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ORIGINAL ARTICLE

Efficiency of leukocyte depletion filters and micro-aggregate filters following intra-operative cell salvage during cesarean delivery

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ABSTRACT
Background: Intra-operative cell salvage is not routinely used during cesarean delivery because it is not cost-effective for patients at low risk of hemorrhage and there are theoretical concerns about amniotic fluid embolism. Some guidelines recommend using leukocyte depletion filters to decrease the risk of amniotic fluid embolism before re-infusing salvaged blood, but these filters are not available in Japan. We compared the efficacy and safety of leukocyte depletion and micro-aggregate filters in combination with intra-operative cell salvage during cesarean delivery.
Methods: Blood was collected in a Cell Saver 5 reservoir during cesarean delivery. Four samples were collected: pre-wash, post-wash, post-filtration with a leukocyte depletion filter and post-filtration with a micro-aggregate filter. Each sample was analyzed for amniotic fluid markers of zinc coproporphyrin-1 and sialyl-Tn, for fetal hemoglobin, and the sample underwent pathological examination for white blood cells and squamous cells. Post-filtration samples were compared using paired t-tests with $P<0.05$ indicating statistical significance.
Results: Zinc coproporphyrin-1 and sialyl-Tn were negative at almost all sample points. Squamous cells decreased by 59.1% post-wash and 91.2% post-filtration using a leukocyte depletion filter. Leukocyte depletion filters removed 99.7% of white blood cells and were more effective in removing white blood cells than micro-aggregate filters ($P=0.02$).
Conclusion: Leucocyte depletion filters are more effective in removing white blood cells and squamous cells than micro-aggregate filters, and their introduction for intra-operative cell salvage during cesarean delivery should be considered in Japanese clinical practice.

Keywords: Amniotic fluid embolism; Cesarean delivery; Intraoperative cell salvage; Leukocyte depletion filter; Micro-aggregate filter

Introduction

Allogenic and autologous blood transfusions have been used for the treatment of severe obstetric hemorrhage associated with placenta accreta, placenta previa, and atonic bleeding. However, allogenic transfusion is associated with the risk of infection and allergic reactions, and there is a limit to the amount of autologous blood that can be collected before delivery. Intra-operative cell salvage (ICS), used in cardiovascular, orthopedic and gynecological surgery, involves use of blood from the bleeding site collected through a heparinised tube, separation of cells by hemoconcentration, differential centrifugation in 0.9% saline, and finally washing of cells in 1–2 L of 0.9% saline. Intra-operative cell salvage removes circulating fibrin, debris, plasma, leucocytes, microaggregates, complement, platelets, free hemoglobin, and most of the heparin.

Intra-operative cell salvage is rarely used in cesarean delivery (CD) in Japan because leukocyte depletion filters are not available and there is a theoretical concern about amniotic fluid embolism (AFE). If its safety can be proven, it could be a useful management option in patients at risk of massive hemorrhage.

According to the guidelines of the National Institute for Health and Care Excellence (NICE)[1] and the National Health Service,[2] ICS during CD is allowed. There is no evidence of a direct relationship between re-infusion of salvaged blood cells and the occurrence

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of AFE. The guidelines recommend the use of a leukocyte depletion filter before re-infusing salvaged blood. However, in Japan, leukocyte depletion filters are not readily available due to low demand since allogenic blood products are almost free of leukocytes at the production phase.[3] The filters were available between 2013 and 2016, but poor clinical demand prompted the manufacturer to cease supply.

Instead, use of a micro-aggregate filter with a 10–40 μm pore size during ICS and autologous blood transfusion is recommended for removing micro-aggregate impurities. To date, there have been no reports of AFE with ICS during CD, when used with or without a micro-aggregate filter.

The aim of this study was to evaluate the efficacy and safety of leukocyte depletion filters and micro-aggregate filters by analyzing salvaged and filtered blood components from women undergoing CD. We hypothesized that a micro-aggregate filter could be used instead of a leukocyte depletion filter during ICS in cesarean delivery.

Methods

After obtaining local research Ethics Committee approval and written informed consent, the data of five women who underwent elective CD between July 30, 2013 and August 18, 2013 were collected. Patients with high fever, suspected intrauterine infection and/or emergency CD due to the onset of labor or premature rupture of membranes were excluded.

During surgery, fluid (blood plus amniotic fluid) was aspirated from the abdomen immediately after rupture of the membranes. The volume of amniotic fluid was estimated visually. The aspirated fluid was collected in the reservoir of a Cell Saver 5® (Hemonetics, Massachusetts, USA). Four samples were collected from each patient:

Sample 1: pre wash (in the collecting reservoir)
Sample 2: post wash
Sample 3: post filtration with a leukocyte depletion filter (Pall RC100DJ®)
Sample 4: post filtration with a micro-aggregate filter (Pall SQ40s®)

Leukocyte depletion filters (Pall RC100DJ®) are 8-μm fiberglass filters. Micro-aggregate filters (Pall SQ40s®) can remove micro-aggregate cells larger than 40 μm.

Sample collection is shown in Fig. 1. All the samples and circuits were shielded with aluminum foil to prevent blood from resolving the zinc coproprphyrin-1 (ZnCP-1) in response to light. Each sample was analysed for amniotic fluid markers ZnCP-1, syalyl-Tn (STN), fetal hemoglobin (HbF) (Kleihauer-Betke test), and underwent

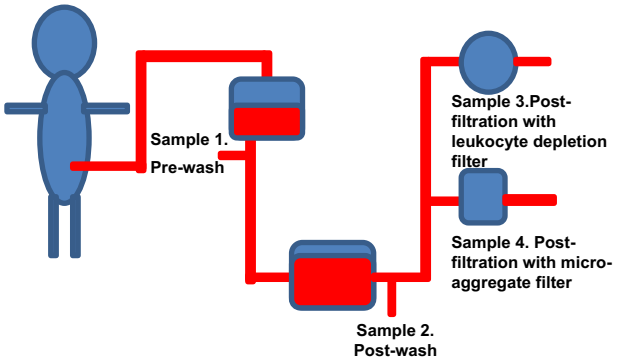

Fig. 1 Flowchart of the sample collection

pathological examination (smear for white blood cells (WBCs), squamous cells (SqCs), hair and meconium; and Alcian blue stain).

Samples for ZnCP-1 were sent, completely shielded with aluminum foil, to the Hamamatsu University School of Medicine, where they were assayed using high performance liquid chromatography.[4] The normal value is <1.6 (pmol/mL), and the sensitivity and specificity for detecting AFE is 46% and 73% respectively.[5] Samples for STN were assayed using competitive radioimmunoassay. The lower limit of detection is 1.2 U/mL, where one unit is equivalent to 25 ng of STN antigen. The sensitivity and the specificity for detecting AFE is 26% and 97% respectively.[5,6]

HbF was analysed using the Kleihauer-Betke method. For pathological examination, we made five blood smear preparations of each sample, counted the number of WBCs and SqCs under a microscope (400×) per mm^2 and calculated the average of five slides. When cells were observed, Alcian blue staining was performed to determine whether they were fetal cells.

The removal rates for pathological examination were calculated according to the following equations:

$$\text{Removal rate 3 (\%)} = (\text{Sample 2} - \text{Sample 3})/\text{Sample 2} \times 100$$
$$\text{Removal rate 4 (\%)} = (\text{Sample 2} - \text{Sample 4})/\text{Sample 2} \times 100$$

The results of samples 3 and 4, which are presented as the numbers of WBC and squamous cells, were compared using the paired t-test in the R statistical programming language. A P-value of <0.05 indicated statistical significance.

Results

The characteristics of the five patients are summarized in Table 1. The mean gestational age at delivery was 38 weeks and 2 days, mean volume of estimated blood loss during CD was 422.6 (188–610) mL, mean volume

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Table 1 Characteristics and data of patients

Case	Age	G	P	Adaptation of the CD	Gestational age (W-D)	Blood loos (mL)	Volume of AF (mL)	Body weight of newborn(g)	Duration of surgery (min)
1	36	3	2	Previous CD	38–4	425	100	3000	50
2	32	1	0	Breech	38–1	490	NA	2118	32
3	40	2	1	Previous CD	38–3	400	200	3722	38
4	33	1	0	CPD	38–3	610	NA	2622	38
5	44	1	0	Breech	38–0	188	300	2814	29
Mean	37	1*	0*		38–2	422.6	200	2855.2	37.4

G: gravida. P: parity. AF: amniotic fluid. CPD: cephalopelvic disproportion. NA: not applicable. CD: cesarean delivery. W-D: weeks-days.
*Values expressed as median.

of estimated amniotic fluid was 200 mL, and mean body weight of the babies was 2855 g. All operations were completed in the routine manner and there were no peri-operative complications.

The pathological results are shown in Figs. 2 and 3 and in Table 3. The median removal rates of SqCs and WBCs after filtration with a leukocyte depletion filter were 91.2% and 99.7%, whereas those after filtration with a micro-aggregate filter were −2.2% and 20.3%, respectively. Two patients had more SqCs and one had more WBCs after filtration with a micro-aggregate filter than before filtration. When the number of WBCs was compared between samples 3 and 4, the *P*-value was 0.02 (95% confidence interval; −135.9 to −17.1), indicating a statistically significant difference (Fig. 4). Alcian Blue staining, which reflects acid mucin in amniotic fluid, was negative in all samples after filtration with either filter (Fig. 3 and Table 4). Neither hair nor meconium were found in any sample.

Table 2 shows the mean values of ZnCP-1, STN, and HbF at each sample point in the five patients. ZnCP-1 levels were <1.6 pmol/mL at all sample points. Similarly, STN levels were <10 U/mL in almost all samples, except in sample 1. Fetal hemoglobin level increased in some samples and decreased in others after filtration with the different filters, without trends.

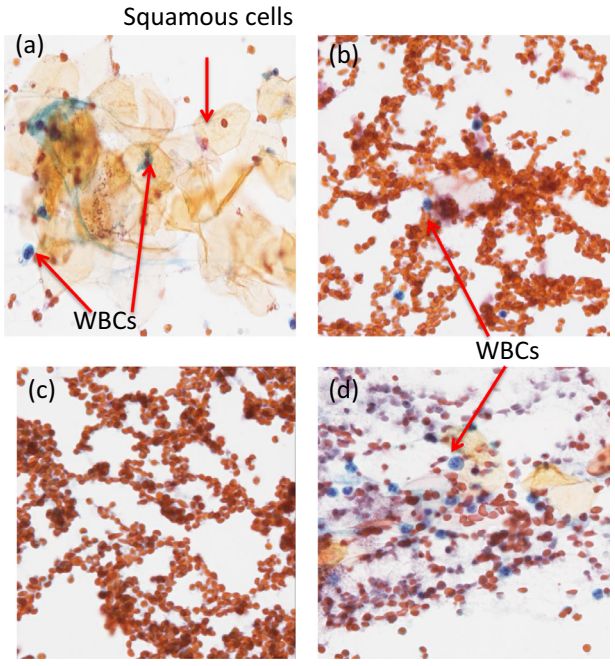

Fig. 2 (a) Pre-wash. There were numerous white blood cells (WBCs) and squamous cells. (b) Post-wash. There were some WBCs. (c) Post-filtration with leukocyte depletion filter. There were few WBCs. (d) Post-filtration with micro-aggregate filter. There were some WBCs

Discussion

Leukocyte depletion filters (Pall RC100DJ®) were more effective at removing WBCs than micro-aggregate filters (Pall SQ40s). In the pathological examination, SqCs decreased by 82.7% in the post-wash stage and further decreased by 91.2% in the post-filtration stage using a leukocyte depletion filter. White blood cells decreased by 99.7% in the post-filtration stage using a leukocyte depletion filter.

Table 2 Mean values of ZnCP-1 and HbF

Test	1	2	3	4
ZnCP-1 (p mol/mL)	<1.6	<1.6	<1.6	<1.6
STN (U/mL)	≦10*	≦10	≦10	≦10
HbF (%)**	0.5	1.2	1.08	1.22

HbF: fetal hemoglobin. STN: sialyl-Tn. ZnCP-1: zinc coproporphyrin-1.
*Most cases are below the detection limit (≤10) except for case 1 (STN 12.7).
**Values expressed as mean.

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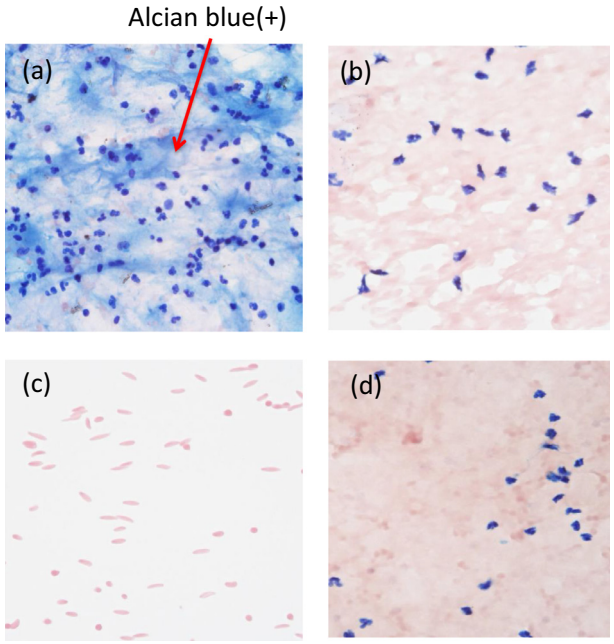

Fig. 3 (a) Pre-wash Alcian blue (+). (b) Post-wash. (c) Post-filtration with leukocyte depletion filter. (d) Post-filtration with micro-aggregate filter. (b–d): Alcian blue (−). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

Leukocyte depletion filters (Pall RC100DJ®) are 8-μm fiberglass filters that act through a combination of passive sieving and active adhesion to a negative surface charge.[7,8] They can remove WBCs, fetal SqCs, trophoblastic cells and activated neutrophils. Micro-aggregate filters (Pall SQ40s®) can remove micro-aggregate cells larger than 20–40 μm. The diameter of red blood cells, WBCs, platelets and SqCs (on the surface) are 6–9 μm, 6–10 μm, 2–4 μm and 50–60 μm respectively.[7] Theoretically, micro-aggregate filters might not effectively reduce WBCs but should be able to remove SqCs.

Catling et al. found fetal SqC contamination in half of the post-filtration samples with ICS and a leucocyte depletion filter (Pall RC100DJ®).[9] In contrast, other studies showed almost no SqCs post filtration with ICS and a leucocyte filter (LeukoGuard® RS),[10] and the same filter alone can remove components of pure amniotic fluid.[7] Filtration had no effect either on the concentrations of α-fetoprotein, tissue factor, or endotherin-1, nor on the presence of meconium or vernix, however lamellar bodies and fetal SqCs (filtration efficacy 96.6% and 99.9%, respectively) and hair were completely removed. The different results, in various reports, regarding removal of SqCs seem to be related to the use of different types of leukocyte depletion filters used in the studies.[10]

A leucocyte depletion filter is recommended during ICS and can decrease the risk of adverse events such as fever, rash, or itching.[1] Leucocyte depletion filters should be used during ICS for blood transfusion based on the results of these studies, and the LeukoGuard® RS filter removes the SqCs more effectively than the RC100DJ® filter.

Amniotic fluid embolism is an anaphylactoid response involving endogenous mediator release following exposure of the maternal circulation to small amounts of amniotic fluid. There is no standard diagnostic test to confirm the presence of AFE, but ZnCP-1 and STN are considered useful markers for its diagnosis.[11] Both ZnCP-1 and STN markers were undetectable in almost all samples even at pre-wash. Few case reports have shown low levels of ZnCP-1 and STN in post-wash materials during ICS or in normal amniotic fluid, so we focused on these markers in this study. Blood contaminated with amniotic fluid was collected to compare the efficiency of removal of fetal components for the two different filters. We anticipated that

Table 3 Results of pathological examination of count per mm^2 of squamous cells (SqC) and white blood cells (WBC)

Case	SqC 1	SqC 2	SqC 3	SqC 4	Removal rate 3*	Removal rate 4**
1	48.2	1.2	0.4	1.8	66.6	−50
2	15	6.8	1.2	2	82.4	70.6
3	11.6	37.2	0	20.2	100	45.7
4	1.8	0	0	0.8	NA	NA
5	40.2	2.6	0	7.6	100	−192.3
Median	15	2.6	0	2	91.2	−2.2
Case	WBC 1	WBC 2	WBC 3	WBC 4	Removal rate 3*	Removal rate 4**
1	9.4	89	0	35.2	100	60.4
2	112.8	223.4	0.6	124.4	99.7	44.3
3	8.8	85.6	0.2	68.2	99.8	20.3
4	161.2	81.4	1.2	129.6	98.5	−59.2
5	12.4	16	0.2	27.2	98.8	−70
Median	12.4	85.6	0.2	68.2	99.7	20.3

*Removal rate 3 (%)=(Sample 2 − Sample 3)/Sample 2 × 100.**Removal rate 4 (%)=(Sample 2 − Sample 4)/Sample 2 × 100.; WBC: white blood cells. SqC: squamous cells. NA: not applicable.

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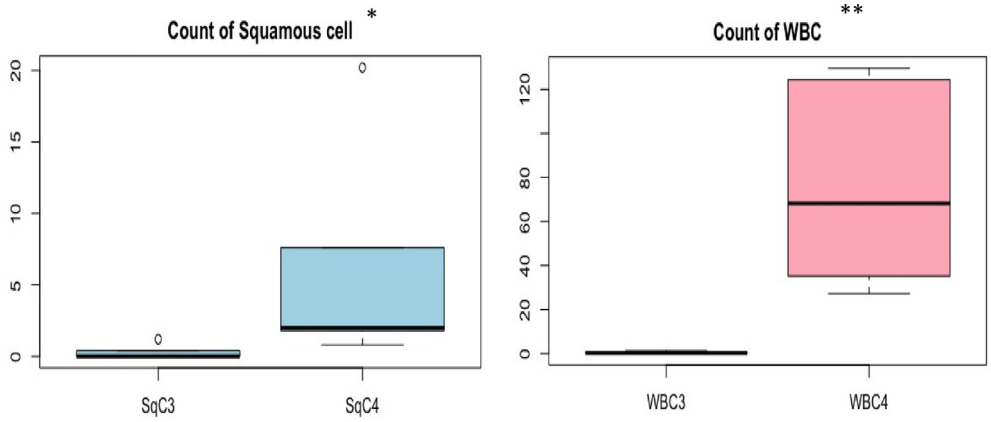

Fig. 4 Results of pathological examination and comparison of post-filtration sample 3 with sample 4. WBC: white blood cells. $^{*}P=0.128$, $^{**}P=0.02$

Table 4 Pathological examinations of Alcian blue

Case	1	2	3	4
1	+	−	−	−
2	+	−	−	−
3	−	+	−	−
4	−	−	−	−
5	+	+	−	−

ZnCP-1 and STN might be detected in sample 1 and then removed after washing (sample 2), but the levels found in almost all samples were too low to permit an assessment of removal efficiency.

ZnCP-1 is not only detected in meconium-contaminated amniotic fluid but also in uncontaminated amniotic fluid.[12] Although it was unclear why ZnCP-1 and STN were negative in this study, these markers might not be detected in elective CD patients whose babies tend to experience less stress.

We could not identify any trends in HbF measurements. In healthy pregnant women, the proportion of HbF is $0.71 \pm 0.51\%$ (non-pregnant women: $0.28 \pm 0.35\%$).[13] We found no difference between the levels of HbF detected in this study and those reported in pregnant women in general, which confirms earlier findings.[9,10] It is difficult to distinguish fetal red blood cells from maternal red blood cells during ICS,[10] and, when salvaged blood product is re-infused into the mother, fetal blood is entrained into the maternal circulation.[9,13] Fetal hemoglobin is less liable to cause embolism and allergic reactions leading to AFE. Except in the case of rhesus incompatibility, HbF contamination of maternal blood is not a significant issue in clinical practice.[11]

Morikawa et al. reported 50 cases of ICS performed in Japan.[14] There were no complications, including AFE or hypotension, associated with ICS blood transfusion using a leucocyte depletion filter. Intra-operative cell salvage may have contributed to the avoidance of allogeneic blood transfusion and to a reduction in the volume of allogeneic blood transfusion in some patients who undergo ICS.

The SALVO study reported 1498 patients who were randomised to receive ICS during CD.[15] Leucocyte depletion filters were used in only 54.9% (782/1498) of the cases because of acute hypotensive events.[15,16] Although it is difficult to completely remove fetal components using ICS with or without a leucocyte depletion filter, there have been no reports of AFE occurring when re-infusing salvaged blood.[17] Some authors do not recommend routine use of leucocyte depletion filters in obstetric practice.[16,18] Further research would be required to reveal whether filtration is needed to make ICS safer during CD.

The study has limitations. First, the number of the participants and tested markers were small due to limited research funding, so the findings must be viewed with caution. In contrast, other studies have included larger sample sizes and more markers.[4,6,13]

Second, the estimated mean amniotic fluid volume (200 mL) was low in our study compared with other publications.[19,20] The volume was estimated visually just after rupture of the membranes and has the potential for inaccuracy.

Thirdly, and importantly, the inconsistent volume of cells seen on pathological examination remains unexplained, especially when using a micro-aggregate filter. This might have resulted from artifacts introduced when preparing the slides or from measurement errors. Such sample handling errors may limit interpretation and applicability of the findings, and mean the results must be viewed with caution.

In conclusion, the AFE markers ZnCP-1 and STN were negative in pre-wash samples at almost all sample points in this study. Leucocyte depletion filters appear more effective at removing WBCs and SqCs than micro-aggregate filters. Introduction of leucocyte depletion filters in Japanese clinical practice for ICS during CD should be considered.

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Declaration of Competing Interest

None.

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