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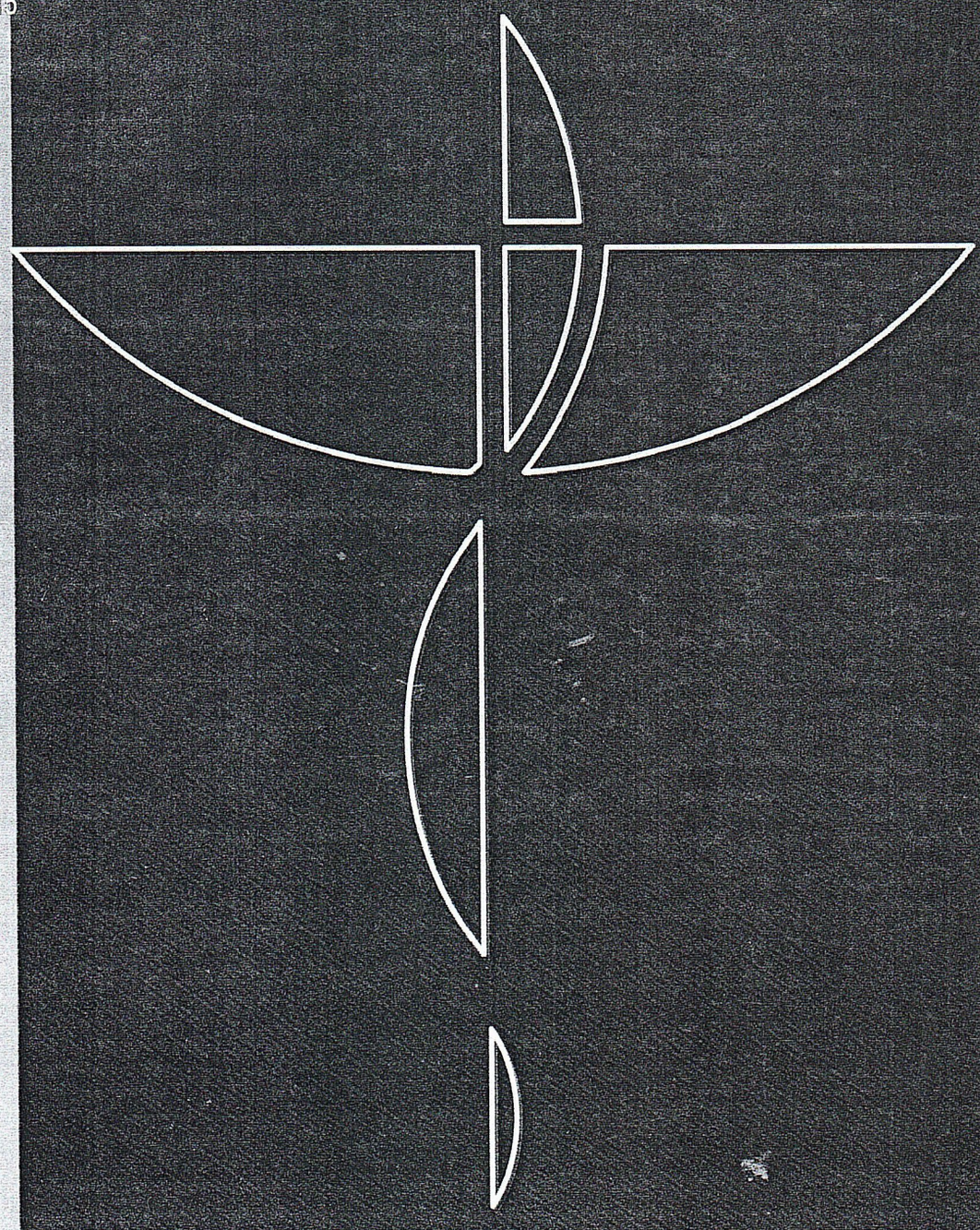

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SIDEROPHAGES AS PARAMETER FOR DETERMING AGE OF CEREBRAL CONTUSIONS

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Abstract
Timing of brain contusions is based on the reparational morphological tissue changes that happen at a certain period after the injury. The earliest appearance of a certain parameter determines the minimal age of the lesion. Siderophages are cellular elements which appearance is time-dependent and hence they can be used in the forensic estimation of the age of injuries along with the other elements of the healing process. The aim of this study was to determine the earliest appearance of siderophages by microscopic analysis of brain contusions with different survival period. In addition, the presence of hematoidin was examined. Our results, both for siderophages and hematoidin, are in agreement with the present knowledge especially in groups with longest surviving time. We can safely use siderophages and hematoidin for determining the age of lesions, in addition to other parameters.

Key words: age of injury, cerebral contusions, siderophages, hematoidin

СИДЕРОФАГИ КАКО ПАРАМЕТАР ЗА ДЕТЕРМИНИРАЊЕ НА ВРЕМЕТО НА НАСТАНУВАЊЕ НА МОЗОЧНИТЕ КОНТУЗИИ

Извадок

Одредување на времето на настанување на мозочните контузии се базира на морфолошките промени во ткивото за време на процесот на заздравување. Најраното појавување на одреден параметар ја одредува минималната старост на одредена лезија. Сидерофагите се клеточен елемент чиешто појавување во лезиите е временски зависно и затоа во склоп со другите елементи при процесот на заздравување можат да се искористат при форензичната проценка на староста на повредите. Целта на овој труд беше, преку микроскопска анализа на мозочни контузии со различен период на преживување, да се одреди најраното појавување на сидерофагите. Дополнително беше испитано и присуството на хематоидинот. Нашите резултати покажаа дека параметрите како што се сидерофагите и хематоидинот слободно можат да се користат во одредување на времето на настанување на мозочните контузии, заедно со другите параметри.

Клучни зборови: време на повредување, сидерофаги, хематоидин, мозочни контузии

Introdaction

Timing of brain contusions is based on the reparational morphological tissue changes that happen at a certain period after the injury. The earliest appearance of a certain parameter determines the minimal age of the lesion [1]. The cerebral contusions are characterized with early morphological changes such as perivascular bleeding, local edema or necrosis of the tissue. These events are usually accompanied by acute inflammatory response as well as by a reaction of microglia and cerebral macrophages with a subsequent reactive gliosis [2-6]. This chain of events is accompanied with proliferation of astrocytes, neoangiogenesis and fibrous glial scar [7]. After acute inflammatory response, clearing process is the next stage of brain injury healing. This process is performed by macrophages and microglial cells. Results from conventional histological studies show varying results concerning temporal appearance of brain macrophages in contused lesions from few hours after injury [8-10], 12-14 hours [11-12] to 1-2 days [13-15].

When a brain injury occurs a process of cleaning up of the dead tissue and other cellular elements begins. Some of the cellular elements that need to be removed are the perivascular erythrocytes and the erythrocytes in the injured tissue. The physical and chemical removal of erythrocytes to hemosiderin is an intracellular process happening in the macrophages. Macrophages containing hemosiderin appear as early as 3-4 days after injury and become most prominent on the 5-7th day. The phagocytosed hemosiderin is a product of the decomposition of iron containing hemoglobin. Siderophages may be present in 20 years old lesions and even longer [8]. Hematoidin is an orange-yellow pigment that does not contain iron generated during the decomposition of hemoglobin due to iron removal. It can be found intra- or extracellularly between the 10th and 12th day after injury. It is easily soluble and it can be quickly removed from the lesion but also it can bind to the connective tissue fibers and persist for a longer time [8].

Siderophages are cellular elements which appearance is time-dependent and hence they can be used in the forensic estimation of the age of injuries along with the other elements of the healing process. The aim of this study was to determine the earliest appearance of siderophages by microscopic analysis of brain contusions with different survival period. In addition, the presence of hematoidin was examined.

Material and methods

We processed a total of 30 brain contusions in individuals with different period of survival whose autopsies were performed at the Institute for Forensic Medicine. The postmortal interval until the autopsy in all of the cases was less than 24 hours. Exclusion criteria were postmortem period of more than 24 hours, and knowledge of any proved former or current pathological changes in the brain tissue. Depending on the survival period the contusions were divided into three groups: survival period up to 100 hours, survival period between 101-200 hours, and survival period of more than 200 hours.
The brain tissue samples were fixed in a 10% buffered formalin for a period of 5 days. After that, they were embedded in paraffin blocks, 5 microns-thick sections were made and stained with H&E and Hematognost Fe kit for iron ions. Siderophages were identified with Hematognost Fe staining kit as ameboid cells which contain hemosiderin granular blue

pigment. Hematoidin was identified with both staining methods as an extracellular yellow pigment.

Counting of siderophages was made on 200x magnification in whole lesion surface and surrounding tissue. For counting we used the microscope NIKON LABOPHOT-2 and for determining the area of the lesion we used the software Visiopharm on microscope Leica 5000 DM.

The results obtained from counting of the siderophages and presence or absence of hemolysis and hematoidin were analyzed with descriptive and nonparametric statistical methods using the commercial software Statistica 6.0 [16].

RESULTS

We stained and analyzed a total of 30 contusions from cases with various survival time. Three of these 30 contusions were excluded due to other pathological process in the brain tissue found in the taken samples.

Descriptive statistics and tests for normality of distribution of examined parameters (survival time, number of siderophages, presence or absence of hemolysis and hematoidin and area of lesion) are shown in Tables 1-3 and Figures 1-4.

Table 1 Descriptive statistics: Siderophages

	Valid N	Mean	Minimum	Maximum	Std.Dev.
survival/h	27	161.2963	1.000000	648.0000	169.4127
siderophages	27	49.8889	0.000000	400.0000	105.7356
lesion/mm2	27	18.0418	0.228000	128.0000	33.4690
siderophages/mm2	27	6.9987	0.000000	68.9000	16.9670

Table 2 Frequency: Hemolysis

:

	Count	Cumulative	Percent	Cumulative
Yes	3	3	11.11111	11.1111
No	24	27	88.88889	100.0000
Missing	0	27	0.00000	100.0000

Table 3 Frequency: Hematin

	Count	Cumulative	Percent	Cumulative
Yes	23	23	85.18519	85.1852
No	4	27	14.81481	100.0000
Missing	0	27	0.00000	100.0000

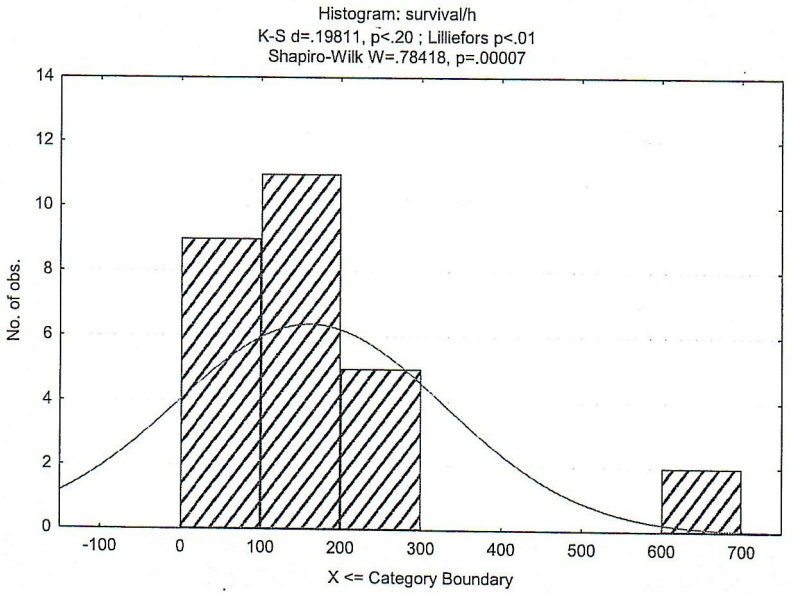


Figure 1 Distribution of survival time

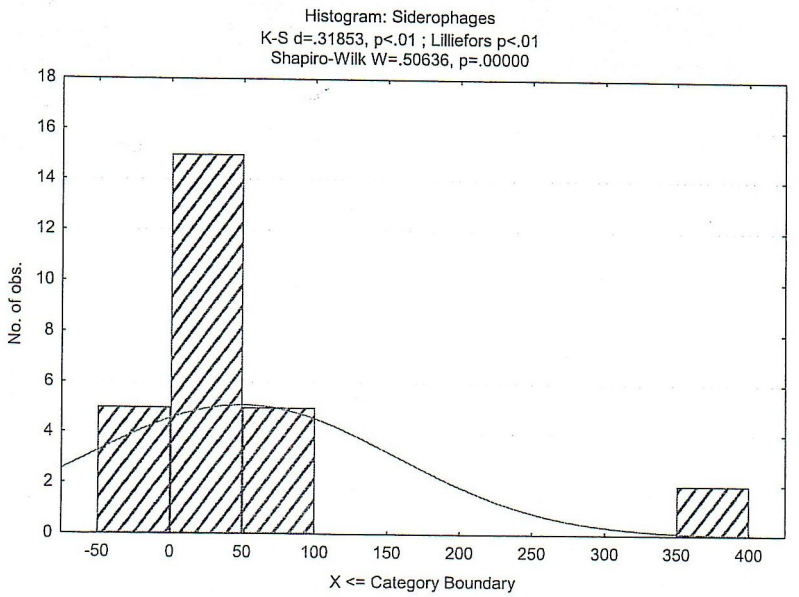


Figure 2 Distribution of siderophages

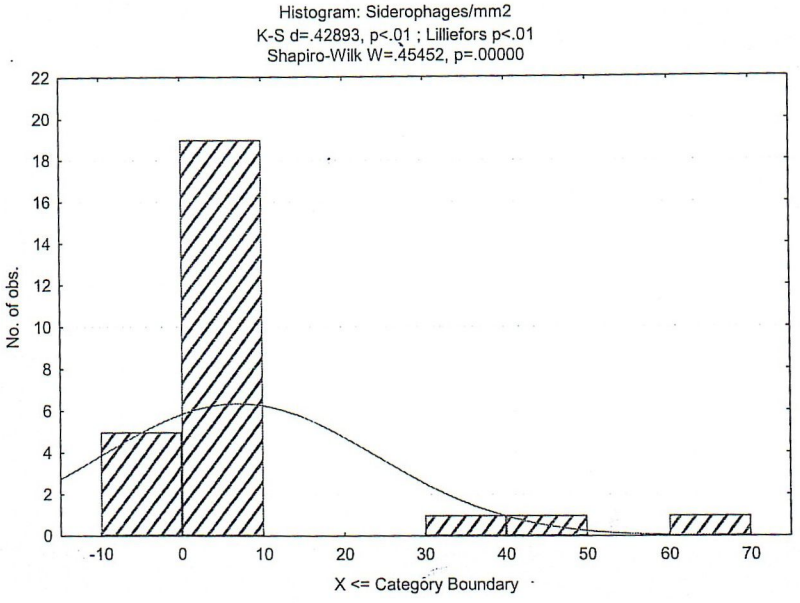


Figure 3 Distribution of siderophages/mm^2

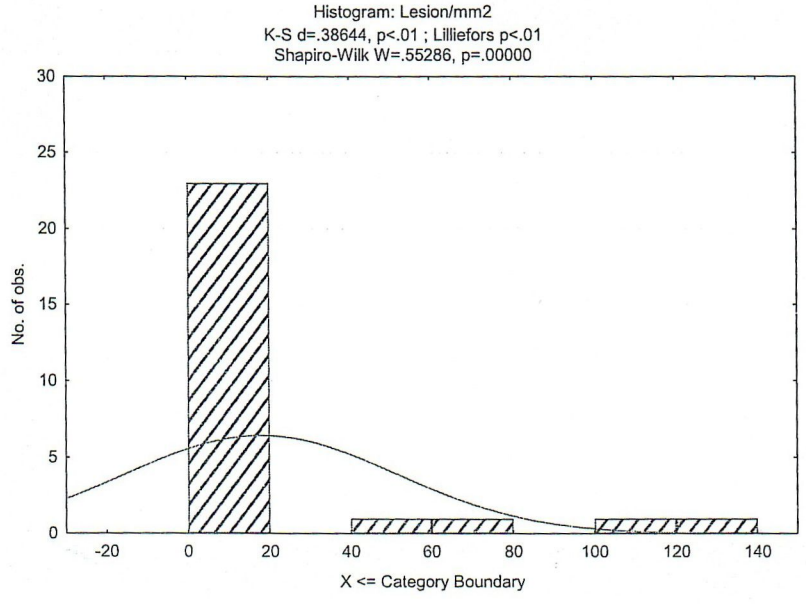


Figure 4 Distribution of lesion/mm^2

Tested parameters showed significance of Kolomogorov-Smirnov test, Lilliefors test and Shapiro-Wilks tests, as well as a significant difference in variance. Therefore, nonparametric tests were used for testing intergroup differences. Level of nonparametric correlation between parameters is shown in Table 4, with significance level of $p<0.05$.

Table 4 Level of nonparametric correlation between parameters

Spearman Rank Order Correlations
MD pairwise deleted
Marked correlations are significant at p <.05000

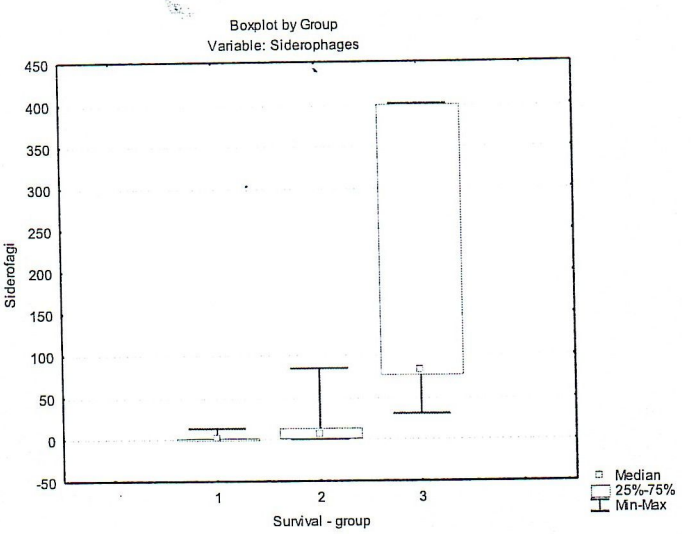
variable	survival/h	siderophages	hemolysis	hematin	lesion/ mm2	siderophages/mm2
survival/h		0.836		0.585	0.458	0.697
siderophages	0.836		0.502	0.518	0.479	0.860
hemolysis		0.502				0.501
hematin	0.585	0.518			0.551	
Lesion/ mm2	0.458	0.479		0.551		
siderophages/ mm2	0.697	0.860	0.501			

In Table 4 only the significant correlations are shown. For testing the intergroup differences of the analyzed parameters 27 samples were divided in three groups. The first group consisted of cases with under 100 hours survival time, the second group consisted of cases with 101-200 hours survival time, and the third group consisted of cases with above 200 hours survival time. Using Kruskal-Wallis test we found a significant difference for presence of siderophages between the three groups, with $p=0.0002$ ($p<0.01$) Table 5, Figure 5. Mann-Whitney test was used to check the difference between the groups. The results obtained have shown a significant difference between the first and the second group ($p<0.05$), as well as between the second and the third group ($p<0.01$) (Figure 6).

Table 5 Presence of siderophages between the three groups

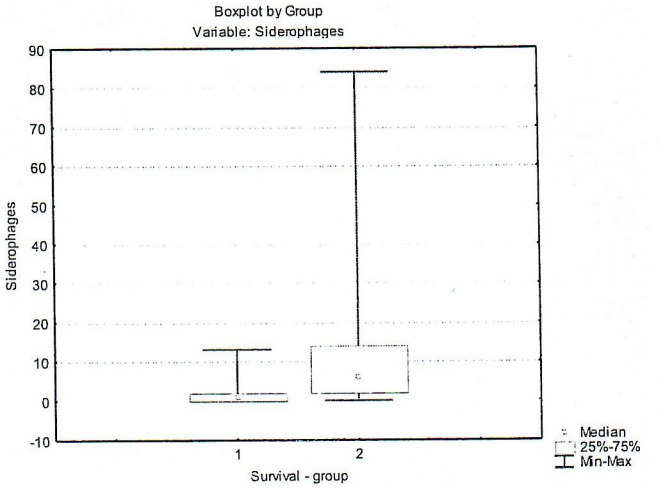
Kruskal-Wallis ANOVA by Ranks; Siderophages
Independent (grouping) variable: Survival - group
Kruskal-Wallis test: H (2, N= 27) =16.74988 p =.0002

Dependent: Siderophages	Code	Valid N	Sum of Ranks
Grp.1	1	9	63.0000
Grp.2	2	11	152.0000
Grp.3	3	7	163.0000



Median Test, Overall Median = 6.00000; Siderophages
Independent (grouping) variable: Survival- group
Chi-Square = 12.51548, df = 2, p = .0019

Figure 5 Presence of siderophages between the three groups



Mann-Whitney U Test: U=18; Z=-2,39; p<0,05

Figure 6 Difference between the groups

There was no significant intergroup difference regarding hemolysis (Table 6), and for presence of hematin there was a significant intergroup difference between the third group in comparison with the first and the second group ($p<0.05$), but there was no difference between the first and the second group since there was no hematin in these samples (Table 7, Figure 7).

Table6 Presence of hemolysis in the three groups

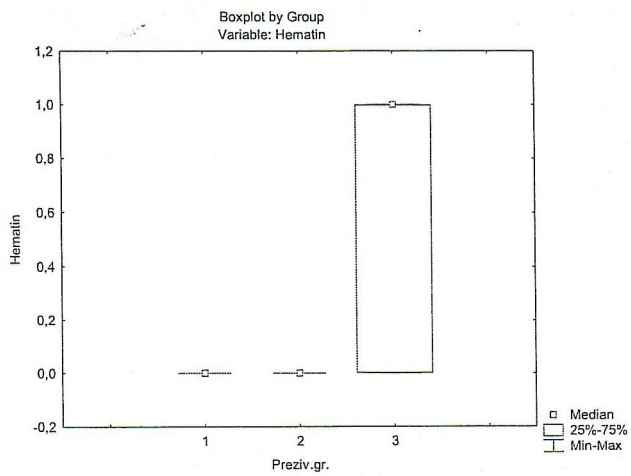
Kruskal-Wallis ANOVA by Ranks;
Independent (grouping) variable: Survival-group
Kruskal-Wallis test: H (2, N= 27) =1.969697 p =.3735

Dependent: Hemolysis	Code	Valid N	Sum of Ranks
Grp.1	1	9	112.5000
Grp.2	2	11	157.0000
Grp.3	3	7	108.5000

Table 7 Presence of hematin in the three groups

Kruskal-Wallis ANOVA by Ranks; Hematin
Independent (grouping) variable: Survival-group
Kruskal-Wallis test: H (2, N= 27) =12.91925 p =.0016

Dependent: Hematin	Code	Valid N	Sum of Ranks
Grp.1	1	9	108.0000
Grp.2	2	11	132.0000
Grp.3	3	7	138.0000



Median Test, Overall Median = 0.00000; Hematin
Independent (grouping) variable: Survival-group
Chi-Square = 13.41615, df = 2, p = .0012

Figure 7 Comparison of hematin between the three groups

Table 8 Lesion/mm^2

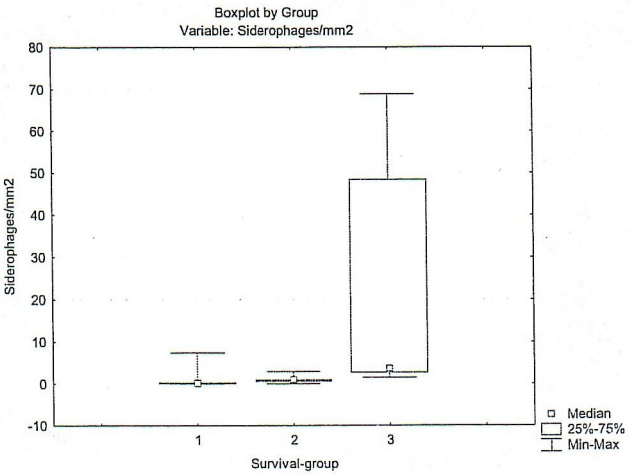
Kruskal-Wallis ANOVA by Ranks; Lesion/mm2
Independent (grouping) variable: Survival-group
Kruskal-Wallis test: H.(2, N= 27) =2.780518 p =.2490

Dependent: Lesion/mm2	Code	Valid N	Sum of Ranks
Grp.1	1	9	94.0000
Grp.2	2	11	170.0000
Grp.3	3	7	114.0000

Table 9 Siderophages/mm2

Kruskal-Wallis ANOVA by Ranks; Siderophages/mm2
Independent (grouping) variable: Survival-group
Kruskal-Wallis test: H (2, N= 27) =14.45309 p =.0007

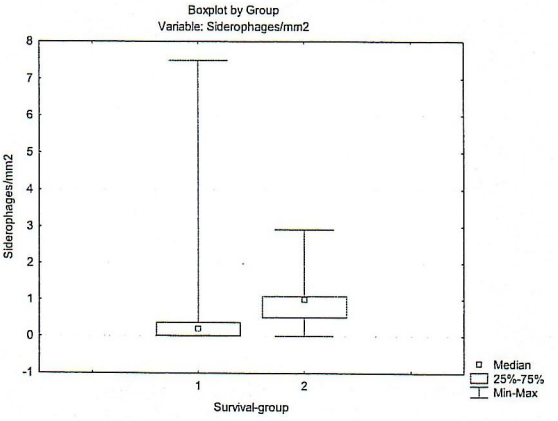
Dependent: Siderophages/mm2	Code	Valid N	Sum of Ranks
Grp.1	1	9	71.5000
Grp.2	2	11	145.5000
Grp.3	3	7	161.0000



Median Test, Overall Median = 1.00000; Siderophages/mm2
Independent (grouping) variable: Survival-group
Chi-Square = 14.28099, df = 2, p = .0008

Figure 9 Siderophages/mm2

There was no significant difference between the lesion/mm^2 and survival time (Table 8). There was a significant difference between the second and the third group concerning the presence of siderophages/mm^2 as well as between the third and the first group (Table 9, Figure 9), although the difference between the first and the second group was based upon presence of siderophages in one case only (Figure 10).



Mann-Whitney U Test: U=22,5; Z=-2,05; p<0,05

Figure 10 Siderophages/mm2

Figures 11 and 12 show siderophages as ameboid cells, which contain hemosiderin granular blue pigment in case with survival time of 27 days.

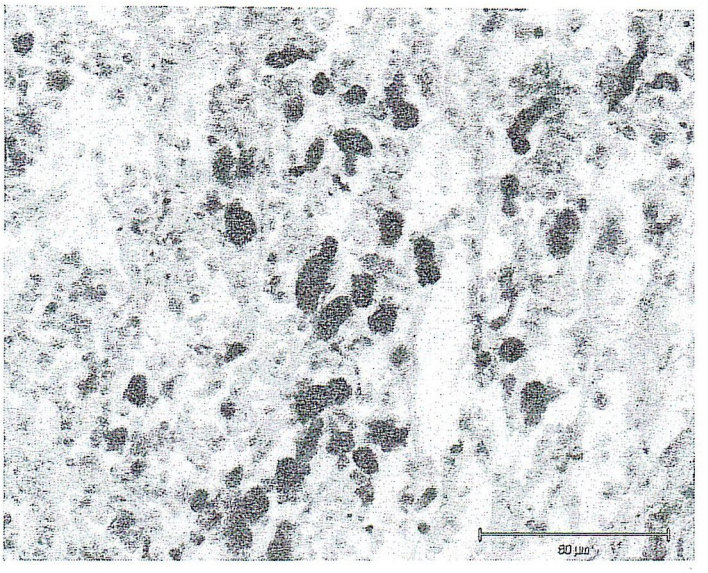


Figure 11 Siderophages contain *hemosiderin granular blue pigment in case with survival time of 27 days (magnification 200x)*

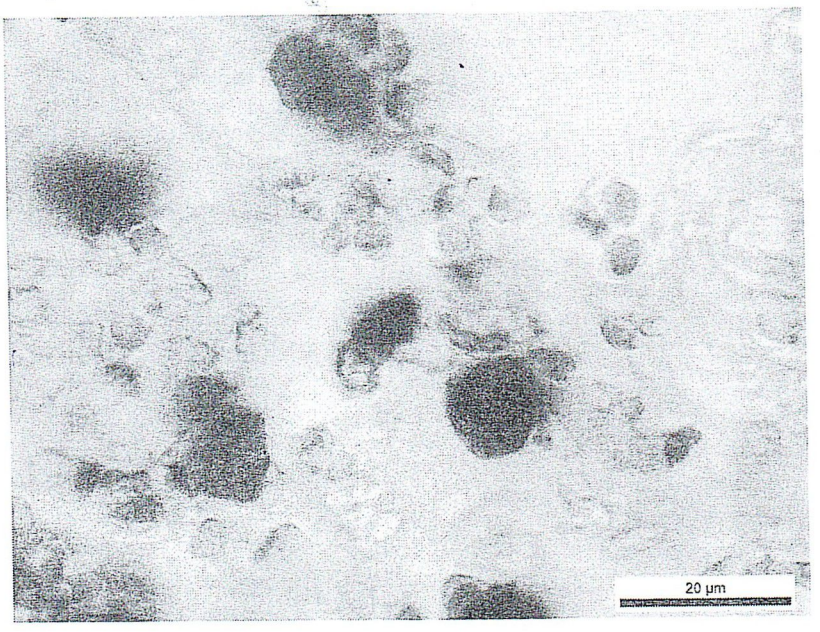


Figure 12 Siderophages contain *hemosiderin granular blue pigment in case with survival time of 27 days (magnification 1000x)*

Discussion

Bleeding as a part of every contusion presents a sufficient stimulus for inflammatory response. The brain macrophages have an active role in the cleanup of necrotic tissue within the inflammatory response. Their presence and distribution are dependent on the time interval after the occurrence of injury. Their morphology and intracellular content changes relative to the time.

In this study, based on the microscopic analysis of samples obtained from cerebral contusions, we showed the time dependency of the appearance and distribution of siderophages (macrophages containing hemosiderin) in and around the lesion. We compared our results with those reported in similar studies. We also checked for presence of hematoidin and hemolysis. Former studies showed similar and consistent results related to the earliest appearance of siderophages. The results ranged from 70 - 90 hours [12], 3 – 6 days after the injury [11] or 3 – 4 days, and the most developed response was seen after 5-7 days [8]. Concerning hematoidin, its appearance was noted from 10-12 days after the injury. [8]. In our study the first appearance of siderophages was in the group with the shortest time of survival (under 100 hours), but there was a significantly larger number of siderophages in the group with the longest survival period (more than 200 hours). As for the presence of hematoidin, its first and the only appearance was in the group with the longest survival period. It is well known that siderophages appearance is time-dependent thus it can be used as a parameter for determining the age of injuries. Our results

correspond with the findings in the literature for survival times above 200 hours regarding hematoidin presence [8,11-12].

Conclusions

Macrophages play a significant role in the healing process after brain injury. Depending on their presence and intracellular content, we could differentiate between lesions that have been inflicted more than 200 hours before death and more recent lesions. Our findings show that we can use them as a parameter for determining the age of an injury especially in cases with longer survival period.

References

1. Hausmann R. Timing of Cortical Contusions in Human Brain Injury Morphological Parameters for a Forensic Wound-Age Estimation. Forensic Pathol Rev. 2004; 1:53-75.
2. Andersson PB, Perry VH. The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. Neuroscience. 1992;48(1):169-86.
3. Hausmann R. Age Determination of Brain Contusions. Forensic Sci Med Pathol.2006;2(2):85-93.
4. Holmin S, Mathiesen T, Shetye J, Biberfeld P. Intracerebral inflammatory response to experimental brain contusion. Acta Neurochir Wien. 1995; 132(1-3): 110-9.
5. Persson L. Cellular reaction to small cerebral stab wound in the rat frontal lobe. Virch Arch B Cell Pathol Mol Pathol. 1976; 22(1):21–37.
6. Spatz H. Von der Morphologie der Gehirnkontusion (besonders der Rindenprellungsherde). Münch Med Mschr. 1951; 93:1.
7. Peters G. Uber gedeckte Gehirnverletzungen (Rindenkontusionen) im Tierversuch. Zentralbl Neurochir. 1943; 8: 172-208.
8. Cervós-Navarro L, Lafuente JV. Traumatic brain injuries: structural changes. J Neurol Sci. 1991;103 Suppl:S3–14.
9. Carmichael AE. Microglia: an experimental study in rabbits after intracerebral injection of blood. J Neurol Psychopathol. 1929;9:209–16.
10. Hammes EM. Reaction of the meninges to blood. Arch Neurol Psychiat 1944;52:505–14.
11. Oehmichen M, Raff G. Timing of cortical contusions. Correlation between histomorphological alterations and post-traumatic interval. Z Rechtsmed 1980;84:79–94.
12. Oehmichen M, Eisenmenger W, Raff G, Berghaus G. Brain macrophages in human cortical contusions as an indicator of survival period. Forensic Sci Int. 1986;30(4):281–301.
13. Macklin CC, Macklin MT. A study of brain repair in the rat by use of trypan blue, with special reference to the vital staining of macrophages. Arch Neurol Psychiat Chic. 1920;3:353–93.

14. Masuda Y. Histological and histochemical study of cortical lesion of brain with special reference to the alteration in compressed area. Jpn J Legal Med. 1969;23(2):139–169.
15. Nevin NC. Neuropathological changes in white matter following head injury. J Neuropath Exp Neurol 1967;26(1):77–84.
16. StatSoft, Inc. STATISTICA (data analysis software system), version 6. 2001. Available from: www.statsoft.com.