

# Impact of Intraoperative Cell Salvage on Circulating Tumor Cells and Cellular Activity in Patients with Hepatocellular Carcinoma Undergoing Curative Resection: A Prospective Comparative Study

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## Abstract

This study investigates the effects of intraoperative cell salvage (IOCS) on cell survival rates, apoptosis levels, and circulating tumor cell (CTC) counts in patients with hepatocellular carcinoma (HCC) undergoing curative resection. A combination of immunofluorescence, Western blot, flow cytometry, and qRT-PCR was employed to assess the impact of IOCS on cellular activity and CTC dynamics in these patients. No significant differences were found in demographic characteristics, including gender, age, body mass index (BMI), Child-Pugh classification, and liver function markers (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) between the experimental and control groups. Preoperatively, both groups exhibited low cell survival rates without statistical differences ( $P > 0.05$ ), and cell survival remained similarly low during surgery. However, 6 h post-surgery, the experimental group showed a significant increase in cell survival rates compared with the control group ( $P < 0.05$ ), suggesting that IOCS enhances postoperative cell viability. Apoptosis levels were similarly high in both groups before and during surgery ( $P > 0.05$ ), but notably, 6 h post-operation, apoptosis levels in the experimental group were significantly reduced ( $P < 0.05$ ), indicating effective prevention of cell death. Although preoperative CTC counts were low and increased during surgery, no significant differences were observed between groups during surgery. However, 6 h post-surgery, the experimental group exhibited a marked decrease in CTC counts ( $P < 0.05$ ), indicating a reduction in tumor cell dissemination. Although these findings suggest that IOCS improves cell survival and reduces apoptosis and CTC counts, there is a potential concern regarding the possibility of IOCS preferentially preserving viable cells with metastatic potential. The long-term impact of this intervention on tumor recurrence or metastasis requires further investigation. In conclusion, IOCS appears to offer short-term benefits in enhancing postoperative cell survival and reducing CTC dissemination in patients with HCC; however, the potential risk of promoting metastatic cell viability warrants additional study before broader clinical application.

## Keywords

Intraoperative cell salvage • Circulating tumor cell • Hepatocellular carcinoma • Cellular activity

Received: 18 December 2024 / Accepted: 11 March 2025 /

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## 1. Introduction

Intraoperative cell salvage (IOCS) is a groundbreaking and efficient method utilized during surgeries to recover a patient's own blood, aiming primarily to decrease the need for transfusions and reduce associated risks (Carroll and Young 2021; Walton et al. 2023). By collecting and reinfusing autologous blood, IOCS boosts patient safety and fosters better postoperative recovery outcomes. This technique is gaining recognition for its potential advantages across various surgical scenarios (Obore et al. 2022). Hepatocellular carcinoma (HCC), the most common primary liver cancer, is on the rise globally, showing significant geographic differences and comprising about 75%–85% of liver cancers (Vogel et al. 2022; Ganesan and Kulik 2023). It originates from hepatocytes, the liver's main functional cells, and is marked by varied morphology and ties to underlying liver

diseases. The application of IOCS is based on specific indications and benefits, along with crucial precautions to ensure patient safety. One surgical context where IOCS may prove highly beneficial is the treatment of HCC (Weller et al. 2021). HCC poses distinct challenges due to its characteristics, incidence, and the risks it presents.

Current treatments for HCC, like surgical resection and liver transplantation, have limitations that highlight the need for improved strategies such as IOCS (Yang et al. 2024). Even after procedures like surgical resection or liver transplantation, the risk of tumor recurrence remains high, especially in patients with underlying liver conditions (Wang and Deng 2023). Targeted therapies and immunotherapies can cause adverse effects that may limit their use or diminish the patient's quality of life. Despite increasing evidence supporting IOCS, especially in resectable HCC cases, traditional beliefs in China significantly hinder its acceptance in surgeries for malignant tumors (Aijtink et al. 2022). The prevalent concern that transfusing autologous blood mixed with cancer cells might trigger tumor recurrence or metastasis has led to hesitancy in adopting IOCS for such cases. However, international studies, including meta-analyses and retrospective reviews, are challenging this viewpoint, emphasizing

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the need to reassess these deep-rooted beliefs (Aijtink et al. 2022). Notably, these retrospective studies often lack a strong theoretical basis, underscoring the necessity for further research into the safety and effectiveness of IOCS in patients with malignant tumors.

Circulating tumor cells (CTCs) are cancerous cells that detach from the main tumor and travel into the bloodstream, playing a pivotal role in the metastatic process (Lin et al. 2021). In HCC, CTCs have drawn considerable attention due to their potential implications in diagnosis, prognosis, treatment response, and understanding tumor biology. Unlike primary tumor cells, CTCs possess unique characteristics. They can undergo epithelial-to-mesenchymal transition, which enhances their ability to migrate and invade (Jiang et al. 2021). This transition is essential for metastasis and often correlates with a more aggressive tumor phenotype. CTCs in HCC are diverse, reflecting the genetic and phenotypic variability of the primary tumor. This diversity can affect their behavior, resistance to therapy, and capacity to initiate metastasis. The presence of CTCs in patients with HCC has been associated with a poorer prognosis. Elevated CTCs counts are linked to advanced disease stages, increased tumor burden, and a higher likelihood of metastasis and recurrence post-treatment (Ahn et al. 2021). Monitoring CTC levels over time can help to assess disease progression or treatment response. A reduction in CTC count during treatment generally indicates a positive response, whereas an increase may suggest disease progression or resistance to treatment.

The objective of this study is to investigate the impact of IOCS on CTCs and cellular activity in patients with HCC undergoing curative resection. By conducting a prospective comparative analysis, we aim to elucidate the effects of IOCS on CTC dynamics and assess its potential to improve patient outcomes in the surgical management of HCC. This study seeks to provide a comprehensive understanding of how IOCS may influence tumor cell behavior and contribute to advancements in treatment protocols for resectable HCC.

## 2. Materials and Methods

### 2.1. Patients

This research is designed as a prospective case-control study, involving 80 patients diagnosed with HCC who received radical resection at our hospital between January 2022 and December 2023. Patients were randomly assigned to either a control group or an experimental group, with each group consisting of 40 participants. Blood samples were collected at three critical time points during the study: preoperative, intraoperative, and postoperative 6 h. In the control group, blood samples directly

collected from the surgical area during the operation were not subjected to any treatment and were analyzed as baseline data. In the experimental group, blood samples from the surgical area were recovered through an IOCS device and then transferred to a storage tank for processing. The processing included centrifugation, separation, and washing and finally filtration through a leukocyte filter to remove potential tumor cells and impurities. All participants in this study were aware of the experiment and gave their written consent, which was authorized by our hospital's Ethics Committee.

#### 2.1.1. Inclusion criteria

- (1) Patients aged  $\geq 20$  years.
- (2) Clear preoperative diagnosis and postoperative histopathological confirmation of liver cancer.
- (3) Undergoing radical resection for liver cancer.
- (4) Significant intraoperative blood loss that requires blood transfusion.
- (5) No anemia before surgery.
- (6) Liver function categorized as Child-Pugh class A or B.

#### 2.1.2. Exclusion criteria

- (1) Presence of concomitant heart or kidney failure.
- (2) Undergoing local resection of the liver surface with minimal blood loss.
- (3) Concomitant coagulation dysfunction or hematological diseases.
- (4) Incomplete clinical data.

Based on these criteria, this study will include 80 patients undergoing radical resection for liver cancer for further research.

### 2.2. Adenosine triphosphate (ATP) cell viability detection

In this research, we employed ATP cell viability detection kits to assess the viability of cells in blood samples from patients undergoing radical liver cancer resection. The control group consisted of blood samples taken directly from the surgical site without IOCS treatment, while the experimental group included blood samples processed through the IOCS device. Blood samples in the experimental group were initially transferred to a storage tank, where they were subjected to centrifugation, separation, and washing. These samples were then filtered using a leukocyte filter to eliminate impurities and potentially harmful cells. The ATP cell viability detection kits were used to determine the cellular activity in both groups by adding reaction reagents as per the kit manual. ATP levels were measured using a fluorescence or luminescence detection instrument to evaluate cell viability, enabling

us to compare the impact of IOCS processing on cell viability in the context of liver cancer surgery.

### 2.3. CTC assay

In this study, we employed the CellSearch Circulating Tumor Cell Detection Kit to assess the residual tumor cell counts. The procedure began with the careful aspiration of 7.5 mL of blood sample into a conical tube designated for the CellSearch kit. Subsequently, 6.5 mL of diluent was introduced into the tube, which was then sealed and inverted five times to achieve complete mixing. The resulting sample was placed in a centrifuge with a radius of 16 cm, properly balanced and subjected to centrifugation at  $800 \times g$  for 10 min at ambient temperature. After centrifugation, the resulting sample was transferred to the CellTracks Autoprep System, where we added a series of antibodies: mouse anti-human EpCAM, CK, and CD45 monoclonal antibody and the nuclear stain DAPI. These components were coincubated to facilitate CTC enrichment. Following the enrichment process, the samples were analyzed. CTCs were defined as cells exhibiting complete membrane and nuclear staining, specifically characterized as EpCAM<sup>+</sup> CK<sup>+</sup> DAPI<sup>+</sup> CD45<sup>-</sup>. The number of CTCs was then recorded, providing insights into the presence of residual tumor cells in the studied groups.

### 2.4. The quantitative detection of CTCs

In this study, we employed an immunomagnetic bead separation method in conjunction with flow cytometry for the quantitative detection of CTCs. The process began with the collection of blood samples from patients. First, immunomagnetic beads coated with specific antibodies targeting tumor cell markers were introduced to the blood samples. These beads selectively bound to the CTCs, allowing for the separation of tumor cells from the rest of the blood components. Once the separation was complete, the samples were subjected to flow cytometry analysis. This technique allowed for the quantitative assessment of CTCs by passing the labeled cells through a laser beam, where they were detected and quantified based on their fluorescence characteristics.

### 2.5. Immunofluorescence assay

Start the immunofluorescence assay by utilizing stabilization and infiltration methods on liver tissues. Subsequently, immerse the tissues in a solution containing primary antibodies. Allow ample time for the primary antibodies to incubate, then promptly wash the samples to remove any unbound antibodies. Then, incubate the liver tissue cells with fluorescent

secondary antibodies and wash again to clear any remaining unbound antibodies. Finally, analyze the processed samples under a fluorescence microscope.

### 2.6. Western blotting

Blood sample protein extracts were subjected to 10% SDS-PAGE for separation, and the resulting proteins were then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes underwent washing with Tris-buffered saline with Tween (TBST) to remove any non-specific bindings. Primary antibodies specific to the target protein and actin, obtained from Bioworld Technology, Inc., China, were applied, and the membranes were stored at 4°C overnight for incubation. After the incubation period, the membranes were washed with TBST to remove non-specifically bound primary antibodies. Then, secondary antibodies from the same supplier were added, and the membranes were kept at room temperature for 2 h. Later, they were washed again with TBST to eliminate any remaining antibodies. Protein bands were detected using an enhanced chemiluminescence reagent and analyzed accordingly.

### 2.7. qRT-PCR

The total RNA was isolated from blood samples following the manufacturer's instructions using TRIzol Reagent (Beyotime, Shanghai, China). The extracted mRNA was then converted into cDNA with the aid of the mRNA Reverse-Transcription Kit (Beyotime). To assess the expression levels of mRNA, quantitative PCR was carried out using SYBR Green PCR Mix (Vazyme Biotech, Shanghai, China) on a real-time PCR system. The relative expression levels were determined by the  $2^{-\Delta\Delta Ct}$  method and were normalized against  $\beta$ -actin. This entire process was conducted in triplicate. The primers utilized in the experiment were as follows: for CTNNB1, Forward: 5'- ATGGCCTGAGAACTGAACTG-3' and Reverse: 5'- CTGAGGAGCCTTGTCTTTCA-3'; for TP53, Forward: 5'- ACCAGCAGCTGACCTGTTTC-3' and Reverse: 5'- GCTGGTCTTGTGACCTCCTG-3'; and for  $\beta$ -actin, Forward: 5'-CGGTCAGGTCATCACTATC-3' and Reverse: 5'-CAGGGCAGTAATCTCCTTC-3'.

### 2.8. Statistical analysis

Statistical analysis was conducted using Prism 8 software. Data measurements were expressed as the mean  $\pm$  standard deviation and were consistently replicated at least three times. To detect differences between two groups, the *t*-test was applied. For comparisons among three or more groups, one-way ANOVA was used. A *P*-value of  $<0.05$  was considered to indicate statistical significance.

### 3. Results

#### 3.1. Participants' general data

This study compared the demographic and biochemical characteristics of patients with HCC. The results showed that there were no notable variations in the distribution of gender, age, or body mass index (BMI), Child-Pugh classification, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels between the two groups. In terms of gender, 60% of the experimental group and 55% of the control group were male, showing no statistical significance ( $\chi^2 = 0.331$ ,  $P = 0.565$ ). The average age was 65.4 years (50.3–73.8) for the experimental group and 64.8 years (50.4–74.5) for the control group, with no significant difference ( $t = 0.319$ ,  $P = 0.750$ ). The average BMI was 26.5 kg/m<sup>2</sup> (24.1–29.3) in the experimental group and 26.9 kg/m<sup>2</sup> (23.2–30.5) without significant difference ( $t = -0.536$ ,  $P = 0.594$ ). Child-Pugh classification showed 65% grade A and 35% grade B, compared to 60% grade A and 40% grade B, with no significant difference ( $\chi^2 = 0.372$ ,  $P = 0.543$ ). The tumor, nodes, metastasis (TNM) staging indicated that there were 18 cases classified as stage I, 20 as stage II, 2 as stage III, and no cases in stage IV within the experimental group, whereas the control group had 20 cases in stage I, 19 in stage II, 1 in stage III, and none in stage IV, with no significant difference ( $\chi^2 = 0.492$ ,  $P = 0.784$ ). Biochemical indicators showed that the ALT level was 61.3 U/L (41.1–82.3) in the experimental group and 61.8 U/L (41.4–81.6) in the control group, with no significant difference ( $t = -0.144$ ,  $P = 0.886$ ). AST levels were 56.2 U/L (40.2–76.1) in the experimental group and 55.6 U/L (40.6–77.3) in the control group, also showing no significant difference ( $t = 0.233$ ,  $P = 0.817$ ). In summary, this study found

no significant differences in demographic characteristics and biochemical indicators between experimental and control groups, indicating that the baseline characteristics of patients in both groups were comparable.

#### 3.2. Cell survival rates in patients with HCC

In this research, we assessed the cell survival rates (%) in two cohorts of patients with liver cancer utilizing the ATP cell viability detection kit (depicted in Figure 1). Preoperatively, the cell survival rates in both the experimental and control groups were relatively low, with no statistically significant difference detected ( $P > 0.05$ ). Intraoperatively, both groups maintained low cell survival rates. Statistical analysis revealed no significant discrepancies between the experimental and control groups ( $P > 0.05$ ). However, 6 h postoperatively, the experimental group demonstrated a marked increase in cell survival rates compared with the control group. This suggests a significant enhancement in cell viability in the experimental group following surgery ( $P < 0.05$ ). These findings underscore the efficacy of the treatment protocol in improving post-operative cell survival rates in the experimental group relative to the control group.

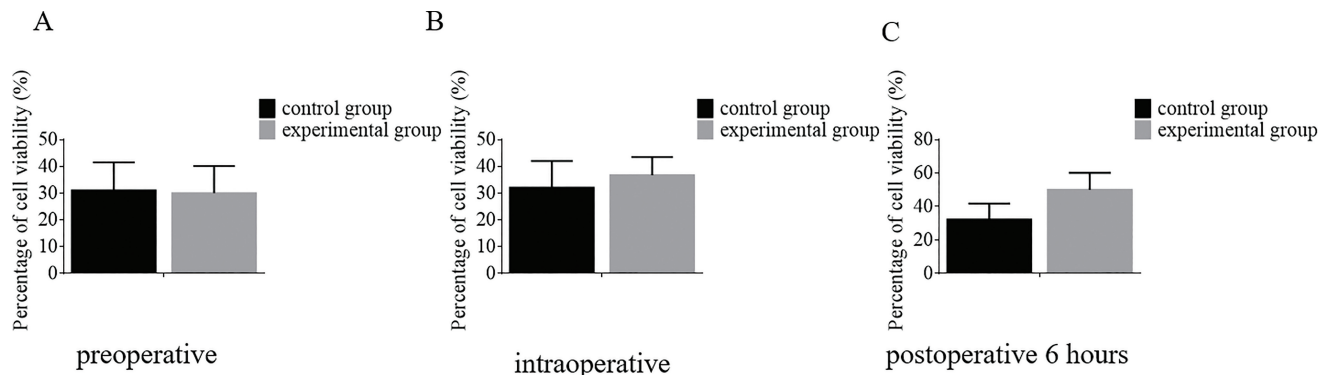
#### 3.3. Comparison of apoptosis levels in patients with HCC

In this investigation, we evaluated apoptosis levels in the blood of patients with HCC using flow cytometry (illustrated in Figure 2). The results are as follows: before surgery, both the experimental and control groups exhibited relatively high apoptosis levels, with no significant statistical difference detected ( $P > 0.05$ ). The results of this study

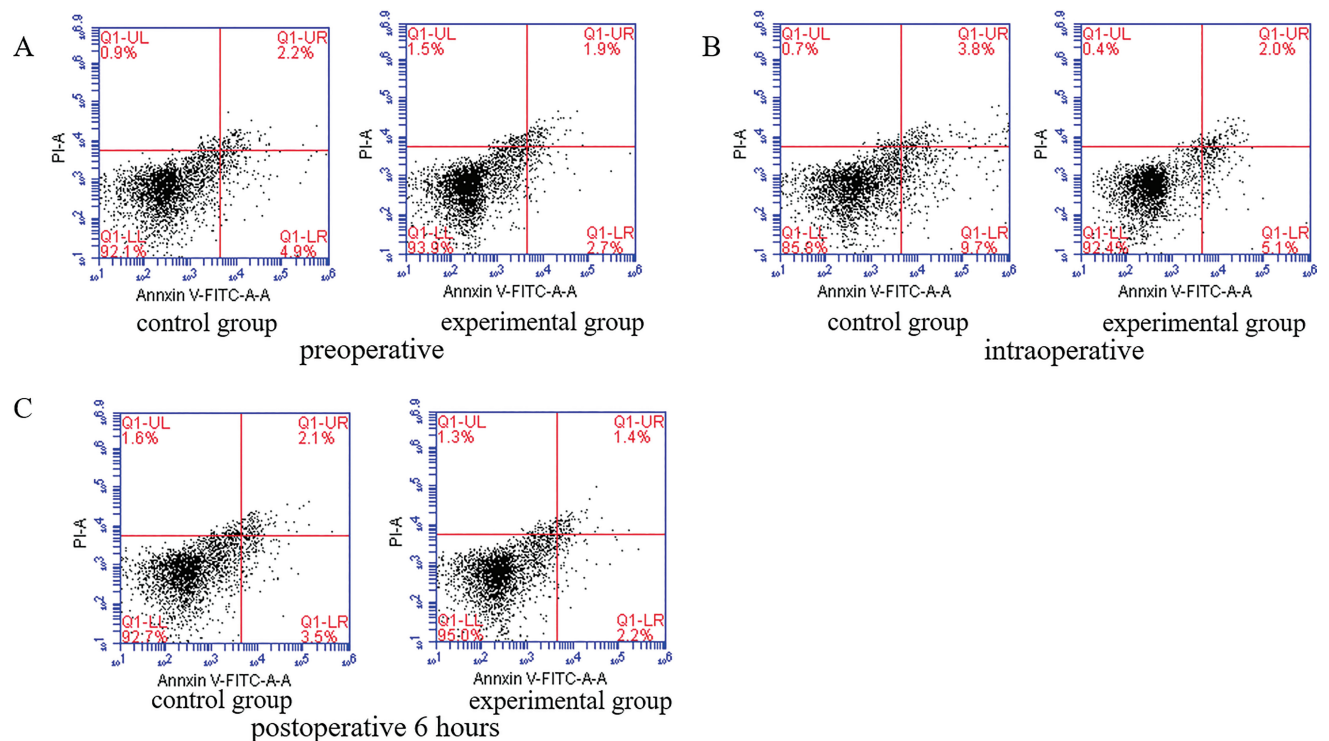
**Table 1.** Demographic, participants' general data

Variable	Experimental group (N = 40)	Control group (N = 40)	$\chi^2/t$	$P$
Gender, male (%)	24 (60%)	22 (55%)	0.331	0.565
Age (years)	65.4 (50.3–73.8)	64.8 (50.4–74.5)	0.319	0.750
BMI (kg/m <sup>2</sup> )	26.5 (24.1–29.3)	26.9 (23.2–30.5)	-0.536	0.594
Child-Pugh classification			0.372	0.543
A	26 (65%)	24 (60%)		
B	14 (35%)	16 (40%)		
TNM stage			0.492	0.78
I	18	20		
II	20	19		
III	2	1		
IV	0	0		
ALT (U/L)	61.3 (41.1–82.3)	61.8 (41.4–81.6)	-0.144	0.886
AST (U/L)	56.2 (40.2–76.1)	55.6 (40.6–77.3)	0.233	0.81

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; TNM, tumor, nodes, metastasis.



**Fig 1.** The apoptosis levels in patients with HCC from different groups were detected at preoperative, intraoperative, and postoperative six points using the ATP cell viability detection assays. ATP, adenosine triphosphate; HCC, hepatocellular carcinoma.



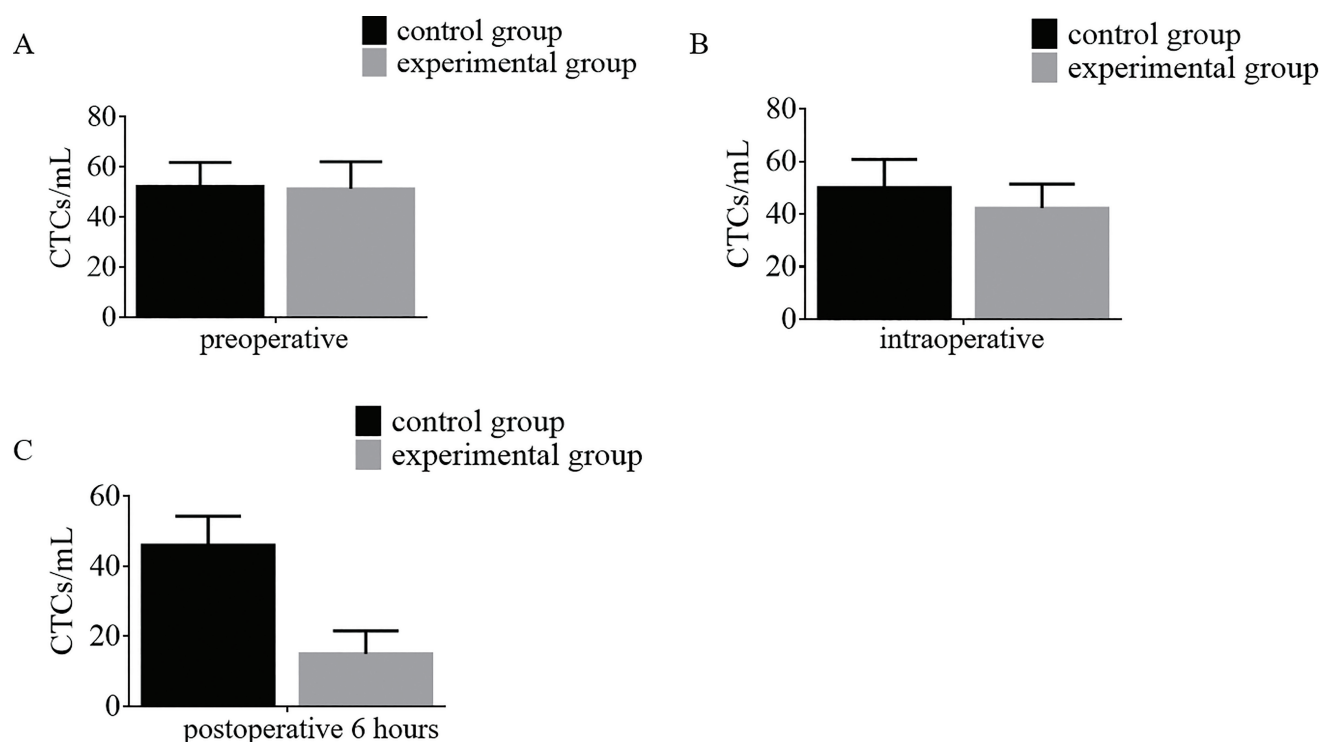
**Fig 2.** The apoptosis levels in patients with HCC from different groups were detected at preoperative, intraoperative, and postoperative 6 h using flow cytometry assays. HCC, hepatocellular carcinoma.

indicate that throughout the operation, high levels of apoptosis were observed in both the experimental and control groups, with no significant differences between them ( $P > 0.05$ ). However, 6 h post-operation, the experimental group demonstrated a significant increase in apoptosis levels compared with the control group ( $P < 0.05$ ). This finding suggests that the treatment administered to the experimental group effectively increased apoptosis levels following the surgical procedure.

### 3.4. CTCs in patients with HCC

In this research, we utilized immunomagnetic bead separation in combination with flow cytometry to quantitatively evaluate CTCs in the blood samples of patients with liver cancer, reported in CTCs/mL (as illustrated in Figure 3). Before surgery, the CTC counts in both the experimental and control groups were relatively low, with no statistically significant differences detected. During surgery, both groups experienced





**Fig 3.** The CTCs in patients with HCC from different groups were detected at preoperative, intraoperative, and postoperative 6 h using an immunomagnetic bead separation combined with flow cytometry assays. CTCs, circulating tumor cell; HCC, hepatocellular carcinoma.

an increase in CTC counts; nevertheless, no statistically significant differences were found between the experimental and control groups. Six hours post-surgery, a substantial reduction in CTC counts was noted in the experimental group compared with the control group ( $P < 0.05$ ). This finding highlights a significant improvement in reducing CTCs in the experimental group following the surgical procedure. These outcomes emphasize the treatment's efficacy in lowering CTC counts postoperatively in the experimental group compared with the control group.

### 3.5. Effect of AFP and GPC3 protein in HCC

Western blotting was utilized to assess the expression levels of AFP and GPC3 proteins among various patient groups, as shown in Figure 4. Six hours post-surgery, the data revealed a notable reduction in AFP and GPC3 protein levels in the model group when compared with the control group ( $P < 0.05$ ). This suggests that the elevated levels of AFP and GPC3 proteins play a role in the development of HCC.

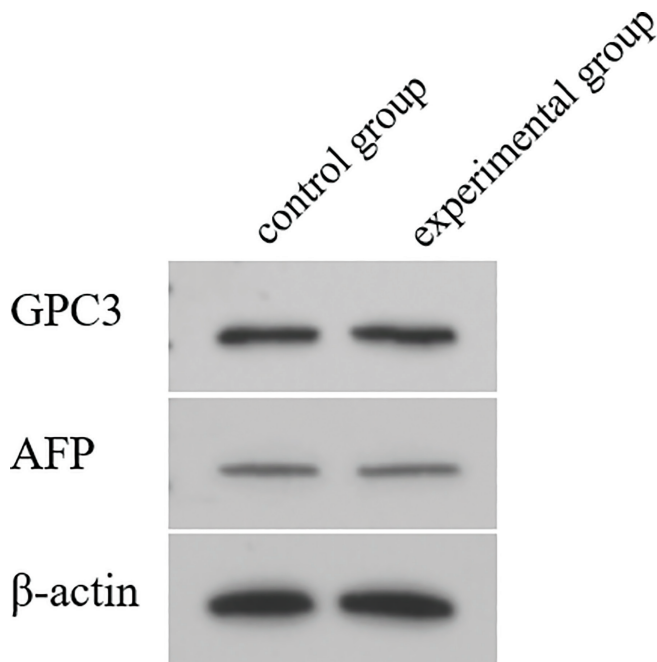
### 3.6. Effect of CTNNB1 and TP53 mRNA in HCC

To evaluate the mRNA expression levels of CTNNB1 and TP53 in different cohorts of patients with HCC, qRT-PCR

was employed, as illustrated in Figure 5. Preoperatively, the results indicated that CTNNB1 and TP53 mRNA levels were elevated in both the control and experimental groups ( $P > 0.05$ ), as shown in Figure 5A. Six hours post-surgery, a significant reduction in CTNNB1 and TP53 mRNA expression levels was observed in the experimental group compared with the control group ( $P < 0.05$ ), as depicted in Figure 5B. These findings imply that the upregulation of CTNNB1 and TP53 mRNA might play a role in the progression of HCC.

### 3.7. The effects of EpCAM in HCC

To assess the impact of EpCAM in patients with HCC, we performed an immunofluorescence assay as depicted in Figure 6. The immunofluorescence analysis revealed EpCAM-positive cells in patients with HCC. Before surgery, the levels of EpCAM-positive cells were comparably high in both the experimental and control groups, with no statistically significant differences ( $P > 0.05$ ). However, 6 h post-surgery, there was a notable decrease in the number of EpCAM-positive cells in the experimental group compared with the control group ( $P < 0.05$ ). These findings suggest that IOCS can lower the count of EpCAM-positive cells in patients with HCC.

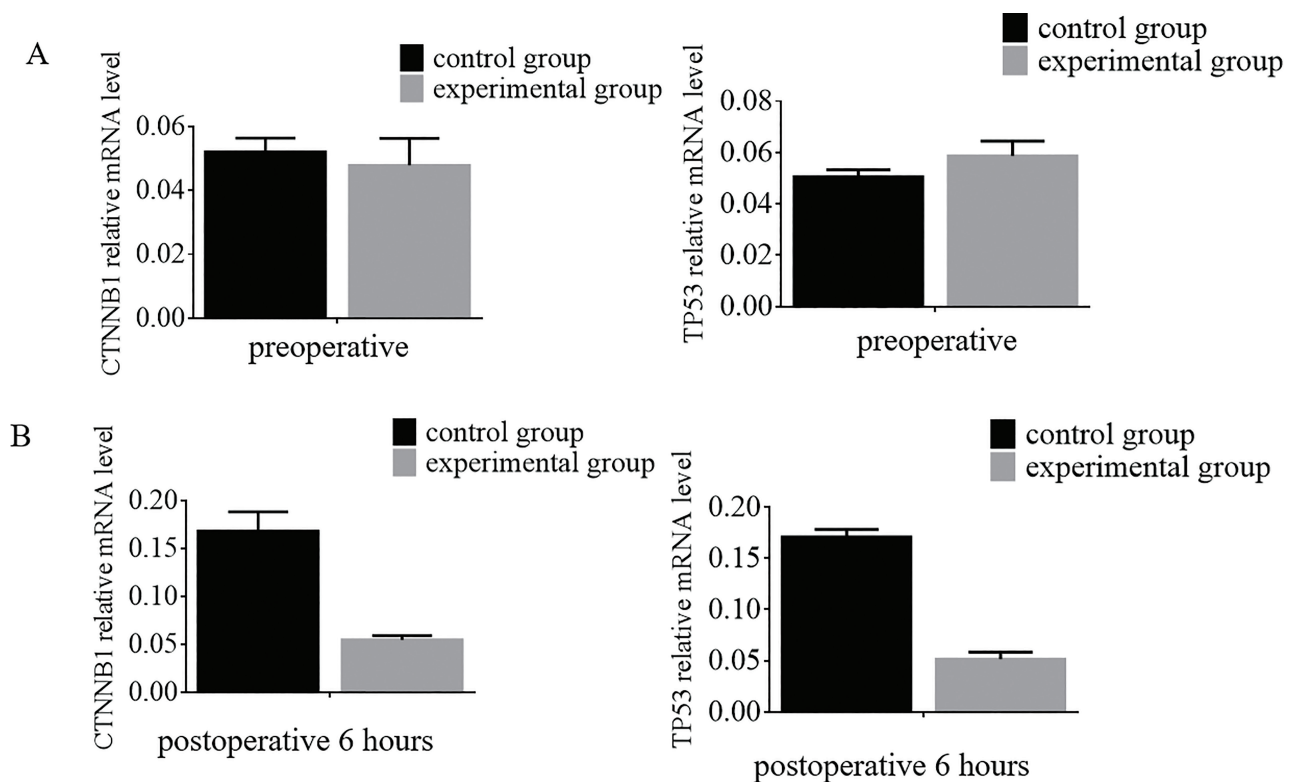


**Fig 4.** The expression levels of AFP and GPC3 proteins in patients with HCC from different groups were determined at postoperative 6 h using Western blotting. HCC, hepatocellular carcinoma,

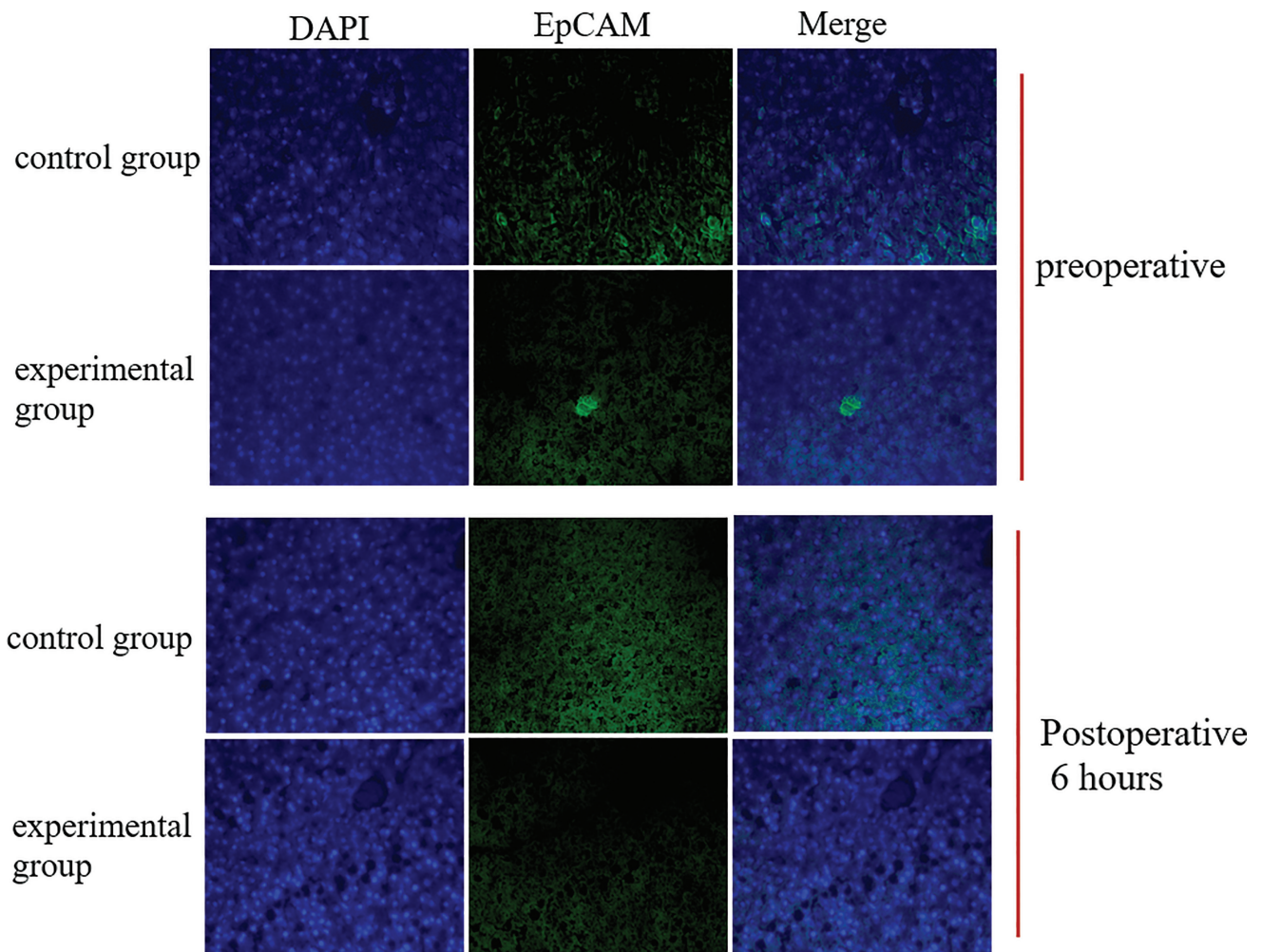
## 4. Discussion

In this study, we investigated various clinical and biological parameters in patients with liver cancer undergoing surgery, focusing on cell survival rates, apoptosis levels, and CTC counts. The findings of this study indicate that there were no significant differences in demographic characteristics, such as gender distribution, age, BMI, Child-Pugh classification, and liver function markers (ALT and AST). This homogeneity strengthens the reliability of this study's results, ensuring that observed differences in treatment outcomes are attributable to the intervention rather than underlying patient characteristics.

IOCS is a beneficial technique for managing HCC surgically (Rajasekaran et al. 2021). It helps to reduce the need for allogeneic blood transfusions, aids in patient recovery, and may improve surgical and oncological outcomes. A retrospective cohort study by Pinto et al. (2021) found that ICOS did not worsen outcomes for patients undergoing curative resection. Similarly, another retrospective study by Ivanics et al. (2021) showed that ICOS and autotransfusion did not negatively impact oncologic outcomes for patients with incidental HCC undergoing curative resection. However, these studies did not detail the mechanisms behind ICOS and autotransfusion in these patients. Further clinical research is needed.



**Fig 5.** The expression levels of CTNNB1 and TP53 mRNA in patients with HCC from different groups were determined at preoperative and postoperative 6 h using the qRT-PCR. HCC, hepatocellular carcinoma.



**Fig 6.** The detection of EpCAM in patients with HCC from different groups at preoperative and postoperative 6 h time points, using an immunofluorescence assay. HCC, hepatocellular carcinoma.

Preoperatively, both groups exhibited low percentages of cell survival rates, a trend that continued during surgery, with no significant differences noted ( $P > 0.05$ ). This aligns with previous studies indicating that surgical stress can lead to compromised cell viability (Liu et al. 2020; Peng et al. 2023). However, 6 h post-surgery, we observed a significant increase in cell survival rates in the experimental group compared with the control group ( $P < 0.05$ ). This improvement underscores the potential of our treatment protocol to enhance cell viability in a postoperative setting, suggesting that it may mitigate some of the cellular stressors typically encountered during and after surgical procedures (Koch et al. 2023). Similarly, we assessed apoptosis levels in both groups, which were found to be relatively high before and during surgery, with no significant differences ( $P > 0.05$ ). However, 6 h post-operation, a significant decrease in apoptosis levels was recorded in the experimental group ( $P < 0.05$ ). This reduction highlights the effectiveness of the treatment in promoting cell

survival and suggests a potential mechanism by which the intervention may exert its protective effects.

Furthermore, the assessment of CTC counts revealed that both groups had relatively low levels preoperatively, reinforcing the idea that the surgical context is characterized by high tumor burden and systemic effects (Espejo-Cruz et al. 2021; Sun et al. 2023). The absence of significant differences in CTC counts underscores the need for interventions that not only improve cell survival rates but also effectively target CTCs to prevent metastasis.

IOCS represents a promising strategy in liver cancer surgery, but its clinical implications are not fully understood, especially in the perioperative setting. This study shows that IOCS significantly enhances postoperative cell survival, reduces apoptosis, and decreases CTC counts in patients with HCC. These findings suggest that IOCS may help to preserve viable cells and reduce tumor cell dissemination during surgery, potentially improving outcomes. However, the effects of IOCS



on tumor growth or metastasis remain unclear, and further research is needed to explore these aspects. Additionally, the potential influence of leukocyte depletion filters in this study warrants further investigation. To clarify the clinical relevance for perioperative care teams, future research should focus on validating these results across different cancer types and tumor models, as well as comparing IOCS with and without LDF to isolate the specific effects of the intervention. This will strengthen the applicability and interpretability of IOCS in clinical practice.

In conclusion, the findings of this study support the hypothesis that the treatment applied in the experimental group significantly enhances postoperative cell survival and reduces apoptosis levels compared with the control group. These results contribute valuable insights into therapeutic strategies aimed at improving patient outcomes in liver cancer surgery and warrant further investigation to explore the underlying mechanisms and long-term implications of these findings.

## 5. Conclusion

This study demonstrates that IOCS significantly enhances postoperative cell survival and reduces apoptosis levels in patients with HCC undergoing surgery. Additionally, a notable reduction in CTC counts was observed in the experimental group 6 h post-surgery, suggesting that IOCS may help to mitigate tumor cell dissemination. However, while these findings suggest beneficial effects of IOCS on cell viability and tumor cell reduction, the potential impact on tumor growth or metastatic potential remains unclear. Further research is needed to directly assess whether the increased survival of viable cells post-IOCS affects tumor growth or metastatic behavior, particularly in relation to the preservation of CTCs. This study contributes to the growing body of evidence on IOCS in liver cancer surgery and highlights the need for further investigation to fully understand the implications of IOCS on tumor biology, as well as its role in the perioperative setting. For clinicians, particularly anesthesiologists and surgeons involved in IOCS, it is essential to consider both the

potential benefits in reducing tumor cell dissemination and the need for careful evaluation of long-term outcomes related to metastatic potential.

## Acknowledgments

We would like to acknowledge everyone for their helpful contributions to this paper.

## Funding

This work was supported by the National Natural Science Foundation of China (No. 82170225), Pudong New Area Science and Technology Development Fund (PKJ2022-Y27), and Key Disciplines Group Construction Project of Pudong Health Bureau of Shanghai (PWZxq2022-5).

## Ethics Approval and Consent to Participate

Ethical approval was given by Gongli Hospital of Shanghai Pudong New Area, and written informed consent was obtained from all patients.

## Author Contributions

Each author has made an important scientific contribution to this study and has assisted with the drafting or revising of the manuscript.

## Competing Interests

All authors declare no conflict of interest.

## Consent for Publish

All authors have consented to publish this research.

## Availability of Data and Materials

The data are freely accessible upon request.

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