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## Original communication

## Dilemmas concerning the diffuse axonal injury as a clinicopathological entity in forensic medical practice

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## ABSTRACT

Dilemmas and discussions concerning the diffuse axonal injury (DAI) and still existing in forensic medical practice are as it follows: 1. Whether the occurrence of DAI can indicate the type of traumatic event that has caused the head trauma, 2. Whether the presence of axonal damage in cases of hypoxia, ischaemia and other pathological conditions casts a shadow on the post-mortem pathological diagnosis of DAI and totally negates it, or there are certain clues in the findings that can point to the aetiology of the axonal damage. This paper discusses our findings based on neuropathological examination of 60 forensic cases of closed head injury. The neuropathological examination included: a macroscopic examination of the coronal sections and a microscopic examination involving an immunohistochemical method with antibody against  $\beta$ -amyloid precursor protein. Our findings indicate that DAI, as a clinicopathological entity, is undoubtedly an acceleration–deceleration injury, predominant in road traffic accidents as it is classically outlined, and cases of falling from a considerable height. Our findings point to a certain difference between the features of traumatic and ischaemic axonal damage. In this paper we also investigate the correlation between pathological grades of DAI and the impairment of the brain function before death.

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## 1. Introduction

Diffuse axonal injury (DAI) as a distinct clinicopathological entity has been introduced in the last 20 years. It has been defined clinically: patients with DAI fall in an immediate and prolonged coma leading to death or severely disabled,<sup>1</sup> and pathologically: a triad of DAI-specific pathoanatomical changes was defined which was the base of the grading system carried out by Adams et al.<sup>2</sup> Although it was classically outlined that mechanism causing DAI is angular acceleration with longer duration,<sup>3</sup> more recent experimental studies showed that a combination of linear and angular accelerations is generally associated with DAI.<sup>4</sup>

The use of monoclonal antibody against  $\beta$ -amyloid precursor protein ( $\beta$ -APP)<sup>5</sup> was a further landmark. It proved to be specific and highly sensitive<sup>6,7</sup> selectively targeting damaged axons. This method was positive even after short period of survival (3 h by Sheriff et al.,<sup>5,7</sup> 1.75 h by Blumbergs et al.,<sup>8</sup> 2 h by McKenzie et al.,<sup>9</sup>

35 min by Hortobagyi et al.<sup>10</sup>). Studies based on the expression of  $\beta$ -APP positivity showed that axonal injuries have no specific biomechanical importance, despite their importance as signs of vitality and indicators of survival time.<sup>7</sup> A terminological confusion also occurred: some authors have used the term DAI while discussing axonal damage to one or two brain samples,<sup>11</sup> whilst other have claimed that the term necessitates widespread sampling of the brain.<sup>12,13</sup> Geddes et al.<sup>14</sup> made an attempt to differentiate the terms: axonal injury (AI), traumatic axonal injury (TAI), and diffuse axonal injury (DAI) as the most severe form of traumatic axonal damage. This has been soon supported by other authors who reported that there is some difference in the histopathological findings that are indicative of the origin of the axonal damage.<sup>15,16</sup>

Still existing dilemmas in forensic medical practice concerning the DAI as a clinicopathological entity are the following:

1. Does DAI has any specific biomechanical relevance which can be of some advantage in the forensic medicine practice? Can the presence of DAI indicate the type of traumatic event that caused the head trauma?
2. Are there certain differences in the pathological features between axonal damage caused by hypoxia and ischaemia and traumatic axonal damage which is the main attribute of DAI?

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## 2. Material and methods

We analysed the appearance and the distribution of DAI on 60 cases with fatal nonmissile head injury (43 male, 17 female, age

ranged from 10 to 94 years, Table 1). Survival times ranged from instantaneous death to 1.5 months.

Inclusion criteria included post-mortem interval up to 24 h and the availability of data concerning: clear evidence of a mechanical

**Table 1**  
Case-material: clinical data, findings from forensic medical autopsy and forensic neuropathological examination.

No	A–G	Trauma	TS	Consciousness	Fr and ICH	FBI	DBI
1	F 13	TA-ped	9 d	Coma, GCS = 7	Fr, EDH, SDH	Cont	DAI 2, Swell, ISH
2	M 48	TA-ped	3–4 h	Concussion	SAH		Swell
3	F 23	TA-cyc	10 d	GCS = 8	SDH	Cont	DAI 1, Swell, ISH
4	M 59	TA-cyc	2 d	Coma	Fr, EDH, SDH, SAH,	Cont c–c	AI-ish, Swell, ISH
5	M 70	TA-ped	1 d	Concussion	Fr, SAH		Swell
6	M 17	TA-motor	3 w	Coma, GCS = 6	SDH, SAH		DAI 2, Swell, ISH
7	F 73	TA-ped	24 h	Coma	SDH, SAH,		DAI 2, Swell, ISH
8	M 41	TA-ped	6 h	Coma, GCS = 3	Fr, SAH		DAI 3, Swell, ISH
9	F 22	TA-ped	1 h		Fr, SAH	Cont	DVI
10	M 39	TA-driver	4–5 h	Coma, GCS = 3	Fr, SAH, IVH	Cont	DVI, DAI 1
11	M 89	TA-ped	3 h	Coma	SAH		DAI 1
12	M 23	TA-driver	2 d	Coma, GCS = 3	SAH, SDH		DAI 3,
13	M 28	TA-pass	Imm		Fr, SAH		Swell
14	F 19	TA-ped	1/2 h		SAH		Swell
15	M ~60	F-height	3.5 w	Sopor	Fr, SDH, SAH	Cont	Swell
16	M 40	TA-ped	11 d	Coma	Fr, SAH		DAI 1, Swell
17	M 33	TA-driver	3 d	Coma, GCS = 3	Fr, SAH		DAI 3, Swell, ISH
18	M 26	F-height	12 d	Coma, GCS = 3	Fr, EDH, SDH, SAH	Cont c–c	DAI 3, Swell, ISH
19	M 45	TA-motor	Imm		Fr, SAH		DVI, signs of DAI
20	M ~50	RA	10 d	Coma	Fr		Swell
21	F 38	TA-pass	Imm		SAH		DVI
22	M 30	TA-motor	10 d	Coma, GCS = 5	Fr, SAH, SDH	Cont c–c	AI-ish, Swell, ISH
23	M 70	F-height	Imm		Fr, SAH		Swell
24	M ~35	F-height	2 d	Coma, GCS = 4	Fr, EDH, SDH, SAH, IVH	Cont c–c	Swell
25	M 55	F-height	1.5 m	Coma, GCS = 3	EDH, SAH	Cont	Swell, ISH, DAI 1
26	M 69	TA-cyc	3 w	Coma	Fr, SDH, SAH	Cont c–c	AI-ish, Swell, ISH
27	F ~70	TA-ped	3 d	Coma	Fr, SDH, SAH, IVH	ICH	DAI 2/3, Swell, ISH
28	F ~60	TA-ped	1–2 h	Coma	SAH		
29	M 10	TA-ped	8 d	Coma, GCS = 4			Swell, ISH, DAI 1
30	F 17	TA-ped	1/2 h		SAH		Swell
31	M 43	F-height	Imm		Fr, SAH		DVI, Swell
32	F 75	F-height	Imm		Fr, SAH	Cont	DVI, Swell
33	M 81	TA-cyc	15 d	Coma	Fr, SDH, SAH	Cont c–c	Swell, DAI 1
34	F 64	TA-ped	4 d	Coma, GCS = 3	Fr, SDH, SAH	Cont	Swell, DAI 2/3, ISH
35	M 61	TA-cyc	5 d	Coma	Fr, SAH, IVH	Cont, ICH	DAI 2, ISH, Swell
36	M 60	F-simple	7 d	Coma	SDH, SAH	Cont c–c	AI-ish, Swell, ISH
37	M ~30	TA-pass	4 h	Coma			DAI 2, Swell, ISH
38	M ~50	Blow	3–5 h	Coma, GCS = 3	Fr, SDH, SAH, IVH	Cont	AI-ish, Swell, ISH
39	M 60	TA-ped	1 m	Coma, GCS = 8	SDH, SAH, IVH		DAI 2, Swell, ISH
40	M 37	F-simple	5 d	Sopor	Fr, EDH, SDH,	Cont	Swell
41	M 26	F-height	Imm		Fr, EDH, SDH, SAH		DVI, Swell
42	M 53	TA-driver	Imm		Fr, SAH		DVI, Swell
43	M 30	TA-driver	2–3 h	Coma	Fr, SAH		DAI 3, Swell, ISH
44	M 29	TA-driver	Imm		Fr fac, SAH		DVI
45	M 60	TA-pass	Imm		Fr, SAH		DVI, DAI 2, Swell
46	F 74	TA-ped	3–4 h	Sopor	SAH		DAI 1
47	M 70	TA-ped	4 h	Coma	Fr, SAH		
48	F 56	TA-ped	1–2 h		Fr, SAH		DVI, DAI 2, Swell
49	F 46	TA-ped	24 h	Coma	SAH		DAI 1, Swell, ISH
50	M 25	TA-motor	4 d	Sopor			DAI 1, ISH
51	M ~50	TA-cyc	7 h	Coma, GCS = 4	Fr, SAH	Cont c–c	DAI 3, Swell, ISH
52	F 94	Blow	1–2 h		Fr, SAH, IVH		
53	M 63	F-simple	10 d	Coma, GCS = 7	Fr, EDH, SAH	Cont c–c	ISH, Swell, AI-ish
54	M 67	TA-ped	2.5 d	Sopor	Fr, SDH, SAH	Cont c–c	Swell
55	F 72	TA-ped	6 d	Coma, GCS = 6	Fr, SDH, SAH	Cont	DAI 2/3, Swell, ISH
56	M 55	B–F	5 d	Coma	Fr, SDH, SAH	Cont	Swell
57	M 58	F-height	6 d	Coma	Fr, SDH, SAH	Cont c–c	AI-ish, Swell, ISH
58	M 59	F-height	24 h	Coma, GCS = 4	Fr, SDH, SAH	Cont c–c	Swell, DAI 2/3, ISH
59	F 20	TA-ped	7 d	Coma	Fr, EDH, SDH, SAH	Cont	DAI 2, Swell, ISH
60	M 50	RA	2 d	Coma	Fr, SAH		DAI 1, Swell

A–G – age and gender; Trauma – type of traumatic event where closed head injury occurred: TA – traffic accident (ped – pedestrian, cyc – cyclist, mcyc – motorcyclist; pass – passenger; RA – railway accident), F – fall (F-simple – fall from one's own height, F-height – fall from a height of more than 2 m), B–F – blow and fall; TS – time of survival (Imm – immediate death; h – hour; d – day, w – week, m – month); Consciousness – state of consciousness immediately after the impact (GCS – Glasgow Coma Score); Fr – fractures of the skull, ICH – intracranial haemorrhage (SAH – subarachnoidal haemorrhage, EDH – epidural haematoma, SDH – subdural haematoma, IVH – intraventricular haemorrhage); FBI – focal brain injury (cont – contusion, cont c–c – contusion coupcontra – coup, ICH – intracerebral haemorrhage); DBI – diffuse brain injury (DAI 1, 2, 3 – diffuse axonal injury – Adams grading system (Adams 1989)); AI-ish – microscopically diagnosed axonal injury with ischaemic pattern; DVI – diffuse vascular injury; ISH – ischaemia; Swell – brain swelling.

Note: This table of the whole examined material has been published in the Romanian Journal of Legal medicine in 2010.

impact to the head and the type of the traumatic event: road traffic accident (RTA), falling of a small or considerable height (considered as height of more than 2 m) or assault cases; the time of survival and full autopsy information. Clinical information was obtained for cases that survived long enough to be clinically investigated.

The injury mechanism was analysed during the forensic autopsy, based on the injuries of the scalp, skull, intracranial structures (epidural, subdural and subarachnoidal haemorrhage) and the brain tissue (focal and diffuse brain injuries). This was followed by a forensic neuropathological examination<sup>17</sup> of fixed brains in 10% buffered formalin. Macroscopic examination of 1 cm thick coronal sections has been documented in photographs. Sampling has been done from the following brain areas: the body and the splenium of the corpus callosum with parasagittal white matter; the posterior limb of the internal capsule; the pons and superior cerebellar peduncles.

In addition to the conventional haematoxylin–eosin staining, immunohistochemical staining was performed with the application of antibody against  $\beta$ -APP, by the method of Sheriff et al.<sup>5</sup> [antigen retrieval in citrate buffer (pH 5.0), incubation with antibody against  $\beta$ -APP (Mouse anti-Alzheimer precursor protein A4 monoclonal antibody, clone 22 C 11, diluted 1:200, Chemicon International, Temecula, CA) overnight at 4°C. The enzyme complex used was ABC (Universal VECTASTAIN ABC-Peroxidase kit, Vector Labs, Burlingame, CA) with a secondary antibody – biotinylated anti-mouse IgG (Biotinylated Anti-mouse IgG, produced in horse, Vector Labs). Diaminobenzidine (Peroxidase Substrate Kit (DAB) Vector Labs) was used for visualisation].

In the process of DAI diagnosing pathological criterion was based on the grading system of Adams et al.<sup>2</sup> according to which: focal lesion in the corpus callosum was regarded as DAI 2; focal lesion in the rostral brainstem was regarded as DAI 3; and microscopic finding of widespread axonal damage without any macroscopic feature was regarded as DAI 1. Focal lesion in corpus callosum or in the brainstem was graded as DAI 2 or DAI 3, only if the macroscopical lesion has been attributed to the microscopic feature of traumatic axonal damage. A microscopic finding of widespread axonal damage had to be with typical traumatic pattern and distribution (single or small groups of  $\beta$ -APP positive axons scattered and diffusely distributed throughout the white matter), in at least three different brain regions, of which at least one located above and one below the tentorium.<sup>12</sup>

Additionally, we investigated the correlation between different pathological grades of DAI and the severity of the impairment of the brain function for cases that survived long enough to be clinically

**Table 2**  
Cases with diagnosed DAI.

Type of traumatic event	Total	DAI	%	No DAI	AI-ish
Traffic accident	44	27	61	14	3
Pedestrian	22	14	64	8	0
Cyclist	6	4	67	0	2
Motorist	4	2	50	1	1
Driver	6	4	67	2	0
Passenger	4	2	50	2	0
Railroad accident	2	1	50	1	0
Fall	13	3	23	7	3
Simple fall (<2 m)	3	0	0	1	2
Fall of a height (>2 m)	10	3	30	6	1
Blow-assault	3	0	0	2	1
<b>Total</b>	<b>60</b>	<b>30</b>	<b>50</b>	<b>23</b>	<b>7</b>

investigated (17 cases). As most indicative parameters of the impairment of the brain function we analysed: the depth of the coma occurring immediately after the trauma expressed as a Glasgow Coma Score (GCS) and the survival time.

Statistical evaluation was made using Pearson Chi-Square test of independence and Kaplan–Mayer procedure for the analysis of the time of survival.

### 3. Results

Using the criteria given above, DAI was diagnosed in 50% of cases (Table 2). Having excluded the cases with a survival time shorter than 2 h, as it was impossible to confirm histological presence of DAI, the frequency of DAI was 73%. It was detected only in the RTA and the falls of a considerable height. It was not detected in the simple falls and in the assault cases.

An association between DAI and the type of injury was explored by statistical evaluation, clarifying that DAI more typically occurs in RTA than in cases of a blow or a fall, (Chi-Square = 9.042,  $p = 0.011$ ) Table 3.

Also, results shown in Table 3 demonstrate that the occurrence of DAI does not depend on age (Pearson Chi-Square = 1.310,  $p = 0.52$ ) or gender (Pearson Chi-Square = 0.7387,  $p = 0.284$ ).

In order to investigate the correlation between different pathological grades of DAI and the severity of the impairment of the brain function, one analysis was made to compare different grades of DAI with the depth of coma occurring immediately after the trauma, shown in Table 4. Evidently, two cases who clinically showed somnolence had grade 1 of DAI, and in the majority of cases

**Table 3**

Association between the occurrence of DAI and the type of the traumatic event, age and gender.

DAI*Type of traumatic event, Gender, Age Crosstabulation		DAI cases		Non DAI cases		Total	
		Count	%	Count	%	Count	%
Type of traumatic event	Traffic accident	27	61.36	17	38.64	44	100
	Fall	3	23.08	10	76.92	13	100
	Blow	0	0	3	100	3	100
	Total	30	50	30	50	60	100
Gender	Female	10	58.82	7	41.18	17	100
	Male	20	46.51	23	53.49	43	100
	Total	30	50	30	50	60	100
Age	<25	6	66.67	3	33.33	9	100
	25–50	11	50	11	50	22	100
	>50	13	44.82	16	55.17	29	100
	Total	30	50	30	50	60	100

There is a significant interdependence between the occurrence of DAI and the type of traumatic event, with higher incidence of traffic accidents and lower incidence of falls in DAI group (Pearson Chi-Square 9.042;  $p$ -value = 0.011, when the significance level of the test is  $\alpha = 0.05$ ). The occurrence of DAI is not gender dependent. (Pearson Chi-Square 0.7387,  $p = 0.284$ , when the significance level of the test is  $\alpha = 0.05$ ), and is not age dependent (Pearson Chi-Square 1.310,  $p = 0.52$ , when the significance level of the test is  $\alpha = 0.05$ ).

**Table 4**  
The interdependence of the grade of DAI and the depth of the coma.

Grade of DAI*GCS Crosstabulation	GCS = 10–13	GCS = 6–9	GCS = 3–5	Total
DAI 1	2	1	3	6
DAI 2	0	3	0	3
DAI 3	0	1	7	8
Total	2	5	10	17

who were in a deep coma (GCS = 3–5), pathologically was perceived DAI 3. Additionally, we explored the association between the grade of DAI and the survival time with statistical analysis (Fig. 1). The Kaplan–Mayer analysis indicated that this association was not statistically significant (Log rank = 1.72 with 1 df,  $p = 0.1892$ ) However, although there is no statistical significance, it is evident from the chart that the lower survival time values are more typical of DAI 3.

**4. Discussion**

Most of the dilemmas concerning the DAI in the forensic practice arise from different criteria in the diagnosing of DAI and equalising DAI with axonal injury (AI).

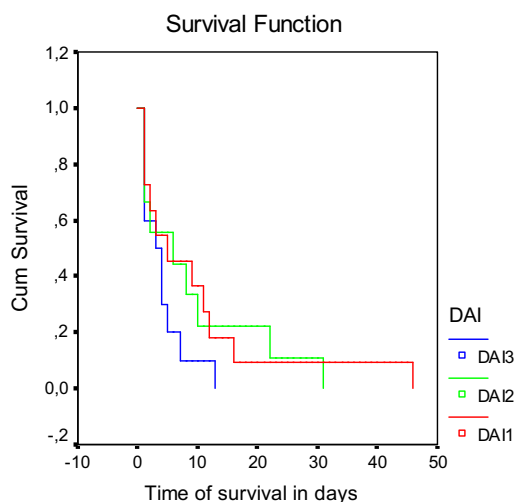
AI is the damage of the axons of any aetiology (ischaemia or other pathological condition), has no specific biomechanical relevance, and can be perceived focally as a local isolated phenomenon. Axonal injury is a pathological feature, and must be distinguished from DAI which is a clinicopathological entity, defined both clinically and pathologically. For the diagnosis of DAI, in addition to the traumatic history, axonal damage has to be perceived widespread and must involve single white matter bundles like corpus callosum and internal capsule.<sup>12–15</sup>

Furthermore, recent studies point to the certain difference in the appearance, distribution and the pattern of the damaged axons that

are suggestive of the origin of the axonal damage.<sup>14–16</sup> Based on those descriptions, in our pathological determination of DAI, in addition to the macroscopic features, only the finding of single or small groups of scattered and diffusely arranged  $\beta$ -APP positive axons, seen as “varicosity-like” swollen axons or as “retraction balls”, was considered as traumatic aetiology of axonal damage<sup>14</sup> (Fig. 2). The finding of circumscribed foci of  $\beta$ -APP accumulation, or linear and geographical patterns, frequently described as a “zig-zag” or “Z-shaped” pattern,<sup>15</sup> where in our experience damaged axons are never large and are not as neatly shaped as the traumatically damaged axons, was considered as hypoxic-ischaemic finding (Fig. 3).

In cases with predominantly ischaemic axonal injury (Table 2, last column), common observation was the presence of intracranial haemorrhage (EDH in three cases and SDH in four cases) and clear signs of raised intracranial pressure. In those cases  $\beta$ -APP immunoreactivity was mostly found in pons (only in pons in two cases, and more widespread in rest of the cases). The pons is obviously most vulnerable of ischaemic axonal damage and this region alone should be avoided for assessment of traumatic axonal damage. Occasionally, ischaemic axonal damage may mask or mimic the traumatic axonal damage,<sup>13</sup> which is documented in the case with the longest survival time (No 25, 1.5 months) (Fig. 4).

As an underlying pathological mechanism of traumatic axonal damage first was introduced a concept of primary axotomy.<sup>18</sup> But, later studies showed that axonal damage is not the immediate but rather delayed consequence of the impairment of axoplasmic transport, which is responsible for the accumulation of  $\beta$ -APP to a level that allows its detection by immunohistochemistry (secondary axotomy).<sup>19</sup> As some axons are not permanently injured, but only disrupted in their function, in this process some authors see a “potential time-window for therapeutic intervention”.<sup>19</sup> More recent studies do not reject completely the concept of primary axotomy, but consider it as a rare phenomenon probably restricted to the highest levels of injury to the axon.<sup>14,20</sup>



	DAI 1	DAI 2	DAI 3
N	11	9	10
Mean (SE)	8.77 (3.94)	8.14 (3.54)	3.08 (1.17)
95%CI	(1.05; 16.50)	(1.21; 15.07)	(0.78; 5.37)
Median (SE)	4.00 (3.85)	5.00 (5.96)	2.00 (1.43)
95%CI	(0.00; 11.55)	(0.00; 16.69)	(0.00; 4.80)

**Fig. 1.** Kaplan–Mayer procedure for the analysis of the survival time. Comparison of the survival times was made for different grades of DAI. The graph represents the proportion of survived patients in the specified period. The Log rank statistics is used to test the equality of the distributions of survival time for the different grades of DAI. It shows no significant difference, (Log rank = 1.72 with 1 df,  $p = 0.1892$ ).





**Fig. 2.** Traumatic pattern of axonal damage: single or in small groups arranged  $\beta$ -APP positive axons are scattered and diffusely distributed (case No 3). b. ( $\times 200$ ).

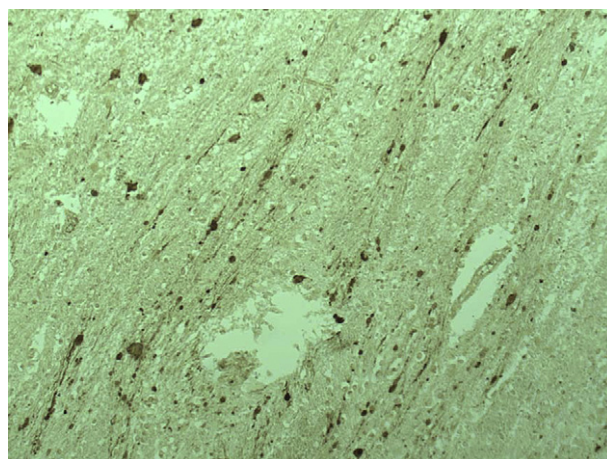
Analysing the association between DAI and the type of trauma, our results show the presence of DAI in RTA and falls of a considerable height, as it was classically outlined.<sup>1–3</sup> Sporadic cases of simple fall and assault with a diagnosed DAI have been reported on several occasions,<sup>21–24</sup> but caution is needed for two things: first, these cases were reported prior to widespread recognition of other causes of axonal damage, particularly ischaemia, and second, the diversity of the yielded results in great part depends on the different criteria of the diagnosing of DAI.<sup>13</sup>

Analysing cases of blow in this study, in one case (case 52 Table 1) which was an assault with multiple kicks to the head and a survival of up to two hours, no  $\beta$ -APP accumulation was found, which can be also attributed to short survival. But, another case of blow (case 56) who was hit by stone, even after 5 days of survival, didn't show any  $\beta$ -APP immunoreactive axons. In the third case of blow to the head with a heavy object (case 38) and a survival of up to 5 h there were found  $\beta$ -APP positive axons in pons, which by the location and the appearance was a typical ischaemic feature.

Analysing cases of fall in this study, in three out of ten cases of fall from a considerable height, the diagnosis of DAI was confirmed, but must be noted that four out of seven cases without DAI died immediately, not allowing the diagnosis of DAI by given criteria. Other three cases showed some  $\beta$ -APP immunoreactivity but not sufficient for the diagnosis of DAI. In one out of three cases of



**Fig. 3.** Linear and geographical patterns of  $\beta$ -APP accumulation are a predominantly hypoxic-ischaemic finding ( $\times 200$ , case No 36).



**Fig. 4.** In a case with a longest survival time of 1.5 month (case No 25), there are well-defined  $\beta$ -APP positive "retraction balls" mixed with few small granules which can be due to ischaemia. Many of the primary damaged axons due to trauma are masked or mimicked by a  $\beta$ -APP accumulation which is not related to the original incident, but is a result of ischaemia.

simple fall (case 4), even after 5 days of survival has not been found  $\beta$ -APP immunoreactivity, and in two other cases (Cases 36 and 53) ischaemic axonal injury was found mainly in pons. In case 53, even predominantly in pons, occasional  $\beta$ -APP positive axons were found in corpus callosum and internal capsule, which can also be attributed to diabetes with unregulated blood glucose level before death.

Hence, results of the present study do not support the assertion of finding DAI in blow cases and cases of simple fall. This might be giving a rise to a biomechanical importance of DAI, but, as stated before, additional studies are required where DAI should be diagnosed by strict criterions.<sup>14</sup> What we know at the moment is the almost exclusive occurrence of DAI in vehicular traffic accidents and falls of considerable height, i.e. traumatic events with a longer duration of the acceleration forces.

Finally, there is no predisposition towards DAI in any particular gender or age group. It appeared that there is no interdependence between survival time and the grade of DAI, but the depth of the coma proved to be the most constant accompaniment to DAI and perhaps the best clinical indicator of the grade of DAI. Survival time obviously does not depend solely on the extensity of the pathological lesion, but it probably depends much more on secondarily occurring brain damage as hypoxia, ischaemia and brain swelling.

#### Conflict of interest

We guarantee that there isn't any conflict of interests in this paper. All analyses have been done inside the Medical faculty of Skopje and this research hasn't been supported by another organisation.

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#### Ethical approval

None.

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