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Review Article

# Pathomorphological staging of subdural hemorrhages: Statistical analysis of posttraumatic histomorphological alterations

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ABSTRACT

We examined 10 histomorphological alterations of 222 cases of subdural hemorrhages following mechanical closed brain injury (MBI) to determine the posttraumatic interval (PTI). These morphological features included red blood cells (RBCs), polymorphonuclear leukocytes (PMNs), macrophages (Ms), RBC-containing Ms, hemosiderin-containing macrophages, hematoidin, fibroblasts, endothelial cells, collagenous fibers and membrane formation. The interval between the time of brain injury and death ranged from a few minutes to 33 years. Following routine staining and immunohistochemical staining of macrophages (CD68), paraffin sections were examined by light microscopy for the presence of the selected histomorphological features. An apparent correlation was found between the frequency of a given histomorphological phenomenon and the length of the PTI. Half of the cases (group 1; $n = 111$) were used to develop a multistage evaluation system, the other half (group 2; $n = 111$) to check its accuracy of prediction. Applying this multistage evaluation model and a special software, 85 of the 111 control group cases (76.6%) could be correctly classified and further 21 cases (18.9%) being assigned to an interval close to the correct interval. Therefore, classification of the correct PTI or an interval close to the correct PTI could be achieved in 95.5% of all cases.



## 1. Introduction

Hemorrhages into the space between dura mater and arachnoid are commonly associated with mechanical brain injury (MBI) as a result of (rotational) acceleration/deceleration forces stretching and tearing the bridging veins. The resulting so-called "subdural hemorrhages" (SDH) are associated with a high mortality rate. SDH may be documented by means of neuroradiology, neurosurgery as well as on occasion of autopsies. From a forensic-neuropathological perspective these findings often raise the question on the time of the traumatic event. This problem typically arises, whenever several traumatic events at various times of a given case need being discussed. For forensic reasons in these cases the respective survival time must be estimated in order to determine to which of the various events SDH can actually be attributed.

Several studies have described the pathologic alterations during the interval between mechanical injury and neurosurgery or death, the so-called posttraumatic interval (PTI) in case of cortical hemorrhages [1–5]. However, only three experimental studies have been carried out for timing the pathomorphological alterations in cases of SDH, one by Leestma [6] on the neuropathological level, and the others by Teasdale and Hadley [7] and by Mori [8] on the MRI level. Yet, so far any statistical analysis on the posttraumatic histomorphological alterations in SDH cases in relation to survival time is still lacking. Three studies already exist that allow an estimation of the time course of cortical hemorrhages after closed traumatic brain injury based on a statistical analysis [5,9,10], which also provides the methodical basis of the analysis presented here.

In the following the correlation of histomorphological alterations with the PTI of 222 cases of SDH is performed in order to develop a software program that allows an estimation of survival time of SDH based on the relative frequency of 10 morphological features. It is aimed at estimating the PTI of SDH following MBI in cases of unknown survival time on the basis of pathomorphological criteria by applying special software.

## 2. Materials and methods

### 2.1. Case material

Two hundred twenty two cases were studied ranging in age from 1 to 88 years with PTI times between a few minutes up to 30 years (Fig. 1). The study included both sexes. The cases were selected according to different criteria. Inclusion criteria were as follows:

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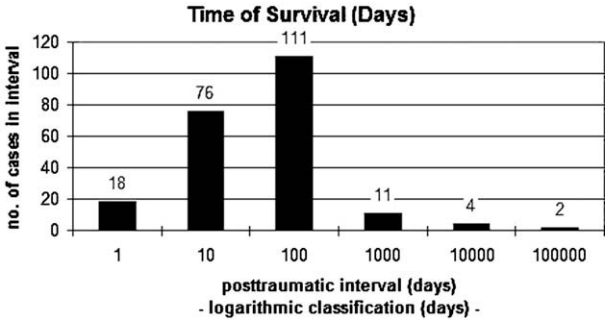


**Fig. 1.** Classification of all the cases according to survival time.

1. closed MBI combined with a macroscopically identified SDH of the brain, sometimes associated also with cortical hemorrhages, lacerations, subarachnoidal hemorrhages and/or epidural hemorrhages;
2. known interval between traumatic event and death
3. inclusion criteria do not presuppose that the cause of death has been the result of SDH

Exclusion criteria are as follows:

1. intracranial circulatory arrest (respirator brain)
2. recurrent intracranial bleeding – as deduced from clinical data (clinical symptoms, CT or MRI) or gross autopsy findings
3. coagulation diseases (hematological disease, liver cirrhosis, anti-coagulation medication)
4. bacterial or viral infection
5. known prior MBI and/or epilepsy

### 2.2. Brain tissue and dura mater

The brains and the dura mater were fixed for 3–4 weeks in 10% buffered paraformaldehyde before dissection. The cerebral hemispheres were sliced in the coronal plane and the brain stem horizontally. The macroscopic findings of the dura and brain were documented (including photographing) according to the protocol. Neither the dural hemorrhage, nor the brain damage [11,12] was quantified. In all cases two samples were taken from the dura hemorrhage as well as the saggital sinus and each of a sample from the following brain regions, regardless of the presence or absence of macroscopically evident changes: frontal lobe, corpus callosum, neostriatum, thalamic nuclei, hippocampus, cerebellum, pons and medulla oblongata. Samples were also taken from brain tissue exhibiting bleeding at the crest of a gyrus (contusional hemorrhages) and in contact with the brain surface (subarachnoidal hemorrhages). The blocks were embedded in paraffin employing routine methods.

### 2.3. Histological procedure

Five micrometers sections were stained according to the following methods: hematoxylin and eosin (H&E), collagenous fibers (trichrome stain – Azan [13], reticular fibers [14], iron (Prussian blue reaction), polymorphonuclear leukocytes (PMNs) – naphthol AS-D chloracetate esterase (NAS-DClAE [15]). A positive macrophage (Ms) reaction was revealed applying the monoclonal antibody CD68 (Dako GmbH, Hamburg, code M-0876) according to standard methods.

### 2.4. Evaluation procedure

Cases were selected according to the macroscopic and microscopic features. 10 histomorphological features of the dura and the clot like red blood cells (RBC), PMNs, Ms, RBC-containing Ms, hemosiderin-containing Ms, hematoidin, fibroblasts, collagenous fibers, capillary proliferation and membrane formation were examined by two independent observers blinded to survival time. A finding was rated as positive if a given histomorphological feature could be identified at least twice on each section. An immediate PMN-reaction was sometimes only identified by a discrete perivascular accumulation of leukocytes within the dura mater and the clot. Hemosiderin, a product of RBC breakdown as demonstrated by Prussian blue reaction, was present in the cytoplasm either homogeneously, in form of blue granules or as fine coarse, especially with increasing survival period. Within the meningeal dura, only extravascular reactive cells were considered. This applies especially to PMNs and Ms. Moreover, demonstration of RBC-containing Ms, hemosiderin- and hematoidin-containing Ms was also considered positive if they were located within the clot of SDH. If the morphological findings of the two observers differed, each observer performed an additional examination.

### 2.5. Statistical evaluation

The posttraumatic interval was recorded in minutes, hours, days, or years. The graphs display a logarithmic presentation of the time axis. The data were subjected to statistical analysis in order to obtain data about the observation period, the relative frequency of a histomorphological criterion, the estimated limits of confidence for this frequency according to Clopper and Pearson [16,17] and the distribution-free (independent) intervals [17].

A logistic regression of forensic relevant posttraumatic intervals was performed applying SPSS 8.0 (SPSS GmbH, Munich, Germany). Therefore, cases were divided into the two groups of cases either with a "short" survival time ($\leqslant$7 days, $n = 72$) or cases with a "longer" survival time (>7 days, $n = 150$).

### 2.6. Multistage evaluation system

Half of the cases (group 1; $n = 111$) were used to develop the multistage evaluation system, the other half (group 2; $n = 111$) to check its accuracy of prediction. These data were used to develop a software program for estimating the survival time of a traumatic event associated with SDH employing a database containing all information of the 222 cases. The software program estimates the survival time of SDH based on the relative frequency of the 10 morphological variables in each of the eight posttraumatic intervals. Because histomorphological criteria are not equivalent in their significance for the calculation, factors representing the contribution of each criterion were calculated using logistic regression analysis [18] and values for each variable were multiplied by an empirical weighting factor. Based on these frequencies, the survival time of new cases can be calculated by entering the data of each of the 10 histomorphological criteria. The development of the software has been described elsewhere in detail [5] and is available at t.walter@individualhealth.de.

## 3. Results

The distribution of the 222 cases to the PTI is presented in a semilogarithmic form in Fig. 1. The number of persons surviving the trauma for less than 2 months was considerably greater than the number surviving for longer periods (up to 30 years). It may be assumed that the death of the subjects with shorter survival periods was directly or indirectly related to the effects of the traumatic event, while most of the deaths occurring after a PTI exceeding 2 months can be attributed to unrelated causes.