

## Elevation of Neutrophil Elastase Activity in Intra-Operative Recovered Shed Blood

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### Abstract

**Background and Aim:** Neutrophil elastase releasing from activated polymorphonuclear neutrophils (PMN) may have a central role in acute lung injury. Thus, we examined whether neutrophil elastase activity (NEA), a crucial parameter for organ damages from stresses, may increase in intra-operative recovered blood and also whether the storage duration of the bank bloods may affect such changes in NEA.

**Methods:** In thirty-two packed red cells of bank bloods, NEAs were measured by ELISA on several day points after the storage. Intra-operative salvaged red cell concentrates (RCCs) were obtained from thirteen patients undergoing spinal surgery. The salvaged bloods from the patients were divided into two groups: NE activities in autologous RCCs obtained from eight patients were examined soon after the preparation (Group A); such activities in RCCs from five subjects were determined post-operatively (Group B).

**Results:** In the bank bloods, NEAs gradually increased after the storage, showing a plateau at the day 10 if not specifically filtered leukocytes before the preparation. In Group B, NEA levels in RCCs were significantly higher than those in Group A. In Group B (RCCs stored for four hours), NEAs were nearly two times higher than those of the peak NEA values of the bank blood at the day 21.

**Conclusion:** Thus, we need to pay attention the occurrence of lung injury even after the transfusion of autologous bloods collecting by the intra-operative shed blood recovered system.

**Keywords:** Neutrophil Elastase Activity; Shed Blood; Red cell concentrates

### Background

Autologous blood transfusion is widely performed for the purpose of reducing the possible adverse reactions, such as transfusion-related acute lung injury (TRALI) from allogeneic transfusion [1]. Intra-operative blood salvage system is a tool for the autologous blood transfusion [2], but the preparation in the system might cause hematological changes in the salvaged bloods. However, there is the limited information regarding such as mediator changes in recovered blood collecting through the system.

Polymorphonuclear neutrophils (PMNs) may be involved in the development of lung injury after allogeneic transfusion [3]. Furthermore, neutrophil elastase (NE) releasing from activated PMNs may have a central role in the acute lung injury (ALI) [4]. Therefore, we examined whether neutrophil elastase activity (NEA), a crucial mediator activity for organ damages after stress situations, may increase in intra-operative recovered blood and also whether the storage duration of bank bloods may affect such changes in NEA.

### Subjects and Methods

Bank red cell concentrates (RCCs): In thirty-two banked packed red cells provided from the Japan Red Cross Society, NEAs were measured on 3, 5, 10, 14, 21 days after the standard storage keeping at 4°C. Eight RCCs were stored for 10, 14, 21 days after the filtering of white blood cells (WBCs) to evaluate the effects of leukocytes on NEA changes in salvaged bloods. Intra-operative salvaged RCCs were obtained from fourteen patients undergoing spinal surgery in the operating theater. The salvaged bloods from the patients were divided into two groups: NE activities in autologous RCCs from eight patients were measured soon after the preparation (Group A: Table 1); such activities in RCCs from six subjects were determined post-operatively (Group B: Table 2). In Group A, bloods were salvaged from shed blood at the room

temperature and transfused during the operation; In Group B, those were recovered also during the operation and infused to be stored at the room temperature four hours after the operation.

Inclusion criteria for this study: adult patients of 20 to 80 years of age undergoing spinal operation using a shed blood recovery system. Exclusion criteria: patients who have severe complications such as diabetes mellitus and organ abnormal functions including coagulopathy. Neutrophil elastase activities (NEA) in the bank and salvaged bloods were measured by ELISA (Mitsubishi Chemical Medicine Co., Ltd. Tokyo JAPAN). The NEA is measured as the concentration of complex of neutrophil elastase combined with alpha 1-antitrypsin. Statistical analysis was done by Mann-Whitney U-test or ANOVA with Scheffé's F-test ( $p < 0.05$ ) as appropriate. This study has been approved to use blood samples obtained from patients in this study by the IRB of Ehime University Hospital (EHIME UNIV IRB, Approvable #1410020).

### Results

In the bank bloods, NEAs increased remarkably at ten days after the storage if not specifically pre-filtered WBCs (Figure 1).

There are no differences in the demographic data between the two

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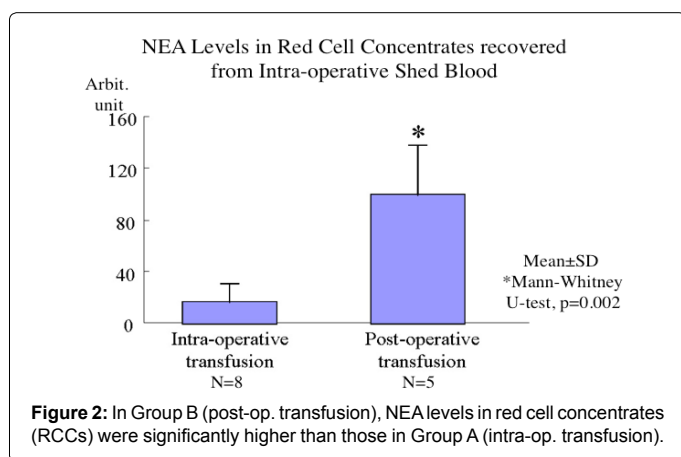
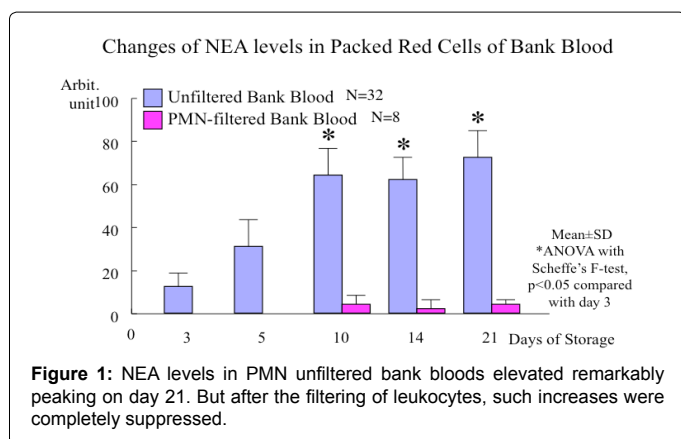
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No of Case	Age	Gender	HT/HW (cm)/(kg)	Vol. of bleeding (ml)	Vol. of RCC transfusion (m1)	Hb of RCC (g/d1)	Timing of transfusion
1	57	Female	147/60	700	120	8.9	Infra-ope.
2	51	Male	160/65	1160	360	11.7	Infra-ope.
3	65	Female	158/65	750	130	9.8	Infra-ope.
4	57	Male	160/60	800	220	12.0	Infra-ope.
5	63	Female	147/58	1200	350	19.5	Infra-ope.
6	50	Male	161/25	550	120	6.9	Infra-ope.
7	21	Male	170/58	850	130	11.7	Infra-ope.
8	50	Female	150/42	200	110	6.8	Infra-ope.
Average (SD)	51.8 +13.7	M:F 4:4	156.6 ± 8.6/6 0.4 ± 9.3	776.3 ± 321.3	192.5 ± 106.1	10.9 ± 4.0	

Table 1: Clinical characteristics of Group A patients.

No. of Case	Age	Gender	HT/BW (cm)/(kg)	Vol. of bleeding (m1)	Vol. of RCC transfusion (m1)	Hb of RCC (g/d1)	Timing of transfusion
1	62	Male	169/72	450	120	99	Post-ope.
	68	Female	149/83	850	210	116	Post-ope.
3	67	Male	163/65	1250	370	117	Post-ope.
4	IS	Female	160/70	500	100	8.6	Post-ope.
5	71	Male	168/53	300	120	103	Post-ope.
Average (SD)	56.6 ± 23.5	M:F 3:2	161.8 ± 8.0 /68.8 ± 10.9	670.0 ± 381.8	184.0 ± 289.8	10.8 ± 1.9	

Table 2: Clinical characteristics of Group B patients.



groups. In Group B (RCCs stored for four hours), NEA levels in red cell concentrates (RCCs) were significantly higher than those in Group A (Figure 2). In Group B, NEA was nearly two times higher than the peak NEA value of the bank blood at the day 21 (Figures 1 and 2).

## Discussion

In this study, we demonstrated the time-dependent elevation of NEAs in the bank bloods without specific filtering of WBCs, but if filtered, such elevations of NEAs were abolished, suggesting that increases in NEA in the bank bloods after the storage for at least more than ten days may be due to PMNs in the bank bloods. Furthermore, we found nearly two-times higher levels of NEAs in the intra-operative recovered autologous bloods as compared those in the bank bloods. Therefore, we need to be aware of a potential occurrence of lung injury even by the transfusion of autologous bloods collecting by the intra-operative shed blood recovered system if stored for several hours. Furthermore, there is need to test a hypothesis that a specific neutrophil elastase inhibitor [5] might have a certain preventive effect on the lung injury after transfusion of recovered shed bloods during the operation.

One might speculate that even if blood containing high levels of NE was transfused, NE would result in the complex of neutrophil elastase-alpha1-antitrypsin (EAC), thereby NE was inactivated [6]. However, it was indicated that EAC itself might activate monocytes, rendering to increase in NE production in humans [7]. In fact, we have reported that in rabbits, EAC itself causes acute lung injuries [8]. Recently, it has been reported that recovered shed bloods after leukocyte filtering could be a safer method even for obstetric or infected patients who were prohibited to use this autologous salvaged blood [9]. In this regard, the present results may support this expansion of the indication of intra-operative recovered shed blood. Furthermore, future research is warranted to define the role of NE contained in the autologous intra-operative salvaged blood in patients undergoing the operation.

In conclusion, in intra-operative salvaged red cell concentrates, augmentation of NE activity occurred. Especially such changes were prominent if stored even for several hours. These results suggest that autologous transfusion of intra-operative salvaged blood should be administered soon after the preparation for preventing potential lung injuries. This concept supports the statement of the autologous blood transfusion guidance [10]. A recent review for TRALI indicated that non-antibody mediated lung injuries might be due to cell derived

substances in donor blood after storage [11], so in the future, we need to clarify definitive causes of TRALI and seek a better method for blood preservation.

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