The immunohistochemical localization of alpha\textsubscript{1}-antichymotrypsin and fibronectin and its meaning for the determination of the vitality of human skin wounds

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Summary. A total of 39 vital human skin wounds (20 cases with short survival times ranging from a few seconds to approximately 30 min and 19 cases with wound ages between 50 min and 13 days) were investigated. Alpha\textsubscript{1}-antichymotrypsin (A\textsubscript{1}-ACT) was visualized by immunohistochemistry. Additionally, fibronectin was localized in 13 of these wounds (8 cases with short and 5 cases with longer survival times). Furthermore, 13 postmortem lesions (stab wounds) were removed from corpses approximately 4 h after infliction and analyzed for A\textsubscript{1}-ACT and fibronectin. The "vital" reaction previously described for A\textsubscript{1}-ACT in form of a band-shaped staining pattern at the wound edges was observed in both vital wounds and in most postmortem lesions. A similar reaction was also obtained for fibronectin in wounds inflicted after death, but could be unambiguously distinguished from vital fibronectin staining by morphological criteria. Therefore, it seems questionable that the vitality of skin wounds can be determined by the immunohistochemical detection of A\textsubscript{1}-ACT and probably other proteinase inhibitors. The meaning of the localization of fibronectin for the determination of the vitality of human skin wounds with a survival time of at least a few minutes could be confirmed.

Key words: Alpha\textsubscript{1} – Antichymotrypsin – Fibronectin – Vital Reaction – Wound Age – Immunohistochemistry

Introduction

The question whether an injury is inflicted before or after death is one of the central problems in forensic medicine. There are a large number of studies dealing with the determination of vital signs in human skin wounds. These include reports on biochemical changes \cite{1, 6, 7, 9, 11, 13, 15, 16, 25} and in particular on morphological phenomena as vital signs. Histochemical methods mainly applied by Raekallio \cite{21, 22, 23} have been used, although
the results have been discussed controversially [for review see 24], as well as routine histological techniques. In short-term wounds the latter methods seem to be restricted to the detection of infiltrating granulocytes. But these cells first appear after survival times of at least 20–30 min and considerable variations have been reported by different authors [for review see 20]. The previously suggested “signs of vitality” such as bleeding and the development of fibrin clots in the wound area [4, 5, 27] have also been questioned [14, 26, 28] and the detection of hemostatic plugs in skin wounds after short survival times is also of very limited use [12]. Furthermore, it has been reported that information on wound vitality can be obtained by changes in the staining pattern of the basic connective tissue substances [17], but on the basis of routine histology this approach provided no valuable parameters for estimation of wound vitality.

The introduction of immunohistochemical techniques, however, seemed promising to enable the detection of vital signs also in wounds after short survival times. Oehmichen et al. [18, 19] described the early release of several proteinase inhibitors and interpreted their findings as an indicator of vitality during the early post-traumatic interval in human skin wounds. Fechner et al. [8] showed that the immunohistochemical localization of fibronectin was suitable for indicating vitality of skeletal muscle damage. The advantage of the detection of this glycoprotein as a vital sign in human skin wounds with a survival time of at least a few minutes was demonstrated by our own previous results [3].

The present study was performed to elucidate the role of the immunohistochemical localization of the proteinase inhibitor A1-ACT as compared with fibronectin, which has previously been shown to be a useful parameter for the determination of the vitality of human skin wounds.

Material and methods
A total of 39 vital and 13 postmortem skin wounds was investigated. In 20 out of the 39 vital wounds (lacerations, stab wounds) the survival time ranged between a few seconds and approximately 30 min. The remaining cases were characterized by wound ages of 50 min up to 13 days (surgical wounds). The postmortem interval did not exceed 3 days and the cadavers were refrigerated (4°C) within a few hours after death. The postmortem wounds (stab wounds on the thigh) were removed during autopsy 4 h after infliction. The specimens were prepared as previously described [3].

Sections were enzymatically pretreated and A1-ACT was visualized using a polyclonal antibody (Fa. Dako, Hamburg, Germany) according to the ABC-method [10]. Fibronectin was localized in serial sections from 13 of the vital skin wounds and in all postmortem lesions as previously described [3].

Results
A1-ACT
In all specimens investigated a positive reaction (“internal control”) was seen in mast cells, macrophages and in endothelial cells.

In only 1 of the 20 cases (5%) with short survival times up to approximately 30 min could a band-shaped positive reaction at the wound edge be observed which was clearly distinguishable from the weak band-shaped staining patterns found at other margins of the specimens. In 3 cases (15%) a questionable “positive” reaction was found and the remaining 16 cases (80%) showed no distinct positive staining at the wound edges when compared to the other margins of the specimens.

In 5 out of 19 skin wounds (26%) with a wound age between 50 min and 13 days, a positive staining was seen at the wound margin and in 3 of these 5 “positive” cases the reaction was restricted only to the coagulated exudate which had developed on the epithelial layer. In only 1 of these 19 cases (survival time 3 h; Fig. 1) a distinct positive reaction was seen at the wound edges but not in other marginal regions of this specimen. In this case the positive staining was detectable around disconnected fiber bundles of the dermis, extending into inner areas of the specimen and was therefore not only restricted to a band localized at the wound edge. In 3 out of the 19 cases (16%) a questionable and in the remaining 11 skin wounds (58%) no staining pattern distinguishable from that of the other margins was demonstrable.