



## **FORENSIC MEDICAL ASPECTS OF DETERMINING THE DURATION OF INJURY DEVELOPMENT**

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### **Abstract**

As in the 19th century, forensic medical professionals are now primarily focused on the macroscopic picture of hemorrhage with subsequent microscopic examination. When studying micropreparations, the cellular composition of the hemorrhage is assessed based on known data, personal knowledge and experience, circumstances of the incident (if indicated in the referral for forensic histological examination), and the victim's age.

**Keywords:** forensic medical histology; immunohistochemical research methods; duration of injury; hemorrhage; damage.

For centuries, forensic doctors have been looking for ways to solve the problem of determining the lifetime of traumatic hemorrhage. The most pressing and complex issue remains the timing of the formation of injuries from the moment of their receipt until the death of the victim. Despite the fact that researchers began studying this problem at the initial stages of the development of forensic medical practice, it has always remained relevant and was repeatedly emphasized at all-Russian scientific and practical conferences (1973-2022), congresses, and plenums of the Society of Forensic Medicalists.

This review summarizes existing literature data on the problem of studying the lifetime and duration of traumatic hemorrhages and the use of various research methods in forensic medical practice. I.P. Shishkin, analyzing the works of other authors and his own research, came to the conclusion that hemorrhages are accompanied by certain tissue reactions that increase over time; tissues have "experience." The "survival" of tissues and the insufficiently pronounced manifestations of the initial period of reparative processes in the damaged area are evidenced by the results of experimental studies, which show the similarity of changes in tissues in terms of morphological indicators in life- and death-related injuries. Both in the studied sample and in the specific case, after determining the expression of pro-inflammatory cytokines, which increased to 72 hours of the postmortem period in the damaged tissues, and the simultaneous increase in IL-6, tumor necrosis factor alfa (TNF- $\alpha$ ), it can be said that





the damage was less than 30 minutes. Also, the main mediators of inflammation (cytokines, growth factor), collagen, when studying lesions, can be reliable indicators and markers for determining the viability or age of the wound. Some authors, having analyzed the data of previous studies, paid attention to the peculiarities of the course, the traumatic process depending on the influence of the external environment, and came to the conclusion that in damaged tissues, a sequence of morphological processes is observed, changing under many conditions, which can affect the degree of their severity and cause significant difficulties in diagnosis.

When studying injuries on corpses found in water bodies, there is no clear understanding of the methods for examining injuries exposed to prolonged water exposure. However, the experience of using a monoclonal anti-human antibody to glycophorin A (anti-GPA) to assess the presence of erythrocytes and their breakdown products has shown that extravasated erythrocyte residues can be detected in hemorrhages for at least 15 days outdoors and up to 1 week in water. The importance and reliability of studying anti-GPA and immunohistochemical staining to assess the viability of injuries in cadavers at various stages of decomposition have been confirmed.

The study of aquaporins 1 and 3 in various types of mechanical and thermal injuries did not give either positive or negative results, which indicates the need for further research and establishing sufficient sensitivity and specificity of this method.

Ponseau/Victoria blue B staining for collagen fibers and muscles was applied to a severely decomposed corpse (1.5 years after death). The method proved to be simple and suitable for diagnosing life trauma as an alternative.

Biophysical parameters (electrical resistance and electrical capacitance) allow for a high degree of accuracy in differentiating hemorrhage in putridly altered tissues. In putridly altered skin, the markers of triptase, glycophorin, IL-15, CD 15, CD 45, and MMP9 showed a high degree of expression within 15 days.

When examining frozen corpses, known algorithms should be followed, relying on already studied traits. It should also be remembered that during the rapid freezing of the corpse (during the first hours after death), the resulting hemorrhages in the soft tissues are microscopically almost indistinguishable from those during life, and to this day, this problem remains unresolved.

L.I. Gromov, N.A. Mityaeva (1965-1966) were among the first domestic histologists who began to observe cellular, vascular reactions in response to mechanical damage, including acute blood loss, under conventional light microscopy, and focused attention on the speed of death after injury, as well as on the general condition of the





body at the time of injury and after it. Subsequently, E.A. Kireeva, in her dissertation research, based on knowledge of blood filling in vessels, capillaries, cellular composition, reactive and proliferative changes in hemorrhages in soft tissues from the fracture area of the ribs, derived qualitative morphological indicators of the age of formation of fractures of the ribs.

The likelihood of postmortem chemotaxis of leukocytes was studied based on known data that neutrophils, detected 6-24 hours after damage, and a little later, 24-48 hours later, - monocytes and lymphocytes - are the first to strive towards the inflammation focus. In the conducted scientific research, such a process was not detected.

When studying hemorrhages in soft tissues, to assess morphological changes in them, the histologist relies not only on existing research results obtained by various authors but also on their own experience.

According to the conclusions of M.N. Chepurnenko and D.A. Chepurnenko, "reactive changes in cells and tissues in the wound process are based on the patterns of embryonic and postembryonic histogenesis; activation and proliferation of low-differentiated cells, their differentiation and interaction with subsequent adaptive restructuring of the regenerate."

As can be seen from Table. 1, there is no single approach or precise, unified time frame that accurately indicates the time elapsed from the moment of injury to the moment of the victim's death. The data obtained by authors over a long period of observation in this area do not always coincide, and sometimes differ significantly when morphologically assessing changes and cellular composition in lesions of both different and the same localization. D.V. Bogomolov and co-authors based on the RSME, V.E. Yankovsky and co-authors based on the KGUZ "Altai Regional Bureau of Forensic Medical Examination," S.V. Liskova based on the OBUZ "Bureau of Forensic Medical Examination" of the Kursk region compiled methodological recommendations based on the provisions of current regulatory documents and literature data, information letters. Domestic scientists have written original scientific articles analyzing and summarizing literary sources on the issue of the duration and lifetime of hemorrhages, showing the state, prospects, and problems in this matter, and noting the need to adhere to the principles of a systematic approach in the study of injuries.

In addition to standard histological staining, histochemical methods are used, which show the state of fibrin as an important factor in comprehensively assessing the duration of hemorrhages in soft tissues in rib fractures, mechanical injuries of soft tissues of various localizations, as well as for histological assessment of fibrin maturity





in blood clots, thrombi, and thrombotic emboli; these methods are based on age-related differential staining of fibrin (fibrin threads stain different colors over time).

The histomolecular method of mRNA research makes a significant contribution to forensic examination. Using a histomolecular approach based on mRNA expression showed that the differential expression of mRNA was demonstrated in premature and postmortem wounds. The expression of mRNA-205 and 21, detected using polymerase chain reaction, was significantly increased and increased within 24 hours after wound application, then sharply decreased. In damaged skin, an increase in CXCL1 mRNA levels was detected - up to 48 hours from the moment of injury and an increase in CXCR2 mRNA levels - after 48 hours. A study of the expression of chitinase-3-like protein 1 (CHI3L1) showed that wounds from the fourth to sixth day after injury can be clearly distinguished from other wounds based on a cutting value of 2.75, sensitivity of 92.31%, and specificity of 85.14%.

The analytical method (Western-Blotting) showed a marked decrease in LC3-II and a reciprocal increase in p62 compared to intact skin tissues, indicating a decrease in the level of autophagy in damaged tissues.

Also, one of the promising laboratory research methods is immunohistochemical immunohistochemical.

These research methods, according to A.M. Khromova and Yu.P. Kalinin, possess high informativeness, and according to V.P. Novoselova et al., allow for improving the quality of forensic medical examinations and objectifying expert conclusions. D.V. Bogomolov and co-authors, based on the analysis of literary sources, argue that the implementation of immunohistochemical methods in scientific and practical forensic medical examination will allow us to answer many questions that remain unanswered today. For a number of objective reasons, immunohistochemical methods have not yet been widely used in practical forensic medicine. It should also be noted that immunohistochemical methods "are additional to traditional histological research and do not replace it. Nevertheless, these methods help to identify pathological processes occurring at a deeper than tissue level and thus increase the accuracy and reliability of forensic histological diagnostics."

A.A. Khalikov and co-authors have written a number of scientific articles reflecting the provisions of his dissertation research, resulting in the development of a multifactorial regression formula that allows determining the duration of injury based on the complex of biophysical characteristics of tissue damage in the period of 10-60 hours, taking into account the influence of the age factor and alcohol concentration in the victim's blood at the time of death.





When studying the duration of hemorrhages using the impedance measurement method, an original method was developed, which uses the values of the skin's electrical resistance measured at different alternating current frequencies using an original measuring device. It has been established that there is a more intensive increase in the thermal conductivity of the skin in the area of hemorrhage in elderly people. This is determined by biophysical methods, therefore, it is clear that it is advisable to consider the age-related characteristics of the body when determining the age of hemorrhages.

When objectifying the assessment of hemorrhages by determining their thermal conductivity coefficient, the thermophysical properties of human skin were assessed. The obtained data required further research and refinement. The study of the chemiluminescence method to determine the lifetime and duration of skeletal muscle mechanical injury showed that the parameters of induced chemiluminescence of damaged muscle homogenates change regularly as the post-traumatic period increases.

Studying the duration of bodily injuries by contactless thermometric method in living individuals demonstrates its high accuracy and prospects for use. Sonography reliably identifies hematoma in soft tissues regardless of its duration; The "age" of the hemorrhage is determined up to 1 month and depends on the location; the use of ultrasound histography allows, with high probability, to determine the timing of hemorrhage formation in vivo. The results of ultrasonography examination of victims with post-traumatic hemorrhages lasting up to 1 month and more than 1 month, using such parameters as sensitivity, specificity, accuracy, demonstrate a high degree of reliability of this method. Studying the features of thermodynamics in the hemorrhage area and intact areas of symmetrical localization in living individuals using original objective methodology and original instrument gives grounds to assume that this research method is accessible and objective, but requires consideration of the individual characteristics of the examined person.

To correctly assess the changes occurring in the body after injury, it is necessary to know and understand the mechanism of the response reaction and its dynamics. The rate, dynamics, sequence, and nature of reactive changes are determined not only by the time of the post-traumatic period but also by the severity of injuries, the location of the injury, as well as concomitant pathology, the presence of chronic diseases, sex and age, which alter the immune system's response, and intoxications of various origins. It is also important to consider the scope of surgical interventions, drug therapy, etc.





At the present stage of forensic medicine development, there are no so-called "standards for conducting microscopic examination," the absence of a standardized protocol complicates the interpretation of the obtained data.

**Conclusion** To date, certain progress has been made in studying the lifetime and duration of damage formation, new research methods have been developed and implemented, both laboratory and instrumental, which have significantly influenced the understanding of reactive changes occurring in tissues. The application of the latest methods will make a great contribution to the development of forensic medicine. Nevertheless, some of these methods are complex and labor-intensive, requiring appropriate material and technical equipment, which is not available in all forensic histology departments and laboratories. Thus, the problem of lifetime and duration of damage formation cannot be considered solved and requires further study.

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