Original Article

# Removal of Hepatocarcinoma Cells From Blood via Cell Washing and Filtration Techniques

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## ABSTRACT

Utilization of autotransfusion during tumor resection remains controversial due to viability of carcinoma cells remaining in collected blood. The purpose of this study was to evaluate autotransfusion techniques combined with leukocyte depleting filters (LDF) for removal of hepatocarcinoma cells from autotransfusate.

An *in vitro* model was created by contaminating expired human erythrocytes with cultured hepatocarcinoma (HEP G2) cells. Autotransfusion devices evaluated were Cobe BRAT2, Sorin STAT-P, and Fresenius CATS. Autotransfusate collected from varying processing conditions were filtered using the Pall Leukoguard RS or Pall Purecell RCQ LDF. Carcinoma concentrations were quantified via Coulter Counter technology.

The CATS exhibited higher concentrations of cancer cells in the autotransfusate prior to washing, a 449% increase. This was significantly higher than either the BRAT2 or STAT-P, 350% and 315% respectively. Post washing HEP G2 concentrations in the BRAT2 were significantly higher than the STAT-P and CATS. Doubled wash volumes removed more HEP G2 cells in all trials, reaching statistical significance only in the CATS. LDF resulted in a significant 75% reduction of HEP G2 cells, with no difference between filters.

While combination use of autotransfusion devices and leukocyte depleting filters did result in a product with concentrated hematocrit, no technique removed all hepatocarcinoma cells.

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# INTRODUCTION

Hepatocellular carcinoma (HCC) is the eighth most common form of cancer worldwide, with over one million cases reported annually (1). Hepatic resection is the common intervention for HCC, with a mortality rate of less than 6%. However, liver resection is one of the most hemorrhagic elective abdominal procedures, with reported transfusion rates of 29 to 98%. Average operative blood loss has been reported at 1872 mL, with a mean transfusion rate of 5.5 units of packed red cells (2).

The transfusion of autogeneic blood products bears many deleterious effects and cancer patients are especially at risk. Studies, including a recent meta-analysis, have documented that intraoperative autogeneic transfusion positively influences tumor recurrence (3, 4). Specifically, transfusion during partial hepatectomy procedures has been associated with marked increases in tumor growth compared to non-transfused groups (5).

The transfusion of donor blood also predisposes the cancer patient to an increased risk of postoperative infections. Banked blood exacerbates immunosuppression, which places the patient at a 20 to 30% greater risk of postoperative infection (6). The immunosuppressive effects of autogeneic transfusions have been documented in oncologic procedures (7). The same study has shown that patients receiving autologous transfusion had no observed adverse immune reactions. Another study has shown that immune profiles of cancer patients undergoing surgical procedures did not differ statistically between the autologous group and those not transfused, however there was a significant decrease noted in the immune response of the group receiving autogeneic transfusion (8). A prospective study of 120 colorectal cancer patients was conducted with patients randomized to receive either autologous predeposit or donor transfusions. Of the autologous group, 12% developed postoperative infection compared to a significantly higher 27% of the autogeneic group (9).

Intraoperative autotransfusion through cell washing techniques seems a likely alternative to homologous transfusion. In instances when blood loss exceeds 1000 mL in adults, use of the autotransfusion device has been shown to be cost effective (10). The use of intraoperative autotransfusion has also been shown to decrease intra and postoperative autogeneic transfusions, making the patient four times less likely to require donor blood (11). Investigations have shown a statistically significant reduction in the amount of donor blood required during hepatic resection procedures, from 3466 to 814 mL when autotransfusion is used (12).

Autotransfusion during oncologic procedures is contraindicated due to the risk posed by viable cancer cells present in the washed product (13). In a recent study of blood samples collected from the surgical sites of patients undergoing various abdominal, orthopedic, urologic and gynecologic cancer procedures, 57 of 61 samples contained viable cells that colonized and proliferated in culture (13). However, recent studies have demonstrated that autotransfusion does not seem to reinfuse cancer cells capable of metastasis (11). In addition, no statistical difference was seen in metastatic occurrence in patients undergoing hepatic resection who received either homologous transfusion or autotransfusion, placing question on the premise of this contraindication (14).

Data from several studies regarding the use of autotransfusion in oncologic procedures have produced contradictory results. In one study, 20 trials were performed using centrifugal cell washing in hepatic resection procedures for hepatocellular carcinoma, with no cells present in the autotransfusate (12). However, in various studies on cells more buoyant than hepatocarcinoma, autotransfusion failed to remove the cancer cells from the washed salvaged blood. Studies on malignant epithelial (10), breast, colon (15), and osteosarcoma cells (16) demonstrated a failure to remove the carcinogenic cells from salvaged blood with the use of a cell processing device.

Recently, much attention has been focused on the possible use of leukocyte depleting filters (LDF) in hemorrhagic cancer procedures for the removal of carcinoma cells. The use of LDF has been shown to successfully remove tumor cells from solution (17). A study performed on urologic cancer cells showed an inability by cell washing and standard transfusion filters to remove the carcinogenic cells from salvaged blood, but when passed through a LDF no cells were detected (18). Likewise, studies on osteosarcoma (16), breast and colon (15) tumor cells found that cell washing of salvaged blood was unable to remove cancer cells from red cell concentrate, but when the concentrate was filtered with a LDF, no tumor cells remained.

The purpose of this study was to evaluate the efficacy of combining various cell processing techniques with leukocyte depleting filtration for removal of malignant liver cells from blood in an *in vitro* setting.

# MATERIALS AND METHODS

### *SALVAGED BLOOD MODEL*

A cell line of human hepatocarcinoma (HEP G2) was cultured by standard practice (10). The cells were maintained in a five percent media of horse serum at 37°C. The cultured cells were harvested with trypsin, washed with phosphate buffer saline (PBS), and resuspended in PBS. Cells were counted using a hemocytometer.

Salvaged blood was simulated by diluting expired units of packed red blood cells (PRBC), less than 3 days past their expiration, with saline to achieve a hematocrit of 25 ± 2%. The mixture was pH balanced by use of sodium bicarbonate and allowed a 30 minute mixing period.

### *CIRCUIT*

A 20 liter container served as a reservoir for the reconstituted blood mixture. Constant admixture was achieved via a ⅜-inch polyvinylchloride recirculation line propelled by a twin

**Table 1. Specific device, speeds, and wash volumes evaluated**

| Device | Centrifuge RPM | Fill Rate (mL/min) | Wash Rate (mL/min) | Wash Volume |
|---|---|---|---|---|
| Cobe BRAT2 | 4400 | 200 | 400 | 1500 or 3000 |
| Sorin STAT-P | 2400, 4400, or 5600 | 400 | 300 | 1500 or 3000 |
| Fresenius CATS | 1600 or 2400 | N/A | N/A | 3:1 or 7:1 |

roller head pump. A connector was placed in line which served as the drawing point for the autotransfusion devices.

Three autotransfusion devices were evaluated: Cobe BRAT 2[a], Sorin STAT-P[b] and the Fresenius CATS[c] (Table 1). Pediatric autotransfusion sets were utilized to effectively reduce the large quantity of blood and tumor cells required by this study. Due to the design of the Fresenius device, which continuously draws and washes the salvaged blood and expels the processed blood, a variable wash volume with respect to the amount of blood volume passing through the device is possible. The fill speed for the Fresenius CATS device is dictated by the wash volume being used. Thus, wash volumes are measured in ratio. The two ratios evaluated for this study were three mL saline wash for every one mL of blood processed as well as a seven to one ratio.

After the product was processed by the various devices, one half of each bowl volume was filtered through either the Pall Purecell RCQ or Pall Leukoguard RS[d]. The Purecell RCQ leukocyte depleting filter is a single use blood filter, while the Leukoguard RS is also a leukocyte depleting filter intended to filter multiple units of blood. Filters were changed after each trial.

### *SAMPLING AND COUNTING*

Each trial consisted of sampling five selected points in the autotransfusion/filter circuit during processing. Two separate three mL samples were drawn at each point. Samples were drawn from the simulated salvaged blood as it entered the device, serving as the baseline HEP G2 concentration for each trial. Samples were also drawn from the concentrate before it was washed, the concentrate after it was washed, and after one half of the product was filtered through the Purecell RCQ with the other half filtered by the Leukoguard RS.

After the reconstituted blood mixture had circulated for 30 min, one trial was performed for each device, centrifuge speed, wash volume, and filter combination prior to the addition of the HEP G2 cells. These samples were quantified using the Coulter Counter. This step was necessary because the counting apparatus included white cells in the HEP G2 counts due to size similarities. The baseline counts quantified white cell concentrations in every trial, which were subtracted from the final counts to equate the actual number of HEP G2 cells present in each given sample.

Once the baseline samples were obtained to quantify white cell concentrations, the salvaged blood was seeded with 3,000 HEP G2 cells per mL of salvaged blood solution. The contaminated blood mixture was then allowed to circulate for 30 min to allow for adequate distribution of the cancer cells. Following the recirculation period, three trials were performed for every combination of device, centrifuge speed, and wash volume.

Cell counts were performed on the samples by use of a Coulter Counter[e] cell counting device and confirmed by manual cytometry. Hematocrit was determined for pre-centrifugation and post-washing samples.

### *STATISTICAL ANALYSIS*

All data was entered into a spreadsheet format. Inter-autotransfusion device as well as intra-device variability was analyzed with regard to centrifuge speed, wash volume, and leukocyte depleting filters. All data is displayed as the mean ± standard deviation. The data was analyzed via analysis of variance (ANOVA) using commercially available statistics software[f]. Statistical significance was observed at $p \leq 0.05$.

## RESULTS

The average cell concentrations for the three trials for every combination were recorded (Table 2). These represent the HEP G2 concentrations found at each sampling point in the combined autotransfusion/filtration circuit.

The effect of centrifuge speed on HEP G2 concentrations seen in the packed red cell layer is shown as percent increase from HEP G2 concentrations in the salvaged blood entering the autotransfusion device (Figure 1). A significantly higher increase in concentration of carcinoma cells was observed in the STAT-P operating at 4400 RPM (322% ± 63.7%), and the CATS operating at 1600 RPM (525 ± 82.4%) and 2400 RPM (418 ± 53.5%), when compared to all other device/speed variations.

The inter-device increases in HEP G2 cell concentration

[a] Cobe Cardiovascular Inc., Arvada, CO
[b] Sorin Biomedical, Irvine, CA
[c] Fresenius USA Inc., Walnut Creek, CA
[d] Pall Biomedical Products Company, East Hills, NY
[e] Coulter Corporation, Hialeah, FL
[f] SuperAnova, Abacus Concepts, Berkeley, CA

**Table 2. Average HEP G2 cell concentrations at each sample point**

| | Sample Points | | | | |
|---|---|---|---|---|---|
| Device/RPM/Wash | Pre Centrifugation | Post Centrifugation | Post Washing | Post Leukoguard RS | Post Purecell RCQ |
| STAT P/2400 RPM/750 mL Wash | 780 ± 432 | 1926 ± 717 | 1227 ± 285 | 939 ± 285 | 216 ± 162 |
| STAT P/2400 RPM/1500 mL Wash | 2403 ± 408 | 2670 ± 545 | 1485 ± 678 | 336 ± 107 | 714 ± 247 |
| STAT P/4400 RPM/750 mL Wash | 2160 ± 729 | 7536 ± 3582 | 3939 ± 1102 | 1197 ± 588 | 1020 ± 321 |
| STAT P/4400 RPM/1500 mL Wash | 2820 ± 606 | 8368 ± 2730 | 3825 ± 1911 | 486 ± 179 | 930 ± 232 |
| STAT P/5600 RPM/750 mL Wash | 2781 ± 898 | 7515 ± 1643 | 3009 ± 1431 | 1143 ± 465 | 1443 ± 456 |
| STAT P/5600 RPM/1500 mL Wash | 4935 ± 1095 | 5946 ± 2115 | 3486 ± 2079 | 1134 ± 497 | 1224 ± 414 |
| BRAT 2/4400 RPM/750 mL Wash | 2718 ± 978 | 4290 ± 834 | 9762 ± 1338 | 528 ± 115 | 660 ± 119 |
| BRAT 2/4400 RPM/1500 mL Wash | 4022 ± 600 | 6030 ± 4500 | 10992 ± 5082 | 1290 ± 296 | 1956 ± 586 |

HEP G2 concentrations presented as mean ± standard deviation.

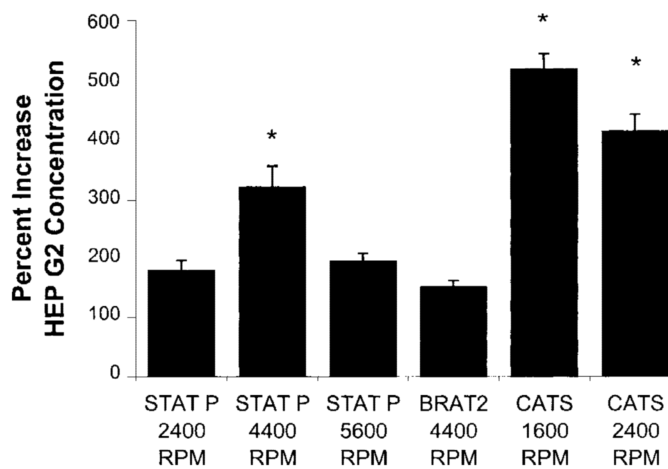


**Figure 1. Effect of centrifuge speed on HEP G2 concentrations in the blood prior to 750 mL wash. *p < .05 versus STAT 2400, 5600, BRAT 2.**

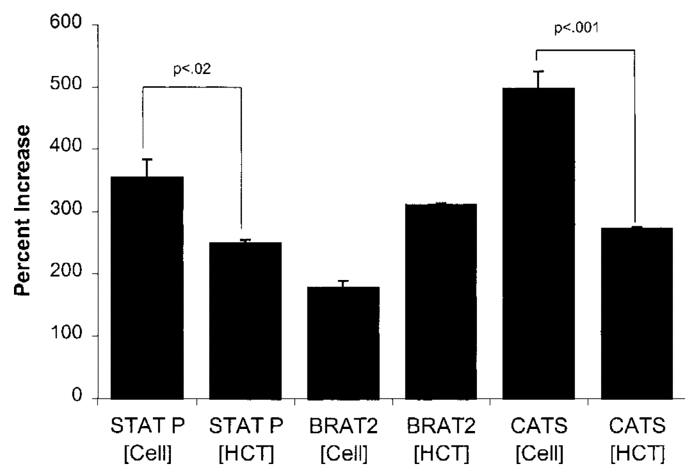


**Figure 2. Increase in hematocrit versus HEP G2 concentrations prior to washing. [Cell] = HEP G2 cell concentration and [HCT] = Hematocrit.**

versus the increase in hematocrit is depicted as percent increase from concentrations entering the respective devices (Figure 2). The percent increases are a mean of the varied speeds for each device. The Sorin STAT-P and the Fresenius CATS concentrated HEP G2 cells at a higher rate ($p < .02$) when compared to the increase in hematocrit.

The effect of wash volume on cancer cell removal was determined from the pre and post wash cell concentrations (Figure 3). In the BRAT 2 and the STAT-P no significant difference was observed in removal with varied wash volumes. However, a significant difference in removal was noted in the Fresenius CATS. The three to one ratio resulted in 6305 ± 1362 cells remaining after washing, while the seven to one contained 2460 ± 735 HEP G2 cells after washing.

A variance was found with regard to percent removal between the devices. The mean pre wash concentrations for all trials for a given device were compared to mean post washing concentrations. The STAT-P removed approximately 58% of the HEP G2 cells with a wash volume of 750 mL, and 67% of the cells when the wash volume used was 1500 mL. The CATS removed approximately 59% of the tumor cells with the three to one wash ratio, and removed 83.5% of the cells with a seven to one wash ratio. The BRAT2, when utilizing 750 mL of wash volume did not decrease the concentration of HEP G2 cells in the concentrate, when the wash volume was increased to 1500 mL, seven percent of the tumor cells were removed.

The efficiency of leukocyte depleting filters to remove remaining HEP G2 cells from the red cell product is depicted as mean percent removal. The Leukoguard RS removed a mean 72.7 ± 13.7% in all trials while the Purecell RCQ filtered 70.2 ± 16.6% of HEP G2 cells that remained after washing. No statistical significance was observed between the performance of the two filters.

## DISCUSSION

Autogeneic transfusion is the current standard for meeting the needs of the hemodynamically challenged cancer patient.

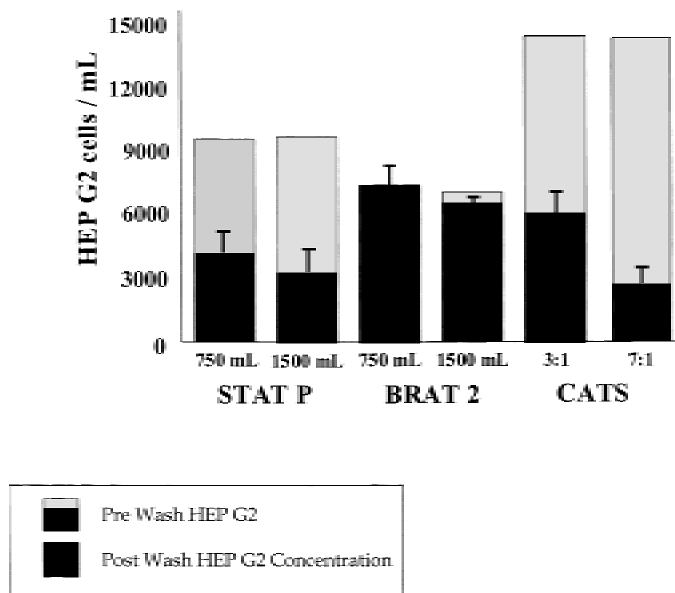


**Figure 3. Wash volume effect on HEP G2 removal.**

Most cancer patients are immunosuppressed prior to surgery due to chemotherapeutic drugs, and transfusion of autogeneic blood has well documented immunosuppressive effects which compound the dilemma (7). Immunosuppression results in an increase of up to 30% in the rate of postoperative infection (6).

Cancer patients receiving donor transfusion experience higher rates of tumor recurrence, most likely due to immunosuppression (3). Recent studies have collaborated these findings, showing a marked increase in tumor recurrence with intraoperative autogeneic transfusion when compared to patients who were not transfused (4). In addition, exposure to donor blood has also been shown to stimulate growth of remaining tumor (5).

The risks associated with autogeneic transfusion during cancer surgery are well documented, and is related to an additional $1,480 increase in hospital cost per unit transfused when compared to autologous transfusion (19). These facts suggest that autologous transfusion can enhance patient outcomes for those undergoing surgical cancer procedures, while reducing hospital costs. Autologous pre-deposit, however, is questionable due to cost and waste issues. It is reported that over 70% of all pre-deposited blood is never used, making the cost per quality-adjusted year of life saved as high as $1 million (20).

Transfusion of donor blood during oncologic procedures, while eliciting many deleterious effects, is the transfusion option of choice because autotransfusion during these procedures remains highly contraindicated due to risk of reinfusion of viable cancer cells (13). However, the use of intraoperative autotransfusion would be a great benefit for this patient group. It has been demonstrated that with the use of autotransfusion a patient is four times less likely to require a unit of donor blood. Autotransfusion, while contraindicated in manufacturer guidelines, is being used in certain centers for hepatic resection procedures, and has reduced the need for banked blood from 3466 mL to 814 mL (11).

There is little debate that blood salvaged during oncologic procedures contains carcinoma cells. In a blinded study involving abdominal, orthopedic, urological and gynecological cancer procedures, 94% of the blood samples salvaged intraoperatively contained carcinoma cells capable of proliferation, colonization, and tumorigenecity (13). If a cell processing procedure could be devised that would eliminate these cells, as well as other debris from salvaged blood, the product would be safe for reinfusion and beneficial for the patient.

The potential of intraoperative blood salvage to reinfuse cancer cells was originally documented by Yaw et al (21). The results from the present investigation support the findings of Yaw et al, such that none of the cell processing devices tested were able to completely remove HEP G2 cells from the simulated salvaged blood.

Centrifugation increased the concentration of HEP G2 cells 150 to 525% above carcinoma concentrations found in the simulated salvaged blood. Differences in bowl design appeared to have an effect on carcinoma concentrations found in the autotransfusate prior to washing. The Cobe BRAT2, utilizing the square shouldered design from Baylor consistently contained the lowest concentration of HEP G2 cells, while the CATS device, a doughnut shaped disk which is a continuous processing device consistently concentrated the carcinoma cells at higher rates (Figure 1). There were differences noted between devices in regard to the increase of carcinoma concentration versus increase in hematocrit (Figure 2). The Sorin STAT-P, utilizing the bell shaped bowl designed by Latham, as well as the Fresenius CATS both increased carcinoma concentrations significantly higher than hematocrit when compared to the respective concentrations entering the device. The Cobe BRAT2 was the only device of the three that increased hematocrit at a higher proportion than cancer cells. The ability to concentrate hematocrit seen in the BRAT 2 may also hinder the ability to remove HEP G2 cells, as is seen in the final product. While bowl design did affect the HEP G2 concentrations seen after centrifugation, these results did not seem to have any influence on the final HEP G2 concentrations in the washed product.

Centrifuge speeds also affected carcinoma concentrations seen in the samples. HEP G2 cells are slightly more dense than white cells, even though they are almost identical in size. They are also slightly less dense than red blood cells, suggesting that they should be removed in the buffy coat with platelets and white cells. However, increases in HEP G2 concentration were not consistent with increases in centrifuge speed, suggesting that factors other than density, such as adhesion qualities and membrane charges may affect the removal of cancer cells. The Sorin STAT-P, operating at a centrifuge speed of 4400 RPM

contained a significantly higher concentration of HEP G2 cells when compared to the same device operating at centrifuge speeds of 2400 or 5600 RPM. Centrifuge speed did not show any statistically significant change in carcinoma concentrations found in the CATS operating at 1600 versus 2400 RPM. The centrifuge rate in the BRAT2 is set at 4400 RPM, thus variable speeds were not examined for this device.

The volume of wash solution has varied effects on the amount of HEP G2 cells removed (Figure 3). Increasing the wash ratio from a three to one saline to blood ratio (approximately 675 mL wash), to a seven to one ratio (approximately 1350 mL of wash) in the CATS resulted in a statistically lower concentration of cancer cells detected in the red cell product. Although statistical significance was not achieved in the STAT-P, increasing wash volumes resulted in fewer hepatocarcinoma cells remaining in the product, suggesting that while increased wash volumes washed out more cells, washing alone could not remove all HEP G2 cells from the product.

Varied removal rates were observed for the different devices. The STAT-P removed approximately 58% of HEP G2 cells when washing with 750 mL, and the removal increased to 67% with a wash volume of 1500 mL. The CATS, when utilizing a three to one wash ratio removed approximately 59% of the tumor cells found in the bowl, with the seven to one ratio removing 83.5% of the cells, the highest removal observed in the study. The BRAT2 did not demonstrate the removal capabilities demonstrated by the other devices. Utilizing a wash volume of 750 mL, no decrease in HEP G2 concentrations was observed, while a wash volume of 1500 mL resulted in a 7% removal. While the BRAT 2 had the best ability to avoid retaining HEP G2 cells with the red cells, it also had difficulty removing the cancer cells once they were in the red cell pack. This data suggests that bowl design may play a role in the removal of tumor cells, as well as the amount of wash volume.

The results of this study do not agree with the data presented by Fujimoto et al, in which 20 trails demonstrated no hepatocarcinoma cells present in the red cell product (12). The results of this study do correlate with the majority of current studies, such as those performed by Leach et al (10), and Edelman et al (17). Leach and associates demonstrated an 86 ± 13% removal rate of malignant epithelial cells. It should be noted that epithelial cells are extremely buoyant in nature due to a high proportion of cytoplasm, and are more buoyant than nearly all other neoplastic cells.

Density is the main principle involved in centrifugation. During centrifugation the more dense cells are retained while the less dense, or more buoyant, cells escape into the effluent line as waste. As mentioned earlier, the HEP G2 cells used in this study were slightly less dense than red blood cells, and are more dense while similar in size to a white blood cell. Edelman and associated performed a series of *in vitro* studies on renal cell carcinoma (786-Q), transitional cell carcinoma (5637) and prostate carcinoma (DU145), in which the results demonstrated an inability of cell processing devices to remove carcinoma cells from salvaged blood (17). The size and densities of the cells used in this study are similar to those of the HEP G2 cells, and results were similar to those seen in all devices tested prior to filtration. The aforementioned studies correlate with the majority of recent studies, demonstrating the ineffectiveness of autotransfusion devices to remove cancer cells from salvaged blood.

The autotransfusion data from this study suggests that factors other than cell density play a role in the removal of tumor cells by mechanical autotransfusion. The data from the varied centrifuge speeds, especially in the STAT-P and CATS devices, demonstrated that at higher centrifuge speeds lower concentrations of HEP G2 cells were found. If cell removal was purely driven by density gradients, a linear concentration increase would have been observed with increased centrifuge rates, however, HEP G2 concentrations were lower at 5600 RPM than at 4400 RPM, suggesting that factors other than density affect the removal of these cells from salvaged blood, such as adhesive properties, membrane charge and buoyancy.

Device design also played a role in the removal of hepatocarcinoma cells by washing. The continuous processing of the CATS device contained the lowest concentration of HEP G2 cells in the autotransfusate. The bowl designs of the STAT-P and BRAT2 which require filling of the bowl followed by the washing phase had lower removal rates.

The use of leukocyte depleting filters in this study resulted in a significant decrease in carcinoma concentrations seen in the final filtered product. No difference in performance was seen between the Pall Purecell RCQ LDF and the Pall Leukoguard RS LDF. The Purecell RCQ LDF removed approximately 70% of the HEP G2 cells that remained in the autotransfusate. The Leukoguard RS removed approximately 73% of the hepatocarcinoma cells. These results, while showing a significant decrease in the number of hepatocarcinoma cells found in the final filtered product, do not agree with recent data demonstrating complete removal of cancer cells by the use of LDF (15, 16, 17, 18). The discrepancy could be related to a difference in cells and cell lines, as well as filters, as the leukocyte depleting filters used in this study were not the same as used in prior studies. It was expected that the Purecell RCQ, a single use filter would remove more cells than the Leukoguard RS, a multiple use filter. However, only one-half of each bowl volume was filtered through each filter, with the filter then being disposed. Multiple units were not filtered using the Leukoguard RS, so no conclusions regarding filtering effectiveness after multiple uses could be made.

Edelman and associates have reported that the use of LDF removed three lines of urologic carcinoma; renal cell, transitional cell, and prostate (17). This study utilized the trypsin blue exclusion method as well as cell culture for the detection of carcinoma cells. It was demonstrated that autotransfusion as well as standard blood filters failed to remove the cells from

the red cell product, while the LDF removed all cells in all trials. Similar work completed by Muller et al demonstrated complete removal of osteosarcoma cells from salvaged blood via the use of LDF (16). A study performed by Miller et al revealed the ability of LDF to remove both breast (SKBr3) and colon (COLO320) carcinoma cells, as quantified by cell culture (17). Leukocyte depleting filters operate on size as well as adhesive properties. Since the adhesiveness of cultured cell lines are likely to be different than that of tumor cells in the intraoperative salvaged blood, more clinical studies are required to test the efficiency of these filters.

Reports concerning the metastatic potential of carcinogenic cells remaining in cell processing product have been varied. Data collected on a variety of carcinogenic cells sequestered from intraoperative salvaged blood demonstrated a proliferation capacity, invasiveness and tumorigenecity. This raises concern that the salvaged blood may cause hematogenic metastasis after retransfusion (13). However, other retrospective reviews consisting of patient follow-ups have found contrary results (11).

Shed blood seeded with A431 human vulva carcinoma cells was studied in a laboratory setting. It was estimated that 55 to 76% of the A431 cells were removed by a cell processing autotransfusion system, and of those cells remaining in the red cell product 62% suffered lethal trauma and the remainder showed morphological change. The conclusion of this study was that of the cancer cells that passed through the autotransfusion device, none were capable of metastasis and proliferation (22).

The data for hepatic resection has also been comparable. A retrospective review of patients undergoing liver resection for malignant tumor revealed a total of 39 patients in whom intraoperative autotransfusion was used (14). The two year actuarial survival rate of the patients in this series, as well as disease free survival and the risk of lung metastasis was not different than the data for patients in whom autotransfusion was not utilized. In a similar study of 104 patients undergoing hepatic resection, no difference was noticed between the 54 patients who received autotransfusion and the 50 who received only autogeneic transfusion (12). Recurrence rates and cumulative survival rates for the autotransfused group were 62.8 and 61.9% respectively, not statistically different from those of the patients receiving only donor blood (67.3 and 52.8%). The data from this study would suggest that there is no added risk of metastasis when using intraoperatively collected blood.

Although the metastatic potential of carcinoma cells remaining in salvaged blood is not well known, recent trials have demonstrated techniques which cause lethal damage to these tumor cells, thus limiting metastatic potential. Irradiation of blood salvaged during cancer surgery has proven to render cancer cells nonviable, allowing for reinfusion of autologous blood that is safe for the patient (23). As little as 50 Gray of radiation has been shown to alter the genetic matrix of cancer cells, rendering them unable to proliferate. While this a promising answer to the dilemma faced when carcinoma cells are present in autotransfusate, it is not necessarily an option when faced with an intraoperative hemodynamic crisis. The time required to irradiate the salvaged blood is the limiting factor in this scenario, and while it may not prevent intraoperative autogeneic transfusion, it most certainly can reduce demands for postoperative donor transfusions.

Recent advances in hepatic resection techniques have shown promise for reducing intraoperative hemorrhage. Various improvements are evident in the dissecting apparatus, liver hepatic inflow clamp, cold hepatic perfusion technique, and intraoperative ultrasonography, as well as advanced assessments of liver function (24). Also, a recent study utilizing an ultrasonic dissector for hepatocellular carcinoma has shown a marked decrease in intraoperative as well as postoperative blood loss (25). Utilized in 69 procedures, blood loss was reduced from 3400 to 2400 mL, and an increase in the percentage of patients not requiring transfusion from 8 to 32% was noted.

The practice of processing intraoperative salvaged blood during cancer procedures with a combination of autotransfusion as well as leukocyte depleting filtration techniques has been shown to remove carcinogenic cells. If an autologous product that was free of all contaminants could be transfused to cancer patients undergoing tumor resection, the amount of donor blood transfused, and the immunosuppressive and tumor enhancing qualities associated with these transfusions could be avoided.

However, in an *in vitro* setting, not all hepatocarcinoma cells were removed from simulated salvaged blood, placing autotransfusion and LDF in question for this application. Limitations to this study include the fact that *in vivo* hepatocarcinoma cells may behave very differently than cultured cells. The recirculation of the HEP G2 cells during the time required to draw and process all samples may also affect the behavior of the cancer cells.

The ability of remaining cancer cells to proliferate and metastasize remains in question. Until facts become apparent, tumor cells remaining in autotransfusate must be recognized to be capable of metastasis, and thus not reinfused to the patient, and unless 100% of carcinogenic cells can be removed by any technique, the processed blood should not be reinfused. In this *in vitro* study the combined use of autotransfusion and leukocyte depleting filter techniques was unable to remove all hepatocarcinoma cells from the product, prompting more thorough tests before these techniques can be applied clinically. Advances in the technology of cell washing devices as well as filters hold hope that removal of all foreign cells from salvaged blood may be possible. From the results of this study, the use of autotransfusion, even when combined with leukocyte depleting filtration failed to remove all hepatocarcinoma cells from the final washed product, and clinical use must remain

contraindicated due to the possibility of reinfusion of metastatic cells.

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