

Commentary: Mast cells and dendritic cells as tools to discriminate vital and post mortem lesions

Authors:

Stefano Bacci (PhD) ⁽¹⁾

Aurelio Bonelli (MD) ⁽²⁾

Departments of ¹Clinical and Experimental Medicine, and ²Health Sciences, University of Florence, Italy

The authors have no conflict of interest to declare.

Financial support was granted by the Italian Ministry of Education, University and Research, and the Ente Cassa di Risparmio di Firenze (grant n. 0082/year 2014).

Corresponding author:

Dr. Stefano Bacci

Department of Clinical and Experimental Medicine,

Research Unit of Histology and Embriology

Viale Pieraccini 6

50139 Florence, Italy

Tel: + 39-55-2758157

Fax: + 39-55-2758160

E-mail: stefano.bacci@unifi.it

Abstract

The determination of vitality and wound age is one of the most central research areas in legal medicine, about which a large amount of scientific work has been submitted for many decades up until now. In the first minutes or hours, standard histological examination may not determine whether the wound was inflicted in the pre- or post-mortem period. Since the study of cellular infiltrate might be a key to discriminate the vitality of lesions and since mast cell is one of the cellular types that participate in the organization of cellular infiltrate, the behaviour of this cell is among the data which can be of help in that respect. Besides dendritic cells, there is another cell type involved in the response to injury even independent of antigen challenge e.g. on the arterial wall and in the heart. This commentary is focused on the recent proposal to use mast cells and dendritic cells as cellular tools for the discrimination of vital and post mortem wounds.

Estimating wound vitality and age is crucial for the proper course of legal proceedings [1-5]. Several scientific studies have been reported using different techniques to solve these problems [1-5]. Historically, Wacker [6] and Orsos [7] first claimed that the determination of wound vitality or wound age was indispensable in forensic practice, besides Raekallio who was the first scientist to address the complex issue of differentiating vital from post-mortal injuries, by investigating the activity of several enzymes at wound sites [8]. Finally, using immunohistochemistry, the study of inflammatory cell infiltrate and the relation with wound healing [9] has expanded dramatically [1-5, 9] and consequently the field of lesions timing investigations.

It is well known that histamine, an important vasoactive amine, participates in an acute inflammatory reaction. Endogenous histamine is responsible for

initiating vascular changes that involve vasodilatation and increased vascular permeability and other mechanisms are then required to maintain them [10]. In 1965 Fazekas and Virágos-Kis [1] observed that there was an increase in the free histamine content in marks caused by hanging. Their work encouraged a number of forensic pathologists to begin biochemical studies on the possible use of the histamine content in the skin to differentiate antemortem from postmortem wounds and to estimate lesion vitality [1]. In particular, Berg and Bonte demonstrated that the histamine levels in vital skin wounds inflicted at least 60 min before death could increase up to 100% [1]. An experimental study with a murine model, using the microfluorometric method, indicated that the skin histamine level was upregulated after 30 min. No statistical relationship was found between MC number and histamine level [11]. To date, histamine is not a reliable marker in

forensic pathology.

Since MC is a source of histamine, Bonelli et al. demonstrated in vital lesions that dermal MC number increased progressively within a few hours from trauma (peak at 1–3 h) [12, 13] and underwent degranulation in the first hour after wounding [14]. In addition, a significantly increased expression of TNF α on MC in vital lesions of 5 min, with a peak at 1 h was found [15]. In other studies, Oehmichen et al. [16] reported early degranulation of MC in intravital wounds. In hard ligatures, Turillazzi et al. [17] showed a strong overexpression of tryptase, i.e. an enzyme localized in MC, in interstitial tissue. Finally, Gauchotte et al., using anti tryptase antibodies, found that MC degranulation rate in stab wounds was higher in wound margins and correlated with the time interval (minimal time, 1 min) [18]. Therefore MC histochemistry has been proposed in addition to classic histological methods to

estimate the course of traumatic events before and after death during forensic expert analysis [19].

Since MC mediators are related to the differentiation and function of dendritic cells (DC) [20] and the close proximity of these cells is often found [14, 21], DC modifications should be useful to recognize vital from post mortem wounds, other than to be involved in the response to injury even independent of antigen challenge as in the arterial wall or more recently in myocardial infarction [22, 23]. Moreover Bacci et al. observed, in vital lesions, that epidermal and dermal MHC-II⁺ cells increased transiently in number within the first hour after wounding, then decreased. In the epidermis, the increase affected also Langerhans cells (LC), which however increased less, earlier and for a shorter time period than MHC-II⁺ cells while the volume density of MHC-II⁺ cells increased to almost twice the baseline 31-60 min after wounding [14].

Therefore these last results show that the ratio between CD1a positive and MHC-II positive cells in the epidermis, the relative volume of MHC-II positive cells in the dermis and the degranulation index of MC can be added to the tools useful to estimate the interval between a lesion and death and, the first two of them, to distinguish vital from post mortem lesions.

Consequently the evaluation of DC can be proposed as complementary to other investigations to discriminate between wounds which occurred in life and those which occurred after death and to estimate the time interval between injury and death.

References

1. Hernandez-Cueto C, Girela E, Sweet D. Advances in the diagnosis of wound vitality: a review. *Am Journal of Forensic Med Pathol* 21:21-31, 2000.
2. Grellner W, Madea B. Demands on scientific studies: vitality of wounds and wound age estimation. *Forensic Sci Int* 165:150-154, 2007.
3. Kondo T. Timing of skin wounds. *Leg Med* 9; 109-114, 2007.
4. Cecchi R. Estimating wound age: looking into the future. *Int J Legal Med* 124: 523-536, 2010.
5. Casse JM, Martrille L, Vignaud JM, Gauchotte G. Skin wounds vitality markers in forensic pathology: An updated review. *Med Sci Law* 56: 128-137, 2016.
6. Walcher K. Über vitale Reaktionen. *Dtsch Z Gesamte Gerichtl Med* 15:16–57, 1930.
7. Orsos F. Die vitalen Reaktionen und ihre gerichtsmedizinische Bedeutung. *Beitr Pathol Anat* 95:163–241, 1935.
8. Raekallio J. Enzyme histochemistry of vital and postmortem skin wounds. *J Forensic Med* 13:85-94, 1966.
9. Fronczek J, Lulf R, Korkmaz I, Witte BI, Van de Goot FR, et al. Analysis of inflammatory cells and mediators in skin wound biopsies to determine wound age in living subjects in forensic medicine. *Forensic Sci. Int.* 247: 7-13, 2015.
10. Mahony LO, Mübeccel A, Cezmi AA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol* 128: 1153-1162, 2011.
11. Zong CF, Zhen ZJ. Localization and quantification of histamine in injured skin as parameters for the timing of

- wounds. *Forensic Sci Int* 51:163–171, 1991.
12. Bonelli A, Bacci S, Norelli GA. Affinity cytochemistry analysis of mast cells in skin lesions: a possible tool to assess the timing of lesions after death. *Int J Legal Med* 117:331–334, 2003.
13. Bonelli A, Bacci S, Vannelli B, Norelli GA. Immunohistochemical localization of mast cells as a tool for the discrimination of vital and postmortem lesions. *Int J Legal Med* 117:14–18, 2003.
14. Bacci S, De Fraia B, Cinci L, Calosi L, Guasti D, et al. Immunohistochemical analysis of dendritic cells in skin lesions: correlations with survival time. *Forensic Sci Int* 244: 179-185, 2014.
15. Bacci S, Romagnoli P, Norelli GA, Forestieri AL, Bonelli A. Early increase in TNF-alpha-containing mast cells in skin lesions. *Int J Legal Med* 120:138–142, 2006.
16. Oehmichen M, Gronki T, Meissner C, Anlauf M, Schwark T. Mast cell reactivity at the margin of human skin wounds: an early cell marker of wound survival? *Forensic Sci Int* 191:1–5, 2009.
17. Turillazzi E, Vacchiano G, Luna-Maldonado A, Neri M, Pomara C, et al. Tryptase, CD15 and IL-15 as reliable markers for the determination of soft and hard ligature marks vitality. *Histol Histopathol* 25:1539–1546, 2010.
18. Gauchotte G, Wissler MP, Casse JM, Pujol J, Minetti C, et al. FVIIIra, CD15, and tryptase performance in the diagnosis of skin stab wound vitality in forensic pathology. *Int J Leg Med* 127: 957-965, 2013.
19. Bacci S, DeFraia B, Romagnoli P, Bonelli A. Advantage of affinity histochemistry combined with

- histology to investigate death causes: indications from sample cases. *J Forensic Sci* 56: 1620-1625, 2011.
20. Dudeck A, Suender CA, Kostka SL, von Stebut E, Maurer M. Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. *Eur J Immunol* 41:1883-1893, 2011.
21. Bacci S, Pimpinelli N, Romagnoli P. Contacts between mast cells and in dendritic cells in human skin. *Ital J Anat Embriol* 115: 25-30, 2010.
22. Bacci S, Focardi M, Pieri L, Defraia B, Pinchi V, et al. Dendritic cells: a candidate cell in injury response to myocardial infarction and a possible tool for sudden death. *Ital. J. Anat. Embriol.* in press., 2016.
23. Bacci S, Pieri L, Buccoliero AM, Bonelli A, Taddei GL, et al. Smooth muscle cells, dendritic cells, and mast cells are sources of TNFalpha and nitric oxide in human carotid artery atherosclerosis. *Thromb Res* 122: 657-667, 2008

Legends

Figure 1. Intercellular contacts between dendritic cells (green labeled) and mast cells (red labeled) in a vital lesion, scale bar = 10 micron

