

# Stab Wound Age Estimation by Changes of Neutrophilic Leukocytes after Injection of Different Substances

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## ABSTRACT

In the present study are described the changes of neutrophilic leukocytes' response on injection of different substances. There are evaluated possibilities to use the changes of these leukocytes as a criterion for injected stab wound age estimation. The experimental study was carried out on formalin-fixed paraffin-embedded material from rat skin. Results show that changes of neutrophilic leukocytes and their response depend on time since injury and injected substance and use of this particular cell response as a criterion for stab wound age estimation is recommended.

**KEYWORDS:** *stab wound age estimation, wound healing, neutrophilic leukocytes*

Stab wound age estimation is the question of great importance in modern Forensic Medicine. Time since injury is possible to detect by the reparative processes of the wound. Unlike other mechanical injuries, the stab wound is characterized by less local inflammatory response and more quick dynamics of reparation. The changes vary according to the size of wound, the tissue, age and health of victim and so on [6]. Unfortunately, biological variability introduces a wide margin of uncertainty, so that a range of probabilities can be offered and definition of time interval is almost always difficult [7]. The subject is of particular interest when the question is associated with different injected substances. There are many publications and experimental works on this subject as it is a favorite topic for research [2,5], but offered schedules for stab wound age estimation are not yet sufficient and particular criteria still should be elaborated and selected.

The aim of the present study was to evaluate the changes of neutrophilic leukocytes and their response on mechanical injury as a possible criterion for injected stab wound age estimation after injection of different substances.

## MATERIALS AND METHODS

**Animals:** Wistar white rats of 11-12 months were obtained from the vivarium of Tbilisi State Medical University and kept under laboratory conditions.

**Injection of Substances:** The substances for injection have been chosen as a positive control (Turpentine), negative control (NaCl) and routine penicillin. Injection of

substances had been performed on the back side of thoracic region of the animal, on midline, under the skin.

**Formalin fixation:** The skin specimens of 10x0,5 mm size have been taken from the back of living animals after injection, respectively, after one hour, three hours, 6 hours, 24 hours, three days, 5 days and 7 days, then put into the buffer: formalin 40% - 100ml; NaH<sub>2</sub>PO<sub>4</sub> - 4g; Na<sub>2</sub>HPO<sub>4</sub> - 6,5g; AD - up 1l; and incubated for 24 hours.

**Paraffin embedding:** Formalin fixed specimens were put for dehydration into the alcohol 70° for 24 hours, then in alcohol 90° - 24h, trice in alcohol 96° for 24 hours each, then on room temperature in solution of alcohol-aether 1:1 for 15 min and twice in Chloroform for 3 min each, then infiltrated with I paraffin on 37°C - 30 min, II and III paraffins on 56°C for 45 min each and then blocked in paraffin on room temperature. The blocks were stored at +4°C.

**Histochemistry:** Paraffin blocks were sectioned on a rotation microtome. Sections were put into the thermostat on 56°C for 20 min and then dewaxed. The slides were stained with routine Hematoxilin-Eosin for histological study [4].

## RESULTS AND DISCUSSION

Results of study revealed different quantitative changes of neutrophilic leukocytes depending on time since injection and injected substance, as shown in *Tab.1* and *Tab.2*.

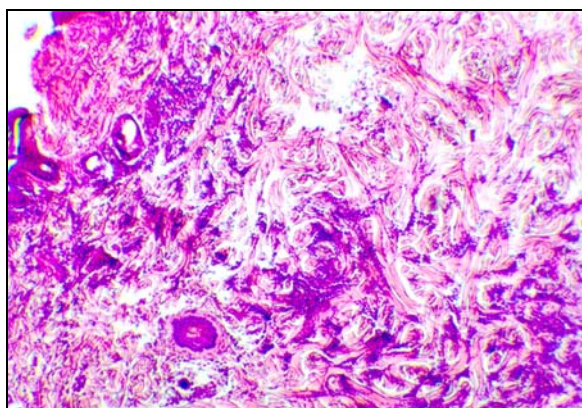
Substance \ Time	Before injection	1 h	3 h	6 h	24 h	3 days	5 days	7 days
Turpentine	1,76±0,21	1,76±0,28	1,88±0,29	94,2±1,96	90,84±3,66	2,36±0,19	1,84±0,16	1,16±0,15
Penicillin	1,76±0,21	1,6±0,14	21,04±0,63	42,92±0,68	20,24±1,02	7,92±0,29	3,32±0,22	1,84±0,15
NaCl	1,76±0,21	1,88±0,17	8,72±0,32	122,8±3,92	90,48±2,69	5,52±0,28	3,92±0,21	2,32±0,21

**Tab.1** Number of neutrophilic leukocytes in defined moments of time after injection of different substances.

Substance \ Time	Before injection	1 h	3 h	6 h	24 h	3 days	5 days	7 days
Turpentine	6	5	5	66	65	4	1	3
Penicillin	6	4	38	48	19	11	6	3
NaCl	6	6	23	72	62	9	7	4

**Tab.2** Share of neutrophilic leukocytes (%) among differentiated cells in defined moments of time after injection of different substances.

To study the structural and metabolic changes of healing process for injected stab wound age estimation has been chosen the model of wounding by injection needle, the "effect" of which by our opinion should be very different from other sharp injuries, that would give us the opportunity to reveal the structural-metabolic characteristics that are specific for injected stab wound and different from others. For complete evaluation of injection stab wounding was considered as very important to foresee both the volume factor (volume increase or swelling due to injection of substance) and chemical structure (feature) of injected substance. So for injection were chosen three different chemical substances: Turpentine - as foreign chemical substance for organism and positive control for acute inflammation, Benzylpenicillinum-natrium - as chemically clean medicine and control sample, and NaCl - as non-pathogenic substance and negative control.



**Fig.1** Neutrophilic infiltration in stab wound area after 6 hours. Rat skin.

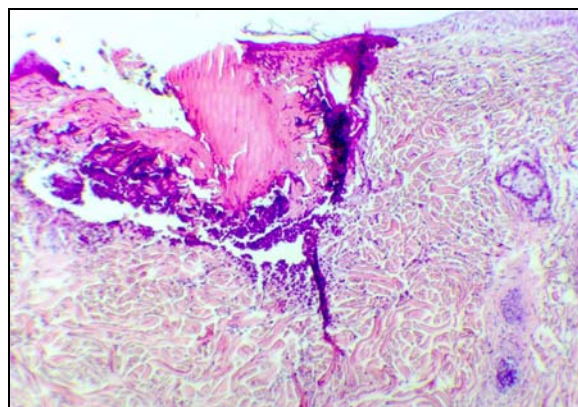
Hematoxilin-Eosin. Microphotography. X75,25.

The results of study revealed that in intact rat skin covering the middle third part of the back surface in spinal column region on square cm. number of differentiated cells is  $31 \pm 0,33$ . In intact skin on square unit the number of neutrophilic leukocytes is 6% from entire quantity of cells.

The results of study show that after injection wounding without substance injection and injection of NaCl the special share of cells in all periods of time are equal, that's why the results of these two groups of study are evaluated together: after three hours from NaCl injection already could be detected increased number of

differentiated neutrophilic leukocytes (23%), then neutrophilic demarcation line enlarges and reaches maximum after 6 hours from injection (72%) and still high indexes of neutrophils (62%) are revealed after 24 hours too. After three days from injection neutrophilic indexes are decreased (9%) and these cells progressively are replaced by macrophages and fibroblasts until in the last time-periods they form the main cell population of injury area.

After penicillin injection very soon just after three hours from injection neutrophils increase in number 6,4 times (38%). It seems that inflammation developing in wounded area rapidly undergoes penicillin protection: after NaCl injection the number of neutrophils is 6,7 times less than after penicillin injection. The data show that after three hours from injury the effect of penicillin is so prominent, that demand to neutrophils is 1,5 times less, and after 24 hours - three times less, than in case without penicillin.



**Fig.2** Neutrophilic infiltration in stab wound area after 24 hours. Rat skin.

Hematoxilin-Eosin. Microphotography. X75,25.

After turpentine injection the number of neutrophilic leukocytes is the same within one and three hours after injection - 5% of differentiated cells. Increase of neutrophilic number begins only after 6 hours from turpentine injection and it is 66% or two-thirds of all cells, almost same index is after 24 hours, but after 3, 5 and 7 days from injection the number of neutrophilic leukocytes is so decreased that it constitutes only 4%, 1% and 3% of all the differentiated cells.

Thus, changes of neutrophilic leukocytes described in present study have considerable value in injected stab wound age estimation. The above-mentioned changes

firmly depend on injected substance. Use of this particular cell response as a criterion for stab wound age estimation is recommended.

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## Установление давности колотой раны по изменениям нейтрофильных лейкоцитов после инъекции различных веществ

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### Р Е З Ю М Е

Целью исследования было определение возможности применения изменений нейтрофильных лейкоцитов в качестве критерия установления давности колотой раны. Были изучены изменения нейтрофильных лейкоцитов в различные интервалы времени соответственно давности повреждения после инъекции разных веществ. В качестве инъекционных веществ использовались скипидар - как позитивный контроль, физиологический раствор - как негативный контроль, и пенициллин. Исследования показали, что изменения нейтрофильных лейкоцитов отчетливо зависят от давности повреждения и характера инъекционного вещества, и могут быть применены для установления давности колотой раны.

**Ключевые слова:** *установление давности колотой раны, заживление раны, нейтрофильные лейкоциты*