Original Article

Study of Histomorphological Changes in the Subarachnoid Hemorrhage at Different Time Intervals Between Injury Infliction and Death

Manoj Hang Limbu[1], Sabnam Shrestha[2], Salmalee Yadav[3], Apurba Acharya[4], Sushila Gurung[5], Shovana Karki[6], Rijen Shrestha[7]

[1]Lecturer, Department of Forensic Medicine, Nobel Medical College, Biratnagar, Nepal, [2]Forensic Medicine Consultant, Bhaktapur Hospital, Bhaktapur, Nepal, [3]Lecturer, Department of Forensic Medicine, Patan Academy of Health Science, Lalitpur, Nepal, [4]Assistant Professor, Department of Forensic Medicine, Karnali Academy of Health Science, Jumla, Nepal, [5]Lecturer, Department of Forensic Medicine, Pokhara Academy of Health Science, Pokhara, Nepal, [6]Associate Professor, Department of Pathology, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal, [7]Assistant Professor, The Maldives National University, Male, Maldives

How to cite this article: Manoj Hang Limbu, Sabnam Shrestha, Salmalee Yadav et. al. Study of Histomorphological Changes in the Subarachnoid Hemorrhage at Different Time Intervals Between Injury Infliction and Death. Indian Journal of Forensic Medicine and Toxicology/Volume 19 No. 1, January - March 2025.

Abstract

Newer biochemical and molecular techniques are under research for wound dating purpose, but histology is still the mainstay of wound dating technique. Significance of knowing how old the wound is, can have major implication in the delivery of justice. Similarly, subarachnoid hemorrhage may need to be dated in cases where other data for wound dating are scarce. This study is designed to study the histomorphological changes in the subarachnoid hemorrhages of different time interval since injury and aim to use this information for dating of subarachnoid hemorrhage for medicolegal purpose. The histology samples of subarachnoid hemorrhage of the deceased with head injuries with known post injury interval were taken during the autopsies and divided into different groups. The gross color changes and histomorphological parameters were evaluated in each group and statistical inference was made. The result showed gross color, RBC lysis, macrophage infiltration, hemosiderin laden macrophage, fibrin deposition, collagen deposition and meningeal reactive changes significantly correlated with post injury interval. Whereas, Neutrophils, lymphocytes and phagocytosis did not correlate. RBC lysis also correlated with gross color change as well. In conclusion, histology of subarachnoid hemorrhage can be a useful tool in dating subarachnoid hemorrhage in cases where it is required.

Key words: Subarachnoid hemorrhage, Wound dating, Histopathology, Forensic pathology

Introduction

Subarachnoid hemorrhage (SAH) can either be traumatic or spontaneous. Of all thetraumatic brain injury, 11 to 60% develops subarachnoid

Corresponding Author: Manoj Hang Limbu, Lecturer, Department of Forensic Medicine, Nobel Medical College, Biratnagar, Nepal.

E-mail: manojfrienddharan@yahoo.com

Submission date: August 4, 2024 **Revision date:** Sept 16, 2024 **Published date:December 3,2024**

hemorrhagewhich is more than spontaneous subarachnoid hemorrhage (incidence of 7.9 per 100000 population)[1,2]. While the mechanism of bleeding in both types are different, the pathophysiology leading to brain injury and death, are similar. [34] This study is about the histomorphology of traumatic subarachnoid hemorrhages and therefore, spontaneous subarachnoid hemorrhages are excluded. Subarachnoid hemorrhage is the second most-common type of intracranial bleed in blunt head trauma cases in Nepal. Meanwhile, common causes of head trauma are road traffic incidents followed by fall and assaults.[56] Post-mortem examination of subarachnoid hemorrhage in these cases can present with a challenge especially when there is a need to know about the age of the subarachnoid hemorrhage.

During the investigation of death, knowing the time since injury can make a difference between delivery and miscarriage of justice, it is also one of the most researched topics in the field of forensic medicine. Even after decades of study on dating of the wound, wound dating has still not gained the reliability as its importance. The principle of dating of wound is based on the examination of the stages of wound healing. The studies on vitality and wound healing have led to the development of guidelines for establishing time since injury. The histological examination of the wound also helps to identify the wound as antemortem or postmortem nature by studying the vitality of the wound. The wound can also be dated by observation of the gross color change. Numerous techniques have evolved over time, yet, histological method is still the gold standard for dating of injury. [7] While abundant literatures regarding extradural and subdural hemorrhages can be found but the study regarding the dating of subarachnoid hemorrhage is scarce. Therefore, study of this kind broadens our knowledge in wound dating work as well as gives valuable information for the future research.

Material and Methods

This study was conducted at Tribhuvan University, Institute of Medicine, Maharajgunj Campus at the Department of Forensic Medicine from the year 2019 to 2021. Ethical clearance was obtained from the Institutional Review Board of the Tribhuvan University. (Dated 16th August, 2019, Ref No.55/(6-11)E2/077/078) Study was conducted on the autopsy cases of traumatic head injury. The inclusion criteria for this study included; all cases with traumatic subarachnoid hemorrhage, both isolated and associated with other intracranial injuries, with known post-injury interval well-preserved brain, and exclusion criteria included; liquefactive changes, spontaneous subarachnoid hemorrhage, unknown post-injury interval and those with signs or history of disease affecting circulatory system. Accordingly, 120 deceased were studied. According to issues discussed in previous studies such as hospital presentation time, survival time anddelayed deaths and according to the availability of the cases in the department,the deceased were grouped according to following post trauma intervals; less than 4 hours, 4 to 12 hours, 12 to 24 hours, 1 to 5 days, 5 to 14 days and 14 to 30 days.[8–11]

During autopsy, brain with subarachnoid hemorrhage were cut into 3 cm x 2 cm x 2 cm block and fixed in a 10% formalin. The sample containers werelabeled and stored until next day.

The gross color *(Red, Purplish, purplish black, Rusty brown, yellowish)*, of the samples was noted before fixing the samples. After the samples were fixed for a minimum of 12 hours, the samples were grossed and processed at the Department of Pathology, Institute of Medicine, where it was processed for slide preparation. After the slides were prepared, the slides were evaluated by two pathologists and scored for each parameters *(RBC lysis, Neutrophils, Lymphocytes, Macrophages, Hemosiderin laden macrophage, Fibrin deposition, Phagocytosis, Meningeal reactive changes)* according to the technique described by P. Tak et al. (1995).[12] The data were analyzed in PASW using Chi Square test. The statistical significance between the groups were analyzed using post hoc test, wherever applicable.

Results

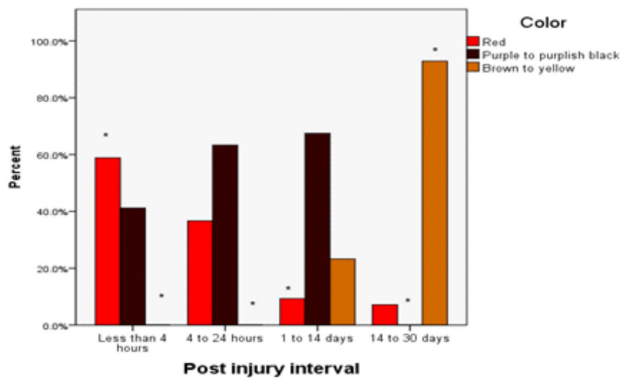


Figure 1: Correlations between the post injury interval and gross color change. (c) (* = p < 0.004)

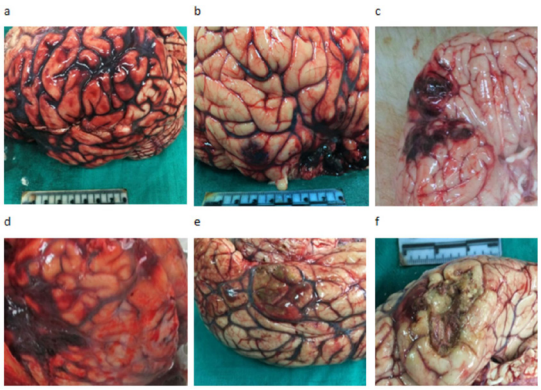
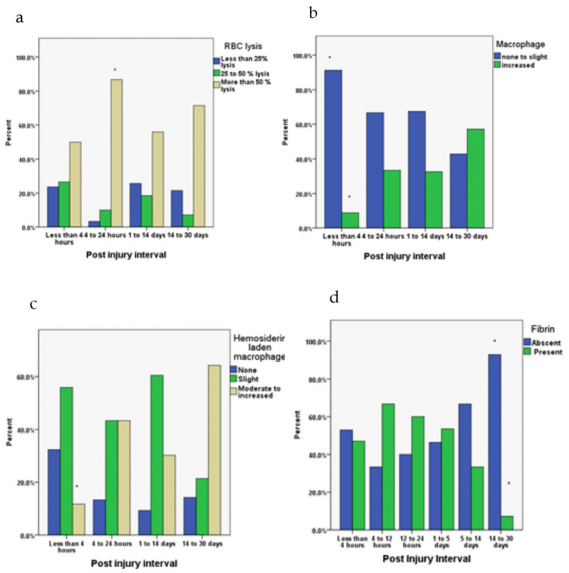


Figure 2: Illustration of SAH of less than 4 hours. (a) purple to purplish black colored subarachnoid hemorrhage of 12 to 24 hours' time interval (b) and 1 to 5 days (c) time interval. Illustration of brown to yellow colored SAH in 1 to 14 days interval group(d) and (e) and in 14 to 30 days interval group (f). Brownish to yellowish transition can be seen on the same case.

Gross color changes

The total of 121 cases were studied. Among them, purple to purplish black color (62, 51.2%) was the most commonly observed, followed by red (36, 29.8%), yellow (13, 10.7%) and brown (10, 8.3%). Post injury interval and gross color changes showed significant correlation. Color red showed decreasing trend with increase in interval up until day 14.The presence of brown and yellow color was significantly associated with interval of 14 to 30 days. Absence of purple and purplish black color was significantly associated with interval of 14 to 30 days. Figure 1 and Figure 2.



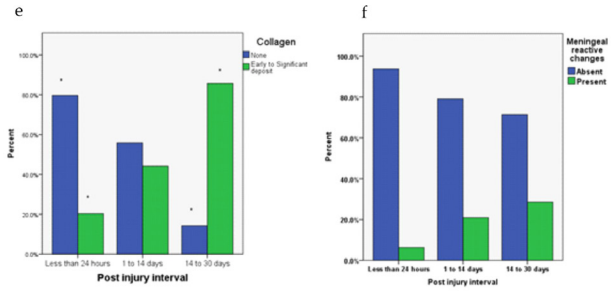


Figure 3: Correlation between post injury interval and RBC lysis. (* = (p < 0.0042) (a). Correlation between the post injury interval and macrophage infiltration. (* = p < 0.0063) (b). Correlation between the post injury interval and amount of hemosiderin laden macrophage. (* = p < 0.0042) (c). Correlation between the post injury interval and level of fibrin deposition. (* = p < 0.0042) (d). Correlation between post injury interval and collagen deposition (* = p < 0.0083) (e). Correlation between the post injury interval and meningeal reactive changes. No significant correlations were seen between the variables.(f)

Histomorphology

Each slide was analyzed for degree of RBC lysis, slides were semi quantified for lysis of less than 25%, 25 to 50% and more than 50%. Post injury interval and RBC lysis showed significant correlation but the correlation was significant only between interval of 4 to 24 hours and more than 50% lysis. Figure 3a, Figure 4

The slides were scored as slight infiltration if neutrophil or lymphocytes were seen as few scattered cells and as increased if aggregates of cells were seen. The analysis between post injury interval and neutrophil and between post injury interval and lymphocytes showed no significant correlation.

The cells were analyzed for both phagocytic and non-phagocytic macrophages and were designated as slight infiltration if less than 10 macrophages were seen in a high-power field and as increased infiltration if more than 10 macrophages were seen in a high-power field in an active field. There was a significant association between post injury interval and macrophage. On post hoc test, none to slight infiltration was significantly associated with post injury interval of less than 4 hours and Increased infiltration was significantly associated with post injury interval of less than 4 hours. Figure 3b

Hemosiderin laden macrophage was evaluated as none if absent, slight if less than 10 per high power field and moderate to increase if more than 10. There was a significant correlation between post injury interval and detection of hemosiderin laden macrophage. The post hoc test showed moderate to increase hemosiderin laden macrophages were less likely to be seen in post injury interval of less than 4 hours. Figure 3c5a

There is a significant correlation between post injury interval and fibrin deposition. On post hoc test, the post injury interval of 14 to 30 days is more likely to have absent fibrin deposition. Figure 3d5b

Slides were evaluated for absence or presence (early to significant) of collagen deposit. There was a significant correlation between post injury interval and collagen deposition. On post hoc test, absence of collagen deposition was more likely to be seen in post injury interval of less than 24 hours and presence of collagen deposition were more likely to be seen in post injury interval of 14 to 30 days. Figure 3e5c

Slides were evaluated for presence or absence of meningeal reactive changes. Changes observed in arachnoid were mesothelial cell proliferation, leucocytes infiltrations, fibrosis and subarachnoid space obliteration. In the early groups the changes were predominantly cellular proliferations, swelling and leucocyte infiltrations where as in the later groups, the changes were collagen deposition and thickening of membranes. Detection of meningeal reactive changes showed increasing trend with increase in post injury interval. Figure 3f, Figure5c

a. b.

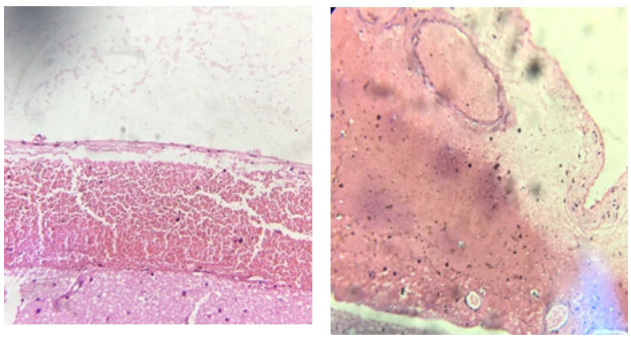
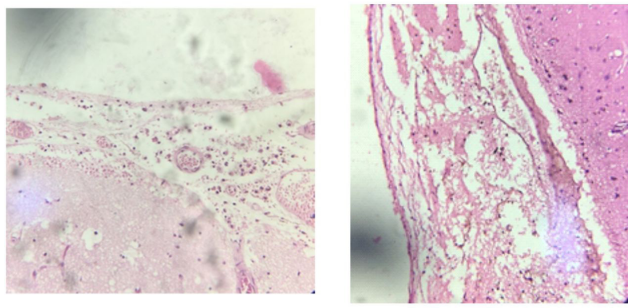


Figure 4: The images show different levels of RBC lysis within the hemorrhage of the same time interval of 4 hours. Fresh RBC (a), more than 75 % lysis with hemosiderin pigments (b)

a. b.



c.

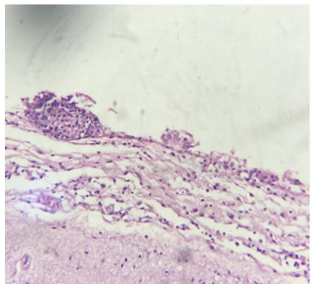


Figure 5: a:Hemosiderin-laden macrophages seen as a golden-brown pigment containing cells, in a 5 to 14 days old SAH. b: Fibrin deposition seen in a 4 to 12 hours old SAH. c:Meningeal reactive changes and collagen deposition seen in a 15 to 30 days old SAH.

Discussions

In this study, postinjury interval significantly correlated with color changes in the SAH, especially red and brown-yellow color, as the injury gets older the red color began disappearing while the brownish to yellow began appearing, the finding were consistent with previous studies. [13,14,15]However, the color interpretation was done with naked eyes, therefore it could not be free of interobserver bias, especially during interpretation of different shades of red color. According to P. Vanezis (2001), naked-eye interpretation of color is highly subjective and use of colorimetry or spectrophotometry is advised. [16] The effect of amount and duration of bleeding on color appearance was not studied. According to J. Ross et. al. (2012), the purplish black color can persist for days if the SAH is large.

On histological evaluation, RBC lysis of 50% or more was significantly associated with SAH of 4 to 24 hours, which is consistent with other studies. [17]Even though, RBC lysis correlated with post injury interval, but it did not correlate with gross color, which could be due to the size of hemorrhage, where RBC in small hemorrhagehemolyze late. It is also seen in a large bleeding where the RBCs in the

center of the hematoma undergo early deformation than the RBCson the periphery. [18]The association in some of the cases was not seen may be because of re bleeding episode, such cases were mostly seenin the post injury interval of 14 to 30 days which featured mixed features of later stages of healing with fresh RBCs. According to the study, rebleed episodes are not uncommon in traumatic SAH. [19–21]

Macrophage pattern seen in this study was consistent with the general principle of wound healing.[22] Neutrophils and lymphocytes did not show correlation similar to study of J. Alpers (1945), where occasional phagocytosis was seen as early as after 12 hours and increased after 3 days, but appearance of leucocytes did not correlate with any time interval. D. Munro (1936) also reported the appearance of phagocytosis in a SDH specimen collected 5days after bleeding. The detection of siderophages was consistent to the pattern described by J. Lee (2010), the author reported that the enzyme responsible for degrading hemoglobin was detected in the SAH as early as 6 hours and peaks at day 3. [23]

Fibrin is an important biological compound that plays an important role in hemostasis, wound healing and other biological functions. [24] During the wound healing process, the fibrin begins to appear as fibrin strands from 12 hours onward and later serves as a framework for connective tissue organization. Slight connective tissue organization begins after 4 days and becomes marked at around day 28, and as the wound organization continues, the fibrin becomes less marked. [25]Decrease in fibrin deposition in this study corresponds to the time of wound organization and might as well becomes useful for estimating the age of the wound, especially if it is nearing later stage of wound healing.

Absence of collagen was associated with early hours of hemorrhage, whereas presence was associated with later days of hemorrhage. According to literatures, collagen begins to appear from 4 to 5 days and becomes marked at 13 to 14 days. [13,19,25] However, in this study, collagen was present even in the SAH of less than 3 days, which was not reported previously. These collagens could betype 3 collagen that were deposited earlier, which later gets replaced by type 1 collagen or it could just be a disrupted subarachnoid trabecula, which are also a collagen structure.[26.27] Therefore, presence of organized collagen supported by ancillary test may give us hint of significantly older hemorrhage.

In order not to over interpret or misinterpret, it is also worthwhile to note that collagen deposition is not only limited to the site of the SAH but can also be at sites remote to the SA Hand also its distribution can be patchy, both of which could lead to miss sampling. Also, some of the post SAH fibrosis could be senility changes which could be easily over interpreted.[28,29] Meningeal reactive changes were also significantly correlated with post injury interval; the pattern shows increasing trend with increase in duration. The studies show, meningeal reaction persists as long as there is blood or blood related product present in the subarachnoid space. Also, meningeal reactions can be present in the form of polymorphonuclear cell infiltration, mesothelial cell proliferation and fibrosis in varying degree according to the post injury interval. Therefore, caution should be applied against coming to conclusion by simply observing presence or absence of reaction.[30] The overall findings are summarized in table 1.

Table 1: **Summary of gross and histological findings across the post-injury intervals.**

Post injury interval	**Gross findings**	**Microscopic findings**
Less than 4 hours interval	Red color, occasional purple to purplish black color	More than 50 % RBC lysis in most of the cases. Few macrophages. * Few hemosiderin laden macrophages. * Moderate to increased fibrin. Absent collagen. Meningeal reaction only in a few cases.
4 to 24 hours	Purple to purplish black color	More than 50 % lysis in more than 80 % of the cases. * Moderate macrophage. Mild to moderate hemosiderin laden macrophages. Moderate to increased fibrin. Absent to few collagens. * Meningeal reaction in only 6 % of the cases.

Continue.....

1 to 14 days	Purplish black color Brownish color in the later phase	More than 50 % lysis in 55 % of the cases. Moderate macrophages. Mild to moderate hemosiderin laden macrophages. Moderate to increased fibrin. Few collagens. Meningeal reaction in 20 % of the cases.
14 to 30 days	Exclusively brown to yellowish color Occasionally red color in case of re-bleeding	More than 50 % lysis in 72 % of the cases. Increased macrophages. Moderate to increased hemosiderin laden macrophages. Absent to few fibrins. * Moderate to increased collagen. * Meningeal reaction in 30 % of the cases.

Note: Polymorphonuclear cellsdid not correlate with any post-injury intervals.

"*" – Statistically significant

Conclusions

Histological examination of wounds can be very useful in medicolegal investigation by providing the age of the wound and correlating it with the chain of events. Histology also helps investigation by telling the antemortem vs postmortem nature of the wounds. This information will be helpful to answer questions such as how old is the wound, is the wound inflicted before or after death? Even though the histological method of dating wound will not yield an exact answer to the above questions, but it can sure narrow down the possibilities. Both gross and histological parameters were analyzed in this study, and not all the parameters' reactions were consistent during the reparative process. Influencing factors such as age, size, health and individual variations becomes determining factors. Therefore, in future studies it is advised to design a study with proper control of the confounding factors.

Funding Sources

Conducted ot subsidized COST from the university and portly self-funded.

Conflict of interest: None

References

1. García-Ballestas E, Durango-Espinosa Y, Mendoza-Flórez R, Moscote-Salazar L, Keni R, Deora H, et al. The puzzle of spontaneous versus traumatic subarachnoid hemorrhage. Apollo Medicine. 2019;16(3):141.
2. Claassen J, Park S. Spontaneous subarachnoid haemorrhage. Vol. 400, The Lancet. Elsevier B.V.; 2022. p. 846–62.
3. Modi N, Agrawal M, Sinha V. Post-traumatic subarachnoid hemorrhage: A review. Neurol India. 2016;64(7):8.
4. Lantigua H, Ortega-Gutierrez S, Schmidt JM, Lee K, Badjatia N, Agarwal S, et al. Subarachnoid hemorrhage: Who dies, and why? Crit Care. 2015;19(1):1–10.
5. Stenholm E, Project D. Analysis of Traumatic Head Injury in Kathmandu, Nepal.
6. Jha S, Yadav BN, Agrawal A, Thakur D, Karna A, Subedi N, et al. The pattern of fatal head injury in a teaching hospital in Eastern Nepal. Journal of Clinical and Diagnostic Research. 2011;5(3):592–6.
7. Grellner W, Madea B. Demands on scientific studies : Vitality of wounds and wound age estimation. 2007;165:150–4.
8. Alexis R, Jagdish S, Sukumar S, Pandit V, Palnivel C, Antony M. Clinical profile and autopsy findings in fatal head injuries. J Emerg Trauma Shock [Internet]. 2018 Jul 1 [cited 2020 Nov 11];11(3):205–10. Available from: /pmc/articles/PMC6182969/?report=abstract
9. Zimmerman A, Fox S, Griffin R, Nelp T, Fonseca Thomaz EBA, Mvungi M, et al. An analysis of emergency care delays experienced by traumatic brain injury patients presenting to a regional referral hospital in a low-income country. PLoS One. 2020 Oct 1;15(10 October 2020).
10. Dell'aquila M, Maiese A, De Matteis A, Viola RV, Arcangeli M, La Russa R, et al. Traumatic brain injury: Estimate of the age of the injury based on neuroinflammation, endothelial activation markers and adhesion molecules. Vol. 36, Histology and Histopathology. Histology and Histopathology; 2021. p. 795–806.
11. Esterov D, Bellamkonda E, Mandrekar J, Ransom JE, Brown AW. Cause of Death after Traumatic Brain Injury: A Population-Based Health Record

Review Analysis Referenced for Nonhead Trauma. Neuroepidemiology. 2021 Jun 1;55(3):180–7.

12. Tak PP, Thurkow EW, Daha MR, Kluin PM, Smeets TJM, Meinders AE, et al. Expression of adhesion molecules in early rheumatoid synovial tissue. Vol. 77, Clinical Immunology and Immunopathology. 1995. p. 236–42.
13. Cummings PM, Trelka DP, Springer KM. Atlas of Forensic Histopathology [Internet]. Cambridge: Cambridge University Press; 2011 [cited 2019 Jan 21]. Available from: http://ebooks.cambridge.org/ref/id/CBO9780511920905
14. Ross JL, Sandberg GD, Powell SZ. Forensic Evaluation of Subarachnoid Hemorrhage. Acad Forensic Pathol. 2012;2(1):30–5.
15. Itabashi HH, Andrews J, Tomiyasu U, Erlich S, Sathyavagiswaran L. Forensic Neuropathology. Forensic Neuropathology. Elsevier Inc.; 2007.
16. Vanezis P. Interpreting bruises at necropsy. J Clin Pathol. 2001;54(5):348–55.
17. Cummings PM, Trelka DP, Springer KM. Post-injury intervals. In: Atlas of Forensic Histopathology [Internet]. Cambridge: Cambridge University Press; [cited 2019 Jan 21]. p. 1–27. Available from: https://www.cambridge.org/core/product/identifier/CBO9780511920905A010/type/book_part
18. Cao S, Zheng M, Hua Y, Chen G, Keep RF, Xi G. Hematoma Changes during Clot Resolution after Experimental Intracerebral Hemorrhage. Stroke. 2016;47(6):1626–31.
19. Alpers BJ FFM. The reparative processes in subarachnoid hemorrhage. J Neuropathol Exp Neurol. 1945;(4):262–8.
20. Naidech AM, Janjua N, Kreiter KT, Ostapkovich ND, Fitzsimmons BF, Parra A, et al. Predictors and impact of aneurysm rebleeding after subarachnoid hemorrhage. Arch Neurol. 2005;62(3):410–6.
21. Kim J, Lee SJ. Traumatic subarachnoid hemorrhage resulting from posterior communicating artery rupture. Int Med Case Rep J. 2020;13:237–41.
22. Puhaindran ME. Principles of wound healing. In: Diabetic Foot Problems [Internet]. World Scientific Publishing Co.; 2008 [cited 2020 Nov 13]. p. 395–402. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534261/
23. Lee JY, Keep RF, He Y, Sagher O, Hua Y, Xi G. Hemoglobin and iron handling in brain after subarachnoid hemorrhage and the effect of deferoxamine on early brain injury. Journal of Cerebral Blood Flow and Metabolism [Internet]. 2010;30(11):1793–803. Available from: http://dx.doi.org/10.1038/jcbfm.2010.137
24. Rustem I. Litvinov, Weisel JW. What Is the Biological and Clinical Relevance of Fibrin? Rustem. PhysiolBehav [Internet]. 2016;176(10):139–48. Available from: file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentarnaqualificação/The impact of birth weight on cardiovascular disease risk in the.pdf
25. Itabashi HH, Andrews JM, Tomiyasu U, Erlich SS, Sathyavagiswaran L. Dating/aging of common lesionsin neuropathology. In: Forensic Neuropathology [Internet]. Elsevier; 2007 [cited 2019 Jan 21]. p. 49–122. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780120585274500055
26. RE P, FC B, DK A, TR B, D D, J H, et al. The cellular, biochemical, and mechanical phases of wound healing. Schwartz's Principles of Surgery, Ninth Edition. McGraw-Hill Professional. ISBN 978-0-07-154769-7. 2009. Fig. 9-1.
27. Suzuki S, Ishii M, Ottomo M, Iwabuchi T. Changes in the subarachnoid space after experimental subarachnoid haemorrhage in the dog: Scanning electron microscopic observation. Acta Neurochir (Wien). 1977;39(1–2):1–14.
28. BAGLEY C. Blood in the Cerebrospinal Fluid: Resultant Functional and Organic Alterations in the Central Nervous System a. Experimental Data. Blood in the Cerebrospinal Fluid: Resultant Functional and Organic Alterations in the Central Nervous System a Experimental Data. 1928;17(1):18–38.
29. Hammes EM. Interest in subarachnoid hemorrhage. 2015;11.
30. HAMMES Jr EM, in Neurology F, Foundation ROCHESTER M. REACTION OF THE MENINGES TO BLOOD [Internet]. Available from: http://archneurpsyc.jamanetwork.com/