High-Monocyte ACP Max™ System Spin Regimens

Arthrex Research and Development

OBJECTIVE

This study aimed to determine a spin regimen using 60 and 90 mL of blood in the ACP Max system that significantly concentrates platelets and monocytes above circulating levels while reducing neutrophils to below-baseline levels.[1,2]

MATERIALS AND METHODS

Blood Collection

Blood was collected from donors (N = 6) using 13.3% acid citrate dextrose solution A (ACD-A) as the anticoagulant. A small volume of anticoagulated blood from each donor was aliquoted for baseline complete blood count (CBC) analyses.

Platelet-Rich Plasma (PRP) Preparation

PRP was prepared for each donor as described below.

› 60 mL High-Monocyte ACP Max PRP

60 mL of anticoagulated blood was moved to the ACP Max device, which was then counterbalanced in a Drucker Horizon 24 Flex-AH centrifuge and spun at 3200 rpm for 6 minutes. The device was removed, and the platelet-poor plasma (PPP) was extracted from the top using a 30 mL syringe until the bottom of the ACP Max plunger was three tick marks (6 mL) above the buffy coat. The PPP syringe was removed, and the Arthrex ACP® double syringe was attached to the top of the device. The next 15 mL of fluid was collected into the outer syringe of the double syringe. The double syringe was then removed, capped, and inverted approximately 20 times before being spun at 1500 rpm for 3 minutes in the same counterbalanced centrifuge. The device was removed from the centrifuge, and the PRP was collected into the inner syringe until the red blood cell layer was reached. Then, the volume was noted on the inner syringe, and an additional 0.8-1 mL of buffy coat and red blood cells was collected into the inner syringe.

› 90 mL High-Monocyte ACP Max PRP

90 mL of anticoagulated blood was moved to the ACP Max device, which was then counterbalanced in a Drucker Horizon 24 Flex-AH centrifuge and spun at 3200 rpm for 12 minutes. The device was removed, and the PPP was extracted from the top using a 30 mL syringe until the bottom of the ACP Max plunger was three tick marks (6 mL) above the buffy coat. The PPP syringe was removed, and the Arthrex ACP double syringe was attached to the top of the device. The next 15 mL of fluid was collected into the outer syringe of the double syringe. The double syringe was then removed, capped, and inverted approximately 20 times before being spun at 1500 rpm for 3 minutes in the same counterbalanced centrifuge. The device was removed from the centrifuge, and the PRP was collected into the inner syringe until the red blood cell layer was reached. Then, the volume was noted on the inner syringe, and an additional 0.8-1 mL of buffy coat and red blood cells was collected into the inner syringe.

The PRP volumes were recorded. A small aliquot of PRP was collected from each device, and a CBC with differential was performed.

Data Analysis

The following analyses were performed on all CBC results, focusing on the platelet (PLT), neutrophil (NE), white blood cell (WBC), and monocyte (MONO) groups.

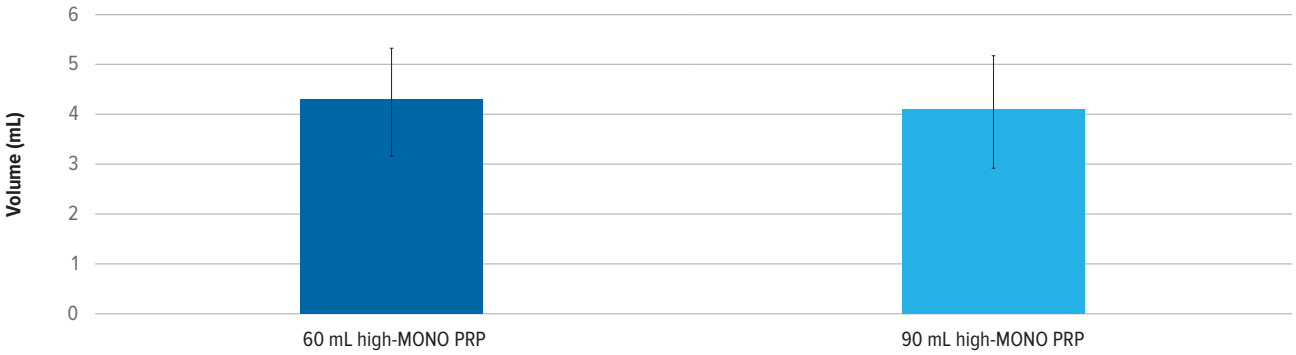
› The fold change in the concentration of each cell type relative to baseline was calculated by dividing the values obtained from the PRP by the corresponding values from the respective whole blood.

› The dose was calculated by multiplying the PRP concentrations by the recovered fluid volume.

Following the calculations for each device, the data were averaged across the 6 donors for each group. To determine the monocyte and neutrophil fold changes, a one-sample T-test was performed comparing the experimentally determined concentrations against a baseline value of 1. Significance was set at $\alpha = .05$ for all analyses.

RESULTS

Figure 1. Recovered volume of high-MONO PRP, with standard deviation, for 60 and 90 mL spin regimens (N = 6).



Using the CBC values, the fold change relative to baseline was calculated for platelets, white blood cells, monocytes, and neutrophils (Figure 2). The average fold change and standard deviation are listed in Table 1.

Figure 2. Average fold change, with standard deviation, of PLT, WBC, MONO, and NE (N = 6).

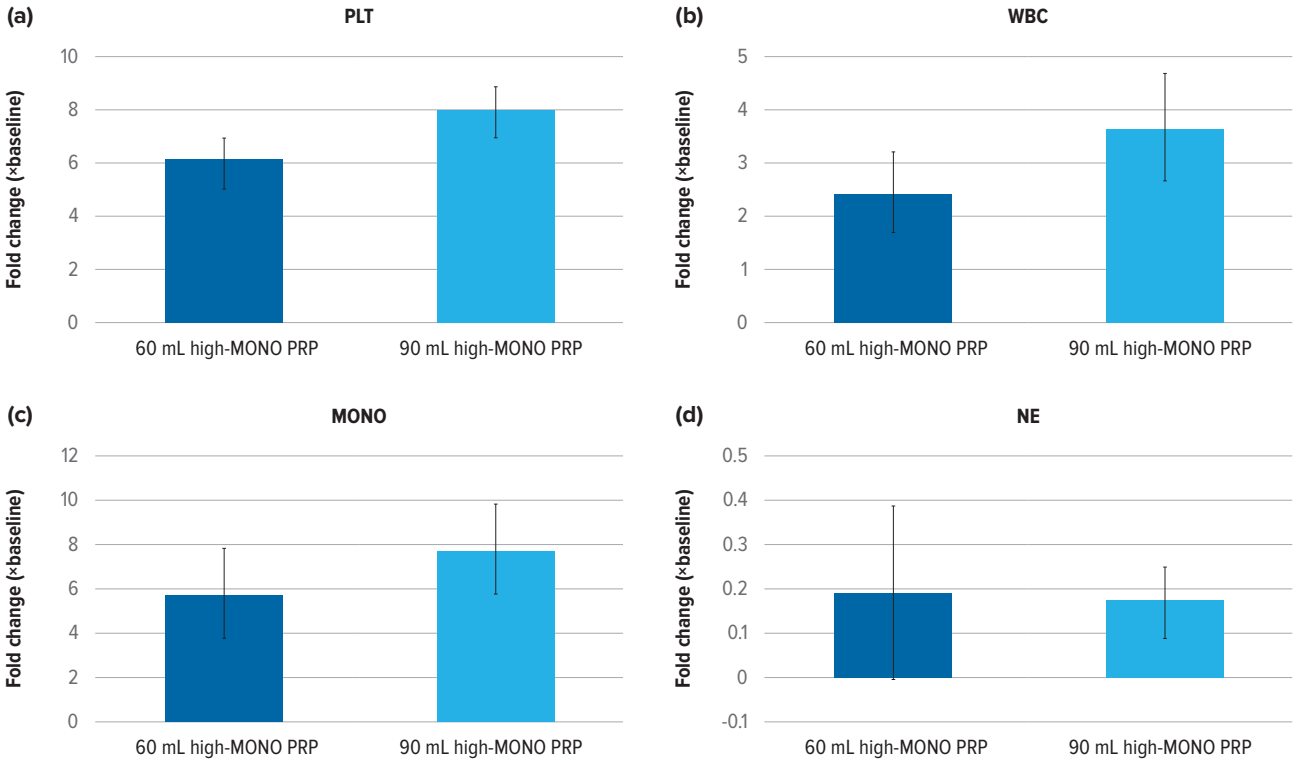


Table 1. Average fold change, with standard deviation, of the analyzed cells.

	60 mL	90 mL
PLT	6.11 ± 0.70	7.97 ± 0.74
WBC	2.46 ± 0.75	3.71 ± 0.99
MONO	5.71 ± 2.03	7.66 ± 2.09
NE	0.19 ± 0.20	0.17 ± 0.08

The expected dose of each cell type was calculated based on the final PRP volume and the concentrations of each cell type (Figure 3). The average delivered dose and standard deviation are listed in Table 2.

Figure 3. Average expected dose, with standard deviation, of PLT, WBC, MONO, and NE and the ratio of delivered MONO:NE (N = 6).

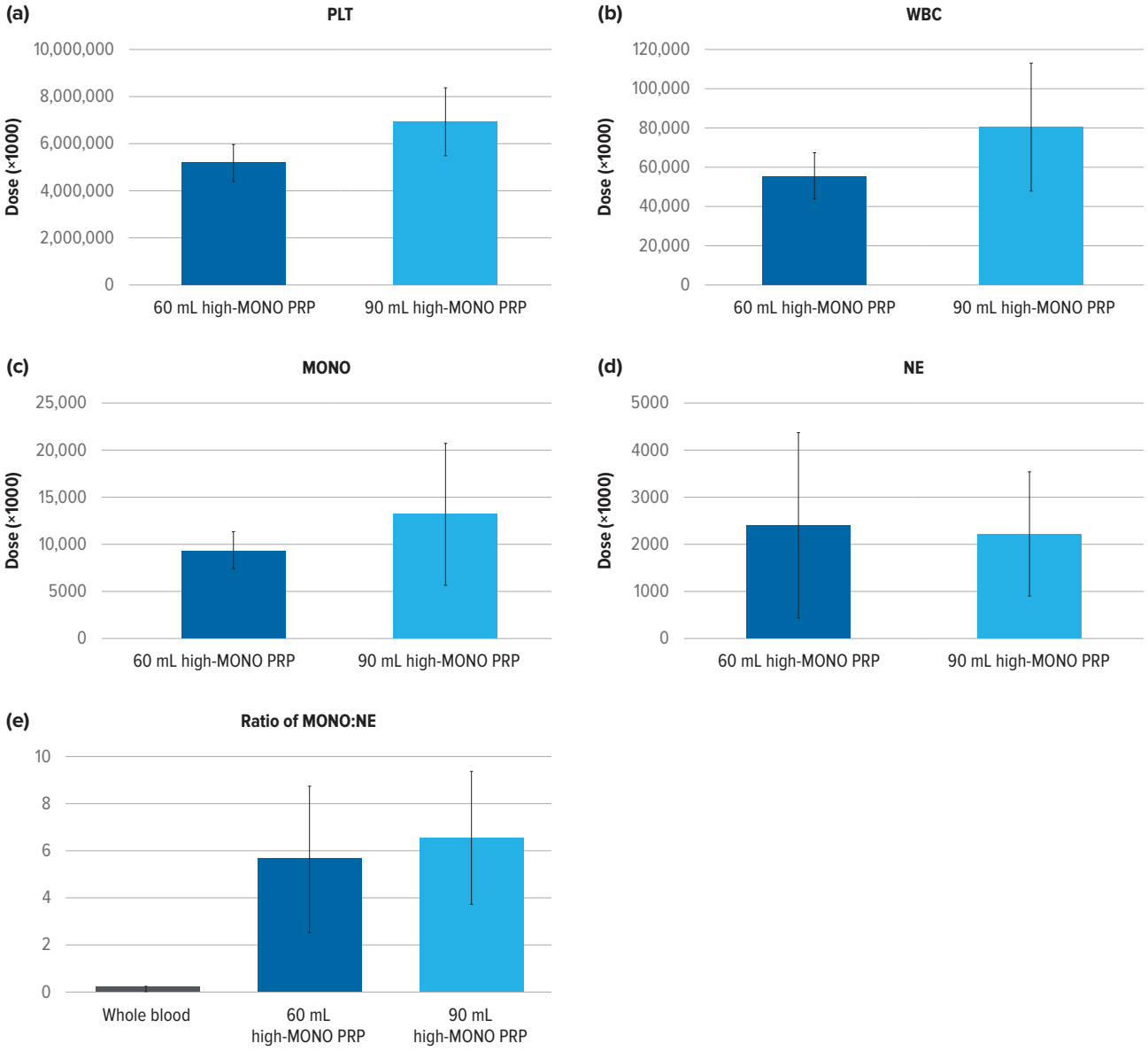


Table 2. Average delivered dose, with standard deviation, of the analyzed cells and the ratio of MONO:NE.

	60 mL	90 mL
PLT (×1000)	5,218,967 ± 691,005	6,958,792 ± 1,523,410
WBC (×1000)	55,310 ± 11,329	80,646 ± 31,609
MONO (×1000)	9319 ± 2219	13,281 ± 7509
NE (×1000)	2417 ± 1926	2204 ± 1256
MONO:NE	5.67 ± 3.03	6.54 ± 2.67

DISCUSSION

Based on the data presented here, a new spin regimen to optimize for high-monocyte ACP Max™ PRP was successfully achieved. The output volumes of both high-monocyte groups were similar to those of the normal ACP Max spin regimen. PRPs were created that concentrated white blood cells 2.46× and 3.71× over baseline for 60 and 90 mL regimens, respectively. More specifically, these regimens aimed to concentrate monocytes while maintaining neutrophils below baseline levels. This was accomplished, with monocytes concentrated 5.71× and 7.66× over baseline for the 60 and 90 mL regimens, respectively, while neutrophils were concentrated only 0.19× and 0.17× over baseline levels. This resulted in a change in the ratio of monocytes to neutrophils from 0.13 in whole blood to 5.67 for the 60 mL regimen and 6.54 for the 90 mL regimen. These regimens also maintained platelet concentration, with fold increases of 6.11× and 7.97× above baseline.

According to the PAW classification of PRP, these new PRP formulations would be classified as P4-Aβ, as the platelet concentrations exceed 1.25M/µL, while the white blood cells are above baseline and the neutrophils are below baseline.[3] The data presented here show that the ACP Max system can alter the concentration of red blood cells and certain white blood cells, such as monocytes and neutrophils, without sacrificing volume or platelet concentration.

While the role of monocytes in inflammation is ever-evolving, monocytes are believed to modulate both pro- and anti-inflammation and tissue remodeling.[4,5] Immediately following tissue damage or disease, monocytes and resident macrophages can increase inflammation to recruit other cell types to the site and fight infection or repair tissue.[4,5] Once the tissue is ready for repair, the monocytes can switch to an anti-inflammatory phenotype and begin orchestrating the repair and reduction of inflammation processes.[4,5] Neutrophils, conversely, are potent innate immune cells believed to orchestrate the inflammatory environment.[6,7] Thus, specific combinations of monocytes and neutrophils may be useful in certain instances in which an initial inflammatory response may be beneficial, followed by an increased reparative response led by the monocytes.

References

1. Arthrex, Inc. Data on file (APT-06729). Naples, FL; 2023.
2. Arthrex, Inc. Data on file (APT-06879). Naples, FL; 2023.
3. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. *Arthroscopy*. 2012;28(7):998-1009. doi:10.1016/j.arthro.2012.04.148
4. Kratofil RM, Kubes P, Deniset JF. Monocyte conversion during inflammation and injury. *Arterioscler Thromb Vasc Biol*. 2017;37(1):35-42. doi:10.1161/ATVBAHA.116.308198
5. Austermann J, Roth J, Barczyk-Kahlert K. The good and the bad: monocytes' and macrophages' diverse functions in inflammation. *Cells*. 2022;11(12):1979. Published 2022 Jun 20. doi:10.3390/cells11121979
6. Wright HL, Moots RJ, Bucknall RC, Edwards SW. Neutrophil function in inflammation and inflammatory diseases. *Rheumatology (Oxford)*. 2010;49(9):1618-1631. doi:10.1093/rheumatology/keq045
7. Mócsai, A., Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med*. 2013;210:1283-1299. doi:10.1084/jem.20122220