Journal of Forensic Pathology

Stefano and Aurelio, J Foren Path 2016, 1:1

Commentary Open Access

Immunohistochemical Analysis of Dendritic Cells in Skin Lesions: Correlations with Survival Time

Stefano Bacci[1*] **and Aurelio Bonelli**[2]

[1]*Department of Clinical and Experimental Medicine, University of Florence, Italy*

[2]*Department of Health Sciences, University of Florence, Italy*

[*]**Corresponding author:** Stefano Bacci, Department of Clinical and Experimental Medicine, Research Unit of Histology and Embryology, Viale Pieraccini 6, Florence 50139, Italy, Tel: 39-55-2758157; E-mail: stefano.bacci@unifi.it

Rec date: Apr 19, 2016; **Acc date:** Apr 25, 2016, **Pub date:** May 02, 2016

Abstract

Wound age evaluation is one of the most challenging issues in forensic pathology. In the first minutes or hours, standard histological examination may not determine whether the wound was inflicted in the pre- or post-mortem period. Numerous studies about wound vitality are available in the literature. They have evaluated markers involved in coagulation or inflammation, using various methods such as enzymology, molecular biology or immunohistochemistry. 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.

Keywords: Dendritic cells; Immunohistochemical analysis; Skin lesions; Wound age; Enzymology; Post-mortem period

Commentary

Deciding whether a skin wound occurred before or after death may be hard for a forensic pathologist when the survival time is short and an inflammatory reaction has not yet started. During the past 50 years, the development of histochemistry, enzymology and biochemistry and their application to investigations on wounds has offered partial solution to the problem [1,2].

Historically, Walcher [3] and Orsos [4] first claimed that the determination of wound vitality or wound age was indispensable in forensic practice; Raekallio investigates the activity of several enzymes at a wound site by histochemistry [5] and thereafter, the application of immunohistochemical techniques opened up a new field of wound age investigation [6-8].

During the past 10 years, wound age determination has been one of the most popular themes in forensic research. In particular, growth factors, cytokines, extracellular matrices, and adhesion molecules [1,9-12], have been intensively investigated, resulting in a significant advancement of the determination of wound vitality or wound age; in this context the behavior of mast cells (MC) and their mediators are among the data which can be of help in that respect.

Histamine is a vasoactive amine released by basophils and MC. In acute inflammatory reaction, histamine induces vasodilatation, vascular permeability increase and leukocyte extravasation. Raekallio et al. [13], reported an overexpression after 15-30 min, Berg and Bonte [14] evaluated the levels of serotonin and histamine at the wound edges, which gave evidence of vital phenomena. 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 [15]. To date, histamine is not a reliable marker in forensic pathology.

Avidin, chymase and tryptase are three of the most used MC markers. Avidin tags MC specifically with high sensitivity; the binding of the molecule is attributable to electrostatic attraction; hence, it has the same meaning as basophilia but with much higher sensitivity than conventional microscopic dyes as toluidine blue or Giemsa [16], chymases are a family of serine proteases found primarily in MC and released upon challenge with parasite antigens, tryptase is a serine protease predominantly expressed in MC and is an important mediator in anaphylactic reactions [17].

Bonelli et al. used anti-tryptase and chymase antibodies or avidin to evaluate mast cell density in skin wounds by immunofluorescence [18,19]. The dermal MC number increased progressively within a few hours from trauma (peak at 1-3 h) [18,19]. Besides a significantly increased expression of TNF-α is seen on skin MC in lesions of 5 min, with a peak at 1 h was found [20]. 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 [21].

In other studies, comparing the staining for tryptase and naphtol AS-D chloroacetate esterase, Oehmichen et al. [22], reported early degranulation of MC in intravital wounds. In hard ligatures, Turillazzi et al. [23], showed a strong overexpression of tryptase localized in interstitial tissue. Finally Gauchotte et al. in stab wounds using anti tryptase antibodies found that MC degranulation rate was higher in wound margins and correlated with the time interval (minimal time, 1 min) [24].

Since MC mediators are related to differentiation and function of dendritic cells (DC) [25] and close proximity of these cells are often found (Figure 1) [26-28]. 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. Dermal MHC-II+ cells became part of a perivascular mononuclear cell infiltrate visible in the sub-papillary dermis by 60 min after wounding, which contained also MC. MC underwent degranulation, other than increase in number, in the first hours after wounding [27-29].

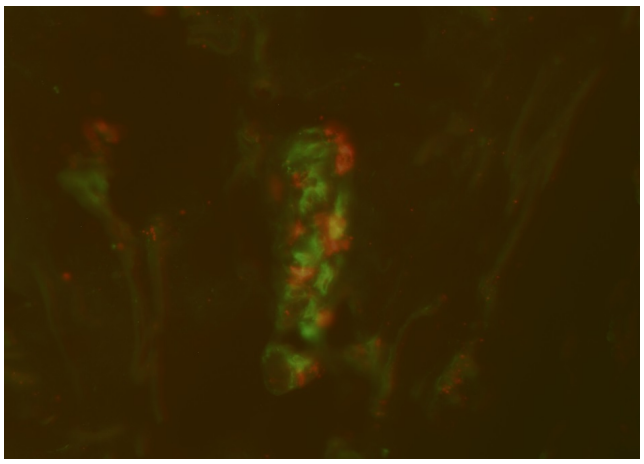

Figure 1: Intercellular contacts between dendritic cells (green labeled) and mast cells (red labeled) in a vital lesion. Fluorescence Microscopy 400X.

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. Dermal MHC-II+ cells became part of a perivascular mononuclear cell infiltrate visible in the sub-papillary dermis by 60 min after wounding, which contained also MC. MC underwent degranulation, other than increase in number, in the first hours after wounding [27-29].

In conclusion 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.

Grant

Financial support was granted by the Italian Ministry of Education, University and Research, and the Ente Cassa di Risparmio di Firenze (grant n. 3681/year 2012).

References

1. Cecchi R (2010) Estimating wound age: looking into the future. Int J Legal Med 124: 523-536.
2. Casse JM, Martrille L, Vignaud JM, Gauchotte G (2016) Skin wounds vitality markers in forensic pathology: An updated review. Med Sci Law 56: 128-137.
3. Walcher K (1930) Vital reactions. Dtsch Z Gesamte Gerichtl Med 15: 16-57.
4. Orsos F (1935) The vital reactions and their forensic significance. Beitr Pathol Anat 95: 163-241.
5. Raekallio J (1966) Enzyme histochemistry of vital and postmortem skin wounds. J Forensic Med 13: 85-94.
6. Hernández-Cueto C, Girela E, Sweet DJ (2000) Advances in the diagnosis of wound vitality: a review. Am J Forensic Med Pathol 21: 21-31.
7. Betz P (1995) Immunohistochemical parameters for the age estimation of human skin wounds. A review. Am J Forensic Med Pathol 16: 203-209.
8. Oehmichen M (2004) Vitality and time course of wounds. Forensic Sci Int 144: 221-231.
9. Grellner W, Madea B (2007) Demands on scientific studies: vitality of wounds and wound age estimation. Forensic Sci Int 165: 150-154.
10. Kondo T (2007) Timing of skin wounds. Int J Legal Med 116: 87-91.
11. Maeda H, Ishikawa T, Michiue T (2014) Forensic molecular pathology: its impacts on routine work, education and training. Leg Med (Tokyo) 16: 61-69.
12. Dressler J, Bachmann L, Strejc P, Koch R, Müller E (2000) Expression of adhesion molecules in skin wounds: diagnostic value in legal medicine. Forensic Sci Int 113: 173-176.
13. Raekallio J, Mäkinen PL (1970) Serotonin and histamine contents as vital reactions. II. Autopsy studies. Zacchia 6: 403-414.
14. Berg S, Bonte WS (1971) Practical experience of biochemical wound age determination. Beitr Gerichtl Med 28: 108-114.
15. Zhong FC, Zhen ZJ (1991) Localization and quantification of histamine in injured skin as parameters for the timing of wounds. Forensic Sci Int 51: 163-171.
16. Tharp MD, Seelig LL Jr, Tigelaar RE, Bergstresser PR (1985) Conjugated avidin binds to mast cell granules. J Histochem Cytochem 33: 27-32.
17. Irani AM, Bradford TR, Kepley CL, Shechter NM, Schwartz LB (1989) Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal antitryptase and anti-chymase antibodies. J Histochem Cytochem 37:1509-1515.
18. Bonelli A, Bacci S, Norelli GA (2003) 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.
19. Bonelli A, Bacci S, Vannelli B, Norelli GA (2003) Immunohistochemical localization of mast cells as a tool for the discrimination of vital and postmortem lesions. Int J Legal Med 117: 14-18.
20. Bacci S, Romagnoli P, Norelli GA, Forestieri AL, Bonelli A (2006) Early increase in TNF-alpha-containing mast cells in skin lesions. Int J Legal Med 120: 138-142.
21. Bacci S, DeFraia B, Romagnoli P, Bonelli A (2011) Advantage of affinity histochemistry combined with histology to investigate death causes: indications from sample cases. J Forensic Sci 56: 1620-1625.
22. Oehmichen M, Gronki T, Meissner C, Anlauf M, Schwark T (2009) Mast cell reactivity at the margin of human skin wounds: an early cell marker of wound survival? Forensic Sci Int 191: 1-5.
23. Turillazzi E, Vacchiano G, Luna-Maldonado A, Neri M, Pomara C, et al. (2010) Tryptase, CD15 and IL-15 as reliable markers for the determination of soft and hard ligature marks vitality. Histol Histopathol 25: 1539-1546.
24. Gauchotte G, Wissler MP, Casse JM, Pujo J, Minetti C, et al. (2013) FVIIIra, CD15, and tryptase performance in the diagnosis of skin stab wound vitality in forensic pathology. Int J Legal Med 127: 957-965.
25. Dudeck A, Suender CA, Kostka SL, von Stebut E, Maurer M (2011) Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. Eur J Immunol 41: 1883-1893.
26. Bacci S, Pimpinelli N, Romagnoli P (2010) Contacts between mast cells and dendritic cells in the human skin. Ital J Anat Embryol 115: 25-30.
27. Bacci S, De Fraia B, Cinci L, Calosi L, Guasti D, et al. (2014) Immunohistochemical analysis of dendritic cells in skin lesions: correlations with survival time. Forensic Sci Int 244: 179-185.
28. Bacci S, Focardi M, Pieri L, Defraia B, Pinchi V, et al. (2016) Dendritic cells: a candidate cell in injury response to myocardial infarction and a possible tool for sudden death. Ital J Anat Embriol.
29. Bacci S, Pieri L, Buccoliero AM, Bonelli A, Taddei GL, et al. (2008) 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.