

Death due to crush injuries in a compactor truck: vitality assessment by immunohistochemistry

R. Cecchi · M. Aromatario · P. Frati · D. Lucidi · C. Ciallella

Received: 30 March 2012 / Accepted: 31 July 2012 / Published online: 25 August 2012
© Springer-Verlag 2012

Abstract We present a case in which the timing of injuries was requested to determine whether the death of a man found in a landfill was due to homicide or accident. The use of immunohistochemistry to detect P-selectin and E-selectin on endothelial cells of vessels in damaged skin samples, and compare them with intact skin samples, as well as the presence of lung adipose embolism provide information on the timing of the injury, thereby helping substantially to identify the dynamics of death.

Keywords Wound age · Immunohistochemistry · Selectins · Crush injury · Compactor truck

Introduction

The golden goal for forensic histopathologists in wound-timing cases is to find histological parameters that can be presented in court as evidence. It should be borne in mind that the optimal condition for the timing of a lesion, thus providing stronger evidence in the trial, is that in which the parameter used (i.e., cell type or substance) is *absent in physiological conditions, appears regularly in time, and disappears after a given time*. Knowledge of the regularity of the occurrence, presence, and disappearance (time limits) of the chosen parameter provides reliable information, particularly when it is absent as it indicates that the lesion occurred either before or after the known time limit for that specific parameter [1]. Although reliable immunohistochemical

wound age determination for the early posttraumatic interval has not been established as yet, in specific cases the immunohistochemical findings can be of indicative significance together with all other available information [2].

Cell adhesion molecules are proteins that are expressed by endothelial cells and allow leukocyte recruitment during inflammatory response. Their activation depends on the type of stimulus they receive (blunt trauma, bruises, bacterial infection, allergic reactions, etc.) [3–6]. Given their fundamental role in the activation of vessels that initiate the inflammatory process and the regularity of the time in which they appear and disappear, adhesion molecules may be considered as good markers for wound timing.

P-selectin (CD62P) is a glycoprotein that is naturally present in Weibel and Palade corpuscles of endothelial cells and in the platelet secretory granules. When endothelial cells or platelets are stimulated by histamine or thrombin, there is a rapid melting (1–2 min) of the bodies of Weibel–Palade with the plasma membrane that causes the translocation of P-selectin on the surface within a few minutes (peak 10–15 min), which then decreases over an hour to physiological levels within 2–4 h. Once it reaches the cell surface average, it binds the integrins (Lex) of neutrophils and monocytes [4, 7, 8]. It is not induced by cytokines such as IL-1 and TNF and is, therefore, an essential cell adhesion molecule for the early binding of leukocytes in sites of inflammation. This characteristic makes it a good parameter for assessing vitality of injuries inflicted in *liminae vitae*

E-selectin (CD62E) is a glycoprotein that is synthesized by endothelial cells of post-capillary venules and of lymph-node venules only after inflammatory stimuli such as cytokines IL-1, TNF, or LPS. Its role is to bind the integrins (sLex) in neutrophils, monocytes, and T lymphocytes of memory [4, 8–10]. Its expression is dependent upon protein synthesis and it is the first molecule to be induced by TNF within 1–2 h, reaching a peak after 4–6 h of stimulation and

R. Cecchi (✉) · M. Aromatario · P. Frati · D. Lucidi · C. Ciallella
Department of Anatomical, Histological, Legal Medical and
Orthopaedic Sciences, Faculty of Medicine and Pharmacology,
Sapienza University of Rome,
Rome, Italy
e-mail: rossana.cecchi@uniroma1.it

then declining until it can no longer be measured after 8–10 h. Finally, it can be traced for a period not exceeding 24 h after the inflammatory irritation.

On the basis of these considerations, we applied immunohistochemical techniques with selectin P and E antibodies, as well as hematoxylin–eosin (HE), to a court case to address the questions that had arisen.

Case report

A male corpse was found in a landfill on a pile of waste, where it had been discharged by a garbage compactor truck. The man, identified as foreign and homeless, was in a prone position and was surrounded by waste. The man's clothing and the part of the body that was exposed were partially covered in dirt and waste material, while his face was splattered with blood. Hypostasis was scarce and masked by the large areas of bleeding, which was abundant on the neck, trunk, and limbs. Numerous injuries and fractures were present on the jaw bone, ribs, right femur, and right foot. Given the heterogeneity of the lesions and the way in which the corpse was discovered, the court wished to establish whether the man's death had resulted from homicide or an accident, i.e. whether the subject had first been subjected to beatings and then placed in a dumpster, or whether he had sought shelter in it for the night and then died after being crushed by the compactor truck.

Autopsy findings

The autopsy revealed craniofacial trauma with subcutaneous hemorrhage of the scalp, massive subarachnoid hemorrhage, and marked cerebral edema, chest trauma with multiple bilateral rib fractures and bilateral signs of pulmonary contusion, abdominal trauma with lacerations of the liver, fracture of the right femur and foot. There were no other pathological changes in the organs tested.

Materials and methods

Samples were taken from the brain, cervical medulla, heart, lungs, kidneys, and liver for histological examination with hematoxylin–eosin (HE).

Skin samples from lacerations of the scalp in the parietal and frontal regions and right fronto-temporal region as well as from esoriations of the right ankle were taken for HE and Elastica van Gieson, and for immunohistochemistry (IHC) with Monoclonal Mouse Anti-human CD62P (P-selectin) Clone 1E3 (Dako), Monoclonal Mouse Anti-human CD62E (E-selectin) NCL-CD62E-382 (Dako), and Monoclonal Mouse Anti-human CD15 Clone MMA (Roche Ventana). Each

sample was compared with a sample of intact skin taken from the same part of the body. Blood samples were taken for toxicological tests.

The specimens were prepared as follows: after being fixed in 4 % PBS-formaldehyde solution, 3–5- μ m-thick paraffin-embedded sections were cut and stained with HE and Elastica van Gieson. For the IHC, samples were dewaxed, hydrated, and subjected to heat-induced epitope retrieval (HIER) using Target Retrieval Solution (Dako) for 20 min in thermostatic bath at 98 °C. The staining procedure was performed using a Dako Autostainer Instrument as follows: incubation in peroxide for 10 min; incubation with primary antibody for 30 min in Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse; incubation for 5 min using the Dako DAB-AWAY detection system; wash in distilled water; and nuclear staining for 1 min with hemalaum.

The primary antibody was replaced with Dako wash buffer 10 \times in the negative control test, while tonsil samples were used for the positive control experiment.

The staining intensity was assessed semi-quantitatively under an optical microscope using a four-category ordinal scale (\emptyset =negative; +=low expression; ++=moderate expression; +++=strong expression). The location of the vessels in the dermis and the number of vessels were compared with the corresponding sample of healthy skin.

Results

The histological examination of both lungs revealed multifocal interstitial and intra-alveolar hemorrhages, alternating areas of alveolar collapse and over-insufflation, few bone marrow emboli (Fig. 1a, b); the brain displayed subarachnoid hemorrhage while the cervical tract medulla displayed acute hemorrhage of the white matter.

With regard to the IHC on the skin samples:

- Lacerations of the scalp from the frontal and parietal region revealed large hemorrhagic areas with a moderate (++) staining reactivity with P-selectin in the endothelial cells of many small- and medium-caliber vessels in the dermis and in few vessels of the subcutaneous adipose tissue (Fig. 2). P-selectin-positive staining thrombocytes were present in the large vessels. There was no positive reaction with E-selectin. CD15 staining revealed sporadic neutrophils in the vessels, but no recruitment.
- Lacerations taken from the right fronto-temporal region and esoriations from the right ankle: small hemorrhagic areas in the dermis and hypodermis with rare small vessels with low positive staining with P-selectin. There

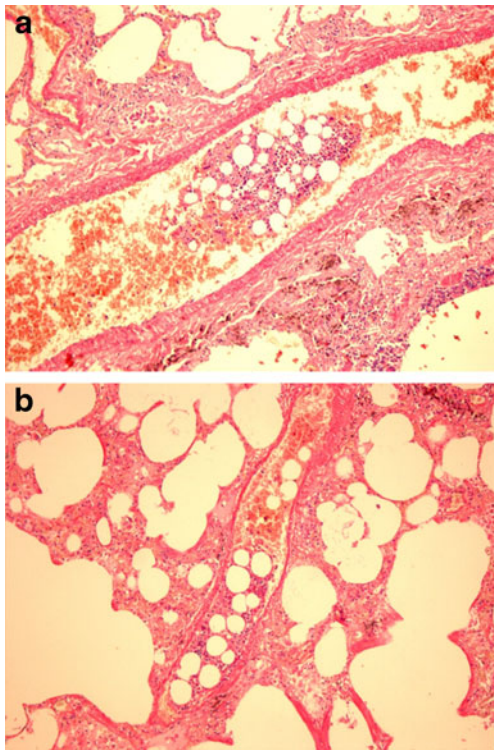


Fig. 1 a, b Bone marrow emboli in blood vessels ($\times 20$)

was no positive reaction with E-selectin and CD15 staining.

- Intact skin displayed a low positive staining reaction with P-selectin in the endothelial cells of few vessels from the papillary dermis and no positive staining with E-selectin and CD15 staining.
- HE and Elastica van Gieson: all the specimens displayed hemorrhagic areas with no signs of fibrin deposition or leucocyte recruitment (Fig. 3).
- Toxicological tests revealed high alcohol levels (4 g/l).

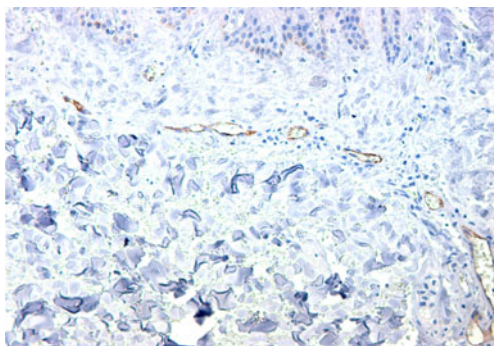


Fig. 2 Moderate staining with P-selectin of endothelial cells of many small- and medium-caliber vessels in the reticular dermis

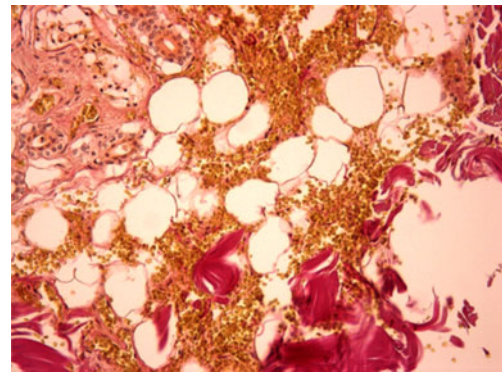


Fig. 3 Elastica van Gieson: all the specimens displayed hemorrhagic areas with no signs of fibrin deposition or leucocyte recruitment ($\times 20$)

Discussion

The search for a good marker of wound timing is still in progress. The cross-over of various parameters whose time of appearance and disappearance is known can help to circumscribe the time in which the injury was inflicted. In a previous paper, we applied IHC with P-selectin to define the survival time of a man after a gunshot to the head [11]. In this paper, P-selectin and E-selectin were used to make a differential diagnosis between ante-mortem and post-mortem injuries and to determine their timing. One important finding that emerged from our investigation was the constant absence of E-selectin in intact skin, which documents, when present, the vitality of the lesion and a chronology of more than 30 min [12–14]. The intensity of the reaction and the type and number of vessels that stain positive for P-selectin, which is constitutively present in healthy skin, represent a reference model for the assessment of injured skin [2]. The crossing-over of the reaction with both antibodies allows the timing the lesion to be circumscribed more effectively, even when HE and van Gieson seems to be sufficient.

Conventional histology (HE and van Gieson) did not reveal any signs of fibrin deposition, and CD15 staining no sign of cell movement (neutrophils). The positive staining reaction for P-selectin in many of small- and medium-caliber vessels in two specimens of the scalp skin, compared with the low positive staining of few vessels in the intact skin, in the fronto-temporal region and in the right ankle, indicates that the first two lesions were inflicted contemporaneously a few minutes before death, and that the man was thus alive when he entered the dumpster. The absence of a positive staining reaction for E-selectin in all samples can exclude that the time elapsed from one injury to the other has exceeded 30 min–1 h; the moderate positivity of P-selectin in the two samples of the scalp skin documents their vitality; the absence in these vital skin wounds of neutrophil recruitment and the rare presence of neutrophils in vessels,

can limit the chronology of these for few minutes. The action of the compactor truck, into which the dumpster was emptied, crushed the man's head and thorax, causing his death. The other two lesions were instead inflicted after death because the morphological picture was comparable to that of intact skin. In particular, the fronto-temporal region lesion may be a cut injury; indeed, a fragment of glass found in the man's temporal muscle is likely to have penetrated the body either in the truck or in the landfill.

The presence of a bone marrow embolism in the lung vessels confirmed the vitality of the bone fractures. Small bone marrow emboli, due to the passage of bone marrow debris into the circulation that ended up in the pulmonary vessels, have been reported within the lung vasculature after trauma or vigorous cardiopulmonary resuscitation. Such a finding should be considered a sign of vitality even in cases in which traumatic death occurs almost rapidly.

The autopsy and IHC data taken as a whole ruled out the hypothesis that the man had been beaten before entering the dumpster. Indeed, the high alcohol levels (4 g/l) revealed by the tests confirmed the hypothesis that he was drunk and had sought shelter there for the night. The case was filed by the judge in that investigations have confirmed our hypothesis of an accidental death.

References

- Dettmeyer RB (2011) Forensic histopathology. Springer, New York. ISBN 9783642206580
- Cecchi R (2010) Estimating wound age: looking into the future. *Int J Legal Med* 124:523–536
- Burch EE, Patil VR, Camphausen RT, Kiani MF, Goetz DJ (2002) The N-terminal peptide of PSGL-1 can mediate adhesion to trauma-activated endothelium via P-selectin in vivo. *Blood* 100:531–538
- Hirata T, Furie BC, Furie B (2002) P-, E-, and L- selectin mediate migration of activated CD8+ T lymphocytes into inflamed skin. *J Immunol* 169:4307–4313
- Subramaniam M, Suffaripour S, Van der Water L, Frenette PS, Takeuchi F, Streilein RD, Hall RP (2003) Increased E-selectin, IL-8 and IL-10 gene expression in human skin after minimal trauma: a potential explanation of regional distribution of skin lesions. *Exp Dermatol* 12:777–783
- Van der Laan N, de Leij LFMH, ten Duis HJ (2001) Immunohistopathological appearance of three different types of injury in human skin. *Inflamm Res* 50:350–356
- Lorant DE, Topham MK, Whatley RE, McEver RP, McIntyre TM, Prescott SM, Zimmerman GA (1993) Inflammatory roles of P-selectin. *J Clin Invest* 92(2):559–570
- Mayadas TN, Hynes RO, Wagner DD (1997) Role of endothelial selectins in wound repair. *Am J Pathol* 150:1701–1710
- Jiao A, Fish SC, Mason LE, Shelling SH, Goldman SJ, Williams CM (2007) A role for endothelial selectins and nonallergic inflammatory disease. *Ann Allergy Asthma Immunol* 98(1):83–88
- Picker L, Kishimoto TK, Smith CW, Warnock RA, Butcher EC (1991) ELAM-1 is an adhesion molecule for skin homing T-cells. *Nature* 349:796–799
- Cecchi R, Cipolloni L, Sestili C, Aromatario M, Ciallella C (2012) Pulmonary embolisation of bone fragments from penetrating cranial gunshot wounds. *Int J Legal Med*. doi:10.1007/s00414-011-0643-9
- Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP (1990) Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* 343:757–760
- Cecchi R, D'Annibale C, Valente S, Ferrera V (2004) La vitalità delle lesioni da arma bianca: studio sperimentale. *Zacchia* 77:49–59
- Dreßler J, Bachmann L, Koch R, Müller E (1999) Enhanced expression of selectins in human skin wounds. *Int J Leg Med* 112:39–44