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# Interleukin (IL)-8 immunoreactivity of injured axons and surrounding oligodendrocytes in traumatic head injury



Takahito Hayashi<sup>\*</sup>, Kazutoshi Ago, Takuma Nakamae, Eri Higo, Mamoru Ogata<sup>\*</sup>

Department of Legal Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

#### ARTICLE INFO

#### ABSTRACT

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Keywords: Traumatic axonal injury Beta-amyloid precursor protein (AβPP) Interleukin (IL)-8 Remyelination Immunohistochemistry Postmortem diagnosis Interleukin (IL)-8 has been suggested to be a positive regulator of myelination in the central nervous system, in addition to its principal role as a chemokine for neutrophils. Immunostaining for beta-amyloid precursor protein (ABPP) is an effective tool for detecting traumatic axonal injury, although ABPP immunoreactivity can also indicate axonal injury due to hypoxic causes. In this study, we examined IL-8 and ABPP immunoreactivity in sections of corpus callosum obtained from deceased patients with blunt head injury and from equivalent control tissue. ABPP immunoreactivity was detected in injured axons, such as axonal bulbs and varicose axons, in 24 of 44 head injury cases. These ABPP immunoreactive cases had survived for more than 3 h. The A $\beta$ PP immunostaining pattern can be classified into two types: traumatic (Pattern 1) and non-traumatic (Pattern 2) axonal injuries, which we described previously [Havashi et al. Int. J. Legal Med. 129 (2015) 1085-1090]. Three of 44 control cases also showed ABPP immunoreactive injured axons as Pattern 2. In contrast, IL-8 immunoreactivity was detected in 7 AβPP immunoreactive and in 2 non-ABPP immunoreactive head injury cases, but was not detected in any of the 44 control cases, including the 3 ABPP immunoreactive control cases. The IL-8 immunoreactive cases had survived from 3 to 24 days, whereas those cases who survived less than 3 days (n = 29) and who survived 90 days (n = 1) were not IL-8 immunoreactive. Moreover, IL-8 was detected as Pattern 1 axons only. In addition, double immunofluorescence analysis showed that IL-8 is expressed by oligodendrocytes surrounding injured axons. In conclusion, our results suggest that immunohistochemical detection of IL-8 may be useful as a complementary diagnostic marker of traumatic axonal injury.

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#### 1. Introduction

Diffuse traumatic axonal injury is an important cause of traumatic coma in the absence of an intracranial mass lesion [1]. In forensic practice, the recognition of traumatic axonal injury is particularly important, since it signifies a rotational injury of considerable severity as may be observed in a road traffic accident, a fall from a height, or an accelerated blow by a punch. However, it is difficult to diagnose traumatic axonal injury in postmortem examination because macroscopic changes may be minimal or even trivial, and most changes can only be recognized microscopically [2]. Under the electron microscope, ultrastructural changes, such as focally enlarged axons with axolemmal infolding or disordered neurofilaments, can be observed within fields of axons

exhibiting neither apparent macroscopic nor light microscopic abnormalities at 6 h survival after head injury [3,4].

Immunostaining for a protein transported along axons, betaamyloid precursor protein (A $\beta$ PP), is the most effective tool for detecting traumatic axonal injury in forensic practice [1,2,5–11]. A $\beta$ PP immunoreactivity can detect injured axons as axonal bulbs, varicose axons (sinusoidally swollen axons), and wavy axons as early as 2–3 h after head injury [1,2,10–12]. Moreover, some axonal bulbs can be detected at shorter survival times after head injury depending on the case [13,14]. However, A $\beta$ PP immunoreactivity also detects axonal injuries due to hypoxic insults [1,10–17]. Several previous studies, including ours [1,17–20], have described the different patterns of A $\beta$ PP immunoreactivity between traumatic and non-traumatic (hypoxic) axonal injuries.

Interleukin (IL)-8, a glutamic acid-leucine-arginine (ELR) motifpositive chemokine (chemotactic cytokine), is a major chemotactic factor for neutrophils in acute inflammation [21,22]. Results of recent studies suggest that IL-8 also functions as a promoter of the myelination of injured axons by mediating the recruitment of oligodendrocyte progenitor cells [23,24]. Incomplete disruptive

<sup>\*</sup> Corresponding authors. Tel.: +81 99 275 5313; fax: +81 99 275 5315. *E-mail addresses*: takahito@m2.kufm.kagoshima-u.ac.jp (T. Hayashi), ogatam@m.kufm.kagoshima-u.ac.jp (M. Ogata).

axonal injury, which usually occurs as diffuse axonal injury, causes a sequence of changes that result in secondary axotomy (complete axonal disconnection) [5]. The initial axon cytoskeletal changes, which result from calcium (Ca<sup>2+</sup>)-mediated ultrastructural collapse, may accompany the demyelination of axons, and, thus, the myelination of injured axons may be required for regeneration. Recent evidence also suggests that myelin debris (degradation products of myelin) from injured axons can trigger inflammation with the up-regulation of a variety of proinflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , and IL-6 [25]. In the present study, we compared IL-8 and A $\beta$ PP immunoreactivities in the corpus callosum of deceased patients with blunt head injury and investigated whether the immunohistochemical detection of IL-8 is useful in the forensic diagnosis of traumatic axonal injury.

#### 2. Materials and methods

#### 2.1. Antibodies

The following monoclonal antibody (mAb) and polyclonal antibodies (pAbs) were used for immunohistochemical and immunofluorescence analyses in the present study: mouse anti-AβPP mAb

Table 1

Details of the cases with head injury.

(EMD Millipore, Darmstadt, Germany), mouse anti-IL-8 mAb (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit anti-myelin basic protein (MBP) pAbs (Thermo Fisher Scientific, Waltham, MA, USA), biotinylated goat anti-mouse IgG (Santa Cruz Biotechnology, Inc.), cyanine dye 3 (Cy3)-conjugated donkey anti-mouse IgG pAbs, and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG pAbs (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA).

#### 2.2. Autopsy samples

Forty-four cases with blunt head injury (age range, 2–93 years; mean age, 61.0 years; 34 males and 10 females; range of survival time after injury, short time (<10 min) to 90 days) and the same number of control cases with hypoxia/brain ischemia without head injury (age range, 2–92 years; mean age, 54.9 years; 25 males and 19 females) were collected from the Department of Legal Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University. The details of the head injury and control cases are summarized in Tables 1 and 2, respectively. This cohort of head injury cases and control cases is identical to that examined in our previous study [20]. From each deceased patient, the corpus

1       80x M       90 d       Falling       SDH $++$ ? $-$ 2       90x F       24 d       Assault       SDH $++$ ? $-$ 3       80x M       18 d       Assault       SDH, SAH, CC $ -$ 4       80x M       18 d       Assault       SDH, SAH, CC $ -$ 5       80x M       12 d       Falling       SDH, SAH, CC $+$ 1.2 $+$ 6       60x M       9 d       Assault       SDH, SAH, CC $+$ 1.2 $+$ 7       40x M       9 d       Assault       SDH, SAH, CC $+$ 1.2 $+$ 8       30x M       7 d       Falling       SDH, SAH, CC $+$ 1.4 $+$ 9       66x M       7 d       Falling       SDH, SAH, CC $+$ 1.4 $+$	Case no.	Age range and gender	Survival time	Cause of head injury	Brain lesions	aAβPP	Pattern	<sup>a</sup> IL-8
2905 Fi24 dAssault50H+1++3805 M21 dTraffa cacidentSDH, CC, ICH4805 M18 dAssaultSDH, SAH5805 M12 dAssaultSDH, SAH++1	1	80s M	90 d	Falling	SDH	++	?	_
3808 M21 dTaffic accidentSDH, SAH, C4808 M14 dAssaultSDH, SAH, C+1, 2+-5808 M12 dFallingSDH, SAH, CC+-1, 2+-7408 M9 dAssaultEDH, SAH, CC+-1, 2+-7408 M9 dAssaultSDH, SAH, CC+-1, 2+-9608 M7 dFallingSDH, SAH, CC+-1, 2+-10608 M7 dFallingSDH, SAH, CC+-1, 2+-11308 F6 dAssaultSDH, SAH, CC+-1, 2+-12608 F5 dAssaultSDH, SAH, CC+-1, 2+-13606 M3 dAssaultSDH, SAH, CC+-1, 2+-142 M3 dAssaultSDH, SAH, CC15668 M2 5 dAssaultSDH, SAH, CC+-1, 216508 M2 5 dAssaultSDH, SAH, CC+-1, 217408 M2 5 dAssaultSDH, SAH, CC+-1, 218708 M1 dAssaultSDH, SAH, CC+-1, 220408 M1 dAssaultSDH, SAH, CC+-1, 221308 M1 dAssaultSDH, SAH, CC+-<	2	90s F	24 d	Assault	SDH	+	1	++
480s M18 dAssaultSDH, SAH, CC	3	80s M	21 d	Traffic accident	SDH, CC, ICH	_		_
5         80 M         14 d         Assault         DI, SAH         ++         1, 2         ++           6         606 M         12 d         Falling         DIH, SAH, CC         ++         1, 2         ++           7         406 M         7 d         Falling         DIH, SAH, CC         +         1, 2         ++           9         606 M         7 d         Falling         SDH, SAH, CC         +         1, 2         +           11         306 F         6 d         Assault         DIH, SAH, CC         +         1, 2         +           12         606 M         7 d         Assault         SDH, SAH, CC         +         1, 2         +           13         606 M         4 d         Assault         SDH, SAH         -         1, 2         +           14         2 M         3 d         Assault         SDH, SAH, CC         +         1, 2         +           14         2 M         3 d         Assault         SDH, SAH         -         -         -         -           14         2 Mo         Assault         SDH, SAH, CC         +         1, 2         -         -           15         606 M         1	4	80s M	18 d	Assault	SDH, SAH, CC	_		_
6     60s M     12 d     Falling     SDH, SAH     ++     1     -       7     40s M     9 d     Assult     EDH, SDH, SAH, CC     ++     1, 2     ++       8     30s M     7 d     Falling     SAH, CC     +     1, 2     +       9     60s M     7 d     Falling     SAH, CC     +     1, 2     -       10     60s M     7 d     Falling     SDH, SAH, CC     +     1, 2     +       11     30s F     6 d     Assult     SDH, SAH, CC     ++     1, 2     +       13     60s M     4 d     Assult     SDH, SAH, CC     ++     1, 2     +       14     2 M     3 d     Assult     SDH, SAH, CC     -     -     -       15     60s M     2,5 d     Assult     SDH, SAH, CC     +     1, 2     -       16     S0s M     2,5 d     Assult     SDH, SAH, CC     +     1, 2     -       17     40s M     1, 4     Assult     SDH, SAH, CC     +     1, 2     -       18     70s M     3 d     Assult     SDH, SAH, CC     +     1, 2     -       21     30s M     1 d     Assult     SDH, SAH, CC     + <td>5</td> <td>80s M</td> <td>14 d</td> <td>Assault</td> <td>SDH, SAH</td> <td>++</td> <td>1, 2</td> <td>+</td>	5	80s M	14 d	Assault	SDH, SAH	++	1, 2	+
7     40s M     9 d     Assault     Shu, SAH, CC     +     1, 2     ++       9     60s M     7 d     Falling     SDH, SAH, CC     +     1     +       9     60s M     7 d     Falling     SDH, SAH, CC     +     7     -       10     60s M     7 d     Falling     SDH, SAH, CC     +     7     -       11     30s F     6 d     Assault     SDH, SAH, CC     ++     1, 2     +       12     60s F     5 d     Assault     SDH, SAH, CC     ++     1, 2     +       13     60s M     4 d     Assault     SDH, SAH, CC     ++     1, 2     +       14     2 M     3 d     Assault     SDH, SAH, CC     ++     1, 2     -       16     50s M     2.5 d     Assault     SDH, SAH, CC     +     1, 2     -       17     40s M     2.5 d     Assault     SAH, CC     +     1, 2     -       18     70s M     2.5 d     Assault     SAH, CC     +     1, 2     -       20     40s M     1 d     Assault     SAH, CC     +     1, 2     -       21     30s M     1 d     Assault     SDH, SAH, CC     + <td>6</td> <td>60s M</td> <td>12 d</td> <td>Falling</td> <td>SDH, SAH</td> <td>++</td> <td>1</td> <td>_</td>	6	60s M	12 d	Falling	SDH, SAH	++	1	_
830 sM7 dFallingSDH, SAH, CC+1+960s M7 dFallingSDH, SAH1060s M7 dFallingSDH, SAH1130s F6 dAssaultSDH, SAH, CC++1, 2++ </td <td>7</td> <td>40s M</td> <td>9 d</td> <td>Assault</td> <td>EDH, SDH, SAH, CC</td> <td>++</td> <td>1, 2</td> <td>++</td>	7	40s M	9 d	Assault	EDH, SDH, SAH, CC	++	1, 2	++
960s M7 dFallingSAH, CC+?-1060s M7 dFallingSDH, SAH+1130s F6 dAssaultSDH, SAH, CC++1, 2++1260s M4 dAssaultSDH, SAH, CC++1, 2++1360s M4 dAssaultSDH, SAH, CC+++	8	30s M	7 d	Falling	SDH, SAH, CC	+	1	+
1060 M7 dFalling5DH, SAH1130s F6 dAssaultSDH, SAH, CC++1++1260s F5 dAssaultSDH, SAH, CC++1, 2+1360s M4 dAssaultSDH, SAH, CC++1, 2+142 M3 dAssaultSDH, SAH, CC-++1560s M3 dFallingSDH, CC1650s M2.5 dAssaultSDH, SAH, CC++1, 21740s M2.5 dAssaultSDH, SAH, CC++1, 21870s M2.5 dAssaultSDH, SAH, CC++1, 22040s M1 dAssaultSDH, SAH, CC++1, 22130s M1 dAssaultSDH, SAH, CC++1, 22250s M1 dAssaultSDH, SAH, CC++1, 22430s M1 dAssaultSDH, SAH, CC++1, 22550s M1 dAssaultSDH, SAH, CC++1, 22650s M9 hAssaultSDH, SAH, CC++1, 22760s M7 hFallingSDH, SAH, CC+12850s M8 hFallingSDH, SAH, CC <td>9</td> <td>60s M</td> <td>7 d</td> <td>Falling</td> <td>SAH, CC</td> <td>+</td> <td>?</td> <td>_</td>	9	60s M	7 d	Falling	SAH, CC	+	?	_
1130s F6 dAssaultSDH, SAH, CC $ +$ $  -$ <th< td=""><td>10</td><td>60s M</td><td>7 d</td><td>Falling</td><td>SDH, SAH</td><td>_</td><td></td><td>_</td></th<>	10	60s M	7 d	Falling	SDH, SAH	_		_
1260s F5 dAssaultSDH, SAH, CC++1++1, 2++1360s M3 dAssaultSDH, SAH, CC++1, 2+142M3 dFallingSDH, CC+1560s M2.5 dAssaultSAH1740s M2.5 dAssaultSAH, CC++1, 21870s M2.5 dAssaultSAH, CC2040s M1 dFallingSDH, SAH, CC++1, 22130s M1 dAssaultSDH, SAH, CC++1, 22250s M1 dAssaultSDH, SAH, CC++1, 22350s M1 dAssaultSDH, SAH, CC++1, 22430s M1 dAssaultSDH, SAH, CC++1, 22550s M1 dAssaultSDH, SAH, CC++1, 22650s M9hAssaultSDH, SAH, CC++1, 22760s M9hAssaultSDH, SAH, CC++1, 22850s M9hFallingSDH, SAH, CC+12960s M7hAssaultSDH, SAH, CC+13060s M7hFalling <t< td=""><td>11</td><td>30s F</td><td>6 d</td><td>Assault</td><td>SDH, SAH</td><td>-</td><td></td><td>+</td></t<>	11	30s F	6 d	Assault	SDH, SAH	-		+
1360s M4 dAssaultSDH $++$ 1, 2 $+$ 142M3 dAssaultSDH, SAH $++$ 1, 2 $+$ 1560s M3 dFalingSDH, CC $  -$ 1650s M2.5 dAssaultSDH, CC $+$ 1, 2 $-$ 1870s M2.5 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 1920s M32bAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2040s M1 dFalingSDH, SAH, CC $+$ 1, 2 $-$ 2130s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2250s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2350s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2430s M13hFallingSDH, SAH, CC $+$ 1 $-$ 2550s M12hFallingSDH, SAH, CC $  -$ 2650s M8hFallingSDH, SAH, CC $  -$ 2760s M7hAssaultSDH, SAH, CC $  -$ 2870s M8hFallingSDH, SAH, CC $  -$ 3180s F6hTraffic accidentSDH, SAH, CC $  -$ 3370s M4hTraffic accidentSDH, SAH, CC $  -$ 34<	12	60s F	5 d	Assault	SDH, SAH, CC	++	1	++
14     2 M     3 d     Assault     SDH, SAH     ++     1,2     +       15     60s M     3,d     Falling     SDH, CC     -     +       16     50s M     2,5 d     Assault     SDH, CC     +     ?     -       17     40s M     2,5 d     Assault     SAH     -     -     -       18     70s M     2,5 d     Assault     SDH, SAH, CC     ++     1,2     -       19     20s M     32,h     Assault     SDH, SAH, CC     ++     1,2     -       20     40s M     1 d     Assault     SDH, SAH, CC     ++     1,2     -       21     30s M     1 d     Assault     SDH, SAH, CC     ++     1,2     -       23     50s M     1 d     Assault     SDH, SAH, CC     ++     1,2     -       24     30s M     12,h     Falling     SDH, SAH     +     1     -       25     50s M     12,h     Assault     SAH, CC     +     1     -       27     60s M     8,h     Falling     SDH, SAH, CC     +     1     -       28     70s M     8,h     Falling     SDH, SAH, CC     +     1     -	13	60s M	4 d	Assault	SDH	++	1, 2	+
15 $60  M$ $3  d$ $Falling$ $SDH, CC$ $  -$ 16 $50  SM$ $2.5  d$ $Assault$ $SDH$ $  -$ 18 $70  sM$ $2.5  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 19 $20  sM$ $2.5  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 20 $40  sM$ $1  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 21 $30  sM$ $1  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 22 $50  sM$ $1  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 23 $50  sM$ $1  d$ $Assault$ $SDH, SAH, CC$ $+$ $1  c2$ $-$ 24 $30  sM$ $13  h$ Falling $SDH, SAH$ $  -$ 25 $50  sM$ $12  h$ Falling $SDH, SAH, CC$ $+$ $1  c2$ $-$ 26 $50  sM$ $9  h$ $Assault$ $SH, SAH, CC$ $+$ $1  c2$ $-$ 27 $60  sM$ $7  h$ Falling $SDH, SAH, CC$ $+$ $1  c2$ $-$ 28 $70  sM$ $8  h$ Falling $SDH, SAH, CC$ $+$ $1  c2$ $-$ 39 $60  sM$ $7  h$ $7  saintSDH, SAH, CC+1  c2-3180  sF6  hTaffic accidentSDH, SAH, CC  -3370  sM<$	14	2 M	3 d	Assault	SDH, SAH	++	1, 2	+
1650 M2.5 dFalling5DH+P-1740 S M2.5 dAssaultSAH	15	60s M	3 d	Falling	SDH, CC	-		+
1740s M2.5 dAssaultSAH $  -$ 1870s M2.5 dAssaultSDH, SAH, CC $  -$ 1920s M32hAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2040s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2130s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2250s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2350s M1 dAssaultSDH, SAH, CC $+$ 1, 2 $-$ 2430s M13hFallingSDH, SAH $  -$ 2550s M12hFallingSDH, SAH, CC $+$ 1 $-$ 2650s M9hAssaultSDH, SAH, CC $+$ 1 $-$ 2760s M8hFallingSDH, SAH, CC $+$ 1 $-$ 2870s M8hFallingSDH, SAH, CC $+$ 1 $-$ 2960s M7hAssaultSDH, SAH, CC $+$ 1 $-$ 3180s F6hTraffic accidentSDH, SAH, CC $+$ 1 $-$ 3280s M6hTraffic accidentSDH, SAH, CC $+$ 1 $-$ 3370s M4.5hTraffic accidentSDH, SAH, CC $+$ 1 $-$ 3450s M4hFallingSDH, SAH, CC $+$ 1 $-$ 35	16	50s M	2.5 d	Falling	SDH	+	?	_
1870s M2.5 dAssaultSDH, SAH, CC++1, 2-1920s M32 hAssaultSDH, CC2040s M1 dFallingSDH, CC++1, 2-2130s M1 dAssaultSDH, SAH, CC++1, 2-2250s M1 dAssaultSDH, SAH, CC++1, 2-2350s M1 dhFallingEDH, SDH, SAH2430s M13 hFallingSDH, SAH, CC++12550s M12 hFallingSDH, SAH2650s M9 hAssaultSAH, CC<	17	40s M	2.5 d	Assault	SAH	_		_
1920s M32hAssaultSAH, CC2040s M1 dFallingSDH, CC++1, 2-2130s M1 dAssaultSDH, SAH, CC++1, 2-2250s M1 dAssaultSDH, SAH, CC++1, 2-2350s M1 dAssaultSDH, SAH, CC++1, 2-2430s M13hFallingSDH, SAH, CC+1-2550s M12hFallingSDH, SAH, CC2650s M9hAssaultSAH, CC2760s M8hFallingSDH, SAH, CC2870s M8hFallingSDH, SAH, CC+1-2960s M7hFallingSDH, SAH, CC+1-3060s M7hAssaultSDH, SAH, CC+1-3180s F6hTaffic accidentSDH, SAH, CC+1-3370s M4.5hTaffic accidentSDH, SAH, CC+1-3450s M6hTaffic accidentSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5hFallingSDH, SAH, CC+1-3750s M2.5hFallingSDH, SAH, CC+1 <td>18</td> <td>70s M</td> <td>2.5 d</td> <td>Assault</td> <td>SDH, SAH, CC</td> <td>++</td> <td>1, 2</td> <td>_</td>	18	70s M	2.5 d	Assault	SDH, SAH, CC	++	1, 2	_
2040s M1 dFallingSDH, CC+11-2130s M1 dAssaultSDH, SAH, CC++1, 22250s M1 dAssaultSDH, SAH, CC++1, 22350s M14 hFallingEDH, SDH, SAH, CC++1, 22430s M13 hFallingSDH, SAH2550s M12 hFallingSDH, SAH, CC+12650s M9 hAssaultSAH, CC2760s M8 hFallingSDH, SAH, CC+1 <td< td=""><td>19</td><td>20s M</td><td>32 h</td><td>Assault</td><td>SAH, CC</td><td>_</td><td></td><td>_</td></td<>	19	20s M	32 h	Assault	SAH, CC	_		_
2130s M1 dAssaultSDH, SAH, CC++1, 2-2250s M1 dAssaultSDH, SAH, CC++1, 2-2350s M14 hFallingEDH, SDH, SAH+1, 2-2430s M13 hFallingSDH, SAH2550s M12 hFallingSDH+1-2650s M9 hAssaultSAH, CC+1-2760s M8 hFallingSDH, SAH, CC2870s M8 hFallingSDH, SAH, CC+1-2960s M7 hFallingSDH, SAH, CC+1-3060s M7 hFallingSDH, SAH, CC3180s F6 hTraffic accidentEDH, SDH, SAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M3 hFallingSDH, SAH, CC+13680s F2.5 hFallingSDH, SAH, CC+13750s M4.5 hFallingSDH, SAH, CC3870s F1.hFallingSAH, CC3980s F1.h	20	40s M	1 d	Falling	SDH, CC	+	1	_
2250s M1 dAssaultSDH, SAH, CC++1, 2-2350s M14 hFallingEDH, SDH, SAH2430s M13 hFallingSDH, SAH2550s M12 hFallingSDH, CC+1-2650s M9 hAssaultSAH, CC+1-2760s M8 hFallingSDH, SAH, CC2870s M8 hFallingSDH, SAH, CC+1-2960s M7 hFallingSDH, SAH, CC+1-3060s M7 hFallingSDH, SAH, CC3180s F6 hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6 hTraffic accidentSDH, SAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M3 hFallingSDH, SAH, CC+13680s F2.5 hFallingSAH, CC3750s M2.5 hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 h <td< td=""><td>21</td><td>30s M</td><td>1 d</td><td>Assault</td><td>SDH, SAH, CC</td><td>++</td><td>1, 2</td><td>_</td></td<>	21	30s M	1 d	Assault	SDH, SAH, CC	++	1, 2	_
2350s M14 hFalingEDH, SDH, SAH+1-2430s M13 hFalingSDH, SAH2550s M12 hFalingSDH, SAH-1-2650s M9 hAssaultSAH, CC+1-2760s M8 hFalingSDH, SAH, CC2870s M8 hFalingSDH, SAH, CC+1-2960s M7 hFalingSDH, SAH, CC3060s M7 hAssaultSDH, SAH, CC3180s F6 hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6 hTraffic accidentSDH, SAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M3 hFalingSDH, SAH, CC+13680s F2.5 hFalingSAH, CC+13750s M2.hFalingSAH, CC3870s F1 hFalingSAH, CC3980s F1.hFalingSAH, CC3980s F1.hFaling	22	50s M	1 d	Assault	SDH, SAH, CC	++	1, 2	_
2430s M13 hFalingSDH, SAH2550s M12 hFalingSDH+12650s M9 hAssaultSAH, CC+12760s M8 hFalingSDH, SAH, CC2870s M8 hFalingSDH, SAH, CC+12960s M7 hFalingSDH, SAH, CC+13060s M7 hAssaultSDH, SAH, CC+13180s F6 hTraffic accidentEDH, SAH, CC+13280s M6 hTraffic accidentSDH, SAH, CC3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M6 hTraffic accidentSDH, SAH, CC3590s M3 hFalingSDH, SAH, CC3680s F2.5 hFalingSDH, SAH, CC3750s M2 hFalingSDH, SAH, CC3870s F1 hFalingSDH, SAH, CC3980s F1 hFalingSDH, SAH, CC3980s F1 hFalingSDH3980s F<	23	50s M	14 h	Falling	EDH, SDH, SAH	+	1	_
2550s M12 hFallingSDH+1-2650s M9 hAssaultSAH, CC+1-2760s M8 hFallingSDH, SAH, CC2870s M8 hFallingSDH, SAH, CC+1-2960s M7 hFallingSDH, SAH, CC+1-3060s M7 hAssaultSDH, SAH, CC3180s F6 hTraffic accidentEDH, SNH, SCH+1-3280s M6 hTraffic accidentSAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4.5 hTraffic accidentSDH, SAH, CC3590s M3 hFallingSDH, SAH, CC3680s F2.5 hFallingSAH, CC3750s M2.5 hFallingSAH, CC3980s F1 hFallingSDH, SAH, CC3980s F0.5 hAssaultSAH, CC3980s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH, SAH, CC4060s F	24	30s M	13 h	Falling	SDH, SAH	_		_
2650s M9hAssaultSAH, CC+1-2760s M8hFallingSDH, SAH, CC2870s M8hFallingSDH, SAH, CC+1-2960s M7hFallingSDH, SAH, CC+1-3060s M7hAssaultSDH, SAH, CC3180s F6hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6hTraffic accidentSDH, SAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSDH, SAH, CC+1-3750s M2.5 hFallingSAH, CC3870s F1hFallingSDH, SAH, CC3980s F1hFallingSDH4060s F0.5 hAssaultSAH, CC4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4440s FShortAssaultSAH	25	50s M	12 h	Falling	SDH	+	1	_
2760s M8 hFallingSDH, SAH, CC2870s M8 hFallingSDH, SAH, CC+1-2960s M7 hFallingSDH, SAH, CC+*1-3060s M7 hAssaultSDH, SAH, CC3180s F6 hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6 hTraffic accidentSDH, SAH, CC+1-3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M4.5 hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSDH, SAH, CC+1-3750s M2.5 hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH, SAH, CC4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH, CC<	26	50s M	9 h	Assault	SAH, CC	+	1	_
2870s M8hFallingSDH, SAH, CC+1-2960s M7hFallingSDH, SAH++1-3060s M7hAssaultSDH, SAH, CC3180s F6hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6hTraffic accidentSDH, SAH, CC3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4hFallingSDH, SAH, CC+13590s M3hFallingSDH, SAH, CC+13680s F2.5 hFallingSAH, CC3750s M2.5 hFallingSAH, CC3870s F1hFallingSAH, CC4060s F0.5 hAssaultSAH, CC4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4440s FShortAssaultSAH, CC4170s F0.5 hAssaultSAH, CC4370s M0.5 hAssaultSAH, CC44 <td>27</td> <td>60s M</td> <td>8 h</td> <td>Falling</td> <td>SDH, SAH, CC</td> <td>_</td> <td></td> <td>_</td>	27	60s M	8 h	Falling	SDH, SAH, CC	_		_
2960s M7hFallingSDH, SAH++1-3060s M7hAssaultSDH, SAH, CC3180s F6hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6hTraffic accidentSAH+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4.5 hTraffic accidentSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH, CC+1-3750s M2.5 hFallingSAH, CC3870s F1hFallingSDH, SAH, CC3980s F1.5 hAssaultSAH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH4440s FShortAssaultSAH4440s FShortAssaultSDH	28	70s M	8 h	Falling	SDH, SAH, CC	+	1	_
3060s M7hAssaultSDH, SAH, CC3180s F6hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6hTraffic accidentSAH+1-3370s M4.5 hTraffic accidentSDH, SAH, CC+1-3450s M4hFallingSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH, CC3750s M2hFallingSAH, CC3870s F1hFallingSDH, SAH, CC3980s F1hFallingSDH, SAH, CC4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH, CC4440s FShortAssaultSAH4440s FShortAssaultSH4440s FShortAssaultSDH	29	60s M	7 h	Falling	SDH, SAH	++	1	_
3180s F6hTraffic accidentEDH, SDH, SAH, CC+1-3280s M6hTraffic accidentSAH+1-3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4hFallingSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH, CC3750s M2hFallingSAH, CC3870s F1hFallingSDH, SAH, CC3980s F1hFallingSDH, SAH, CC4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH4440s FShortAssaultSAH4440s FShortAssaultSDH	30	60s M	7 h	Assault	SDH, SAH, CC	_		_
3280s M6hTraffic accidentSAH+1-3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4hFallingSDH, SAH, CC+1-3590s M3hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH3750s M2hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH, SAH, CC4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH, CC4440s FShortAssaultSAH, CC4440s FShortAssaultSAH4545ShortAssaultSAH4440s FShortAssaultSDH	31	80s F	6 h	Traffic accident	EDH, SDH, SAH, CC	+	1	_
3370s M4.5 hTraffic accidentSDH, SAH, CC3450s M4hFallingSDH, SAH, CC+1-3590s M3 hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH3750s M2.hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH, SAH, CC4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH, CC4440s FShortAssaultSAH, CC4440s FShortAssaultSAH, CC4570s MShortAssaultSAH, CC4640s FShortAssaultSAH, CC4770s MShortAssaultSAH4840s FShortAssaultSH4940s FShortAssaultSDH	32	80s M	6 h	Traffic accident	SAH	+	1	_
3450s M4 hFallingSDH, SAH, CC+1-3590s M3 hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH3750s M2 hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH4440s FShortAssaultSDH	33	70s M	4.5 h	Traffic accident	SDH, SAH, CC	_		_
3590s M3 hFallingSDH, SAH, CC+1-3680s F2.5 hFallingSAH3750s M2 hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortAssaultSAH4440s FShortAssaultSDH	34	50s M	4 h	Falling	SDH, SAH, CC	+	1	_
3680s F2.5 hFallingSAH3750s M2 hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH, CC4370s MShortFallingSAH4440s FShortAssaultSDH	35	90s M	3 h	Falling	SDH, SAH, CC	+	1	_
3750s M2hFallingSAH, CC3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	36	80s F	2.5 h	Falling	SAH	_		_
3870s F1 hFallingSDH, SAH, CC3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	37	50s M	2 h	Falling	SAH, CC	-		_
3980s F1 hFallingSDH4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	38	70s F	1 h	Falling	SDH, SAH, CC	_		_
4060s F0.5 hAssaultSAH4170s FShortAssaultSAH, CC4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	39	80s F	1 h	Falling	SDH	_		_
4170s FShortAssaultSAH, CC4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	40	60s F	0.5 h	Assault	SAH	-		_
4220s MShortAssaultSAH4370s MShortFallingSAH4440s FShortAssaultSDH	41	70s F	Short	Assault	SAH, CC	-		_
43         70s M         Short         Falling         SAH         -         -           44         40s F         Short         Assault         SDH         -         -         -	42	20s M	Short	Assault	SAH	-		-
44 40s F Short Assault SDH – –	43	70s M	Short	Falling	SAH	-		_
	44	40s F	Short	Assault	SDH	-		-

EDH, epidural hematoma; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; CC, cerebral contusion; ICH, intracerebral hemorrhage.

 $^{a}\,$  -, 0 axonal bulbs (AB); +, 1–5 AB; ++, >5 AB per 200 $\times$  microscopic field.

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