Original Article

# A laboratory study of the effects of processing blood through a cell salvage device and leucocyte depletion filter on levels of pro-inflammatory cytokines and bradykinin

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## Summary

We investigated changes in concentrations of interleukin-1β, interleukin-6, tumour necrosis factor-α and bradykinin in blood during passage through a cell salvage device and a leucocyte depletion filter, with or without application of subatmospheric pressure across the filter. Blood samples from 19 healthy women undergoing scheduled caesarean section showed concentrations of cytokines and bradykinin in blood filtered under gravity flow that were equal to or significantly lower than those of pre-operative venous blood samples. They were also significantly lower than that in postoperative orthopaedic shed blood, which is commonly reinfused after orthopaedic surgery. A minority of samples taken from blood that had been filtered using subatmospheric pressure showed raised interleukin-6 concentrations. We suggest that use of a leucocyte depletion filter for cell-salvaged blood with gravity flow is likely to be safe with regard to concentrations of cytokines and bradykinin. However, this may not hold true for the filter used with sub-atmospheric pressure. If transfusion of salvaged blood using a leucocyte depletion filter seems to induce hypotension, elevation of interleukin-6 should be suspected.

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Allotransfusion carries risks of infection, blood group mismatch, acute and chronic haemolytic complications, and transfusion-related acute pulmonary damage. Given these risks, autologous transfusion methods, which can reduce the amount of allotransfusion, have been in the spotlight. Among various autologous transfusion methods, cell salvage is considered a safe and physiological method for intra-operative use.

Bleeding is a significant cause of maternal mortality and morbidity during caesarean section. The use of cell salvage during caesarean section can reduce allotransfusion, increase postoperative haemoglobin and shorten the hospitalisation period [1]. However, its use in this situation is controversial because of fears of amniotic fluid embolism. A leucocyte depletion filter can reduce contamination of fetal materials [2, 3] and the American Association of Blood Banks recommends the use of a leucocyte depletion filter at caesarean section [4]. However, hypotension has been reported within 5 min of transfusing salvaged blood when a leucocyte depletion filter was used with a cell salvage device [5–7].

The cause of hypotension during the use of cell salvage in caesarean section is yet to be determined. A recent study suggested that amniotic fluid embolism may be caused either by an anaphylactic reaction or a systemic inflammatory reaction of the maternal immune system against the amniotic fluid [8]. In addition, the cell salvage process might generate cytokines during the extracorporeal circulation. Finally, although it is assumed that the cause of hypotension during transfusion of salvaged blood with a leucocyte depletion filter is the production of bradykinin [6], this has not been investigated.

The aim of this study was to evaluate the safety of a cell salvage device with a leucocyte depletion filter during caesarean section by measuring the level of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and bradykinin in blood taken at various stages in the cell salvage process, including after passage through a leucocyte depletion filter under gravity or subatmospheric pressure. We compared our results to published reference values for cytokines and bradykinin in normal healthy adults and third trimester parturients. In addition, we identified published data on retrieved blood after arthroplasty, as this is sometimes transfused without the processing that occurs during cell salvage.

## Methods

This study was approved by the Seoul National University Hospital Institutional Review Board. After gaining informed consent, 20 parturients of ASA physical status 1–2 scheduled for scheduled caesarean section were recruited. Parturients were excluded if they had coagulation abnormalities on pre-operative coagulation tests (platelet $< 100.0 \times 10^9.l^{-1}$, prothrombin time INR > 1.2, prothrombin time < 80%, activated partial thromboplastin time > 35.3 s) or suspected infection (WBC $> 10.0 \times 10^9.l^{-1}$, C-reactive protein > 5 $nmol.l^{-1}$ or erythrocyte sedimentation rate > 20 $mm.h^{-1}$).

Parturients were not premedicated. Spinal or combined spinal-epidural anaesthesia was induced using 10 mg hyperbaric bupivacaine 0.5% and 10 μg fentanyl. General anaesthesia was induced with 250 mg thiopental with 100 mg suxamethonium and maintained with nitrous oxide 67% in oxygen, and sevoflurane.

We used a continuous auto transfusion cell salvage device (C.A.T.S., Fresenius AG, Bad Homburg, Germany). Heparin 25 000 U (JW Pharmaceuticals, Seoul, Korea) in 1000 ml saline 0.9% was used as the anticoagulant [9]. Collected blood was washed using the high-quality wash, quality wash or emergency wash programme provided by the C.A.T.S. device (Fig. 1). The procedures were performed by a blinded researcher who was expert at using the C.A.T.S. device. The programme was selected using a random number table [10]. Two leucocyte depletion filters (Bio R 02 BBS plus; Fresenius AG, Bad Homburg, Germany) were installed in parallel to drain the washed blood. The clamp was released on one filter and 50% of the collected blood was drained under gravity. The first filter was then clamped and the second unclamped. The remaining blood was then drained using subatmospheric pressure applied with a 50-ml syringe (Fig. 1).

Paired 3-ml blood samples were collected at four stages of the procedure. A venous blood sample was collected from the parturient before surgery (baseline). Salvaged blood was collected from the cell salvage collection reservoir (reservoir), after the wash process (post-wash), and after passage through the leucocyte depletion filter (post-filter/gravity or post-filter/pressurised). Samples for pro-inflammatory cytokine measurement were transferred to endotoxin-free EDTA bottles, and those for bradykinin measurement to aprotinin-treated test tubes. Sample bottles were stored on ice immediately after collection, and were centrifuged within 5 min. Centrifugation was performed at 1500 **g** for 10 min at room temperature and the samples were then frozen and stored at −70 °C until analysis. Multiplex cytokine assay system Bio-Plex Pro™ Assays (Bio-rad, Philadelphia, PA, USA) were used to analyse IL-1β, IL-6 and TNF-α concentrations. Serum bradykinin concentration was analysed using enzyme immunoassay (Bradykinin (Human, Rat, Mouse) EIA kit; Phoenix Pharmaceuticals, Inc., Burlingame, California, USA).

 

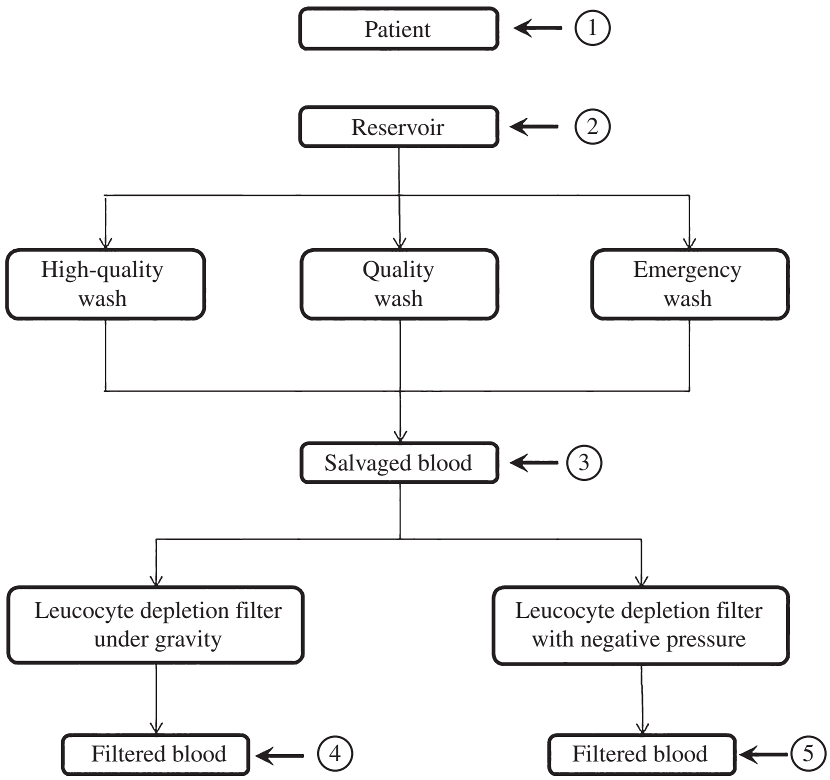


**Figure 1** Overview of blood sampling at numbered points. ① Baseline pre-operative venous blood ② Unprocessed blood from Reservoir; followed by random allocation to one of three wash programmes. ③ post-wash ④ post-filter/gravity ⑤ post-filter/pressurised.

A power analysis was performed after a pilot study of 10 parturients. A sample size of 20 was required to detect a difference of 20 $pg.ml^{-1}$ for IL-1β, 100 $pg.ml^{-1}$ for IL-6, 200 $pg.ml^{-1}$ for TNF-α and 1 $ng.ml^{-1}$ for bradykinin with a power level of 80% and a $p < 0.05$. Statistical analysis was performed using SPSS software (version 19.0: SPSS, Inc; an IBM Company, Chicago, IL, USA). The Kruskal–Wallis test was used for patients' characteristics and Wilcoxon's signed rank test for the effect of pressure as well as comparisons with published results. Friedman's test was used for the combined effects of washing protocols and pressurisation, with Kruskal–Wallis used for a post-hoc test comparing the differences between the wash protocols. Results were considered significant at $p < 0.05$.

We performed a literature search to ascertain published reference values for cytokine and bradykinin concentrations in other patient groups for comparison with our results. The following keywords were used to search for studies in 'PubMed': 'bradykinin'; 'cytokine'; 'interleukin-1 beta'; 'interleukin-6'; 'operative blood salvage'; 'postoperative blood salvage'; 'pregnancy'; 'reference value'; and 'tumour necrosis factor-alpha'.

## Results

The mean (SD) age of 20 parturients was 34.7 (3.4) years, with a height of 160.6 (4.1) cm, weight 71.5 (14.4) kg and gestation 38.2 (1.0) weeks. Four parturients had major placenta praevia, one had hyperthyroidism and two had a history of gynaecological surgery. One parturient had thrombocytopenia and pulmonary oedema. She had abnormal values of cytokines and bradykinin in the baseline blood sample, and was excluded post-hoc.

The salvaged blood from five parturients, all of whom had regional anaesthesia, was treated with high-quality wash. Blood from seven parturients, of whom four had regional and three general anaesthesia, was treated with quality wash, and that from seven parturients, of whom five had regional and two general anaesthesia, was treated with emergency wash. No salvaged blood was re-transfused.

 

**Table 1** Concentrations of cytokines and bradykinin in pre-operative venous blood and during the cell salvage process, according to different wash programmes. Values are as mean (SD).

| | n | Baseline | Reservoir | Post-wash | Post-filter/gravity | Post-filter/pressurised |
|---|---|---|---|---|---|---|
| IL-1β; pg.ml$^{-1}$ | | | | | | |
| High-quality wash | 5 | 2.2 (0.6) | 2.4 (1.0) | 2.4 (1.4) | 2.0 (1.1) | 2.5 (1.8) |
| Quality wash | 7 | 2.3 (0.3) | 1.7 (1.0) | 3.8 (3.1) | 2.2 (0.8) | 2.2 (0.8) |
| Emergency wash | 7 | 2.3 (0.6) | 2.5 (1.5) | 2.9 (1.6) | 2.2 (1.1) | 2.0 (0.7) |
| All | 19 | 2.2 (0.6) | 2.2 (1.2) | 3.1 (2.2) | 2.2 (0.9) | 2.2 (1.1) |
| IL-6; pg.ml$^{-1}$ | | | | | | |
| High-quality wash | 5 | 6.8 (3.8) | 245.2 (268.3) | 18.7 (28.6) | 6.9 (4.9) | 29.7 (48.5) |
| Quality wash | 7 | 4.4 (1.3) | 215.8 (252.7) | 21.6 (20.2) | 23.9 (27.1) | 38.1 (44.9) |
| Emergency wash | 7 | 5.1 (1.5) | 166.9 (209.1) | 18.5 (18.5) | 9.5 (8.9) | 11.4 (8.1) |
| All | 19 | 5.3 (2.4) | 205.5 (230.1)* | 19.7 (20.8)* | 14.1 (18.3)† | 26.0 (36.9)† |
| TNF-α; pg.ml$^{-1}$ | | | | | | |
| High-quality wash | 5 | 136.4 (169.0) | 17.5 (19.7) | 0.1 (0.2) | 0.2 (0.4) | 1.3 (2.8) |
| Quality wash | 7 | 61.4 (53.9) | 7.9 (12.5) | 2.1 (3.7) | 2.5 (4.1) | 2.4 (3.1) |
| Emergency wash | 7 | 85.5 (95.6) | 32.1 (29.5) | 9.1 (19.2) | 1.1 (2.5) | 1.0 (1.5) |
| All | 19 | 90.0 (106.2) | 19.3 (23.3)‡ | 4.1 (11.9)‡ | 1.4 (2.9) | 1.6 (2.5) |
| Bradykinin; ng.ml$^{-1}$ | | | | | | |
| High-quality wash | 5 | 14.9 (23.9) | 24.0 (43.3) | 1.4 (2.6) | 0.2 (0.3) | 1.3 (2.1) |
| Quality wash | 7 | 14.9 (37.5) | 2.8 (2.7) | 0.7 (1.7) | 0.7 (0.8) | 0.5 (0.7) |
| Emergency wash | 7 | 4.8 (4.6) | 1.8 (2.6) | 0.2 (0.4) | 0.0 (0.0) | 0.0 (0.0) |
| All | 19 | 11.2 (25.1) | 8.0 (22.8)§ | 0.7 (1.7)§ | 0.3 (0.6) | 0.5 (1.2) |

IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α.
*$p < 0.001$; †$p < 0.001$; ‡$p = 0.023$; §$p = 0.035$.

Concentrations of cytokines and bradykinin in the samples from 19 parturients are presented in Table 1. There were no differences based on the different washing protocols, and so the results were pooled (Fig. 2). Concentrations of IL-6, TNF-α and bradykinin were reduced by washing. The concentration of IL-6 in post-filter/pressurised blood was significantly higher than that in post-filter/gravity blood ($p < 0.001$). Concentrations of IL-6, TNF-α and bradykinin were significantly lower after the wash process ($p < 0.001$, $p = 0.023$, and $p = 0.035$, respectively; Fig. 2).

Two of the 19 parturients showed outlying values. One parturient underwent a caesarean section owing to major placenta praevia; the IL-6 concentration was 44.2 pg.ml$^{-1}$ in post-wash blood, but 74.4 pg.ml$^{-1}$ and 89.3 pg.ml$^{-1}$ in post-filter/gravity and post-filter/pressurised blood, respectively. The second had a caesarean section due to twin pregnancy; the IL-6 concentration was 42.6 pg.ml$^{-1}$ 7 in post-wash and 42.4 pg.ml$^{-1}$ in post-filter/gravity blood, but 111.4 pg.ml$^{-1}$ in post-filter/pressurised blood.

The excluded patient was carrying male twins at 38 weeks' gestation. She had relative thrombocytopenia ($109 \times 10^9.l^{-1}$) and was diagnosed with pulmonary oedema based on pre-operative chest X-rays. Spinal anaesthesia was induced using bupivacaine and fentanyl. High-quality wash was applied to the reservoir blood. Cytokine concentrations are shown in Table 2.

Published reference values for cytokines and bradykinin from other patient groups are shown in Table 3 [11–18]. Concentrations of IL-6 and bradykinin in post-filter blood with or without pressurisation were similar to, and concentrations of IL-1β and TNF-α were significantly lower ($p < 0.001$) than, those in normal healthy adults and third-trimester parturients. Concentrations of cytokines and bradykinin in post-filter blood with or without pressurisation were significantly lower than those of retrieved blood after arthroplasty ($p < 0.001$).

## Discussion

Our results showed that concentrations of cytokines and bradykinin in the blood processed through a cell salvage device with a leucocyte depletion filter were generally within the range of normal values in adults and pregnant women [11–18]. There was no difference according to the washing protocol that was used. Concentrations of IL-6, TNF-α and bradykinin

 

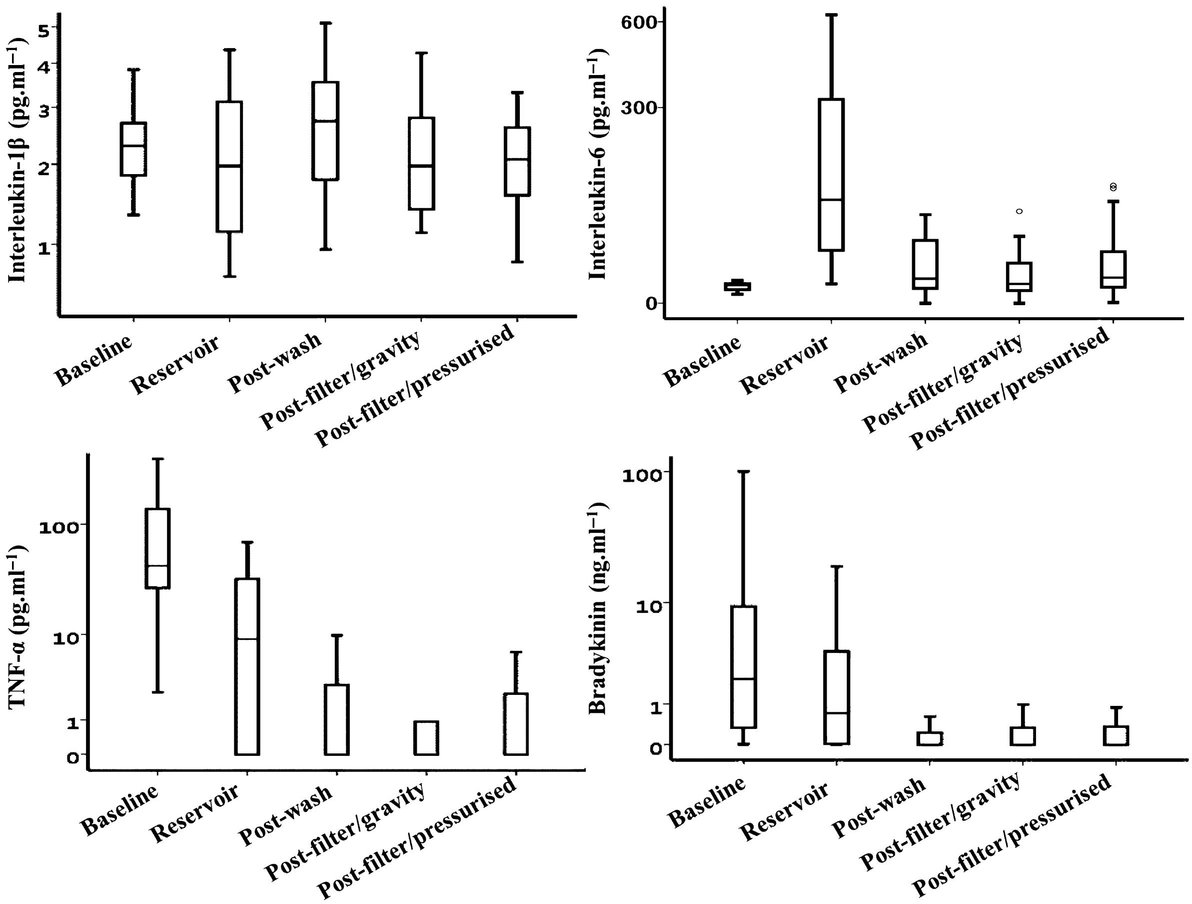


**Figure 2** Concentrations of cytokines and bradykinin at different blood sampling points (pooled results). TNF-α, tumour necrosis factor-α. Values are median (line), IQR (box) and range (whiskers).

**Table 2** Concentrations of cytokines and bradykinin during the cell salvage process in the patient excluded from the pooled results.

| | Baseline | Reservoir | Post-wash | Post-filter/gravity | Post-filter/pressurised |
|---|---|---|---|---|---|
| IL-1β; $pg.ml^{-1}$ | 1.9 | 9.8 | 17.0 | 5.4 | 22.7 |
| IL-6; $pg.ml^{-1}$ | 20.0 | 924.0 | 365.4 | 269.6 | 1501.5 |
| TNF-α; $pg.ml^{-1}$ | 17.4 | 91.2 | 0.9 | 3.6 | 3.6 |
| Bradykinin; $ng.ml^{-1}$ | 0.1 | 9.7 | 0.3 | 0.0 | 0.1 |

IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α.

decreased after the wash process and that of IL-1β increased. These results are congruent with previous studies where relatively large amounts of white blood cells remain after the wash process and where some cytokines other than pro-inflammatory cytokines increase [19, 20]. In addition, there was no significant average change in cytokine and bradykinin levels during passage through the leucocyte depletion filter, also matching previous studies [21, 22].

The Serious Hazards of Transfusion group reported about 10 cases of hypotension between 2008 and 2010 associated with the use of a leucocyte depletion filter with cell salvage [23–25]. Similar reports have occurred in parturients [5–7]. In two healthy parturients, hypotension occurred after transfusion of salvaged blood processed with a leucocyte depletion filter to which pressure had been applied [5, 6]. In a third report, the patient underwent a caesarean section for

**Table 3** Reference values for cytokines and bradykinin in normal healthy adults, in normal third trimester parturients and in retrieved blood after arthroplasty [11–18].

| | IL-1β; pg.ml$^{-1}$ | IL-6; pg.ml$^{-1}$ | TNF-α; pg.ml$^{-1}$ | Bradykinin; ng.ml$^{-1}$ |
|---|---|---|---|---|
| Normal healthy adults | < 16 | < 12 | < 220 | < 0.3 |
| Normal third trimester parturients | < 27 | < 14 | < 51 | NA |
| Postoperative orthopaedic shed blood | < 11 | < 1335 | < 22 | NA |

IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; NA, not available.

intra-uterine fetal demise [7]. Rapid hypotension occurred during transfusion using a leucocyte depletion filter, but blood pressure was maintained when the filter was removed.

There have also been recent reports of acute hypotension when using a leucocyte depletion filter for transfusion of blood products, especially platelet concentrate [26]. The authors of that paper suggested that the cause was stored cytokines or bradykinin. It is possible that bradykinin levels would be raised if using a negative-charged leucocyte depletion filter [25], although we used a neutrally charged filter.

In our study, there was a trend for IL-6, TNF-α and bradykinin concentrations to increase with passage through the filter using subatmospheric pressure. This suggests that the production of inflammatory factors during filtering may be caused by damaged leucocytes residing within the filter or by spilled stored cytokine. One of the cases in our study showed a dramatic increase in IL-6 levels in blood passed through the filter with subatmospheric pressure. We speculate that an idiosyncratic reaction such as this might account for abnormal haemodynamic responses to blood passed through a leucocyte depletion filter. However, our study was too small to determine the frequency of this phenomenon.

We believe that IL-6 is the most likely causative agent of hypotension after transfusion through a leucocyte depletion filter. Interleukin-6 plays a key role in the process of septic shock and anaphylaxis and is correlated with the rate and severity of hypotension as well as duration of anaphylactic reaction symptoms [27, 28]. Hartemink and Groeneveld reported that higher levels of IL-6 in patients with septic shock are correlated with lower mean arterial pressure and systemic vascular resistance, and peripheral vasodilation is very strongly and independently correlated with secretion of IL-6 [29], which is considered a relatively important prognostic factor in septic shock [30].

We excluded results post hoc from one parturient who was not physically healthy before surgery. She had an abnormally high baseline serum IL-6 concentration. She had underlying thrombocytopenia and was suspected of pulmonary oedema based on a pre-operative chest X-ray. As IL-6 is known to stimulate the haemopoietic system and levels are increased in thrombocytopenia patients with complement-mediated reactions [31, 32], we postulate that thrombocytopenia was the cause of the raised pre-operative IL-6 level. Furthermore, there were extremely high levels of IL-6 after the washing process and after pressurised passage through the leucocyte depletion filter.

In summary, when maternal blood is processed with a cell salvage device and leucocyte depletion filter under gravity flow, the levels of cytokines and bradykinin are generally safe. Our results indicate that cytokines may be raised unpredictably if subatmospheric pressure is applied to a leucocyte depletion filter. In addition, it can also be concluded that it is dangerous to apply pressure when fast infusion of blood is needed during surgery because of the possibility of producing large amounts of cytokines and bradykinin. We postulate that IL-6 is the most likely mediator of hypotension in the reported incidents following transfusion of cell-salvaged blood through a leucocyte depletion filter.

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## Competing interests

No competing interests declared.

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