



## Postmortem serum erythropoietin level as a marker of survival time in injury deaths

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### ABSTRACT

Circulating erythropoietin (EPO) is mainly derived from the kidneys, and the serum concentration is rapidly increased in response to anemia and hypoxia. The present study investigated postmortem serum EPO levels in injury death cases ( $n = 185$ , postmortem time  $< 48$  h, survival time  $< 7$  days: sharp instrument injury,  $n = 44$  and blunt injury,  $n = 141$ ) with regard to survival time, compared with C-reactive protein (CRP) as a marker of inflammation. Serum levels of both markers were independent of postmortem time. A survival time-dependent increase in serum EPO up to about 100 mU/ml was seen within 6 h of sharp instrument injury to the heart or a proximal major vessel (thoracic aorta or subclavian/carotid artery) and blunt injury with massive hemorrhages, showing high correlations ( $r = 0.957$  and  $r = 0.822$ , respectively,  $P < 0.0001$ ), whereas the increase was insignificant ( $P > 0.05$ ) for sharp instrument injury to a peripheral vessel or lungs/abdominal viscera and blunt injury with minor hemorrhages over the same survival period. A further increase ( $> 100$  mU/ml) was often detected in cases of death about 24 h after blunt injury, irrespective of the type of injury. In contrast, a gradual increase in serum CRP level was seen about 12–24 h after blunt injury. These findings suggest that serum EPO can be a marker for investigating survival time within 6 h of major injury involving acute massive hemorrhaging.

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## 1. Introduction

Estimating the time since death and survival time after a fatal insult is an important part of forensic pathology to determine the time of an assault and survivability by rescue and medical care. To estimate the survival time in injury death cases, wound timing by histopathological and immunohistochemical procedures has been established [1–10]. In addition, serum C-reactive protein (CRP), as a biochemical marker of inflammatory response, may be helpful for investigating the severity of blunt injury and survival time over a period from about 12 h to several days after injury [11–13]. For injuries involving fatal hemorrhage, serum erythropoietin (EPO) may also be used as a marker for estimating the time after injury. Although EPO is produced in a spectrum of tissue cells depending on the oxygen level [14–16], circulating EPO is mainly derived from

the kidneys, and increases within hours due to anemia and/or systemic hypoxia [17–20]. A previous study showed that serum EPO was stable during the early postmortem period within 48 h, and the elevation was mainly seen for protracted deaths due to injury and fires, although these cases did not show any evident difference in renal EPO immunopositivity compared with acute death cases, suggesting that serum EPO can be used as a marker for investigating anemia and/or hypoxia as a consequence of fatal insult in subacute or prolonged deaths [21]. For injury cases, however, factors that contribute to the elevation in serum EPO level have not been clarified.

The aim of the present study was to investigate postmortem serum EPO levels in injury death cases with special regard to the relationship to injury severity involving bleeding and survival time, in comparison with serum CRP.

## 2. Materials and methods

### 2.1. Autopsy material

Forensic autopsy cases with a postmortem time within 48 h ( $n = 257$ ) over a period of 9 years (2000–2008) at our institute were examined. Sharp instrument injury ( $n = 44$ ) and blunt injury ( $n = 141$ ) were subdivided into two groups, respectively, considering the severity of tissue damage and bleeding: sharp

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**Table 1**  
Case profile.

| Cause of death                   | Case number | Male/female   | Age years (median)  | Survival time (median) (h) | Clinical care yes/CPR/no | Postmortem interval (median) (h) | Erythropoietin 95% confidence limit (median) (mU/ml) | C-reactive protein 95% confidence limit (median) (mg/dl) |
|----------------------------------|-------------|---------------|---------------------|----------------------------|--------------------------|----------------------------------|--|--|
| <b>Injury groups</b>             |             |               |                     |                            |                          |                                  |  |  |
| <b>Blunt injury<sup>a</sup></b>  |             |               |                     |                            |                          |                                  |  |  |
| Massive hemorrhage <sup>b</sup>  | 47          | 27/20         | 19–86 (56.0)        | <0.5–96 (1.8)              | 24/10/13                 | 7–47 (18.9)                      | 20.0–830.0 (52.4)                                    | 0.01–56.10 (0.28)  |
| Minor hemorrhage <sup>c</sup>    | 94          | 81/13         | 18–96 (57.0)        | <0.5–165 (1.5)             | 45/9/40                  | 5–48 (19.2)                      | 5.0–303.0 (25.1)                                     | 0.01–36.3 (0.24)   |
| <b>Sharp instrument injury</b>   |             |               |                     |                            |                          |                                  |  |  |
| Major vessel <sup>d</sup>        | 17          | 10/7          | 19–75 (44.0)        | <0.5–2.5 (0.6)             | 7/4/6                    | 6–47 (15.0)                      | 5.6–88.8 (28.1)                                      | 0.01–0.79 (0.02)   |
| Minor vessel <sup>e</sup>        | 27          | 22/5          | 36–75 (56.0)        | <0.5–10.5 (0.9)            | 12/5/10                  | 6–42 (15.2)                      | 1.2–30.9 (14.5)                                      | 0.01–0.79 (0.08)   |
| <b>Control groups</b>            |             |               |                     |                            |                          |                                  |  |  |
| Mechanical asphyxia <sup>f</sup> | 48          | 23/25         | 19–94 (55.0)        | <0.5                       | 0/7/41                   | 6–48 (21.3)                      | 8.1–37.1 (20.0)                                      | 0.02–1.90 (0.15)   |
| Acute cardiac death              | 24          | 20/4          | 51–87 (63.0)        | <0.5                       | 0/15/9                   | 10–39 (22.1)                     | 7.5–38.0 (15.6)                                      | 0.02–1.86 (0.16)   |
| <b>Total cases</b>               | <b>257</b>  | <b>183/74</b> | <b>18–96 (59.0)</b> | <b>&lt;0.5–165 (1.3)</b>   | <b>88/50/119</b>         | <b>5–48 (18.5)</b>               | <b>1.2–830.0 (20.0)</b>                              | <b>0.01–56.10 (0.16)</b>                                 |

<sup>a</sup> Traffic accidents ( $n=46$ ), falls ( $n=72$ ), blows ( $n=21$ ) and others ( $n=2$ ).

<sup>b</sup> Trauma with hemothorax ( $n=18$ )/peritoneum ( $n=9$ ), pelvic and/or femoral bone fracture ( $n=14$ ) and others ( $n=6$ ).

<sup>c</sup> Non-head trauma without massive hemorrhage ( $n=19$ : chest,  $n=9$ ; abdomen,  $n=6$ ; back,  $n=2$ ; others,  $n=2$ ), and head injury ( $n=75$ : poly trauma,  $n=12$ ; single trauma,  $n=63$ ).

<sup>d</sup> Heart ( $n=9$ ) and proximal major vessel ( $n=8$ : common carotid artery,  $n=7$ ; subclavian artery,  $n=1$ ).

<sup>e</sup> Lungs ( $n=8$ ) and peripheral vessel (abdomen,  $n=10$ ; neck,  $n=4$ ; limbs,  $n=5$ ).

<sup>f</sup> Hanging ( $n=11$ ), strangulation ( $n=21$ ), aspiration ( $n=14$ ) and others ( $n=2$ ).

CPR, cardiopulmonary resuscitation without recovery.

instrument injury to the heart or a proximal major vessel including the aorta and subclavian/carotid artery (major vessel injury group,  $n=17$ ) and peripheral vessel(s) or lungs/abdominal viscera (minor vessel injury group,  $n=27$ ); blunt injury involving polytrauma or a single trauma with hemothorax/peritoneum (massive hemorrhage group,  $n=47$ : with/without head injury,  $n=16/31$ ) and single trauma without massive hemorrhage (minor hemorrhage group,  $n=94$ : head injury,  $n=77$  and others,  $n=17$ ). These cases included those underwent medical care involving critical cardiopulmonary support, surgery to the site of injury, blood transfusion and fluid infusion shock therapy, but without EPO administration. Cases with longer survival time of  $>1$  h were deaths due to prolonged hemorrhagic shock or brain dysfunction, including 5 cases of blunt injury without medical care (massive hemorrhage,  $n=2$  and minor hemorrhage,  $n=3$ ; survival time, about 3–48 h). The control group consisted of acute death cases without evident bleeding ( $n=72$ ), comprising mechanical asphyxiation ( $n=48$ ), and acute cardiac death ( $n=24$ ), irrespective of critical terminal medical care involving cardiopulmonary resuscitation with fluid infusion for cases of cardiopulmonary arrest on arrival (CPAOA) at hospital. Case profiles are shown in Table 1. For all groups, clearly accountable cases with well-documented circumstantial evidence were collected. Blood samples were collected from the left and right cardiac chamber and peripheral external iliac vein using sterile syringes. Serum was immediately separated by centrifugation and stored at  $-20$  °C until use. Case history, and pathological and toxicological data were collected from autopsy documents. Cases with complications that may have affected serum EPO or CRP were excluded. The postmortem interval was defined as the time from estimated time of death to autopsy. Survival time was the period from the onset of the fatal insult to death. For this purpose, we used cases where a witness and/or circumstantial evidence was well established to confirm survival and postmortem times estimated based on their pathological findings, for which the deviation was considered to be within about 1 h including the elapsed time for determination of clinical death.

## 2.2. Biochemical analysis

Serum EPO levels were measured by a specific radio-immunoassay using the Recombigen EPO RIA kit (Nippon DPC, Chiba, Japan) and a standard EPO of recombinant human EPO (epoetin alfa; Kirin Brewery Co., Tokyo, Japan) [22,23]. The minimum detectable quantity of EPO was 5.0 mU/ml. Inter-assay and intra-assay coefficients of variation were 3.5% and 8.9%, respectively. Diluted human serum showed that the displacement curve was parallel to the standard EPO curve. The mean recovery of EPO was 93.3%. The clinical reference range for EPO was  $<36$  mU/ml. Serum CRP levels were measured by nephelometry using NA-latex CRP (Boehringer Diagnostics, Marburg, Germany), in which the clinical reference range was  $<0.2$  mg/dl. In addition, serum total protein and albumin concentrations were measured by dextran with a biuret-type assay (total protein HR-II kit, Wako Pure Chemical Industries, Osaka, Japan) [24] and serum protein fractioning by electrophoresis, in which the clinical reference ranges were 6.7–8.3 and 3.7–5.2 g/dl, respectively.

## 2.3. Statistical analysis

The Scheffe test was used for multiple comparisons among groups, and comparisons between groups were performed by non-parametric Mann–Whitney

*U*-test. Linear regression analyses were used to examine the relationships of serum markers with survival time. Analyses were performed using Microsoft Excel and Statview (version 5.0, SAS Institute Inc., SAS Campus Drive Cary, NC). A *P*-value of less than 0.05 was considered significant. In Fig. 2, the results of data analysis are shown as box-plots, for which 50% of the data are summarized in the box. The line in each box represents the median, and the lines outside of each box represent the 90% confidence interval. Variables that were not normally distributed were log-transformed for statistical analyses.

## 3. Results

### 3.1. Postmortem interval, age and gender of subjects

For all cases ( $n=257$ ), the serum EPO level showed an almost equivalent correlation between left ( $x_1$ ), right ( $x_2$ ) cardiac and peripheral ( $y$ ) blood:  $y = 0.99 x_1 + 1.93$ ,  $r = 0.98$ ,  $P < 0.0001$ ,  $n = 59$ ;  $y = 1.15 x_2 - 2.91$ ,  $r = 0.97$ ,  $P < 0.0001$ ,  $n = 57$ . CRP showed similar correlations:  $y = 1.04 x_1 - 0.02$ ,  $r = 0.99$ ,  $P < 0.001$ ;  $y = 0.96 x_2 + 0.01$ ,  $r = 0.99$ ,  $P < 0.001$ ,  $n = 142$ . EPO and CRP levels showed no relationship to postmortem interval ( $<48$  h) at each site, and were independent of the age or gender of subjects. A moderate to marked correlation was detected between serum EPO and CRP levels at each site ( $r = 0.56–0.74$ ,  $P < 0.001$ ). Considering the topographical stability of serum EPO and CRP levels, further analyses were performed primarily using the data from right cardiac blood, which were partly supplemented by those from peripheral or left cardiac blood for a deficit ( $n=31$ ).

Serum total protein and albumin levels in right cardiac blood were within the clinical reference ranges (6.7–8.3 and 3.7–5.2 g/dl, respectively) in most cases, showing medians of 7.9 and 4.2 g/dl, respectively. There were slight tendencies toward an increase in their levels depending on the postmortem interval ( $<48$  h;  $r = 0.365$ ,  $P < 0.001$  and  $r = 0.258$ ,  $P < 0.01$ , respectively).

### 3.2. Relationship to the medical care

For injury cases, hospital death cases ( $n=88$ ) had significantly higher serum EPO and CRP levels compared with CPAOA cases without recovery ( $n=28$ ) and cases without any medical care ( $n=69$ ) ( $P < 0.05$ ). However, 3 and 4 cases of blunt injury with a longer survival time (about 3–48 h) without medical care showed higher serum levels of EPO (71.1–334.0 mU/ml) and CRP (2.1–13.1 mg/ml), respectively. All CPAOA cases without recovery had

low serum levels of EPO (<50 mU/ml) and CRP (<2.0 mg/ml). There was no difference between cases of CPAOA without recovery and those without any medical care ( $P > 0.05$ ).

### 3.3. Relationship to the cause of death

#### 3.3.1. Erythropoietin

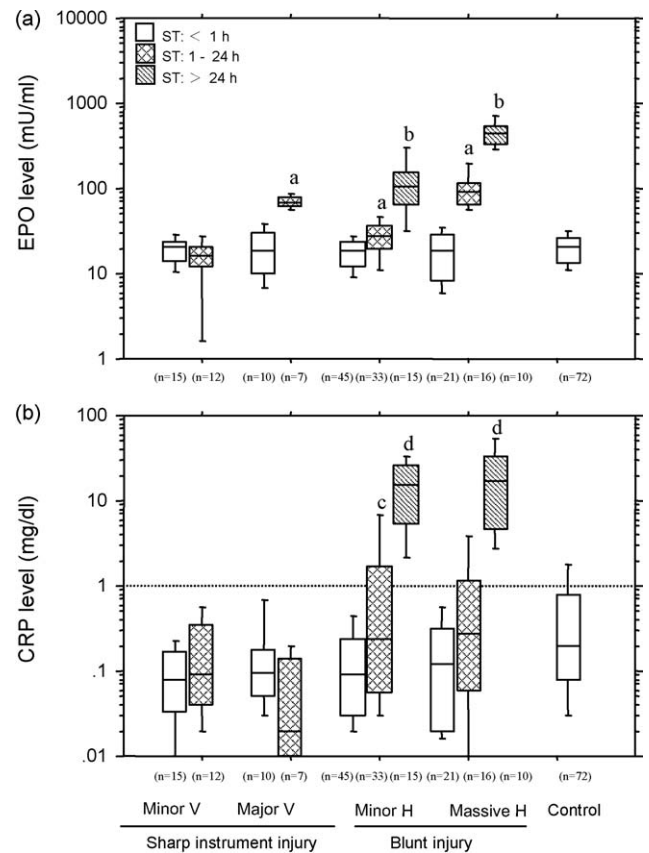
**3.3.1.1. Control groups.** Postmortem serum EPO level (mU/ml) was usually low and within the clinical reference range (<36) in control groups comprising acute deaths due to mechanical asphyxia and myocardial infarction, showing a mean  $\pm$  SD of  $21.3 \pm 10.9$  and a median value (90% confidential range) of 21.0 (7.5–38.0).

**3.3.1.2. Sharp instrument injury groups.** In sharp instrument injury cases, the major vessel injury group ( $n = 17$ , survival time < 2.5 h) showed a higher serum EPO level than the minor vessel injury group ( $n = 27$ , survival time < 10.5 h;  $P < 0.01$ ). For major vessel injury, cases with a survival time of 1–2.5 h ( $n = 7$ ) showed a higher EPO level (median, 69.0 mU/ml; range, 55.0–88.8 mU/ml), compared with the shorter survival group ( $n = 10$ , survival time of <1 h; median, 18.3 mU/ml; range, 5.6–41.7 mU/ml) (Fig. 1a). EPO levels were low for both minor vessel injury cases with a survival time of <1 h (median, 20.0 mU/ml; range, 7.0–29.0 mU/ml,  $n = 15$ ) and those with a survival time of 1–10.5 h (median, 16.7 mU/ml; range, 1.2–30.9 mU/ml,  $n = 12$ ). There was no difference between cases with and without clinical care in the death process ( $n = 28$  and  $n = 16$ ).

In regression equation analyses with regard to survival time ( $x$ , h), serum EPO levels ( $y$ , mU/ml) showed different curves between major and minor vessel injury groups: the correlation coefficient was high for the major vessel injury group with a survival time of <2.5 h ( $y = 31.75x + 10.92$ ,  $n = 17$ ,  $r = 0.957$ ,  $P < 0.0001$ ), but was insignificant for the minor vessel injury group with a survival time of <10.5 h ( $y = -0.45x + 18.22$ ,  $n = 27$ ,  $r = 0.126$ ,  $P = 0.530$ ) (Fig. 2).

**3.3.1.3. Blunt injury groups.** In blunt injury cases, serum EPO levels were mostly low for both minor and massive hemorrhage groups with a shorter survival time of <1 h (a median of 18.1 mU/ml with a range of 5.0–111.0 mU/ml,  $n = 45$  and a median of 20.0 mU/ml with a range of 5.0–60.0 mU/ml,  $n = 21$ , respectively; Fig. 1a). The massive hemorrhage group with a survival time of 1–24 h showed a significantly higher EPO level (median, 93.0 mU/ml; range, 20.0–413.0 mU/ml,  $n = 16$ ) compared with the minor and massive hemorrhage groups with a survival time of <1 h ( $P < 0.05$ ), and also a minor hemorrhage group with a survival time of 1–24 h (median, 27.4 mU/ml; range, 5.0–95.6 mU/ml,  $n = 33$ ;  $P < 0.05$ ). Further analyses of cases with a survival time of 1–6 h presented with significantly higher serum EPO levels in massive hemorrhage group (median, 76.0 mU/ml; range, 20.0–110.0 mU/ml;  $n = 11$ ) than in the minor hemorrhage group (median, 22.0 mU/ml; range, 8.4–57.0 mU/ml;  $n = 20$ ;  $P < 0.0001$ ). For these cases with a survival time of <24 h, there was no difference between cases with and without clinical care in the death process ( $n = 66$  and  $n = 48$ ). Serum EPO levels were markedly higher for cases with a survival time of >24 h in both the minor and massive hemorrhage groups with a median of 71.1 mU/ml (range, 8.3–303.0 mU/ml,  $n = 15$ ) and 437.5 mU/ml (range, 246.0–830.0 mU/ml,  $n = 10$ ), respectively, compared with cases with a shorter survival time of <1 h ( $P < 0.05$ ). Most cases with a survival time of >24 h ( $n = 21/5$ ) were protracted deaths under clinical care. There was no significant difference between cases with and without head injury.

In regression equation analyses with regard to survival time ( $x$ , h), serum EPO levels ( $y$ , mU/ml) in the massive and minor hemorrhage groups showed different curves with a high correlation coefficient:  $y = 6.80x + 39.42$  ( $n = 47$ ,  $r = 0.919$ ,



**Fig. 1.** Serum erythropoietin (EPO, a) and C-reactive protein (CRP, b) levels in sharp instrument and blunt injury cases. <sup>a</sup>Significantly higher: For sharp instrument injury, major vessel injury with a survival time of 1–24 h vs. that with survival time of <1 h, in minor vessel injury groups and the control group ( $P < 0.01$ ). For blunt injury, the minor hemorrhage group with a survival time of 1–24 h vs. that with a survival time of <1 h, and the control group ( $P < 0.01$ ). Massive hemorrhage group with a survival time of 1–24 h vs. minor hemorrhage groups with a survival time of <1 and 1–24 h, massive hemorrhage group with a survival time of <1 h, and the control group ( $P < 0.001$ ). <sup>b</sup>Significantly higher: for blunt injury, the minor hemorrhage group with a survival time of >24 h vs. minor hemorrhage groups with a survival time of <1 and 1–24 h, and the control group ( $P < 0.0001$ ). The massive hemorrhage group with a survival time of >24 h vs. minor hemorrhage groups, massive hemorrhage groups with a survival time of <1 and 1–24 h, and the control group ( $P < 0.0001$ ). <sup>c</sup>Significantly higher: minor hemorrhage group with a survival time of 1–24 h vs. that with survival time of <1 h ( $P < 0.05$ ). <sup>d</sup>Significantly higher: Minor hemorrhage group with a survival time of >24 h vs. minor hemorrhage groups with a survival time of <1 and 1–24 h, massive hemorrhage groups with a survival time of <1 and 1–24 h, and the control group ( $P < 0.0001$ ). Massive hemorrhage group with survival time of >24 h vs. minor hemorrhage groups with a survival time of <1 and 1–24 h, massive hemorrhage groups with survival time of <1 and 1–24 h and the control group ( $P < 0.0001$ ). Control: mechanical asphyxiation,  $n = 48$  and acute cardiac death,  $n = 24$ . ST, survival time; V, vessel; H, hemorrhage.

$P < 0.0001$ ) and  $y = 1.13x + 23.19$  ( $n = 92$ ,  $r = 0.634$ ,  $P < 0.0001$ ), respectively (Fig. 3a). Longer survival cases without any medical care ( $n = 5$ ) showed similar distribution. Further analyses presented a distinct difference between the massive and minor hemorrhage groups within survival time of <6 h, showing a higher correlation coefficient for the massive hemorrhage group ( $y = 16.49x + 19.89$ ,  $n = 32$ ,  $r = 0.822$ ,  $P < 0.0001$ ) and a lower correlation coefficient for the minor hemorrhage group ( $y = 2.42x + 19.33$ ,  $n = 64$ ,  $r = 0.240$ ,  $P = 0.056$ ; insignificant) (Fig. 3b). An analysis of massive hemorrhage cases with a survival time of <2.5 h, which were simulated to the above-mentioned cases of sharp instrument injury to a major vessel, showed a moderate correlation:  $y = 19.98x + 17.18$ ,  $n = 26$ ,  $r = 0.589$ ,  $P < 0.01$ .

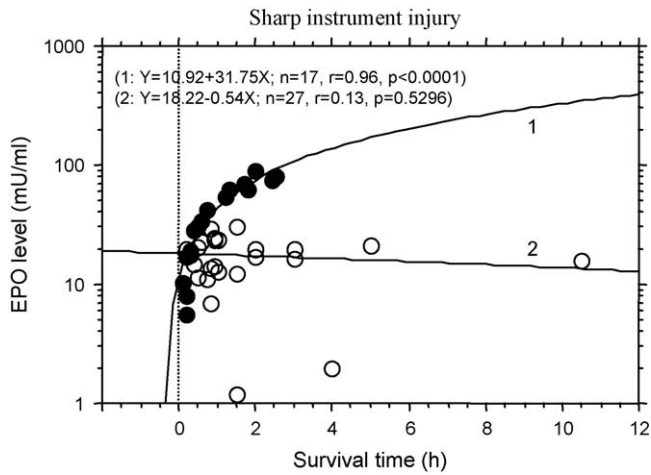


Fig. 2. Correlation between serum erythropoietin (EPO) level and survival time (<12 h) in sharp instrument injury cases (1, major vessel injury group; 2, minor vessel injury group).

3.3.2. C-reactive protein

3.3.2.1. Control groups. Serum CRP level (mg/dl) was usually low in control groups comprising acute deaths due to mechanical asphyxia and myocardial infarction, showing a mean  $\pm$  SD of  $0.93 \pm 2.51$  and a median (90% confidential range) of 0.20 (0.02–1.90).

3.3.2.2. Sharp instrument injury groups. For sharp instrument injury, the serum CRP level was low in both minor and major

vessel injury cases with a survival time of <1 h (median of 0.08 mg/dl, range of 0.01–0.45 mg/dl,  $n = 15$  and a median of 0.10 mg/dl, range of 0.03–0.79 mg/dl,  $n = 10$ , respectively), and was also low in those with a survival time of 1–10.5 h (median of 0.09 mg/dl, range of 0.02–0.79 mg/dl,  $n = 12$  and a median of 0.02 mg/dl, range of 0.01–0.20 mg/dl,  $n = 7$ , respectively) (Fig. 1b).

3.3.2.3. Blunt injury groups. For blunt injury, the serum CRP level was low in both minor and major hemorrhage cases with a survival time of <1 h (a median of 0.09 mg/dl with a range of 0.01–5.69 mg/dl,  $n = 45$  and a median of 0.12 mg/dl with a range of 0.01–0.92 mg/dl,  $n = 21$ , respectively; Fig. 1b), and was slightly higher in those with a survival time of 1–24 h (a median of 0.24 mg/dl with a range of 0.02–26.10 mg/dl,  $n = 33$  and a median of 0.28 mg/dl with a range of 0.01–9.26 mg/dl,  $n = 16$ , respectively), although the elevation was not significant compared with controls ( $P > 0.05$ ). A marked elevation was seen in both minor and major hemorrhage injury cases with a survival time of >24 h (a median of 15.40 mg/dl with a range of 0.93–36.3 mg/dl,  $n = 15$  and a median of 17.2 mg/dl with a range of 1.32–56.10 mg/dl,  $n = 10$ , respectively) with a maximum elevation 3–4 days after injury.

Regression equation analysis of serum CRP levels ( $y$ , mg/dl) for minor and major hemorrhage blunt injury cases with regard to survival time ( $x$ , h) showed a high correlation coefficient:  $y = 0.259x + 0.229$  ( $n = 118$ ,  $r = 0.799$ ,  $P < 0.0001$ ) and  $y = 0.422x - 1.011$  ( $n = 64$ ,  $r = 0.895$ ,  $P < 0.0001$ ), respectively (Fig. 4). In further analyses of cases with a survival time of <24 h, the correlation was high for the major hemorrhage blunt injury group ( $y = 0.241x - 0.191$ ) ( $n = 30$ ,  $r = 0.848$ ,  $P < 0.0001$ ), but was insignificant for the minor hemorrhage blunt injury group ( $y = 0.118x + 0.589$ ) ( $n = 65$ ,  $r = 0.204$ ,  $P = 0.103$ ). There was no correlation for minor or major hemorrhage blunt injury cases with a survival time of <12 h ( $P > 0.05$ ). Longer survival cases without any medical care ( $n = 5$ ) showed similar distribution.

3.3.3. Serum total protein

For right cardiac blood, the serum albumin level showed a slight decrease, depending on survival time, for all injury cases ( $r = 0.216$ ,  $P < 0.05$ ,  $n = 105$ ), although such a decrease was not detected for total serum protein level ( $P > 0.05$ ). In sharp instrument injury cases, serum total protein and albumin levels were lower for subacute death (survival time, 1–10.5 h) than for controls ( $P < 0.05$ ). Blunt injury cases with minor hemorrhage showed a lower level of right cardiac serum total protein for subacute and protracted deaths (survival time, 1–24 and >24 h, respectively), compared with the control ( $P < 0.05$ ), and a lower serum albumin

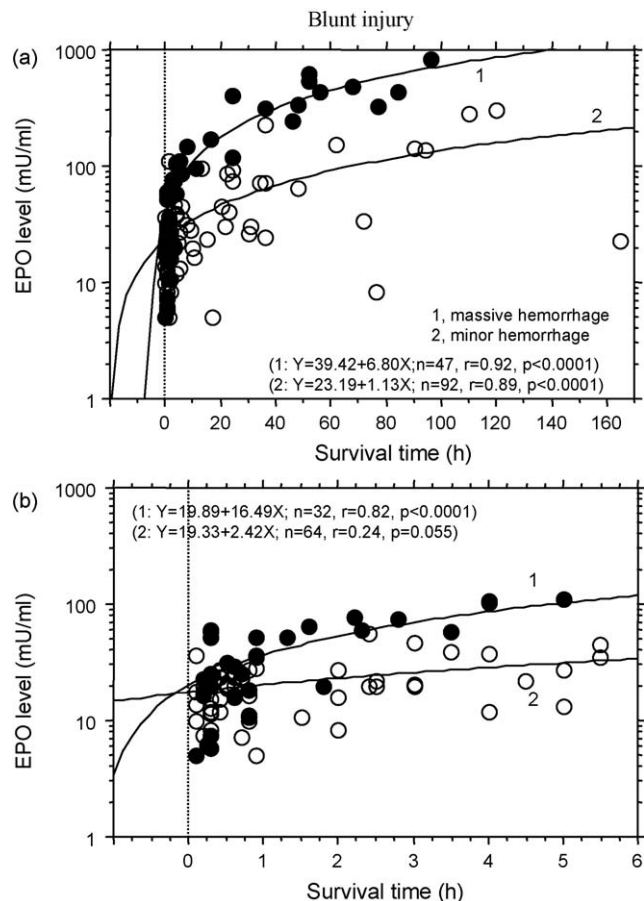


Fig. 3. Correlation between serum erythropoietin (EPO) level and survival time in blunt injury cases (1, massive hemorrhage group; 2, minor hemorrhage group).

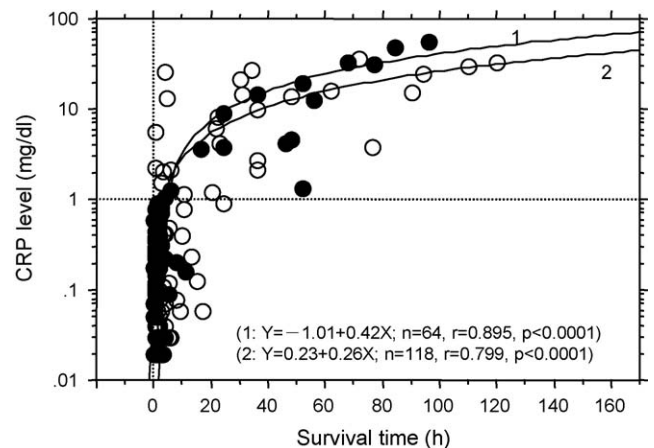


Fig. 4. Correlation between C-reactive protein (CRP) level and survival time (<168 h) in injury cases (1, massive hemorrhage group; 2, minor hemorrhage group).

level for protracted death than for acute death (survival time <1 h) and the control ( $P < 0.05$ ). In major hemorrhage blunt injury cases, right cardiac serum total protein and albumin levels were lower for acute and protracted death, compared with the control ( $P < 0.05$ ).

#### 4. Discussion

EPO is mainly produced by cells close to the proximal tubules in the kidneys, and the production is regulated by blood oxygen level [14–17]. Elevation in the serum EPO level is an indication of anemia or prolonged hypoxia [18–20]. The present study using autopsy material established the stabilities of serum EPO and CRP during an early postmortem period (<48 h), although serum total protein albumin levels showed slight tendencies toward an increase for all cases, possibly due to postmortem water redistribution (hemoconcentration). There was no difference by blood sampling site for these markers, and postmortem reference values were estimated as <38.0 mU/ml for EPO and <1.9 mg/dl for CRP, which approximated the clinical reference ranges, as previously shown [12,13,21].

Findings for CRP with regard to the cause of death were similar to those in previous studies, which showed an increase depending on the severity of tissue damage and survival time in injury and fire fatality cases [11–13]. Serum CRP gradually increased about 12–24 h after blunt injury, showing a maximum elevation after 3–4 days, but the correlation of serum level to survival time within 12 h was not significant. This elevation in serum CRP appeared dependent on the wound healing process during survival period under clinical care in most cases [12,13]. However, some cases without medical care did not show any different findings; thus there was no evidence for the influence of medical intervention. Furthermore, no significant complication was seen in the present study. Such an elevation was not seen for sharp instrument injury (survival time <10.5 h) probably due to minor tissue damage and shorter survival time. However, serum EPO showed an elevation that was closely related to the bleeding nature of the injury as follows.

In sharp instrument injury cases, an elevation in serum EPO level (50–100 mU/ml) was seen during an early survival period (1–2.5 h) for subacute death cases with rapid and massive hemorrhage due to injury to a major vessel injury, showing a high correlation ( $r = 0.957$ ) to survival time. However, such a finding was not noted for cases of minor vessel injury with a survival time of <10.5 h. These findings suggest that in sharp instrument injury cases, bleeding velocity is the main factor for early elevation in serum EPO level, possibly due to rapid progression of renal ischemia/hypoxia. For cases of sharp instrument injury to a major vessel with a survival time of <2.5 h, the rate of increase of serum EPO was estimated to be approximately 30 mU/ml/h by regression equation analysis; the serum level was usually <30 mU/ml within 0.5 h after injury, and around 50, 80 and 100 mU/ml after 1, 2 and 3 h, respectively. Since all of these cases with survival time over 1 h were death under common critical medical care except for surgery to the individual sites of injury, the influence of individual measures is not interpretable. At least, however, serum EPO was not elevated in cases of survival time within 1 h, irrespective of intensive medical care.

For blunt injury, an elevation in serum EPO level was seen within 6 h in the massive hemorrhage group with polytrauma or a single trauma with hemothorax/-peritoneum, while such an elevation was not detected in the minor hemorrhage group with single trauma without hemothorax/-peritoneum. These findings suggest that bleeding velocity is also crucial for early elevation of the serum EPO level in blunt injury cases. By regression equation analysis of massive hemorrhage blunt injury cases with a survival time of <6 h, the estimated rate of increase of

serum EPO after injury was approximately 20 mU/ml/h with a high correlation ( $r = 0.822$ ), which was slightly lower than for sharp instrument injury to a major vessel; the serum level was around 70 and 100 mU/ml after 3 and 6 h, respectively. Some cases without any medical care did not show any different findings for serum EPO; thus there was no evidence for the influence of medical intervention. These findings suggest that increases of serum EPO in early death cases mainly depend on the bleeding velocity, and were independent of clinical care; thus, bleeding was fatally severe. However, the serum EPO level remained lower in the minor hemorrhage group during the period 12 h after injury. Thereafter, serum EPO appeared to gradually increase for longer survival cases in both the massive and minor hemorrhage groups, although the elevation was larger for the massive hemorrhage group. These delayed elevations in serum EPO in blunt injury cases, which were independent of the type of injury, may have been due to anemia and/or hypoxia involved in posttraumatic systemic disorders secondary to injury (e.g., acute respiratory distress, systemic inflammatory response, and/or sepsis) despite intensive clinical care, thus causing death [25–29]. In this respect, decreases in serum total protein and albumin levels according to survival time suggest systemic deterioration after injury, which is similar to posttraumatic clinical findings [30,31].

The present study analyzed primarily fatal injury cases of survival time within 4 days without complications. In this condition, early elevation in serum EPO may be attributed to the vitality after rapid blood loss, partly under critical life support measures involving blood transfusion and fluid infusion shock therapy. Delayed elevations in serum EPO and CRP may be due to prolonged hemorrhagic shock and wound healing process before complication of secondary infection, respectively, mostly under intensive cardiopulmonary support measures. Thus, critical medical care involving artificial ventilation, blood transfusion and fluid infusion shock therapy may have partly contributed to the vitality involving elevations in serum EPO and CRP.

The above-mentioned findings suggest that an early increase in serum EPO up to about 100 mU/ml within about 6 h after injury depends on a rapid loss of circulating blood, accompanied by acute renal ischemia/hypoxia. Longer survival cases under clinical care may show a delayed increase of >100 mU/ml, depending on the severity of anemia and/or hypoxia involved in posttraumatic systemic disorders. However, to evaluate the serum EPO level with regard to the survival time, an elevation due to medical administration of an EPO agent (often >1000 mU/ml) or preexisting diseases involving advanced anemia/hypoxia should be considered [21]. When such specific cases are excluded, postmortem serum EPO may be used to estimate survival time within 6 h after any injury causing rapid, massive bleeding. In blunt injury cases, CRP may be used as an additional serum marker to evaluate survival time over 12 h.

In conclusion, the present study showed that serum EPO was stable during an early postmortem period (<48 h), and that the early elevations in injury cases were closely related to the severity of hemorrhage and survival time. Estimating the survival time may be difficult since so many variables have an influence on increase of serum EPO, for which further animal model investigation is necessary. However, these findings suggest that serum EPO can be used as a marker to investigate survival time within 6 h after major injury involving massive hemorrhage.

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