# 7 Hematopoietic Progenitor Cells, Apheresis and Therapeutic Cells, T-Cells Collection: Instrumentation, Operating Parameters, and Troubleshooting

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## 7.1 Introduction

The focus of this chapter will be to discuss the current instrumentation used for the collection of cellular therapy products from the peripheral blood by apheresis methods, specifically hematopoietic progenitor cells, apheresis (HPC[A]), and therapeutic cells, T cells (TC-T). The chapter will not include a discussion of legacy apheresis instruments, such as the *Fenwal CS3000 Plus* or *COBE Spectra*, as these devices are or will soon no longer be supported by their manufacturers, even though still widely used in some parts of the world. Instead, discussion will focus on the *Fenwal Amicus* and *Terumo BCT Spectra Optia*, including both the MNC and continuous MNC (CMNC) protocols for the latter, with the goals of describing (1) the methods of cell separation and collection for each device/protocol; (2) the advantages, disadvantages, and operating parameters; and (3) troubleshooting for both devices. The content of this chapter is derived from information made available by the instruments' manufacturers, publications in the peer-reviewed medical literature, and the authors' personal experiences in using these devices, including both *Spectra Optia protocols*, in a busy therapeutic apheresis service which collects more than 1000 HPC(A) and TC-T products, annually.

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S. A. Abutalib et al. (eds.), *Best Practices of Apheresis in Hematopoietic Cell Transplantation*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy, https://doi.org/10.1007/978-3-319-55131-9_7

## 7.2 Hematopoietic Progenitor Cells, Apheresis

HPC(A) products are collected from autologous or allogeneic donors in order to restore hematopoiesis following myeloablative (all cases of autologous and select cases of allogeneic transplants) and reduced intensity/nonmyeloablative (select cases of allogeneic transplant) conditioning regimens of chemotherapy with or without concurrent radiation therapy. In the context of allogeneic transplantation, the goal is to replace the recipient's immune system with that of the donor's in the hope of producing an immune response to the recipient's tumor, i.e., graft-versus-tumor (GvT) effect. In the autologous setting, the goal of the graft is to "rescue hematopoiesis" of the patient following high-dose chemotherapy, i.e., myeloablative condition regimen (MAC). Evidence does, however, suggest that there is an immune component to autologous transplants similar to that observed in allogeneic transplant recipients. When collecting $CD34^+$ cells via HPC(A), the goal is to collect sufficient $CD34^+$cells that would result in timely restoration of hematopoiesis. A variety of targets have been used to define an adequate product, but most commonly, it is a dose, on a per kilogram of recipient weight, of $CD34^+$ cells. Stem and progenitor cells represent a subset of $CD34^+$ cells. The usual collection targets in the autologous setting are anywhere in the ranges of $2–5 \times 10^6$ $CD34^+$ cells/kg while that for allogeneic transplants is usually in the range of $4–8 \times 10^6$ $CD34^+$ cells/kg of recipient weight. These doses may be achieved by processing a fixed volume of blood per collection (e.g., 2–3 blood volumes), measuring $CD34^+$ cells within the product during the collection and adjusting the duration of the collection accordingly or by processing blood for a fixed length of time (e.g., 4–6 h). Significant variability in clinical practice means that there is no standard in this regard (see Chaps. 4 and 5).

## 7.3 Therapeutic Cells, T Cells

TC-T are lymphocytes collected either for subsequent genetic modification (e.g., chimeric antigen receptor T cells [CAR-T cells]) or other manipulations or for infusion into patients after allogeneic hematopoietic cell transplantation (allo-HCT) in order to induce a GvT effect. The $CD3^+$ T-cells targets vary depending upon the medical use. The number collected for subsequent modification will vary depending upon the protocols and methods used to modify, and potentially expand, the T cells. When used without modification such as for conventional donor lymphocyte infusion (DLI), typically doses of $1–2 \times 10^8$ $CD3^+$ cells are collected and divided in specific aliquots for cryopreservation; this is usually done after administration of initial first fresh dose of DLI to the transplant recipient. In order to achieve these doses, collections may be performed using a blood volume target (e.g., 2–3 total blood volumes) or procedure length target (e.g., 4-h collection); again, there is no standard but institutional practices vary on the length of procedure.

## 7.4 Instrumentation

### 7.4.1 Fenwal Amicus (Fresenius Kabi, Lake Zurich, IL)

The *Fenwal Amicus* has been capable of TC and HPC(A) collections since clearance by the Food and Drug Administration (FDA) for these procedures in 2002. The separation kit is a disposable flexible plastic belt-shaped chamber that is wrapped around and secured to a rigid spool (Fig. 7.1). There are two chambers in the belt *(separation and collection chambers)*; however, only the *separation chamber* is used for TC and HPC(A) collection. A ramp is molded into the spool which directs flow within the separation chamber (Fig. 7.1).

As whole blood enters the *separation chamber*, the blood separates into the cell and plasma layers. Blood enters at a lower hematocrit (approximately 35%) and flows to the far end of the *separation chamber*. Platelets quickly separate and are returned by the platelet-rich plasma pump (Fig. 7.2). The granulocytes drop down into the RBC layer and flow to the outlet. As the whole blood flows through the chamber, the hematocrit increases to about 80%. As the mononuclear cells (MNCs) enter, they are heavier than platelets and drop to the cellular layer and move toward the outlet. However, as the hematocrit increases, the MNCs separate from the granulocytes and RBC and flow back to the inlet (Fig. 7.2). When they encounter the lower hematocrit, they drop down to the cellular layer resulting in the MNC recirculation in the center of the *separation chamber* (Fig. 7.2). Set volumes of whole blood are processed (usually 1000 mL or 1400 mL, depending on peripheral white blood cell (WBC) count) to allow buildup of the MNC layer prior to MNC harvesting. When the programmed volume has been processed, the *Amicus machine*

**Fig. 7.1** Amicus mononuclear cell (MNC) spool. From left to right, note the slope of the ramp for the separation chamber (see text)

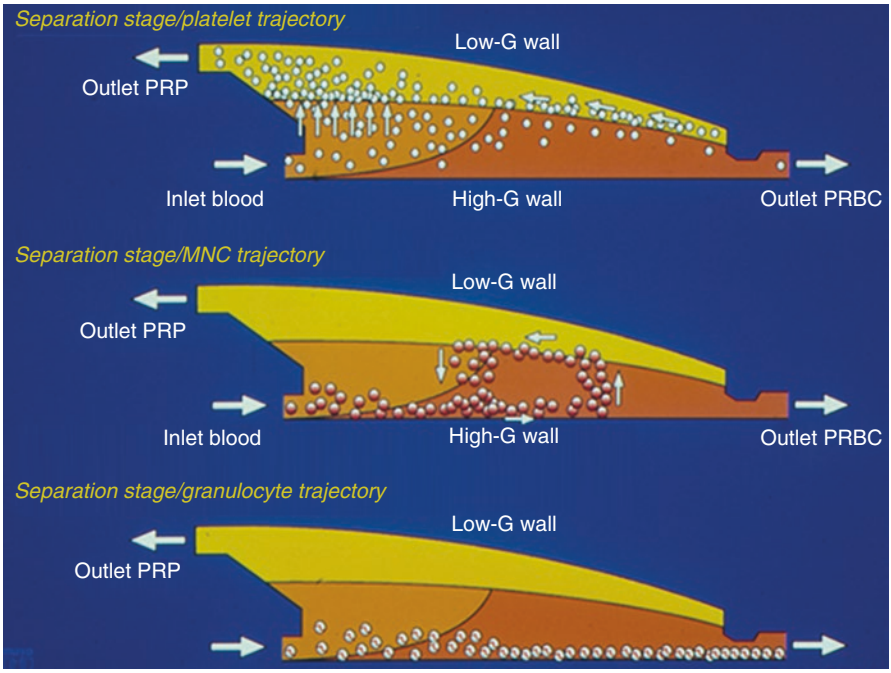


**Fig. 7.2** Amicus blood separation in the centrifuge. Circulation of the various cells during separation and harvest of mononuclear cells (MNC). Platelet-rich plasma is returned to the patient/donor through the platelet-rich port (left). MNCs recirculate in the center of the chamber until harvest, and then they exit out the platelet-rich port (left) with the addition of packed RBC. Packed RBCs and granulocytes are returned to the patient/donor through the RBC port (right). (Used with permission from Fresenius Kabi)

automatically diverts about 30 mL of the high-hematocrit RBC to a small collection bag outside the *separation chamber* to be used to push the MNC out of the centrifuge. The MNC transfer then ensues. The inlet speed is dropped, and the cell/plasma interface that has been maintained on the ramp at about 60% is allowed to rise on the ramp and exit the *separation chamber*. The MNCs then pass through an optical sensor outside the centrifuge. When a designated amount of light is blocked (sense level), a whole blood pumped counter is activated. There are two settings used to open and close the valves to the collection bag: (1) the MNC and (2) the RBC offsets. The *MNC offset* is used to open the valve. It allows the platelet-rich plasma to be diverted back to the donor/patient. When the *MNC offset* is reached, the valve to the collection bag is opened and the MNCs continue to flow into the collection bag until the *RBC offset* setting is reached. When the collection valve closes, a small amount of platelet-rich plasma is flushed back to the donor, and the platelet-poor plasma (about 8–10 mL) is used to flush the MNC in the collection line to the collection bag. A new cycle is then started with cycles continuing until the end of the HPC(A) procedure.

During most of the procedure, plasma leaving the centrifuge is platelet rich. Platelet-poor plasma is usually collected at the very beginning or at end of the procedure that can be added to the product/graft or as a separate collection, if desired.

The settings for the *MNC offset*, *RBC offset*, ramp position, and light blockage (sense level) in the optical sensor can be adjusted by the operator (Burgstaler et al. 2010; Burgstaler and Winters 2014). Various cycle volumes have also been used and are operator programmable (Burgstaler and Winters 2011a).

### 7.4.2 Spectra Optia (Terumo BCT, Lakewood, CO, USA)

The *Spectra Optia* has two HPC(A)/TC collection protocols, each of which has different disposable supplies. The *Spectra Optia* was cleared by the FDA for the collection of TC and HPC(A) in 2012 using the MNC protocol and 2015 using the CMNC protocol. The MNC protocol works in cycles and uses an additional *separation chamber* to separate the MNC from the platelets (Fig. 7.3). The CMNC protocol continually collects MNC, similar to the *COBE Spectra* MNC procedure, and does not utilize the separate *separation chamber* (Fig. 7.4).

#### 7.4.2.1 Spectra Optia MNC

The *Spectra Optia MNC protocol* uses a flexible circular centrifuge chamber that fits in a rigid insert (Fig. 7.5). The centrifuge chamber is the same diameter throughout. Whole blood enters the chamber and flows counterclockwise to the collection ports. There are three ports in the collector area: (1) the plasma port which is closest

**Fig. 7.3** Spectra Optia blood separation in the centrifuge using the MNC protocol. Whole blood flows counterclockwise with separation of the MNC and platelets that are drawn into the conical chamber (left) and then into the collection bag, during the harvest cycles. Platelet-poor plasma is drawn from the plasma port (center) to be returned to the patient/donor. Packed RBCs (right port) are returned to the patient/donor. (Used with permission from Terumo BCT)

**Fig. 7.4** Spectra Optia blood separation in the centrifuge using the CMNC protocol. Whole blood flows counterclockwise with separation of the MNCs that are drawn from the left port and into the collection bag, continuously. Platelet-rich plasma is drawn from the plasma port (center) to be returned to the patient/donor. Packed RBCs (right port) are returned to the patient/donor. (Used with permission from Terumo BCT)

**Fig. 7.5** Spectra Optia MNC insert. Circular channel with vertical walls and the secondary conical chamber holder

to the center, (2) the packed cell (RBC) line which is closest to the outside, and (3) the collection port is the first port (Fig. 7.3). The collection port is connected to the collection pump, while the plasma port is connected to the plasma pump. The remaining blood components are pushed out of the centrifuge chamber by the inlet

pump. The *Spectra Optia* has an optical system, the automated interface management (AIM) system, which monitors and controls the cell/plasma interface. With the MNC program, the centrifuge packing factor is high, driving platelets into the buffy coat layer. The buffy coat is drawn off in a small collect line that is connected to a conical chamber located in the centrifuge (Fig. 7.3). The first stage of separation uses cell density to separate red blood cells from the buffy coat in the flexible circular *separation chamber*. The conical chamber located near the axis of rotation for the centrifuge then separates the platelets and MNC according to cell size using two forces: (1) centrifuge g-force and (2) fluid flow from the collect pump. The centrifuge g-force pushes components away from the axis of rotation, while the collect pump pulls components toward the center of the centrifuge, toward the axis of rotation, causing separation of the cells. First, the chamber fills with platelets. Then as RBC and MNC enter the chamber, based on their sizes, the MNC stays on the inlet side of the chamber while RBC percolates through the MNC and layer on top toward the outlet and the axis of rotation. As the chamber fills with MNC, the RBC eventually reaches the outlet and exits the chamber. An optical sensor detects the RBC-blocking light and triggers a chamber flush. The collect valve opens, and the MNCs are harvested into the collect bag (set volume is collected). A plasma flush then clears the line of MNC, flushing them into the collection bag. This is then followed by the start of a new cycle. The collect volume and plasma flush volume can be adjusted by the operator. The operator can also change the optical sensor control to use either the cycle volume or operator interaction (manual initiation) to trigger the MNC harvest. The operator can also determine the amount of RBC entering the chamber by adjusting the Collection Preference which controls the plasma pump speed. Donors with very high WBC counts will have many cycles because the chamber fills quickly resulting in frequent MNC harvests. This, in turn, results in larger collection volumes due to both the number of cells collected and the plasma flush used to clear the chamber and lines.

#### 7.4.2.2 Spectra Optia CMNC

The *Spectra Optia CMNC* uses a different insert, the Intermediate Density Layer (IDL), for collections (Fig. 7.6). As with the MNC *separation chamber*, the CMNC chamber has the same diameter throughout, but it does not have a chamber bracket or the conical separation chamber present in the MNC kit (Fig. 7.6). The slot that the flexible chamber fits into is pear-shaped rather than rectangular which increases the extracorporeal volume of the device by approximately 100 mL compared to the MNC disposable kit. As with the *Spectra Optia MNC*, whole blood is pumped counterclockwise to the collection area. A lower packing factor is used to prevent platelets from being forced into the buffy coat. The same collection ports present in the MNC disposables are used; however, the collect line does not have a separate chamber attached, as with the *Spectra Optia MNC* kit (Fig. 7.4). The AIM system monitors and adjusts the position of the buffy coat interface. The collect pump continuously draws the MNC off and pumps them into the collection bag. The color of the collection is monitored by the operator, similar to what was done with the *COBE Spectra*, and adjusted by changing the Collection Preference, which adjusts

**Fig. 7.6** Spectra Optia CMNC (IDL) insert. Circular channel with pear-shaped walls and no secondary conical chamber holder

the plasma pump. The plasma is platelet rich and is returned to the donor/patient. For that reason, there are only certain times that platelet-poor plasma can be collected. Separate plasma can be collected for addition to the collection or separate plasma collection. The *Spectra Optia* has a monitoring screen that can indicate if the AIM system is correctly adjusting the cell/plasma interface. The collect pump speed can be adjusted, and the collect volume is more predictable, compared to the MNC protocol, because of the continual collection.

## 7.5 Advantages and Disadvantages

Both of the instruments (described above), as well as the different protocols available for the *Spectra Optia*, offer advantages and disadvantages depending upon the characteristics of the patient/donor undergoing collection, experience of the operators, the processes and workflow of the apheresis collection center, and laboratory responsible for processing the cellular therapy product. Table 7.1 represents the authors' opinions of the advantages and disadvantages of the *Amicus* and the *Spectra Optia* protocols. It is important to acknowledge that one person's advantage may be another's disadvantage and vice versa. It should be noted that the authors utilize both devices in their practice with success, tailoring their use to the characteristics of those undergoing collection in order to maximize the safety and efficacy of the collection procedures. Table 7.2 provides how the authors utilize both devices in tailoring collections based upon patient/donor characteristics.

**Table 7.1** Advantages and disadvantages

| Advantages | Disadvantages |
|---|---|
| *Fresenius Kabi Fenwal Amicus*<br>• Automated with manual adjustments possible<br>• Low extracorporeal volume (ECV)—163 mL<br>• Platelet sparing<br>• Good MNC differentials, low granulocyte content<br>• Low RBC content<br>• Heparin/ACD-A anticoagulant solutions can be used.<br>• $CD34^+$ cell and MNC collection efficiencies (CE) are as expected.<br>• With AC ratios to 26:1 when using heparin/ACD-A anticoagulant:<br>Less citrate toxicity<br>Less volume to patient<br>Higher inlet rates<br>• Can collect separate plasma<br>• Custom prime for low blood volumes | *Fresenius Kabi Fenwal Amicus*<br>• Does not have sterile addition of AC to product bag<br>• Cannot see into centrifuge<br>• Works in cycles, which prolongs procedures due to transfers<br>• Product size can vary slightly.<br>• Slightly harder to control RBC and granulocyte content |
| *Terumo BCT Spectra Optia—CMNC*<br>• Automated with manual adjustments possible<br>• Simple to adjust, provides continuous collection<br>• Very predictable product size<br>• Sterile addition of AC to product bag<br>• Tracking monitor on interface position<br>• Can observe action in centrifuge<br>• Can collect separate plasma<br>• Very good MNC differentials, very low granulocyte content<br>• Very low RBC content<br>• Custom prime for low blood volumes<br>• $CD34^+$ cell and MNC (CE) are as expected. | *Terumo BCT Spectra Optia—CMNC*<br>• Poorly compatible with heparin/ACD-A anticoagulant solutions due to platelet clumping<br>• Without 26:1 AC ratios:<br>More citrate given<br>More fluid to patient<br>Slower inlet rates<br>• Larger ECV—297 mL<br>• Platelet loss is moderate to high. |
| *Terumo BCT Spectra Optia—MNC*<br>• Automated with manual adjustments possible<br>• Low ECV—191 mL<br>• Low RBC content<br>• Good MNC differential; low granulocyte content, but more than CMNC<br>• Custom prime for low blood volumes<br>• Sterile addition of AC to collection bag<br>• $CD34^+$ cell and MNC (CE) are as expected. | *Terumo BCT Spectra Optia—MNC*<br>• Poorly compatible with heparin/ACD-A anticoagulant solutions due to platelet clumping<br>• Without 26:1 AC ratios:<br>More citrate given<br>More fluid to patient<br>Slower inlet rates<br>• Product volume variable due to WBC count<br>• Large products with high WBC counts<br>• Works in cycles, which prolongs procedures due to transfers<br>• Platelet loss is moderate to high.<br>• Requires more manipulation and observation of interface and platelets in chamber |

**Table 7.2** Patient/donor characteristics and optimal apheresis instrument

| Characteristic | Optimal instrument | Rationale |
|---|---|---|
| Concerns about/ sensitivity to excessive fluid | Amicus | Heparin/ACD-A AC and 26:1 ratio will infuse half as much fluid per collection with Amicus, while heparin AC is associated with platelet clumping in the Spectra Optia.<br>Due to the cycles, less blood and AC are processed compared to Spectra Optia CMNC with no cycles. |
| Very lipemic plasma | Spectra Optia CMNC or MNC | Interface detector is more tolerant of high lipids than Amicus. |
| Low platelet count | Amicus | Amicus demonstrates greater platelet sparing. |
| High granulocyte count | Spectra Optia CMNC | CMNC collects the least granulocytes. |
| Small patient with small blood volume | 1st choice: Amicus<br>2nd choice: Spectra Optia MNC | Amicus has the lowest ECV of 163 mL<br>Spectra Optia MNC ECV: 191 mLs<br>Spectra Optia CMNC ECV: 297 mLs<br>Amicus is also more compatible with heparin/ ACD-A AC and less citrate can be given. |

*AC* anticoagulant, *CMNC* continuous mononuclear cell, *ECV* extracorporeal volume

## 7.6 Product Content and Characteristics

Both instruments and protocols can collect HPC(A) and TC products which are pure, potent, and efficacious. There are, however, differences in product content which may influence which device or protocol is used in certain circumstances or patients/donors. Table 7.3 provides a summary of the HPC(A) product content reported in the medical literature for each of the instruments and protocols, while Table 7.4 provides a summary of characteristics reported for TC collections.

## 7.7 Troubleshooting

As indicated above, both devices can collect an appropriate HPC(A) or TC product. However, at times, the content of the product may not be what is expected or desired. When this occurs, it is necessary to examine the collection procedure and the product content to be able to effectively troubleshoot. Etiology of unexpected hematopoietic graft yields and product content can result from patient/donor characteristics, operator errors, and equipment malfunction.

Troubleshooting for poor TC and HPC(A) collection yields can be very complex, especially in patients who have been mobilized with chemotherapy and/or cytokines. Usually, the problem is not the machine or the operator; the biggest variables in suboptimal collections are the donors/patients and the number of $CD34^+$ cells mobilized in the PB or the number of circulating lymphocytes. Factors such as the

**Table 7.3** HPC(A) product content and collection procedure parameters reported in the medical literature

| Source | Proc. N | Pt. N | Pre CD34+ cells/μL | Pre WBC ×10⁹/L | CD34+ cell CE1 % | CD34+ cell CE2 % | CD34+ cell ×10⁶/kg or ×10⁶ | PLT CE % | PLT loss % or ×10¹¹ | RBC mL | Product volume mL | WB processed mL/TBV | Time min | Gran % | Gran ×10⁹ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AMICUS** | | | | | | | | | | | | | | | |
| Tarik et al. (2013) | 17 | | | | 44.4 ± 14.8 | | 6.3 ± 11.0 | | 11.4 ± 11.0% | | | | 295 ± 42 | | |
| Sputtek et al. (2013) | 36 | 15 | | | 64.6 ± 14.6 | | 386 ± 413 × $10^6$ | | 0.7 ± 0.3 × $10^{11}$ | | | 10,900 ± 1900 | 250 ± 15 | | |
| Burgstaler and Winters (2014) | 20 | 20 | 49 | 56.5 | 55.0 | | 3.28 | | 2.2 × $10^{11}$ | 25 | | 15,800 | 300 | | 15.7 |
| Burgstaler and Winters (2014) | 20 | 20 | 51 | 58.0 | 59.6 | | 3.74 | | 1.9 × $10^{11}$ | 28 | | 15,800 | 300 | | 15.3 |
| Burgstaler et al. (2012) | | 30 | | 50.2 | | | 234.2 × $10^6$ | | 1.3 × $10^{11}$ | 30 | | | 300 | 20 | 13.5 |
| Burgstaler et al. (2012) | | 30 | | 44.1 | | | 346.7 × $10^6$ | | 1.3 × $10^{11}$ | 28 | | | 300 | 30 | 14.6 |
| Burgstaler et al. (2010) | 120 | 156 | | 3.0–118.0 | | | 0.89–2.91 | | 1.1–2.3 × $10^{11}$ | 16–33 | | 13,000–22,000 | 300 | 13–36 | 4.8–18.8 |
| Burgstaler and Winters (2011b) | 200 | 63 | | 45.2 | | | 1.5 | | 1.3 × $10^{11}$ | 21 | | | 300 | 24 | 9.8 |
| Burgstaler and Winters (2011b) | 200 | 66 | | 53.6 | | | 0.89 | | 1.2 × $10^{11}$ | 17 | | | 300 | 23 | 7.2 |
| Burgstaler and Winters (2011c) | 50 | 20 | | 51.6 | | | 3.07 | | 23%, 2.3 × $10^{11}$ | 26 | | 15,800 | 300 | 28 | 15.1 |
| Burgstaler and Winters (2011a) | 24 | 12 | 63<br>77 | 50.4<br>52.4 | 37.0<br>46.7 | | 3.48<br>4.04 | | 2.4 × $10^{11}$<br>3.2 × $10^{11}$ | 30<br>36 | 305<br>340 | 15,700<br>14,900 | 300<br>300 | 33<br>41 | 21.9<br>27.8 |
| Gumogda et al. (2015) | | 20 | | | | 37.04 ± 14.8 | | | | | | 2.5 (TBV) | | | |
| Burgstaler et al. (2004) | 80 | 65 | | 33.3<br>36.8 | | | 2.1<br>1.5 | | 1.1 × $10^{11}$<br>1.1 × $10^{11}$ | 14<br>15 | | 13,400<br>20,300 | 300 | 25–40 | 5.3–15.1 |
| Burgstaler and Pineda (2002) | 40 | | | 12.3–43.3 | 37.0<br>46.7 | | 60.5 × $10^6$<br>285 × $10^6$ | | 2.4 × $10^{11}$<br>3.2 × $10^{11}$ | 30<br>36 | | 10,000–29,000 | 300 | 33<br>41 | 21.9<br>27.8 |

(continued)

**Table 7.3** (continued)

| Source | Proc. N | Pt. N | Pre CD34⁺ cells/μL | Pre WBC $\times 10^9$/L | CD34⁺ cell CE1 % | CD34⁺ cell CE2 % | CD34⁺ cell $\times 10^6$/kg or $\times 10^6$ | PLT CE % | PLT loss % or $\times 10^{11}$ | RBC mL | Product volume mL | WB processed mL/TBV | Time min | Gran % | Gran $\times 10^9$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPECTRA OPTIA MNC** | | | | | | | | | | | | | | | |
| Sputtek et al. (2013) | 36 | 15 | | | 71.1 ± 12.0 | | $378 + 404 \times 10^6$ | | $2.6 \pm 1.5 \times 10^{11}$ | | | 9800 ± 1600 | 244 ± 15 | | |
| Burgstaler and Winters (2015) | 80 | | 32–55 | 48–51 | | 50.3–59.2 | $265–379 \times 10^6$ | 17.3–21.2 | 32.2–40.6% | 8–11 | | 12,500–20,700 | 300 | 18–28 | 10.5–19.3 |
| Burgstaler and Winters (2016) | 20 | | 41.0 | 49.9 | | 49.0 | $379.3 \times 10^6$ | 14.5 (CE2) | $4.1 \times 10^{11}$ | 11 | 382 | 20,700 | 300 | 28 | 19.1 |
| Brauninger et al. (2012) | 50 | | 103.6 ± 7.4 | | 68 ± 0.02 | 51 ± 0.01 | 9.4 ± 0.6 | | Approx. 5.4% | 388 ± 9 | | | | 38 | |
| Brauninger et al. (2011) | 30 | | 100.2 ± 8.9 | | 41.1 ± 0.7 | | 8.0 ± 0.7 | | 37.2 ± 1.4% | | 270.8 | 14,100 | | 39.2 | |
| Karafin et al. (2014) | 30 | | | | 77 (43–111) | 65 (12–173) | | | | | | | | 15 (0–48) | |
| Reinhardt et al. (2011) | 35 | | | | 47 ± 1.9 | | | | 36.9 ± 1.5% | | 233.6± 13.3 | 2.8 | | | |
| Lisenko et al. (2017) | 98 | 78 | 6–433 | 3–80 | | 7–116 | 0.5–40.4 | | 1–63% | | | ≤4 TBV | ≤300 | | |
| **SPECTRA OPTIA CMNC** | | | | | | | | | | | | | | | |
| Lisenko et al. (2017) | 94 | 72 | 4–531 | 5–113 | | 8–87 | 0.3–237 | | 0.57% | | | ≤4 TBV | ≤300 | | |
| Lozano et al. (2014) | 10 | 9 | 40.2<br>12.0–252.9 | | 60.7<br>40.5–90.1 | | | | | | 191<br>146–257 | 3.3<br>1.7–4.7 (TBV) | 210<br>111–293 | | |
| Gumogda et al. (2015) | 20 | 20 | | | | 50.2 ± 11.2 | | | | | | 2.5 (TBV) | | | |
| Lamb and Stevens (2015) | 40 | 25 | 39.2<br>7–446 | 35.9<br>4.0–69.6 | | 52<br>29–114 | 2.5<br>0.45–27.4 | | | | 268<br>114–389 | | | | |
| Watts et al. (2015) | 50 | 41 | | | | 54<br>50–62<br>44<br>41–47<br>43<br>38–52 | | | | | 192<br>191–194<br>156<br>152–157<br>112<br>111–113 | | 200 | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marculescu et al. (2015) | 10 | 10 | | | | 47.4<br>34.4–56.5 | 7<br>2.8–8.1 | 12.8<br>(CE1) | | 3.7<br>1.4–6.5 | 9256–18,052 | | | | |
| Toney et al. (2015) | | 11 | 30 ± 5 or<br>421 ± 94 | 38.6 ± 4.9 | | 47.5 ± 4.6<br>36.2 ± 5.6 | 8.75 ± 2.11 | (CE1) | 16.7 ± 3.4% | | 164.4 ± 0.02 | 8090 ± 900 | | | |
| Sanderson et al. (2017) | 39 | 23 | | | | | 2.92 | | 27.3% | 4.4%<br>HCT | 220 | | 239 | | |
| Burgstaler and Winters (2017) | 34<br>63 | | | 41.8<br>51.6 | | | 4.79<br>3.38 | 14.6<br>(CE2)<br>13.4<br>(CE2) | | 7<br>6 | 340<br>339 | 23,300<br>22,300 | 300<br>300 | 13<br>8.9 | 11.1<br>5.9 |
| Burgstaler and Winters (2016) | 20 | | | 45.5 | | 57.1 | $363.0 \times 10^6$ | 14.5<br>(CE2) | 31% | 13 | 340 | 23,900 | 300 | 12 | 9.4 |
| Schmidt et al. (2015) | 17 | | 86.2 ± 47.6 | 43.1 ± 11.2 | | | | | $110 \pm 27 \times 10^9$/L | | 277 ± 30 | | | | |

*Empty cells* no data reported, *Proc* number of procedures, *Pt* number of patients, *WBC* white blood cell count, *CE* collection efficiency, *PLT* platelet, *RBC* red blood cell, *WB* whole blood, *Gran* granulocyte

$$\mathrm{CE1} = \frac{\mathrm{CD34+\,cell/\mu L\,product} \times \mathrm{PV}}{\frac{\mathrm{CD34+\,cell/\mu L\,pre} + \mathrm{CD34+\,cell/\mu L\,post}}{2}} \times \mathrm{TVP}$$

$$\mathrm{CE2} = \frac{\mathrm{CD34+\,cell/\mu L\,product} \times \mathrm{PV}}{\mathrm{CD34+\,cell/\mu L\,pre} \times \mathrm{TVP}}$$

where *PV* product volume, *pre* pre-apheresis, *post* post-apheresis, and *TVP* total volume processed

**Table 7.4** Therapeutic cell product content and collection procedure parameters reported in the medical literature

| Source | Device (# Procedures) | Lymphocyte CE1 % or $\times 10^9$ | Monocyte CE1 | MNC CE1 % or $\times 10^9$ | MNC Purity % | Platelet CE1 % | Volume mL | Throughput MNC $\times 10^6$/mL |
|---|---|---|---|---|---|---|---|---|
| Robitzsch et al. (2015) | AM (40) | 57 ± 19 | 39 ± 16 | 52 ± 16 | 96 ± 3 | | | 53 ± 25 |
| Robitzsch et al. (2015) | SP CMNC (20) | 56 ± 17 | 65 ± 15 | 64 ± 11 | 90 ± 7 | | | 69 ± 34 |
| Robitzsch et al. (2015) | SP MNC (31) | 54 ± 19 | 61 ± 21 | 55 ± 17 | 92 ± 7 | | | 57 ± 22 |
| Steininger et al. (2014) | AM (12) | 1.20 ± 0.37<br>2.80 ± 1.1<br>$\times 10^9$ | | | | | | |
| Steininger et al. (2014) | SP MNC (20) | 1.64 ± 0.70<br>2.36 ± 0.96<br>$\times 10^9$ | | | | | | |
| Punzel et al. (2015) | SP CMNC (17) | 60 ± 13 | | | 85 ± 10 | | 176 ± 54 | |
| Punzel et al. (2015) | SP MNC (18) | 64 ± 15 | | | 87 ± 4 | | 238 ± 47 | |
| Fischer et al. (2013) | AM (40) | 57 | 18% | 51 | | 7 | | |
| Fischer et al. (2013) | SP MNC (10) | 64 | 33 | | | 24 | | |
| Burgstaler et al. (2005) | AM (20) | $9.6 \times 10^9$ | $3.3 \times 10^9$ | $13 \times 10^9$ | 98 | | 155 | |

*Empty cells* no data reported, *AM* amicus, *SP* Spectra Optia, *CMNC* continuous mononuclear cell, *MNC* mononuclear cell

$$\text{CE1} = \frac{\text{Cell count}/\mu\text{L product} \times \text{PV}}{\frac{\text{Cell count}/\mu\text{L pre} + \text{Cell count}/\mu\text{L post}}{2} \times \text{TVP}}$$

where *PV* product volume, *pre* pre-apheresis, *post* post-apheresis, and *TVP* total volume processed

type and extent of previous chemotherapy and radiation treatments, the marrow's ability to mobilize $CD34^+$ cells, the number of cells present in the circulation, the number of unwanted cells such as granulocytes, patient vascular access, the presence of high concentrations of plasma proteins such as seen in some plasma cell disorders, and other patient-specific issues can produce poor collection yields. The influence of these factors is unpredictable and fluctuates, changing over the course of the collections frequently in less than 24 h.

When investigating a suboptimal or poor collection, it is best to consider all of the components of collection rather than just $CD34^+$ cell or MNC yield. The MNC content should indicate if the collection was drawn from the proper layer**.** A product with a high percentage of MNC with a low percentage of granulocytes would indicate that the collection was appropriate. High granulocyte and RBC contents indicate the collection was too deep and that the buffy coat containing the desired cells was not harvested. A high platelet content and low total WBC and RBC contents

would indicate the collection was too high, missing the portion of the buffy coat containing the desired cells.

If a product collected on the *Amicus* has a low WBC content, this can occur due to either a thick layer of platelets or a blush of RBCs triggering the optical sensor in the *Amicus* which opens and closes the valves during the harvest (see previous description). The *Amicus* uses the amount of light detected by the optical sensor (sense level) to open *(MNC offset)* and close *(RBC offset)* the valve to the collection bag. When the amount of light blocked reaches the programed level (sense level), the harvest is initiated with a volume of blood pumped *(MNC offset)* until the valves to the collection bag open. This is then followed by a volume of blood *(RBC offset)* pumped into the collect bag and then the valve closes. Once triggered, further changes in the amount of blocked light during the harvest are ignored by the device. If a thick layer of platelets or an RBC blush passes through the collect line, it can trigger the optical sensor by blocking sufficient light to reach the *sense level* with the valve opening and closing too early resulting in a failure to harvest the desired cells. This will occur even if the platelets or RBC blush clears as once the *sense level* is reached, the offsets are triggered. Using the default MNC (2.3 mL) and RBC (6.8 mL) offsets on *Amicus* without observing the actual color of the collection can result in some very poor collections. The operator monitoring the first cycle (manual monitoring) and making adjustments in the *RBC offset* can produce more consistent yields and lower cross cellular content (Burgstaler and Winters 2011b). This relatively simple procedure requires the operator to observe the upper right pump cassette to ensure that the RBCs make it to the top of the cassette (Fig. 7.7). If this does not occur, then the *RBC offset* can be adjusted by the operator for subsequent cycles (Burgstaler and Winters 2011b).

### 7.7.1 Poor CD34⁺ Cell Yields

Obviously, high PB CD34⁺ cell counts are required for high CD34⁺ cell yields, but many patients do not mobilize well and therefore poor yields are not related to the actual collection procedure. It is thought that the CD34⁺ cells have a specific gravity similar to that of lymphocytes and monocytes; therefore, the goal during collection would be to target that layer of the buffy coat between the platelets and the granulocytes. The only real indication of the layer being harvested is the red color of the collected cells as they are being harvested. Some RBCs are needed in the product in order to collect an appropriate product, but too many or too few indicate that the buffy coat has not been appropriately collected. If the collection contains primarily platelets and lymphocytes, attempts should be made to go deeper (darker red) for the next collection. If the collection contains large numbers of granulocytes and RBC, going lighter in the RBC color should harvest the buffy coat. Unfortunately, at times there are collections with appropriate RBC, granulocytes, and good MNC yield, but poor CD34⁺ cell yields despite adequate numbers of circulating CD34⁺ cells. With the *Amicus machine*, if there are good yields of WBC, but poor yields of CD34⁺ cells, the operator should inspect the window on the spool holder for cracks. Cracks can affect CD34⁺ cell yields by interfering with the interface detector.

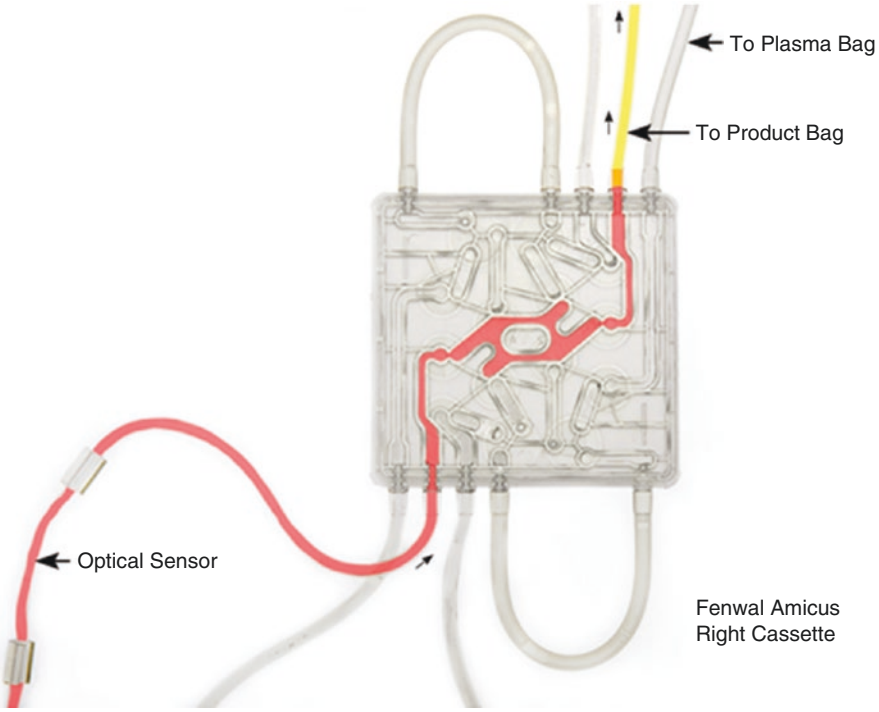


**Fig. 7.7** Amicus right cassette during MNC transfer. Red blood cells should be present to the top of the cassette, prior to plasma flush. (Used with permission of Wiley-Blackwell)

Another factor that can affect $CD34^+$ cell yield is the mean corpuscular volume (MCV) of the RBC. Small RBCs, such as those present in patients/donors with iron-deficient anemia (IDA), can migrate up into the buffy coat layer and be harvested with the MNC. If the operator uses the normal color indicator for these (with low MCV) collections, the product will appear to be sufficiently red, but when cell content is measured, they will have small numbers of $CD34^+$ cells and MNC. In order to correct this problem, the operator must target a darker than normal red color in the product in patients with concomitant IDA with low MCV.

Very lipemic plasma can also adversely affect the performance of the instrument optical sensors and operator's perception of red color. A darker red color should be sought to overcome the white color of the lipemic plasma.

### 7.7.2 Excessive Platelet Loss

The platelets have a specific gravity (1.040) close to that of MNC (lymphocytes 1.050–1.061 and monocytes 1.065–1.066). As a result, there will be platelets in the collection; however, the amount of platelets can be modified. The *Amicus* is more

platelet sparing than the *Spectra Optia* as seen in Table 7.3 (Sputtek et al. 2013). On the *Amicus*, a larger MNC offset will direct more of the platelets back to the donor. Using a high *MNC offset*, >2.3 mL, could, however, reduce the number of $CD34^+$ cells. It has been reported that using an *MNC offset* of 1.5 mL rather than the default setting of 2.3 mL can still achieve similar platelet loss and $CD34^+$ cell yields (Burgstaler et al. 2010). An unexpected finding from this study was that going too deep into the RBC layer also increased platelet loss on the *Amicus* (Burgstaler et al. 2010). Using a very light red color on the *Spectra Optia* can increase platelet loss.

High circulating WBC counts, as well as the combination of high WBC counts and high inlet flow rates, can adversely affect $CD34^+$ cell collection (Burgstaler et al. 2004; Burgstaler and Pineda 2002; Cooling et al. 2010). Therefore, in the presence of high WBC, inlet flow rates should be limited to increase centrifuge dwell time to allow for adequate separation and subsequent collection of the $CD34^+$ cells.

### 7.7.3 Excessive Granulocyte Content

Excessive granulocyte content is usually a result of harvesting too deep into the RBC layer (darker red color). When this occurs, using a lighter red color will decrease granulocyte content. However, even with the correct amount of RBC in the product, high granulocyte content may occur. This can be due to the high content of neutrophil precursors in the products due to the patient's/donor's response to mobilization. Immature, neutrophil precursors such as myelocytes (1.070) and promyelocytes (1.058–1.066) have a similar specific gravity as MNC (lymphocytes 1.050–1.061 and monocytes 1.065–1.066) and will be harvested with the MNC. Since the red color is the operator's only indication of the types of cells being collected, they are not able to prevent the collection of these immature/precursor neutrophils and a higher percent of granulocytes may be present in the collection in this setting.

### 7.7.4 Excessive RBC Content

Excessive RBC content usually indicates that the collect color was too dark. RBCs hemolyze during the freezing process, and usually large numbers of granulocytes in high RBC products are also collected due to the similar densities of granulocytes and RBC. Low red cell MCV is associated with poor $CD34^+$ cell and MNC yields (Cantilena et al. 2017; Panch et al. 2015; Wang et al. 2013; Leitman et al. 2010). If the red cell MCV is low, it may be necessary to go deeper into the RBC layer to get MNC and HPC (discussed above), but the collection will also contain increased numbers of RBC and granulocytes. For the *Terumo Optia*, an increase in the packing factor may help (seek the advice of the manufacturer). If graft appears to contain large volumes of RBCs, then one must consider evaluation of hematology analyzer used to determine the RBC

content of the product. Some analyzers, such as the *Beckman-Coulter ACT 10* (Beckman-Coulter Corp., Miami, FL), are adversely affected by large numbers of WBC when determining the MCV and hematocrit (Seigneurin and Passe 1983). These devices were, after all, designed to measure PB cell counts and not cell counts on highly concentrated cellular therapy products. We have found that by using the donor's peripheral MCV and the collection RBC count, we were able to correct the RBC volume with similar results reported by others for the same apheresis equipment when utilizing the *Beckman-Coulter ACT 10* to measure RBC content.

## 7.8 Conclusion

Both the *Amicus* and *Spectra Optia* are flexible, highly automated apheresis systems capable of collecting high-quality HPC(A) and TC products. Each device, and in the case of the *Spectra Optia* each protocol, collects cells in a slightly different manner resulting in differences in product content, and each is influenced in slightly different ways by patient/donor characteristics such as lipemia and WBC count. These differences can be utilized to tailor the collection to specific patient characteristics. In the absence of both devices being available, either device can be used to effectively collect cellular therapy products as long as procedures are adjusted for variables related to individual patient.

When troubleshooting poor collections, it is important not to focus solely on the $CD34^+$ cell or lymphocyte yields but also to consider the content of other cells including granulocytes, platelets, and red blood cells as their presence, or absence, provides important clues in optimizing subsequent collections (Table 7.5).

**Table 7.5** Clinical pearls

- *Amicus* and *Spectra Optia* are flexible, highly automated apheresis systems capable of collecting high-quality HPC(A) and TC products.
- The *Spectra Optia* was cleared by the FDA for the collection of TC and HPC(A) in 2012 using the MNC protocol and 2015 using the CMNC protocol.
- Etiology of unexpected hematopoietic graft yields and product content can result from patient/donor characteristics, operator errors, and equipment malfunction.
- The MNC content should indicate if the collection was drawn from the proper layer.
- High granulocyte and RBC contents indicate the collection was too deep and that the buffy coat containing the desired cells was not harvested.
- A high platelet content and low total WBC and RBC contents would indicate the collection was too high, missing the portion of the buffy coat containing the desired cells.
- The only real indication of the layer being harvested is the *red color* of the collected cells as they are being harvested.
- If graft appears to contain large volumes of RBCs, then one must consider evaluation of hematology analyzer used to determine the RBC content of the product. Hyperleukocytosis leads to an overestimation of the determination by Coulter Counter Model S of RBC count, hemoglobin, MCV, and packed cell volume.

## References

Brauninger S et al (2011) Mobilized allogeneic peripheral stem/progenitor cell apheresis with spectra Optia v. 5.0. a novel automatic interface controlled apheresis system: results from the first feasibility trial. Vox Sang 101:217–246

Brauninger S et al (2012) Allogeneic donor peripheral blood "stem cell" apheresis: prospective comparison of two apheresis systems. Transfusion 52:1137–1145

Burgstaler EA, Pineda AA (2002) The negative effect of high peripheral white blood cell count on CD34+ cell recovery. J Clin Apher 17:148

Burgstaler EA, Winters JL (2011a) The effects of shorter cycles to improve CD34+ cell yields in patients with elevated circulating CD34+ cell levels using the Fenwal amicus. Transfusion 51(Suppl):226A

Burgstaler EA, Winters JL (2011b) Manual color monitoring to optimize hematopoietic progenitor cell collection on the Fenwal amicus. J Clin Apher 26:123–130

Burgstaler EA, Winters JL (2011c) Comparison of hematopoietic progenitor cell (HPC) collections using the COBE spectra version 7 and amicus version 3.1 for patients with AL amyloidosis. J Clin Apher 26:186–194

Burgstaler EA, Winters JL (2014) The effects of adjusting the mononuclear cell sense level to improve automation of Fenwal amicus hematopoietic progenitor cell collections. J Clin Apher 29:1

Burgstaler EA, Winters JL (2015) Comparison of 3 anticoagulant techniques used during hematopoietic progenitor cell collection using the TerumoBCT spectra Optia. J Clin Apher 30:64

Burgstaler EA, Winters JL (2016) Comparison of hematopoietic progenitor cell (HPC) collection using the new spectra Optia continuous mononuclear cell (CMNC) collection program vs. the spectra Optia mononuclear cell (MNC) program. J Clin Apher 31:23

Burgstaler EA, Winters JL. Comparison of hematopoietic progenitor cell (HPC) collection using the spectra optia continuous mononuclear cell (CMNC) collections with a 26:1 anticoagulant ratio versus an anticoagulant ratio ramping technique. J Clin Apher 2017; publication pending

Burgstaler EA, Pineda AA, Winters JL (2004) Hematopoietic progenitor cell large volume leukapheresis (LVL) on the Fenwal amicus blood separator. J Clin Apher 19:103–111

Burgstaler EA, Winters JL, Pineda AA (2005) Mononuclear cell collection with the Fenwal amicus versus Fenwal CS3000 plus. J Clin Apher 20:57

Burgstaler EA, Porrata LF, Markovic SN, Winters JL (2010) Use of various offset settings in the fenwal amicus during hematopoietic progenitor cell collection to increase lymphocyte yield and reduce cross-cellular contamination. J Clin Apher 25:301–309

Burgstaler EA, Kreuter JD, Winters JL (2012) Plerixafor plus G-CSF mobilization results in similar CD34+ cells to total nucleated cells ratio and number of freezer bags as G-CSF only. J Clin Apher 27:1

Cantilena CC, Yau YY, West K, Klein HG (2017) Red cell mean corpuscular volume predicts mononuclear cell apheresis collection efficiency and yield #47. Special Issue Abstracts From the American Society for Apheresis 38th Annual Meeting May 3–6, 2017 Fort Lauderdale, Florida. J Clin Apher 32:71–142. https://doi.org/10.1002/jca.21537

Cooling L, Hoffman S, Herrst M, Muck C, Armelagos H, Davenport R (2010) A prospective randomized trial of two popular mononuclear cell collection sets for autologous peripheral blood stem cell collection in multiple myeloma. Transfusion 50:100–119

Fischer JC, Wenzel F, Rox JM (2013) Comparison of the apheresis devices amicus, spectra Optia, and COBE spectra in non-stimulated mononuclear cell collections. Transfus Med Hemother 40:75

Gumogda M et al (2015) Comparative study of the collection efficiency of continuous mononuclear cell collection (CMNC) spectra Optia apheresis system device versus amicus cell separator device in mononuclear cell collection (AMNC). Bone Marrow Transplant 50(Suppl 1):5349

Karafin RM et al (2014) Evaluation of the spectra Optia apheresis system for mononuclear cell (MNC) collection in G-CSF mobilized and nonmobilized healthy donors: results of a multicenter study. J Clin Apher 29(5):273–280

Lamb R, Stevens J (2015) Single center experience with the continuous mononuclear cell collection (CMNC) protocol on the spectra optia apheresis system. Bone Marrow Transplant 50(Suppl 1):S349

Leitman SF, Yau Y, Matthews CL, Hopkins JA, Min K (2010) Optimization of unstimulated mononuclear cell collection using the amicus continuous-flow apheresis device. Transfusion 50:15A

Lisenko K, Pavel P, Bruckner T, Puthenparambi J, Hundemer M, Schmitt A, Witzens-Harig M, Ho A, Wuchter P (2017) Comparison between intermittent and continuous spectra Optia leukapheresis systems for autologous peripheral blood stem cell collection. J Clin Apher 32:27–34

Lozano M et al (2014) Evaluation of white blood cell depletion kit in the spectra optia separator for CD34+ collection in mobilized patients. Bone Marrow Transplant 49(Suppl 1):5529

Marculescu A et al (2015) Stem cell apheresis using the spectra optia CMNC protocol-experience of Fundeni Clinical Institute, Bucharest, Romania. Bone Marrow Transplant 50(Suppl 1):S336–S337

Panch SR, Yau YY, Kang EM, DeRavin SS, Malech HL, Leitman SF (2015) Mobilization characteristics and strategies to improve hematopoietic progenitor cell mobilization and collection in patients with chronic granulomatous disease and severe combined immunodeficiency. Transfusion 55:265–274

Punzel M et al (2015) Feasibility and advantages of a novel continuous spectra optia apheresis system (CMNC system) to collect non-stimulated mononuclear cells (MNC) for cellular therapy. Bone Marrow Transplant 50(Suppl 1):S348

Reinhardt P et al (2011) Automatic interface-controlled apheresis collection of stem/progenitor cells: results from an autologous donor validation trial of a novel stem cell apheresis device. Transfusion 51:1321–1330

Robitzsch JT, Rox JM, Fischer JC (2015) Comparison of four apheresis systems (COBE spectra, spectra optia, spectra optia CMNC and amicus) for non-stimulated mononuclear cell collections. Bone Marrow Transplant 50:S344

Sanderson F, Poullin P, Smith R, Nicolino-Brunet C, Philip P, Chaib A, Costello R (2017) Peripheral blood stem cells collection on spectra optia apheresis system using the continuous mononuclear cell collection protocol: a single center report of 39 procedures. J Clin Apher 32(3):182–190. https://doi.org/10.1002/jca.21485

Schmidt JP, Grabowski C, Winterfeld S, Kroll H (2015) Comparison of three different cell separators for the collection of peripheral blood stem cells. Transfus Med Hemother 42:24

Seigneurin D, Passe D (1983) Interference of hyperleukocytosis on coulter model S blood counts: methods for correction. Biomed Pharmacother 37:401–404

Sputtek A, Schubert C, Peine S, Rowe AW (2013) Safe collection of peripheral blood stem cells in patients and donors using the amicus separator and the spectra Optia apheresis system. Vox Sang 105:294–295

Steininger PA, Strasser EF, Weiss D, Achenbach S, Zimmerman R, Eckstein R (2014) First comparative evaluation of a new leukapheresis technology in non-cytokine-stimulated donors. Vox Sang 106(3):248–255

Tarik K, Pascal L, Bouhya S (2013) Evaluation of the amicus separator for mononuclear cell collection. J Clin Apher 28:118–119

Toney I et al (2015) Stem cell apheresis using the new spectra Optia CMNC platform. Bone Marrow Transplant 50(Suppl 1):S343

Wang TF, Chen SH, Yang SH, Su YC, Chu SC, Li DK (2013) Poor harvest of peripheral blood stem cells in donors with microcytic red blood cells. Transfusion 53:91–95

Watts M et al (2015) Predetermined small volume HPC-A harvests for cryopreservation using the continuous mononuclear cell (CMNC) procedure of the Terumo Optia apheresis machine. Cytotherapy 17(6):S59