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Original Research Article

Role of Inflammatory Markers in Estimation of Wound Age: A Clinicopathological and Immunohistochemical Study

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Abstract:

Background: Accurate estimation of wound age is one of the most challenging aspects of forensic pathology. Conventional histopathological methods provide only approximate estimates, particularly during the early post-traumatic period. Inflammatory cytokines such as Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-1β (IL-1β) have emerged as promising biomarkers for wound age estimation owing to their predictable temporal expression during wound healing.

Aim: To evaluate the utility of inflammatory markers IL-6, TNF-α, and IL-1β in determining wound age through histopathological and immunohistochemical examination.

Materials and Methods: A prospective clinicopathological study was conducted on 100 skin wound specimens obtained during medicolegal autopsies. Cases with known survival intervals were categorized into five groups according to wound age. Routine hematoxylin-eosin staining and immunohistochemical analysis for IL-6, TNF-α, and IL-1β were performed. Expression intensity was assessed semi-quantitatively and correlated with known wound age.

Results: A progressive increase in expression of IL-6 and TNF-α was observed with increasing wound age. IL-6 demonstrated the highest diagnostic accuracy with sensitivity of 88.4% and specificity of 81.6%. TNF-α showed sensitivity of 84.2% and specificity of 76.3%. IL-1β exhibited variable expression and lower predictive value. Significant positive correlations were found between cytokine expression and wound age ($p<0.001$).

Conclusion: IL-6 and TNF-α are reliable inflammatory biomarkers for wound age estimation, especially during the early post-traumatic interval. Combining immunohistochemical markers with routine histopathology enhances the accuracy of forensic wound dating.

Keywords: Wound age estimation, IL-6, TNF-α, IL-1β, Immunohistochemistry, Forensic pathology.

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Introduction

Determination of wound age constitutes an essential component of forensic investigations and frequently influences the reconstruction of crime events. Establishing the interval between injury infliction and death assists forensic experts in evaluating wound vitality, survival period, and medicolegal circumstances surrounding death [1]. Traditional histological methods rely on cellular responses such as hemorrhage, leukocyte infiltration, fibroblast proliferation, and collagen deposition. However, these findings often overlap and provide only approximate estimates of wound age [2-4]. The wound-healing process comprises four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. During the inflammatory phase, several cytokines are released from activated platelets, neutrophils, macrophages, endothelial cells, and keratinocytes. These mediators regulate recruitment of inflammatory cells and initiate tissue repair mechanisms. Among these cytokines, IL-6, TNF-α, and IL-1β play critical roles in the early response to tissue injury [5-7].

Experimental and human studies have demonstrated that cytokine expression follows a time-dependent pattern after injury. TNF-α is one of the earliest cytokines released following tissue damage, followed by IL-1β and IL-6. Their expression levels increase progressively during the initial hours after injury and may therefore serve as biological clocks for wound age estimation. Immunohistochemistry (IHC) offers

the advantage of localizing cytokine expression within specific tissue compartments and provides objective evidence regarding wound vitality. Recent advances in forensic pathology have emphasized the use of molecular and immunohistochemical markers to supplement conventional histology. The present study was undertaken to evaluate the diagnostic utility of IL-6, TNF-α, and IL-1β in estimating wound age and to correlate immunohistochemical findings with histopathological changes observed in skin wounds [8-10].

Materials and Methods

Study Design: Prospective observational clinicopathological study.

Study Setting: Department of Forensic Medicine and Toxicology in collaboration with the Department of Pathology at a tertiary care teaching hospital.

Study Duration: January 2024 to December 2025.

Sample Size: A total of 100 medicolegal autopsy cases with known survival intervals following injury were included.

Inclusion Criteria

- Antemortem skin wounds with documented injury time.
- Survival period less than 72 hours.
- Age above 18 years.

Exclusion Criteria

- Burn injuries.
- Septic wounds.
- Chronic ulcers.
- Decomposed bodies.
- Immunocompromised individuals.

Grouping of Cases

Group	Wound Age
I	<30 minutes
II	30 min–2 hours
III	2–6 hours
IV	6–24 hours
V	24–72 hours

Twenty cases were included in each group.

Tissue Sampling: Full-thickness skin specimens were collected from wound margins and corresponding uninjured contralateral skin.

Histopathological Examination: Sections were stained with Hematoxylin and Eosin and evaluated for: Hemorrhage**,** Neutrophilic infiltration**,** Macrophage infiltration**,** Fibroblast proliferation**,** Angiogenesis

Immunohistochemistry: Primary antibodies: IL-6, TNF-α, IL-1β

Scoring System

Score	Percentage of Positive Cells
0	<5%
1	5–25%
2	26–50%
3	51–75%
4	>75%

Statistical Analysis: Data were analyzed using SPSS version 26.

- Chi-square test
- One-way ANOVA
- Pearson correlation
- ROC curve analysis

Significance level: $p<0.05$.

Results

Table 1: Demographic Characteristics of Study Subjects

Variable	Number (%)
Male	76 (76%)
Female	24 (24%)
Mean Age	38.6 ± 14.2 years

Table 1 shows the demographic profile of the study population. Among the 100 autopsy cases included in the study, males constituted the majority with 76% of cases, while females accounted for 24%. The

age of the subjects ranged from 18 to 72 years, with a mean age of 38.6 ± 14.2 years. The predominance of males may be attributed to their higher exposure to occupational hazards, road traffic accidents, interpersonal violence, and other injury-producing situations. No statistically significant association was observed between age or sex and the expression of inflammatory markers.

Table 2: Histopathological Findings in Different Wound-Age Groups

Histological Feature	<30 min	30 min–2 h	2–6 h	6–24 h	24–72 h
Hemorrhage	100%	100%	95%	90%	80%
Neutrophils	10%	40%	75%	100%	100%
Macrophages	0%	5%	20%	65%	100%
Fibroblasts	0%	0%	10%	45%	90%
Angiogenesis	0%	0%	5%	30%	80%

Table 2 demonstrates the chronological histopathological changes observed in wound tissues. Hemorrhage was consistently present in all early wounds and gradually decreased with increasing wound age. Neutrophilic infiltration became evident within 30 minutes to 2 hours and reached maximum intensity after 6 hours. Macrophage infiltration was absent in fresh wounds but increased significantly after 6 hours, reflecting transition to the reparative phase. Fibroblast proliferation and angiogenesis were predominantly observed in wounds older than 24 hours, indicating active tissue repair and remodeling. These findings are consistent with the established chronology of wound healing.

Table 3: Mean Immunohistochemical Scores

Group	IL-6	TNF-α	IL-1β
I	0.8±0.4	1.1±0.5	0.6±0.3
II	1.8±0.6	2.0±0.7	1.2±0.5
III	2.7±0.8	2.8±0.6	1.7±0.7
IV	3.4±0.5	3.2±0.6	2.0±0.6
V	3.8±0.4	3.5±0.5	2.2±0.8

Table depicts the expression pattern of IL-6 across different wound-age groups. A progressive increase in IL-6 immunoreactivity was observed with increasing wound age. Fresh wounds demonstrated weak staining, whereas wounds older than 24 hours showed intense cytoplasmic staining in inflammatory cells and endothelial cells. The gradual rise in IL-6 expression suggests its important role in initiation and propagation of the inflammatory response and supports its utility as a biomarker for wound-age estimation. TNF-α showed detectable expression even in very early wounds, reflecting its role as one of the earliest inflammatory mediators released after tissue injury. Expression increased progressively up to 72 hours. The results indicate that TNF-α may be particularly useful in distinguishing fresh wounds from older injuries and can complement IL-6 in forensic wound dating. Although a gradual increase in expression was noted with increasing wound age, the rise was less pronounced compared with IL-6 and TNF-α. Considerable overlap between groups was observed, reducing its discriminative power. These findings suggest that IL-1β may be more useful when interpreted alongside other inflammatory markers rather than as an independent predictor.

Table 4: ANOVA Analysis

Marker	F-value	p-value
IL-6	62.81	<0.001
TNF-α	54.39	<0.001
IL-1β	18.47	<0.001

Significant differences were observed among wound-age groups. Table 4 summarizes the results of one-way ANOVA used to compare cytokine expression among wound-age groups. Highly significant differences were observed for all three markers ($p<0.001$). IL-6 exhibited the highest F-value, indicating the greatest variation between groups and strongest association with wound age. TNF-α also demonstrated excellent discriminatory ability, whereas IL-1β showed comparatively weaker differentiation.

Table 5: Correlation with Wound Age

Marker	r-value	p-value
IL-6	0.84	<0.001
TNF-α	0.79	<0.001
IL-1β	0.53	0.002

Table 5 shows Pearson correlation analysis between cytokine expression scores and known wound age. IL-6 demonstrated the strongest positive correlation (r=0.84), followed by TNF-α (r=0.79). Both markers exhibited highly significant relationships with wound age. IL-1β showed only moderate correlation. These findings support the use of IL-6 and TNF-α as reliable indicators for estimating the age of wounds.

Discussion

Wound age estimation remains one of the most important and difficult challenges in forensic pathology. Histological evaluation alone is often inadequate during the early post-traumatic interval because cellular infiltration requires time to become evident. Consequently, inflammatory cytokines have gained importance as potential biomarkers for determining wound vitality and age [11-13].

The present study demonstrated a significant increase in IL-6 expression with increasing wound age. IL-6 showed the strongest correlation with survival interval and achieved the highest diagnostic accuracy among all markers studied. Similar observations were reported by Birincioglu et al., who identified IL-6 as a useful marker for wounds less than 30 minutes old and for later inflammatory stages [14].

TNF-α expression was detectable within the earliest post-traumatic period and increased progressively throughout the first 24 hours. Kondo and colleagues demonstrated that TNF-α peaks within hours following injury and plays a central role in initiating inflammation [15, 16].

The present findings also agree with studies reporting significant upregulation of TNF-α and IL-6 in wound margins compared with uninjured skin. These cytokines are released from activated macrophages, neutrophils, keratinocytes, and endothelial cells and contribute to leukocyte recruitment, angiogenesis, and tissue repair [17].

IL-1β showed less consistent expression than IL-6 and TNF-α. Although levels increased with wound age, the correlation was weaker and diagnostic accuracy was lower. Previous investigations have similarly questioned the utility of IL-1β as a standalone marker because of variable expression patterns and overlap between age groups [18].

Histopathological findings observed in this study were consistent with the established chronology of wound healing. Early wounds demonstrated hemorrhage and vascular congestion, whereas older wounds exhibited progressive neutrophil infiltration, macrophage accumulation, fibroblast proliferation, and angiogenesis. These findings support previous forensic studies describing the temporal sequence of inflammatory and reparative responses following injury [19,20].

ROC analysis revealed that IL-6 possessed excellent discriminative ability (AUC 0.91), followed by TNF-α (AUC 0.87). These findings suggest that IL-6 may be the most reliable biomarker for forensic wound dating. Similar conclusions have been reported in immunohistochemical studies where IL-6 exhibited superior sensitivity and specificity compared with TNF-α [21-23].

The principal strength of this study lies in the combined clinicopathological and immunohistochemical approach. Evaluation of multiple markers rather than a single cytokine improves reliability and minimizes false-positive interpretations. Modern forensic practice increasingly favors a multimarker strategy for wound age estimation [24].

Limitations include the relatively small sample size and potential influence of individual biological factors such as comorbidities, medications, and postmortem interval. Future studies incorporating molecular techniques, multiplex cytokine analysis, and artificial intelligence-assisted image quantification may further improve accuracy [25].

Conclusion

Inflammatory cytokines demonstrate significant temporal changes during wound healing and provide valuable information regarding wound age estimation. IL-6 emerged as the most reliable marker, followed by TNF-α. IL-1β showed comparatively lower diagnostic utility. The combination of routine histopathology and immunohistochemical assessment significantly improves the precision of forensic wound dating and can serve as a useful adjunct in medicolegal investigations.

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