sundararajan S, Kiernan MS, Couper GS, Upshaw JN, DeNofrio D, Vest AR. The neutrophil-lymphocyte ratio and survival during left ventricular assist device support. J Card Fail. 2019;25(3):188–94.
11. Woolley JR, Teuteberg JJ, Bermudez CA, Bhama JK, Lockard KL, Kormos RL, et al. Temporal leukocyte numbers and granulocyte activation in pulsatile and rotary ventricular assist device patients. Artif Organs. 2014;38(6):447–55.
12. Granja T, Magunia H, Schüssel P, Fischer C, Prüfer T, Schibilsky D, et al. Left ventricular assist device implantation causes platelet dysfunction and proinflammatory platelet-neutrophil interaction. Platelets. 2022;33(1):132–40.
13. Berk ZBK, Zhang J, Chen Z, Tran D, Griffith BP, Wu ZJ. Evaluation of in vitro hemolysis and platelet activation of a newly developed maglev LVAD and two clinically used LVADs with human blood. Artif Organs. 2019;43(9):870–9.
14. Radley G, Pieper IL, Robinson CR, Ali S, Beshr M, Bodger O, et al. In vitro benchmarking study of ventricular assist devices in current clinical use. J Card Fail. 2020;26(1):70–9.
15. Sansone R, Stanske B, Keymel S, Schuler D, Horn P, Saeed D, et al. Macrovascular and microvascular function after

implantation of left ventricular assist devices in end-stage heart failure: role of microparticles. J Heart Lung Transplant. 2015;34(7):921–32.
16. Westerman M, Porter JB. Red blood cell-derived microparticles: an overview. Blood Cells Mol Dis. 2016;59:134–9.
17. Tuzun E, Roberts K, Cohn WE, Sargin M, Gemmato CJ, Radovancevic B, et al. In vivo evaluation of the HeartWare centrifugal ventricular assist device. Tex Heart Inst J. 2007;34:406–11.
18. Sun W, Zhang J, Shah A, Arias K, Berk Z, Griffith BP, et al. Neutrophil dysfunction due to continuous mechanical shear exposure in mechanically assisted circulation in vitro. Artif Organs. 2022;46(1):83–94.
19. Sun W, Wang S, Zhang J, Arias K, Griffith BP, Wu ZJ. Neutrophil injury and function alterations induced by high mechanical shear stress with short exposure time. Artif Organs. 2021;45(6):577–86.
20. Pieper IL, Radley G, Christen A, Ali S, Bodger O, Thornton CA. Ovine leukocyte microparticles generated by the CentriMag ventricular assist device in vitro. Artif Organs. 2018;42(6):E78–89.
21. Radley G, Laura Pieper I, Thomas BR, Hawkins K, Thornton CA. Artificial shear stress effects on leukocytes at a biomaterial interface. Artif Organs. 2019;43(7):E139–51.
22. Shive MS, Salloum ML, Anderson JM. Shear stress-induced apoptosis of adherent neutrophils: a mechanism for persistence of cardiovascular device infections. Proc Natl Acad Sci USA. 2000;97(12):6710–5.
23. Fukuda S, Yasu T, Predescu DN, Schmid-Schönbein GW. Mechanisms for regulation of fluid shear stress response in circulating leukocytes. Circ Res. 2000;86(1):E13–8.
24. Loebe M, Koster A, Sänger S, Potapov EV, Kuppe H, Noon GP, et al. Inflammatory response after implantation of a left ventricular assist device: comparison between the axial flow MicroMed DeBakey VAD and the pulsatile Novacor device. ASAIO J. 2001;47(3):272–4.

How to cite this article: Awad MA, Sun W, Han D, Griffith BP, Wu Z. Increased phagocytosis capacity of circulating neutrophils in patients on continuous flow ventricular assist device support. Artif. Organs. 2024;48:636–645. https://doi.org/10.1111/aor.14693