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Evaluation of Safety and Preliminary Effectiveness of Point-of-Care Cell Processing System: An Observational Study

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ABSTRACT

The potential of cell therapy in treating incurable and irreversible diseases has been proven to be safe and feasible with varying degrees of success in medical fraternity. In the past decade, there has been a steady increase in the development of advanced point-of-care cell processing technologies and intra-operative procedures that integrate autologous cell-based therapy with conventional surgical procedure in a single sitting, have emerged as an exciting approach in regenerative medicine and cell therapy field. Point-of-care (POC) cell processing devices are automatic, closed systems that allow rapid processing of whole tissue to the desired cell population at the patient's bedside, at an affordable cost. This observational study evaluates the safety and feasibility of a rapid point-of-care technology, the Res-Q™ 60 system used for processing bone marrow and/or peripheral blood intra-operatively for treating patients with various clinical indications who were administered the cell therapy product either as a standalone treatment or in conjunction with standard-of-care treatment. The data from 254 patients treated using either Bone Marrow Concentrate (BMC) or Platelet Rich Plasma (PRP) produced by Res-Q™ 60 devices was observed and analysed for device performance including cellularity, sterility and safety. The cellular output from Res-Q™ 60 BMC and PRP devices showed a significantly high MNC recovery of $71.35\% \pm 2.10$ (SD) and platelet recovery of $78.3\% \pm 3.0$ (SD), respectively. All the samples were sterile with no bacterial or fungal growth and all the patients tolerated the device output i.e. BMC or PRP well with no related adverse events (SAEs/AEs). Therefore, the Res-Q™ 60 BMC and PRP devices were found to be safe, feasible and preliminary effective for autologous cellular therapy at the point-of-care.

Keywords

Cell Therapy, Point-of-care, Bone marrow, Peripheral blood, Intra-operative, Minimal manipulation.

Introduction

Medicine and healthcare industry witnessed a technological revolution in the twentieth century through advanced instruments, information and communication technologies. This transformation has further revolutionized in the twenty-first century with smart cross- and trans- disciplinary technologies and innovations focused on improving medical practice, healthcare delivery and patient outcomes [1,2]. However, despite significant advances in

medical technology there has been paucity in the corresponding improvement in the quality of healthcare delivery. One approach to overcome these failures is to deliver healthcare that is safe, effective, patient centred, efficient and equitable at an affordable cost [3]. The growth of Point-of-care (POC) technology has dramatically changed the way physicians care for patients by enabling patient-centred care at reduced cost, especially in resource-limited settings and providing access to quality and timely medical care [1,2].

Point-of-care healthcare technology refers to portable medical devices used at or near the place of patient care often at the patient's bedside. Most POC devices are simple, can be used in

primary care settings and in remote areas without the requirement of a laboratory infrastructure [2]. The POC market includes a wide range of products diverse both in terms of application and technology, ranging from portable diagnostic and monitoring devices to therapeutic processing and treatment devices, used by healthcare professionals within and outside the traditional care setting. Advanced point-of-care cell processing technologies have emerged as an exciting approach in regenerative medicine and cell therapy field that allows rapid processing of whole (raw) tissue to desired cell population in short time duration at an affordable cost. They could be used for homologous (example, apheresis in haematology) and non-homologous (example, regenerative medicine) cell therapeutic applications [4].

Regenerative medicine offers hope for restoring or establishing normal function in damaged tissues or organs by replacing or regenerating these cells using exogenous cell sources that typically harness stem or progenitor cells [5]. In general, cell based therapies can be classified into two broad categories, that is, autologous therapies (those derived from patient's own cells) and allogenic therapies (those derived from donor cells) [6]. Over the last ten years, there has been a steady increase in the clinical development of autologous cellular therapies and intraoperative point-of-care procedures that integrate autologous cell-based therapies with conventional surgical interventions into a single procedure, have emerged as an important and exciting approach that can potentially treat many unmet medical conditions [5,6].

Clinical use of cell based therapies has been limited due to its requirement for expensive infrastructure i.e. GMP/GTP compliant laboratory; skilled technical staff, stringent regulatory concerns along with high cost are major obstacles [7]. However, with the development of advanced automated point-of-care cell processing devices for isolating and concentrating the desired cell therapy product in a single operative procedure within few hours, which takes it over the 'minimal manipulation threshold' has helped overcome most of these challenges [4,5,7]. Autologous intra-operative cell based therapies are beneficial as they do not trigger an immune response are safe and feasible using patient's own cells, avoid in vitro cell manipulation and costly cell expansion. The final output cell therapy product is likely to be effective due to the presence of progenitor cells, cytokines and growth factors in relative abundance. Additionally, the point-of-care approach avoids the need for a second patient procedure (at a different time point) for delivering the output cell therapy product [5].

Emergence of cell-based therapies, though a relatively new concept, has provided a new hope for treating various incurable or irreversible clinical ailments and the pace of advancement clearly reveals its significant role in the near future. Intra-operative cell processing, using autologous and minimally manipulated cells have been shown to be safe and feasible for treating various clinical indications such as early stage Avascular Necrosis (AVN), atrophic Non-Union Fractures (NUF), Non-Healing Ulcers (NHU), and Peripheral Arterial Disease (PAD), among many others. The primary goal of the treating physicians is to restore the structure

and function of the damaged tissues and organs. The point-of-care cell processing approach typically involves bone marrow or peripheral blood harvesting with subsequent processing using a point-of-care (POC) device at the patient's bedside (in the cases reported herein to be the Res-Q™ 60 system) to obtain the desired autologous cell therapy product followed by delivery at the site of injury. The Res-Q™ 60 system is a closed, sterile, single use point-of-care system for concentrating Bone Marrow or Peripheral Blood to a stem-cell rich buffy coat in a rapid, simple and reliable manner within 15 minutes at the patient's bed side. This system provides consistently high yield in terms of cellularity and cell viability, with low haematocrit content.

Importantly, the US Food and Drug Administration (FDA), the European Medicines Agency, and other regulatory authorities generally consider adult cell products as biological products that can be divided into two categories: minimally manipulated biological products (e.g., autologous blood products, including platelet-rich plasma or platelet concentrate, and autologous bone marrow cell concentrate), and manipulated biological products such as culture-expanded Mesenchymal Stem Cells (MSCs) [5]. The Res-Q™ 60 intra-operative cell processing approach reported here fits under the minimally manipulated biological product category.

The primary focus of this report is to evaluate the safety and feasibility of Res-Q™ 60 rapid point-of-care devices used for processing bone marrow or peripheral blood and treating various clinical indications by analysing the device performance based on output product cellularity, sterility and safety.

Materials and Methods

Res-Q™ 60 system (Thermogenesis Corp., USA) is a rapid, automated, cell capturing system designed to concentrate bone marrow mononuclear cells or platelet rich plasma from bone marrow aspirate or peripheral blood respectively, within 15 minutes at the patient's bedside. The patented system maximizes the percentage yield of Total nucleated cells (TNC) and Mononuclear cells (MNC), and/or platelets at the POC, while reducing sample volume by up to 20x. The ancillary equipment consists of a Res-Q™ 60 sterile disposable device, a portable centrifuge unit and a semi-automated processing tray.

Each sterile disposable device can accommodate a capacity ranging from 30-60 mL and segregate the desired cell population from whole (raw) tissue based on the principle of density gradient centrifugation. The Res-Q™ 3400 Centrifuge is a portable, 4 chamber density gradient centrifuge that processes the samples at 3200 rpm for 12 minutes. During the centrifugation step, cells are separated into three distinct layers, red blood cells (RBCs) settle at the bottom of the device, while the platelet poor plasma is visible as the top most layer above the capturing funnel. The buffy coat consisting of TNCs and platelets suspended in plasma gets collected into the capturing funnel that can be harvested directly through the harvesting port. The processing tray creates a magnetic field, which helps in preparing uniform suspension of

cells or platelet concentrate having visual indicators to notify the user when to harvest the cells by a defined volume. Figure 2 depicts the diagrammatical representation of the cell processing procedure using Res-Q™ 60 devices. Res-Q™ 60 System is a US FDA 510(k) cleared device and is CE marked (CE 0197) class I Exempt Medical Device indicating that the product is in compliance with the essential health and safety requirements of all directives that apply to the product. This System has also been registered with the Central Drugs Standard Control Organization of India (CDSCO) (License #MD-826) allowing its commercial use.

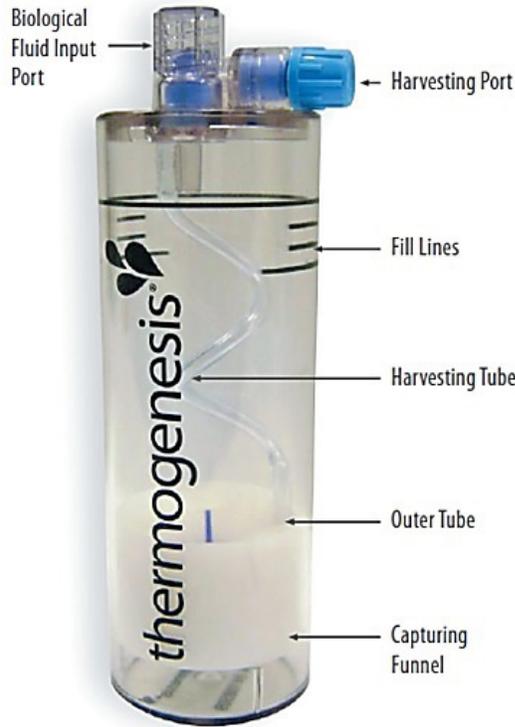


Figure 1: Res-Q™ 60 Sterile Disposable Processing Device.



Figure 2: Cell Processing Procedure using the Res-Q™ 60 POC device.

In a retrospective observational study, we included 254 patients with various clinical indications who were treated with autologous cellular therapy i.e. Bone Marrow Concentrate (BMC) produced by Res-Q™ 60 BMC system or Platelet Rich Plasma (PRP) produced

using the variant device, Res-Q™ 60 PRP system. BMC or PRP product has been administered either as a standalone treatment or in conjunction with standard-of-care treatment. These treatments were carried out between October 2010 and May, 2016, and the average age of the treated patients was 46 years (12-87 years). The study includes 69 femoral head Avascular Necrosis, 59 atrophic non-union fractures, 36 Critical Limb Ischemia, 66 Osteoarthritis and 24 Non-healing ulcer patients. Informed consent was obtained from all the participants prior to treatment and ethical approvals were taken (TIEC/2010/30/04, TIEC20123919).

Preparation of autologous BMC and PRP at the Point-of-Care

Autologous BMC preparation procedure was carried out under conscious sedation and strict aseptic conditions in the operating suite at the patient’s bedside. A total volume of 40-120 mL of bone marrow was aspirated from multiple sites from the posterior superior iliac crest in syringes containing anti-coagulant (either heparin or ACD-A) under local anesthesia using an 11 gauge trocar (Jamshidi) needle. A small aliquot of 1 mL was collected from the pooled aspirated bone marrow and analyzed for cell counts and sterility.

The harvested bone marrow was processed and concentrated using the Res-Q™ 60 BMC system intraoperatively at the point-of-care. The harvested bone marrow was transferred aseptically into Res-Q™ 60 BMC device(s) (capacity of each disposable device is max. 60 mL) using a clot filter to remove any large particulate matter, such as clots, fat and bone chips. The processing was carried out in the operating room at the patient’s bed side and 7-24 mL (based on the indication treated and volume of bone marrow harvested) of BMC enriched in progenitor cells was collected from the device. Aliquot (1 mL) of BMC was collected from the post-processed sample and later analyzed for cell counts and sterility. The remaining volume of autologous bone marrow concentrate (aBMC) was administered appropriately at the diseased site based on the clinical indication being treated.

Autologous PRP preparation procedure was carried out under aseptic conditions at the patient’s bedside, and 40-120 mL of peripheral blood was drawn into syringes containing anticoagulant (ACD-A) from patient’s anti-cubital vein using a 21-gauge needle. Blood and anticoagulant were thoroughly mixed before transferring to the Res-Q™ 60 PRP device, to prevent formation of blood clots, which in turn facilitates higher cell recoveries. 1 mL aliquot of pooled blood was collected and later analyzed for pre-processed cell counts and sterility. The aspirated whole blood was then processed using the Res-Q™ 60 PRP processing device at the patient’s bedside. The device works by separating peripheral blood into three distinct layers; Erythrocytes settle at the substratum, above that the plasma layer containing rich concentrate of platelets (PRP) and mononuclear cells, and platelet poor plasma (PPP) as the top layer. After centrifugation, 7-15 mL of PRP was harvested from the processing device using aseptic technique, of which 1 mL aliquot was analyzed for post-processed cell counts and sterility. The remaining volume of PRP was appropriately administered at the diseased site.

Mode of Administration

The cell concentrate prepared using the Res-Q™ 60 BMC and PRP devices were administered through different routes depending upon type of clinical indication as summarized in Table 1.

Indication	Mode of Administration
Non-Union Fracture	Percutaneous/Intra-osseous
Avascular Necrosis	Intra-osseous
Critical Limb Ischemia	Intra-muscular
Osteoarthritis	Intra-articular
Non Healing Ulcers	Superficial/Subcutaneous

Table 1: Mode of Administration of the Cell Concentrates for the clinical indications treated.

Res-Q™ 60 Device Performance

For every patient treated using the Res-Q™ 60 technology, pre- and post- processed samples were collected to determine the device performance by evaluating cellularity and sterility. The cell counts were analysed using a hematological cell coulter to evaluate the cellular performance of the device in terms of cell recoveries, and cell viability was evaluated using trypan blue dye exclusion method. Microbiological Sterility of the device output product was performed using the BD BACTEC system.

Safety Analysis

Safety analysis of the Res-Q™ device were performed by assessing the number of adverse events (AE's) or serious adverse events (SAEs) classified by different reasons, and their relationship to the device cellular output in all patients post- BMC or PRP treatment.

Statistical Analysis

Descriptive analysis was carried out, clinical event rates are presented in number and percentage (%), and continuous variables are presented as mean ± standard deviation. Statistical significance among treatments was determined using one-way analysis of variance (ANOVA). All reported adverse events (AEs) or severe adverse events (SAEs) are summarized using number and percentage (%). All statistical tests were done using two-sided, 0.05 level of significance.

Results

The Res-Q™ 60 BMC and PRP devices have been used in the treatment of various clinical indications in 254 patients at the point-of-care (Figure 3). Bone marrow processing using Res-Q™ 60 BMC device were performed in 164 patients, out of which 42% (n=69) were suffering from early stage avascular necrosis, 36% (n=59) were suffering from atrophic non-union fracture and 22% (n=36) had critical limb ischemia. While peripheral blood processing to extract Platelet Rich Plasma using the Res-Q™ 60 PRP device were performed in 90 patients, wherein 73% (n=66) had musculoskeletal disorder (osteoarthritis) and 27% (n=24) were being treated for non-healing ulcers of different aetiologies.

For all the patients treated using the Res-Q™ 60 POC technology, pre- and post- Bone marrow or Peripheral blood samples were

analysed for cell counts, percentage cell recoveries, average cellular fold increase and final cell dose administered into patients for evaluating the device performance and effectiveness of the device. Analysis of the bone marrow samples showed consistent MNC recoveries with an average of $71.35\% \pm 2.10$ (SD) with 5-6 fold increase in post-processed MNC counts compared to pre-processed MNC. The analysis of PRP samples showed an average platelet recovery of $78.3\% \pm 3.0$ (SD), with 4-5 fold increase in platelet counts in the final concentrated PRP. The average bone marrow mononuclear cells (BMMNC) and PRP dose administered were 18.34×10^7 and 757.89×10^7 , respectively. Figure 4 summarizes the percent cell recoveries (%) in the BMC and PRP samples used for treating various clinical indications.

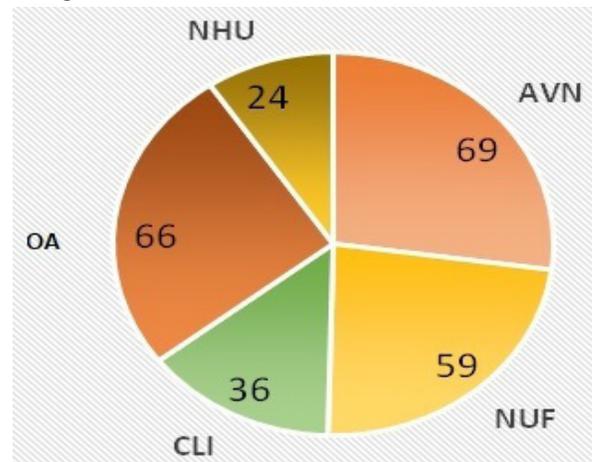


Figure 3: Clinical Indications treated using the Res-Q™ 60 POC devices.

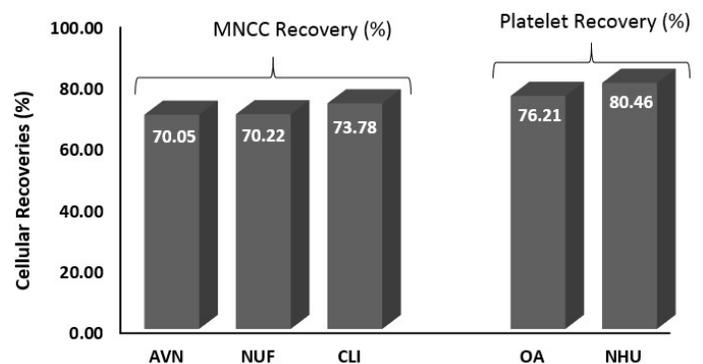


Figure 4: Percentage Cellular Recoveries in BMC and PRP Samples.

The microbiological analysis of the samples prepared using the Res-Q™ 60 devices showed no microbial growth on BD BACTEC system at day 5 incubation, indicating BMC or PRP samples administered into patients were 'sterile' and potentially free from bacteria or fungi in all 254 samples when processed through rapid, single use, closed Res-Q™ devices. The chemical free closed system maintained sterility throughout the intra-operative procedure of cell purification or concentration.

Bone marrow cell concentrate was used for treating a number of orthopaedic indications (homologous use) including atrophic NUF and AVN of femoral head, and in various phases of clinical trials for non-homologous indications like CLI. Additionally,

PRP was used for treating vascular indications (homologous use) which included NHU and Diabetic Foot Ulcers (DFU), and non-homologous use for musculoskeletal disorders like OA. Table 2 summarizes the patient demographics for the indications treated using BMC and PRP.

Indication Treated	No. of Patients	Average Age \pm SD	Male (%)	Female (%)
AVN	69	36.21 \pm 12.6	79.71	20.29
NUF	59	41.32 \pm 16.37	71.19	28.81
CLI	36	52.02 \pm 14.43	86.11	13.89
OA	66	50.28 \pm 15.51	34.85	65.15
NHU	24	62.83 \pm 14.04	66.67	33.33

Table 2: Summary of the indications treated and patient demographics.

A total of 69 patients suffering from early stage AVN of femoral heads were treated using autologous bone marrow concentrate prepared by Res-Q™ 60 BMC device following core decompression, which is a standard of care medical procedure for treating AVN. Out of the 69 patients, 55 (79.71%) were males and 14 (20.29%) females having a mean age of 36.21 \pm 12.6 years. The mean total nucleated and mono-nucleated cell count in post-processed bone marrow sample was 66.29x10³/μl and 21.35x10³/μl respectively, which was almost 5 fold higher than the cell counts in pre-processed sample. An average of 70% MNC recovery was obtained and average MNC dose administered per patient was 18.75x10⁷. Also, patients tolerated the procedure well with no related SAEs or AEs. However, 4 patients had pain at the site of bone marrow aspiration and hematoma at the site of core decompression which were unrelated expected procedural events, each of these resolved quickly with concomitant medications. BMC treatment showed positive outcomes in the patients with reduction in pain and joint symptoms.

Among the 59 patients who had a persistent atrophic non-union fracture for more than 8 months following primary open reduction internal fixation (ORIF) of the bone, treatment included autologous bone marrow cell concentrate (prepared using Res-Q 60 BMC device) implantation either percutaneously or in combination with readily available synthetic graft (tri-calcium phosphate) at the fracture site. The mean age of the treated patients was 41.32 \pm 16.37years, which included 42 (71.19%) male and 17 (28.81%) female patients. All the bone marrow samples showed a significant increase in the post-processed TNC and MNC counts, which were 74.06x10³/μl and 24.37x10³/μl respectively. The post processed cell concentrate (BMC) contained an average MNC dose of 17.66x10⁷ cells and the mean MNC fold increase was 5.92 \pm 2.29. No treatment related SAEs/AEs were observed, however 2 (3.39%) patients developed swelling at local injection site and pain at site of bone marrow aspiration which were considered expected unrelated events that were resolved spontaneously; while bone union was observed in approximately 80% of the patients.

The safety and preliminary effectiveness of autologous BMC in treating “poor option” CLI patients were assessed in a non-

randomized, open label feasibility study and under compassionate use, where 36 patients who had an underlying aetiology due to atherosclerotic arterial occlusive disease or Thromboangitis Obliterans (TAO) or Buerger’s Disease were treated. All the patients were administered with single dose of intramuscular injections of autologous BMC in their afflicted ischemic limbs. The mean age of the patients were 52.02 \pm 14.43 years, where out of 36 patients 31 (86.11%) were males and 5 (13.89%) were females. The mean total nucleated cell (TNC) count and mono-nucleated cell (MNC) count in the bone marrow samples processed using Res-Q™ 60 BMC devices were 36.97x10³/μl and 11.62x10³/μl respectively. The mean MNC dose administered into the ischemic limb of the treated patients was 18.62x10⁷ cells, where an average 4.49 \pm 1.54 fold increases was observed in the post-MNC counts. Out of 36 patients treated, there were no related SAE/AEs reported. However, expected unrelated adverse events were reported in eight (22.22%) patients, where four (11.11%) patients underwent major limb amputation (above the ankle) which were expected outcome of the disease progression, two (5.55%) patients underwent minor amputation (digit/s), and two (5.55%) unrelated deaths were reported at 12 month follow-up. All the reported adverse events were treated as serious adverse events and were testified as expected unrelated AEs/SAEs.

A total of 66 patients with musculoskeletal disorder like knee osteoarthritis (OA), and aged between 18 to 80 years having a mean age of 50.28 \pm 15.51 years were treated with activated autologous PRP injections prepared using the Res-Q™ 60 PRP device. Out of the 66 patients, 23 (34.85%) were males and 43 (65.15%) were females. No related SAEs or AEs were observed during the treatment and follow-up period, only minor expected adverse events (AEs) were reported in 3 (4.54%) patients that were not related to PRP treatment. Minor expected unrelated AEs includes mild pain and swelling at injection site. The mean platelet counts increased from 222.56x10³/μL to 847.27x10³/μL in the post processed PRP sample and the mean platelet dose injected was 814.78x10⁷. The 4 fold increase in the platelet count was observed in the final PRP product with significant reduction in RBC. PRP treatment showed positive effects in patients with diminishing pain, improved symptoms and quality of life.

Further, twenty-four (24) patients, each having single wound/ulcer of varying etiology were treated with single dose of subcutaneous PRP injections around the wound periphery and topical administration of autologous platelet gel (prepared by combining PRP with thrombin and calcium chloride). Among the included patients, 16 (66.6%) were males and 8 (33.33%) were females with an average age of 62.5 \pm 13.53 years. Among the ulcers treated, there were 10 (41.67%) venous ulcers, 9 (37.5%) diabetic ulcers, 3 (12.5%) arterial ulcers and 2 (8.33%) pressure ulcers. PRP processing and administration was accomplished at the patient’s bedside in a single sitting using the Res-Q™ 60 PRP system. Patients tolerated the procedure well, and no adverse or serious adverse events were reported. The mean platelet counts increased from 261.91x10³/μL to 1177.35x10³/μL in the post-processed sample and the mean platelet dose administered to the

patients were 701.01×10^7 . Almost 5 fold increase in the platelet counts were observed in the final PRP product, which was statistically significant ($p < 0.01$) when compared to pre-processed PRP. All the patients showed signs of wound/ulcer healing with significant reduction in wound size and reduction in mean healing time to 8.2 ± 1.9 weeks.

The comparison of the cellular output between pre- and post-processed BMC and PRP samples are given in Figures 5(a), 5(b) and 6 respectively ($***p < 0.001$ compared to pre-processed counts).

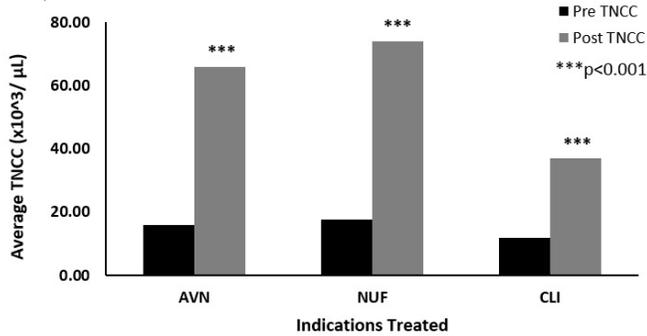


Figure 5(a): Average TNC counts in Bone marrow samples for different indications ($***p < 0.001$ as compared to pre-counts).

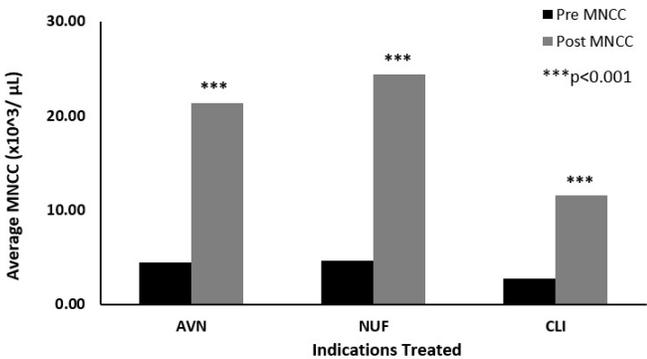


Figure 5 (b): Average MNC counts in Bone marrow samples for different indications ($***p < 0.001$ as compared to pre-counts).

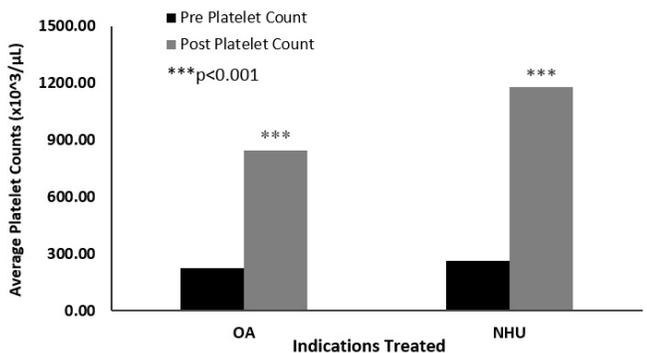


Figure 6: Average platelet counts in PRP sample for different indications ($***p < 0.001$ as compared to pre-counts).

Overall safety analysis following autologous BMC or PRP administration indicated that there are no treatment related SAEs or AEs. However 4% of the 254 treated patients exhibited mild adverse events (AEs) that included pain, headache, nausea,

hypertension and swelling at injection site, while 3% exhibited severe adverse events (SAEs) which included major and minor amputations and death. Unlike death, which was an unexpected SAE, all others are classified as expected adverse events of the respective treatment and are unrelated to the cellular output product. All AEs experienced were mild in nature that lasted for not more than 48 hours and were easily managed using concomitant medications.

Table 3 summarizes the clinical indications treated, the cellular output characterization from Res-Q™ 60 POC devices and adverse events reported.

Discussion

The objective of this report is to present an analysis of the safety and feasibility of the post-marketed Res-Q™ 60 intra-operative, point-of-care devices that aid in processing and concentrating bone marrow or peripheral blood with minimal manipulation. In particular, analysis of the data showed no complications such as excessive new bone formation, infections, tumour induction or morbidity at the aspiration site associated with processing and delivery of the device output product. The automated closed system of Res-Q™ 60 technology used for processing the cellular output avoids or minimizes the risk of infection and helps deliver high cell dose with consistently high cell recoveries as shown in figure 4. Furthermore, the described treatment technique using autologous cell therapy is a minimally invasive procedure resulting in shorter operating time and reduction in the incidence rates of associated co-morbidities and lower cost.

Autologous bone grafting is considered as the gold standard approach in the clinical setting, in order to harness bone's natural regenerative capacity when a bone defect occurs. Treatment of bone defects using autogenous bone graft is not something new as it has been practiced since several years by orthopaedic surgeons. The initial intra-operative cell therapies to promote osteogenic regeneration utilized whole bone marrow injections without concentration. However, most of these therapeutic approaches failed due to requirement of large volumes of bone marrow for successful treatment [7]. In the late 1980s, Connolly et al. proposed a method of concentrating bone marrow nucleated cells by centrifugation that enhanced the osteogenic potential of injected bone marrow cells resulting in improved regeneration as assessed radiographically [8]. The initial studies had shown beneficial effects on a small patient population of non-union and avascular necrosis of the femoral head. However, the traditional cell concentration method had several limitations including the requirement of a Good Manufacturing Practice (GMP) facility for processing the bone marrow samples that increased the cost of the procedure and a second surgical procedure for delivering the cellular therapy. In addition, there is very little clarity on the extent of the laboratory procedure leading to a change in the biological property of the injected progenitor cells and the related risk associated with patient safety. However, with the development of rapid automated intra-operative cell processing devices, processing and concentration of bone marrow cells could be achieved at the patient's bedside with

Clinical Indication	Mode of Administration	Microbiological Sterility Test*	Device Cellular Performance			AEs/SAEs **		
			MNCC/ Platelet Fold Increase \pm SD	Cell Recovery (%)	Numbers reported (n)	Severity	Relatedness	Probability
Avascular Necrosis	Intra-osseous	Sterile	5.86 \pm 4.61	70.05	4	Mild	Not related	Expected
Non-Union Fracture	Percutaneous/ Intra-osseous	Sterile	5.92 \pm 2.29	70.22	2	Mild	Not related	Expected
Critical Limb Ischemia	Intra-muscular	Sterile	4.49 \pm 1.54	73.78	8	Severe	Not related	6 expected & 2 unexpected
Osteoarthritis	Intra-articular	Sterile	3.77 \pm 1.50	76.21	3	Mild	Not related	Expected
Non-Healing Ulcer	Superficial/ Subcutaneous	Sterile	4.34 \pm 1.41	80.46	0	NA	NA	NA

Table 3: Summary of the Clinical Indications Treated, cellular output characterization and adverse events. *Sterile - No Growth, Non-Sterile - Growth; ** AE - Adverse Event, SAE - Serious Adverse Event.

high cell recoveries and minimal risks to patients.

In a review article by Edgar and Einhorn (2011), it has been mentioned that the use of intra-operatively processed autologous bone marrow concentrate for the treatment of Avascular Necrosis (AVN) has led to a symptomatic relief of pain in the patients and also prevented the collapse of the femoral head. They also emphasized that concentrated bone marrow has a heterogeneous mixture of various cell types like hematopoietic stem cells, mesenchymal stem cells and other stromal cells, therefore the cell numbers may be critical for the therapeutic effect essential for the regeneration of bone in orthopedic lesions and promote further angiogenesis [9]. Hwang et al. utilized the COBE®2991 cell separator for concentrating bone marrow of 43 patients with early stage osteonecrosis of the femoral head. The average number of TNCCs obtained after processing bone marrow was $137 \times 10^3/\mu\text{L}$, with 4.9% monocytes and 13.8% lymphocytes on an average. The patients did not experience any side effects and results from a two year follow up period showed that the disease did not progress to later stages [10]. Similarly, in a prospective study by Hernigou et al. (2002) on 116 patients with a vascular necrosis of the femoral head, same cell separation technology was used and it was observed that the mean cell dose administered was 16.4 million cells and the average total number of colony-forming units injected was estimated to be 25×10^3 cells. Also, minor complications were observed which included pneumonia and pain at site of aspiration and delivery [11]. In a controlled study by Gangji et al. 13 patients (18 hips) with necrosis of the femoral head were evaluated and a clear difference was observed between the treatment group (core decompression with BMC) and control group (only core decompression). The mean number of leukocytes injected in the treatment group was 20.3×10^7 cells and after twenty-four months the treatment group showed significant reduction in pain and joint symptoms. In addition, no major side effects were observed, only minor pain and hematoma formation in few patients that was controlled with concomitant medication [12-14]. Similar results have been observed in the AVN patients treated using Res-Q™ 60 BMC technology. The cell concentrate obtained using the Res-Q™ 60 devices had consistent mononuclear cell recovery of more than 70% and the mean mononuclear cell dose administered was 18.76×10^7 cells, and the average TNC counts obtained was $66.29 \times 10^3/\mu\text{L}$. Also, the results showed that autologous bone

marrow cell concentrate implantation was associated with only minor adverse events that resolved spontaneously.

Treatment of non-unions or delayed unions is a challenge, particularly in those patients who have failed multiple operative procedures. Percutaneous autologous bone marrow injections may be a good option for treatment of non-union fracture patients who have previously been treated with internal fixation. The BMC injection procedure has high success rate in appropriately selected patients as demonstrated in many studies. Hernigou et al. (2005) conducted a retrospective study in 60 tibial non-unions to evaluate the effect of progenitor cells present in autologous bone marrow concentrate, in bone healing for atrophic non-union fractures. Their study results indicated that the average nucleated cells present in the bone marrow concentrate were $51 \times 10^3/\mu\text{L}$ and bone union was observed in 53 patients out of the 60 treated, concluding that number of progenitor cells in the injected bone marrow play an important role in safely and effectively treating atrophic non-union fractures [15]. In a prospective study by Goel et al. results of bone marrow grafting in 20 tibial NUF patients were presented. Under local anesthesia, marrow concentrate containing an average of 18 million cells was injected into the non-union site and the process was repeated at 4-6 weeks if there was no radiological evidence of callus formation. The results revealed clinical and radiological bone union in 15 out of 20 patients (75%). There were no cases of infection following the injection, and no complications at the donor site, concluding this therapeutic procedure to be safe and feasible [16]. Another study conducted by Bhargava et al. on 28 atrophic non-union patients, showed accelerated healing of the non-union fracture in 23 patients following single infusion of BMC. Since no major complication was observed, the procedure was deemed as a safe and reliable alternative to traditional techniques of treating non unions [17]. Ponemone et al. conducted a safety and feasibility study on 17 patients with atrophic non-union fracture who were treated with percutaneous injections of bone marrow concentrate. Their results showed a mean TNC and MNC count of $5.54 \times 10^8 \pm 1.99$ and $1.64 \times 10^8 \pm 0.86$, respectively in post-processed bone marrow with 82% union rate, concluding a BMC administration to be safe and effective in treating patients with atrophic non-union [18]. We observed the data from 59 patients with atrophic non-union treated by either percutaneous injections or by combining autologous BMC with tri-calcium phosphate to form a semi-

solid 'putty' using the Res-Q™ 60 BMC intra-operative POC technology. The treated patients exhibited only mild adverse reactions, which included pain, inflammation at injection site and fever, that were controlled easily using concomitant medications. Furthermore, the device delivered a consistent cellular dose of average 17.66×10^7 cells with high MNC recoveries. Thus, the use of Res-Q™ 60 BMC device for concentrating bone marrow at the point-of-care appears to be a safe and feasible.

Bone marrow cell concentrate contains a heterogeneous population of endothelial progenitor cells, mesenchymal stem cells, and hematopoietic stem cells that stimulate angiogenesis and vasculogenesis that can be used to treat disorders of inadequate tissue perfusion. In the event of ischemia, oxygen delivery increases via a network of collateral vessels to stimulate angiogenesis, but this natural capability is impaired in CLI patients. Autologous bone marrow (BM)-derived progenitor cells have been identified as a potential new therapeutic option for CLI patients to induce therapeutic angiogenesis by formation of collateral vessels [19]. A comprehensive review by Campagna et al. showed that several studies have used cell therapy for no-option CLI patients and the results obtained have confirmed the beneficial effects of cell therapy in reducing the major amputation rate, improving distal perfusion, reducing rest pain and claudication pain, and overall improvement in the ischemic symptoms of CLI patients [20]. However, the cellular dose delivered plays a pivotal role in determining the treatment efficacy. Matoba and Matsubara (2009) also reported the beneficial effect of autologous bone marrow cell transplantation on therapeutic angiogenesis for CLI patients [21]. The therapeutic application of bone marrow cell concentrate harvested and processed intra-operatively for the treatment of CLI was first reported in 2002 by Tateishi et al. and their results showed significant improvement in ankle brachial index, rest pain and pain-free walking distance. Since then several studies have validated these results and shown the positive effect of intra-operative preparation of BMC and intra-muscular delivery in the ischemic limb for the treatment of CLI [22]. Benoit et al. (2011), evaluated randomized controlled trials involving bone marrow derived stem and progenitor cells ($n=295$) and found that the amputation rates between the control arms and treatment arms was statistically significant (25.4% vs.14.8% $p=0.02$) demonstrating that bone marrow derived cells do improve outcomes in CLI patients [23]. Furthermore, Wang et al. analysed 31 published Randomized Controlled Trials (RCTs) and non-RCTs, having a total of 1,214 patients, and advantageous effects of autologous bone-marrow cell therapy was reported, where majority of severe adverse events (SAEs) were associated with hospitalization for disease process-related complications and not related to cell therapy [24]. Ponemone et al. evaluated the safety and therapeutic effectiveness of intra-muscular injections of a BMC in 17 no-option CLI patients. Their results showed a significant improvement in ABI, TcPO₂, and rest pain with major amputation free survival rate of 70.6% [25]. Similar results were observed in the CLI patients treated with intra-muscular injections of BMC harvested and processed using the rapid point-of-care device, Res-Q™ 60 BMC system. The treatment significantly facilitated in the reduction

of major limb amputations and at 40 month follow-up, a 70.6% amputation free survival rate was observed in no-option CLI patients. Furthermore, our data suggested that the use of bone marrow-derived cell product could potentially increase limb perfusion and improve claudication symptoms of limb ischemia, by demonstrating a significant increase in ABI, TcPO₂ levels, and pain-free walking distance. The infused cell dose plays a pivotal role in the effectiveness of cellular therapy. The point-of-care device and technology (Res-Q™ 60 BMC system), used in our study for processing the cell concentrate demonstrated several advantages: reduced time, cost and labour-intensive procedure and was capable of delivering a consistent high cell dose of 18.62×10^7 cells.

Platelet rich plasma (PRP) is defined as a rich suspension of platelets in plasma derived from whole blood that has 2-6 fold higher platelet concentrate [26]. Platelets are a reservoir of proteins known as growth factors including platelet derived growth factor (PDGF), transforming growth factor- β (TGF- β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1), and fibroblastic growth factor (FGF). They also contain several cytokines and many other proteins [27]. When platelets come into contact with exposed endothelium within wounds or damaged tissues, these cytokines and growth factors are released that trigger biological effects such as chemotaxis, cell proliferation and differentiation, angiogenesis, extracellular matrix deposition, and remodelling which are key elements in the process of tissue repair and regeneration [28]. Hence, the concept of increasing platelet concentration by injecting PRP in an injured tissue was proposed so to increase the levels of multiple bioactive factors and, subsequently, improve the natural healing process [27,29].

Knee Osteoarthritis is a highly prevalent joint disease affecting the daily lives of millions of people and pain and limited function often become a chronic problem, hindering the day to day activity of the individual. Growth factors derived from platelets obtained from autologous blood have the capability to accelerate and improve healing, and the concept of intra-operative point-of-care delivery of PRP opens up a novel treatment option for this disease. Currently, many published studies support the safety, feasibility and efficacy of PRP therapy for degenerative knee conditions including Osteoarthritis. Sanchez et al. (2008) reported the preliminary results about the effectiveness of intra-articular injections of PRP for knee OA in an observational retrospective cohort study on 30 patients and suggested this approach as safe and feasible [30]. Wang-Saegusa et al. evaluated the treatment efficacy of PRP injections for knee OA in 261 patients and showed a significant improvement in clinical outcome in 73% of the patients at 6-month follow-up [31]. Furthermore, a pilot study conducted by Kon et al. on 100 patients treated with intra-articular injections of PRP reported evidence of safety, pain reduction and improved function [32]. Similar results have been observed in the 66 patients undergoing treatment for musculoskeletal disorders like OA using PRP prepared by the rapid intra-operative POC device Res-Q™ 60 PRP system. All the patients showed improvement

in the disease condition with reduction in pain and only minor adverse events were observed, making it a safe treatment modality. Also, the device were able to efficiently concentrate whole blood with almost 4-5 fold increase in the platelet concentration and an average platelet dose of 814.78×10^8 were injected.

Leg ulcers can be classified as acute or chronic depending on the duration they have persisted. Chronic wounds/ ulcers of different aetiologies come with significant cost and morbidity for the patient and their treatment is a topical issue of modern medicine [33,34]. PRP therapy is the preferable treatment option in patients with chronic non-healing wounds/ulcers of different aetiology, particularly when other more conventional therapies lack evidence of effectiveness. In 1986, Knighton et al. showed that the use of autologous platelet factors accelerated epithelialization of granulation tissue leading to complete repair of chronic non-healing ulcers. This was the first clinical study that demonstrated the promising role of locally acting factors derived from autologous blood in promoting healing of chronic cutaneous ulcers [35]. In the research article by Hong-Bum Park et al. it has been mentioned that the use of platelet rich plasma (PRP) for the treatment of skin wound healing or non-healing ulcers, has shown a considerable safety profile and no adverse effect has been reported [36]. The case series by Suthar et al., showed that a single dose of combination of intra-muscular injections of PRP and topical administration of PRP gel facilitated wound healing in all the 24 treated patients, with reduction in wound size and no side effects [37]. A study conducted by Frykberg et al. on 49 patients with 65 non-healing ulcers showed that 63 of 65 ulcers responded well to PRP therapy with a reduction in area, volume and undermining of the ulcers in a mean duration of 2.8 weeks with 3.2 treatments [38]. Driver et al. conducted a prospective, randomized controlled trial on 40 patients to evaluate the safety and efficacy of autologous platelet-rich plasma gel (treatment group) for the treatment of non-healing diabetic foot ulcers as compared to saline gel (control group). Their study results showed that significantly more wounds healed in patients treated with platelet-rich plasma gel (13 out of 16 or 81.3%) than patients treated with control gel (8 out of 19 or 42.1%). No treatment related serious adverse events were reported and the results showed significantly higher healing in the PRP gel group [39]. PRP prepared using the rapid point-of-care device, Res-Q™ 60 PRP system, was used for treating 24 patients with chronic non-healing ulcers of different aetiologies. The patients received single dose of PRP injections around the wound periphery and topical administration of autologous platelet gel. The device was capable of concentrating platelets almost five (5) fold higher as compared to baseline values in whole peripheral blood with minimal manipulation and no adverse or serious adverse events were reported.

POC cell therapy has several advantages such as rapid, operated at patient's bedside as a practice of medicine within single procedure, deals with autologous cells that could fit into minimal manipulation category, and has short regulatory path to approval, usually does not require IND/ clinical trials, GMP processing and Biological license application (BLA). These point-of-care devices which

allow rapid cell processing from whole (raw) tissue to desired cell populations significantly reduce the cost with low regulatory burden.

Conclusion

The Res-Q™ 60 BMC and PRP devices are sterile, single use, closed system devices safe for use in autologous cellular therapy. These devices are efficient and versatile tools for the preparation of Bone Marrow Concentrate or Platelet Rich Plasma intra-operatively. They are rapid, fit into minimal manipulation, have a low regulatory burden and are cost effective. The data obtained from treated 254 patients was used to analyze the device performance and results suggest that the Res-Q™ 60 devices were able to deliver the desired cell product with consistently high cell recoveries at the point-of-care for the treatment of the mentioned diseases, and no treatment related AEs/SAEs were reported. Therefore, the Res-Q™ 60 BMC and PRP devices were found to be safe, feasible and preliminary effective for autologous cellular therapy at the point-of-care.]

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Authors' Contributions

Kenneth Lee Harris, Mitchel Sivilotti and Venkatesh Ponemone contributed to the concept and design of this study. Suhail Bukhari and Harshavardhan Hegde contributed as study clinical investigators. Saniya Gupta, Manish Suthar, Venkatesh Ponemone and Dalip Sethi contributed in the executed of the study and data analysis. Saniya Gupta contributed in writing the manuscript, reviewed by Manish Suthar and Venkatesh Ponemone.

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