Int J Legal Med (1996) 108:262–264

ORIGINAL ARTICLE

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Morphometrical analysis of hemosiderin deposits in relation to wound age

Received: 19 October 1995 / Received in revised form: 30 November 1995

Abstract A morphometrical analysis of the extent of hemosiderin deposits in 71 human skin wounds with post-infliction intervals between 2 days and 7 months was performed. Earliest positive findings were detectable in a lesion aged 3 days, and with increasing wound age an increase in the amount of hemosiderin occurred. A value of more than 20% of the microscopic field with hemosiderin deposits was found earliest 8 days after wounding and therefore the detection of considerable amounts of hemosiderin (arbitrarily defined as 20% or more of the evaluated area) indicates a minimum wound age of approximately 1 week. Since the extent of hemosiderin formation depends upon the extent of the initial hemorrhage and a "physiological" reduction in the amount of this pigment with advanced wound age, slight or absent hemosiderin deposits cannot provide information on the post-infliction interval.

Key words Wound age · Morphometry · Hemosiderin

Introduction

In addition to immunohistochemical techniques [5] the routine histological detection of hemosiderin deposits in wounds provides information on post-infliction intervals of at least a few days [1–4, 7, 9, 10]. As a wound-age-dependent increase in the activity of the hemoglobin-degrading enzyme microsomal heme oxygenase [11, 12] was shown in an experimental study [8], an increase in the amount of hemosiderin dependent on wound age also seems to be probable. Therefore, we investigated whether a morphometric analysis could provide information on the post-infliction interval in addition to that which can be obtained by the earliest appearance of hemosiderin.

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Materials and methods

A total of 71 human skin wounds (stab wounds, lacerations, surgical wounds) with post-infliction intervals between 2 days and 7 months was investigated. The individual age of subjects ranged between 20 and 73 years, and the postmortem interval did not exceed 3 days in any case. No substances such as cytostatic agents or glucocorticoids, which can influence wound healing, were administered during medical treatment. Furthermore, no severe diseases such as metabolic disorders or malnutrition, which could also have influenced the results, were recorded in our series.

From each skin wound, at least two specimens were fixed in 4% formaldehyde solution and then embedded in paraffin. Sections 3–5 µm thick were prepared and stained with Perls' Prussian blue. A morphometric analysis of 10 randomly selected microscopic fields of the wound area was performed in each specimen using a 100-square grid (magnification 200 ×). Each of the 100 squares of the grid in which hemosiderin could be localized was scored as 1% and the mean value of all evaluated microscopic fields of one skin wound and the maximum value found in a single microscopic field were recorded. A wound-age-dependent relationship and a possible influence of individual age on the extent of hemosiderin deposits were investigated.

Results

Earliest positive findings were detectable in a lesion aged 3 days. In this specimen, however, the mean value was 0.1% since hemosiderin deposits were found in only 1 out of 1000 evaluated squares. A significant increase in the extent of hemosiderin deposits was observed with increasing wound age, but in some cases only low amounts of hemosiderin or none at all were found in spite of longer post-infliction intervals. A hemosiderin level of more than 20% of the evaluated area was first detectable in a wound aged 8 days (see Fig. 1). In this specimen, a maximum value of 37% was found in one microscopic field (see Fig. 2) clearly exceeding those of the specimens with shorter post-infliction intervals. The period between 8 days and 1 month was characterized by considerable interindividual variability, and a mean value of 40% of the evaluated area showing hemosiderin deposits was first exceeded in a specimen aged 11 days (maximum single value: 77%).

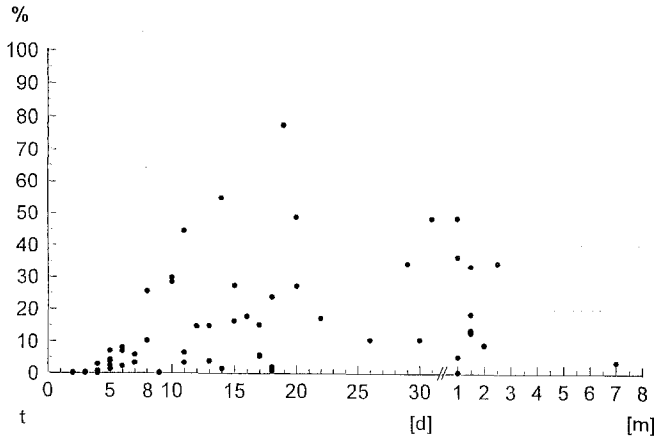

Fig. 1 Extent (x_{mean}) of hemosiderin deposition in human skin wounds ($n = 71$) in relation to wound age (t post-infliction interval, d days, m months, % percentage of evaluated area with hemosiderin deposits)

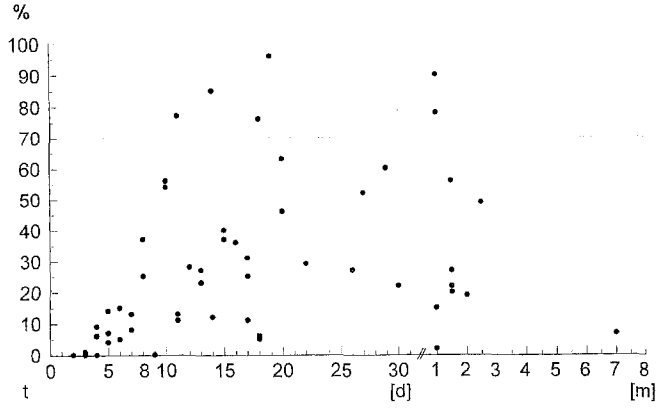

Fig. 2 Extent (x_{max}) of hemosiderin deposition in human skin wounds ($n = 71$) in relation to wound age

The maximum mean and single values in our series were detected in a 19-day-old lesion (x_{mean}: 77.4% – x_{max}: 96%). In wounds with post-infliction intervals of more than 19 days, a decrease in the extent of hemosiderin deposits was observed and the oldest wound investigated (wound age: 7 months) showed a mean value of 3.4% and a maximum single value of 7%.

No relevant differences in the amount of hemosiderin exceeding the interindividual variability were found between younger and older individuals. A differentiation between the time intervals in which extra- or intracellular hemosiderin deposits were detectable also provided no further information on wound age since both forms could be found in wounds with shorter and longer post-infliction intervals.

Discussion

The detection of hemosiderin, a pigment formed in local blood extravasations [13], using Perls' Prussian blue is a simple routine histological technique and has the additional advantage of a good contrast between blue-stained hemosiderin and slightly red-colored surrounding connective tissue. Furthermore, in an experimental study [6] it was shown that the identification of hemosiderin using the Prussian blue reaction was relatively unaffected by putrefaction, indicating that this staining method can also provide information on wound age in skin specimens removed from corpses showing signs of advanced putrefaction. With regard to these practical aspects, the detection of hemosiderin is an important parameter for wound age estimation, and the possibility of obtaining information on the post-infliction interval exceeding that provided by the earliest appearance of this pigment approximately 3 days after wounding would be a great advantage.

The morphometric analysis revealed time-dependent differences in the extent of hemosiderin deposits, which can be useful for a forensic wound age estimation. The results provide evidence for an increase of hemosiderin with advanced post-infliction intervals, which can easily be explained by an increase in the activity of the hemoglobin-degrading microsomal heme oxygenase as reported by Laiho and Tenhunen [8]. In our series, hemosiderin deposits exceeding 20% of the evaluated area were detectable earliest in a wound aged 8 days. A mean value exceeding 40% was first observed 11 days after wounding but with regard to the considerable variation found in a rather limited time interval of 3 days, a reliable differentiation of wound age between 8 and 11 days seems to be doubtful under forensic aspects. This rapid increase in the amount of hemosiderin found in this period of post-infliction interval is in good accordance with the findings of Laiho and Tenhunen [8], who described a ten-fold increase in the activity of the microsomal heme oxygenase between 2 and 9 days after infliction of the hematoma.

On the other hand, slight or no hemosiderin deposits were found up to a wound age of approximately 18 days providing evidence for a considerable interindividual variability as frequently observed in biological processes such as wound healing. The observation of rather limited amounts of hemosiderin or even the lack of this pigment in the area of the lesion could – in addition to methodological factors – be explained at least in part by slight initial traumatic bleeding, since it is easily conceivable that the extent of hemosiderin formation depends on the extent of the hemorrhage. The rather low values found in some wounds aged one month or more, however, could be explained by a transportation of hemosiderin by siderophages migrating from the wound area or by an extracellular degradation of hemosiderin. With regard to such a "physiological" reduction in hemosiderin deposits of older skin wounds, no further conclusions on wound age can be drawn from low values or even by negative findings. The morphometrical analysis of hemosiderin deposits, however, provides information on post-infliction intervals of at least approximately 1 week in cases with extensive hemosiderin deposits arbitrarily defined as involving 20% or more of the evaluated wound area. Therefore, such an analysis is useful for a forensic wound age estimation since further information can be obtained exceed-

ing that provided by the earliest appearance of hemosiderin approximately 3 days after wounding.

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