Int J Legal Med (2001) 115:23–28

ORIGINAL ARTICLE

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Immunohistochemical alterations after intravital and post-mortem traumatic myocardial damage

Received: 23 November 2000 / Accepted: 28 December 2000

Abstract Two series of experiments have been carried out on heart tissue for the occurrence of post-mortem and intravital myocardial damage. The first series was carried out on 18 porcine hearts collected immediately after the pigs were killed in a slaughterhouse. The hearts were subjected to stab wounds post-mortem, varying between 5 min and 140 min after death. The second series investigated were human hearts with intravital damage, i.e. 4 stab wounds, 1 gunshot, 13 contusions and ruptures. The time the trauma occurred before death varied between 0 and 30 min. The investigation comprised the four myocyte structural proteins myoglobin, FABP, troponin C, desmin and the three plasma proteins fibrinogen, fibronectin and C5b-9. Both series exhibited a variety of direct traumatic changes with a much broader zone in vital damage compared to post-mortem damage. In vital damage the zone of direct damage is in continuity with a further zone of indirect damage which is a three dimensional network. The signs of damage are contraction bands, depletion of structure antigens, contraction-associated accumulation of structure proteins, accumulation of plasma proteins on the cell surfaces and in the interstitium. In vital damages there is in addition an intrasarcolemmal accumulation of plasma proteins. The pattern of all damage is much broader and much more variegated in vital damage, thus vital damage can be clearly differentiated from post-mortem damage.

Keywords Myocyte · Structure proteins · Fibrinogen · Fibronectin · C5b-9 · Intravital traumatic damage

Introduction

A series of immunohistochemical markers have recently been reported which are useful for the diagnosis of acute myocardial infarction (AMI) and include C5b-9, myoglobin, heart-type fatty acid binding protein (FABP), troponin, desmin, fibrinogen and fibronectin (Amberg 1995; Brinkmann et al. 1993; Glatz et al. 1994; Kleine et al. 1993; Leadbetter et al. 1989; Ortmann et al. 2000a, 2000b; Osuna et al. 1998; Thomsen and Held 1994, 1995). The aim of the present study was to elaborate reaction patterns of these marker proteins in mechanical heart trauma induced ante- and post-mortem and to explore their value for wound age determination in forensic casework.

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Materials and methods

Series 1

A total of 18 porcine hearts were obtained from a slaughterhouse. Immediately after exenteration the hearts were incubated in donor blood diluted (2:1) with 0.9% saline solution at room temperature for 20 h followed by fixation in 4% buffered formalin for 24 h and then sectioned and embedded in paraffin. During a time interval between 5 and 140 min post-mortem, transmural stab wounds were inflicted to the left ventricle (Table 1). A cut injury to a porcine heart caused operatively 1 h pre-mortem was used as the positive control sample.

Series 2

In 18 deaths due to trauma (Institute of Legal Medicine, Münster, 1994–2000) with fatal heart injuries (4 stab wounds, 1 gunshot wound and 13 contusions with ruptures), the time interval between trauma and cardiac arrest was estimated by evaluation of clinical data, case history and gross pathology (Table 2). Myocardial samples from these cases were fixed in buffered formalin for a period of 1–7 days, sectioned and paraffin embedded.

Immunohistochemistry (IH)

The antibodies, working dilutions, pre-treatment procedures and incubation times are shown in Table 3. Pre-treatment procedures and visualisation steps were processed as described before (Ortmann and Brinkmann 1997).

Evaluating the IH reaction

The intensity and extent of the staining reactions were scored semi-quantitatively. Structural antigens (troponin C, FABP, myoglobin

Table 1 Results of the analysis of porcine heart wounds (*a. m.* ante-mortem, *p. m.* post-mortem infliction)

Number of cases (n = 18)	Wound age	Contraction bands	Myoglobin	FABP	Troponin C	Desmin	Fibronectin	Fibrinogen	C5b-9
1	60 min a. m.	++	++	++	++	++	++	++	++
3	5–10 min p. m.	++	++	++	++/+	+	–	–	–
2	10–20 min p. m.	++	++/+	+	+/–	+/–	–	–	–
2	20–30 min p. m.	+	+	+/–	–	–	–	–	–
2	30–40 min p. m	+/–	–	–	–	–	–	–	–
8	40–140 min p. m.	–	–	–	–	–	–	–	–

Table 2 Case history and results of the antigen reactions (*CPR* professional cardiopulmonary resuscitation attempts, *autolysis* time between death and autopsy)

Case	Wound age a. m. (min)	Mechanical traumatisation	Autolysis (h)	Age (sex)	Myoglobin	FABP	Troponin C	Desmin	Fibronectin	Fibrinogen	C5b-9
1	0	Aeroplane accident, rupture of all cardiac chambers	17	51 (M)	++	++	++	+	–	–	–
2	0	Traffic accident, impalement injury with disconnection of heart	22	24 (M)	++	++	++	++	–	–	–
3	5	Traffic accident, rupture of all cardiac chambers	20	22 (M)	++	++	+	–	–	–	–
4	5	Traffic accident, dismemberment of heart	96	47 (F)	++	++	++	++	–	–	–
5	5	Traffic accident, severe rupture of cardiac septum, right atrium and pulmonary artery	24	13 (M)	++	++	++	++	–	+	–
6	5	Gunshot, disrupted left ventricle	45	56 (M)	++	++	++	+	+	+	–
7	5	Traffic accident, global contusion with ruptures of both ventricles	55	18 (M)	++	++	++	++	–	–	–
8	5	Transmural stab across both atria	18	30 (M)	++	++	++	+	+	+	–
9	5	Transmural stab of left ventricle	14	30 (M)	++	++	++	++	++	++	–
10	10	Traffic accident, contusion with ruptures of both atria	14	44 (F)	++	++	++	+	++	++	–
11	20	Traffic accident, contusion with rupture of right ventricle	36	68 (M)	++	++	++	++	+	+	–
12	20	Fall, contusion with epicardial rupture over right ventricle	48	38 (F)	++	++	++	+	+	+	–
13	20	Traffic accident, contusion with rupture of right atria and right coronary artery	10	17 (F)	++	++	++	+	++	++	+
14	20	Traffic accident, contusion with ventricular intramural ruptures and bleeding	102	26 (M)	++	++	++	++	++	++	++
15	10 (+CPR)	Traffic accident, contusion and rupture of right atrium	40	38 (M)	++	++	++	+	++	++	–
16	10 (+CPR)	Transmural stab of left ventricle, tamponade	21	30 (M)	++	++	++	++	++	++	–
17	30 (+CPR)	Transmural stab of left ventricle	13	21 (M)	++	++	++	+	+	+	–
18	30 (+CPR)	Fall, contusion and intramural ruptures	25	18 (M)	++	++	++	++	++	++	++

and desmin) with loss, displacement or depletion and plasma antigens (fibrinogen, fibronectin and C5b-9) with intrasarcolemmal deposition, were graded as negative (–), weak but distinct (+) and strong reaction (++).

Single cells or cell groups isolated by the trauma and transferred into the area of the rupture did not influence the grading of the IH.

Results

The IH gave positive results for all antibodies applied in the vitally injured porcine control sample. However, compared to the human samples the staining intensity of the structure antigens was generally weaker.

Table 3 Panel of antibodies used in this study

Antibody	Autoclave pre-treatment	Proteinase K pre-treatment	Incubation time of primary antibody, temperature	Concentration of primary antibody
Fibrinogen, (rabbit anti-human, DAKO)	–	–	60 min, 20 °C	1:2,000
Fibronectin (rabbit anti-human, DAKO)	–	30 min	30 min, 20 °C	1:1,000
Desmin (rabbit anti-chicken, Sigma)	+	10 min	60 min, 20 °C	1:80
Myoglobin (rabbit anti-human, DAKO)	+	10 min	60 min, 20 °C	1:500
C5b-9 (mouse anti human, DAKO)	+	10 min	16 h, 4 °C	1:25
Troponin C (rabbit anti-human, Novo Castra)	+	–	60 min, 20 °C	1:100
FABP (rabbit anti-human)	+	10 min	60 min, 20 °C	1:400

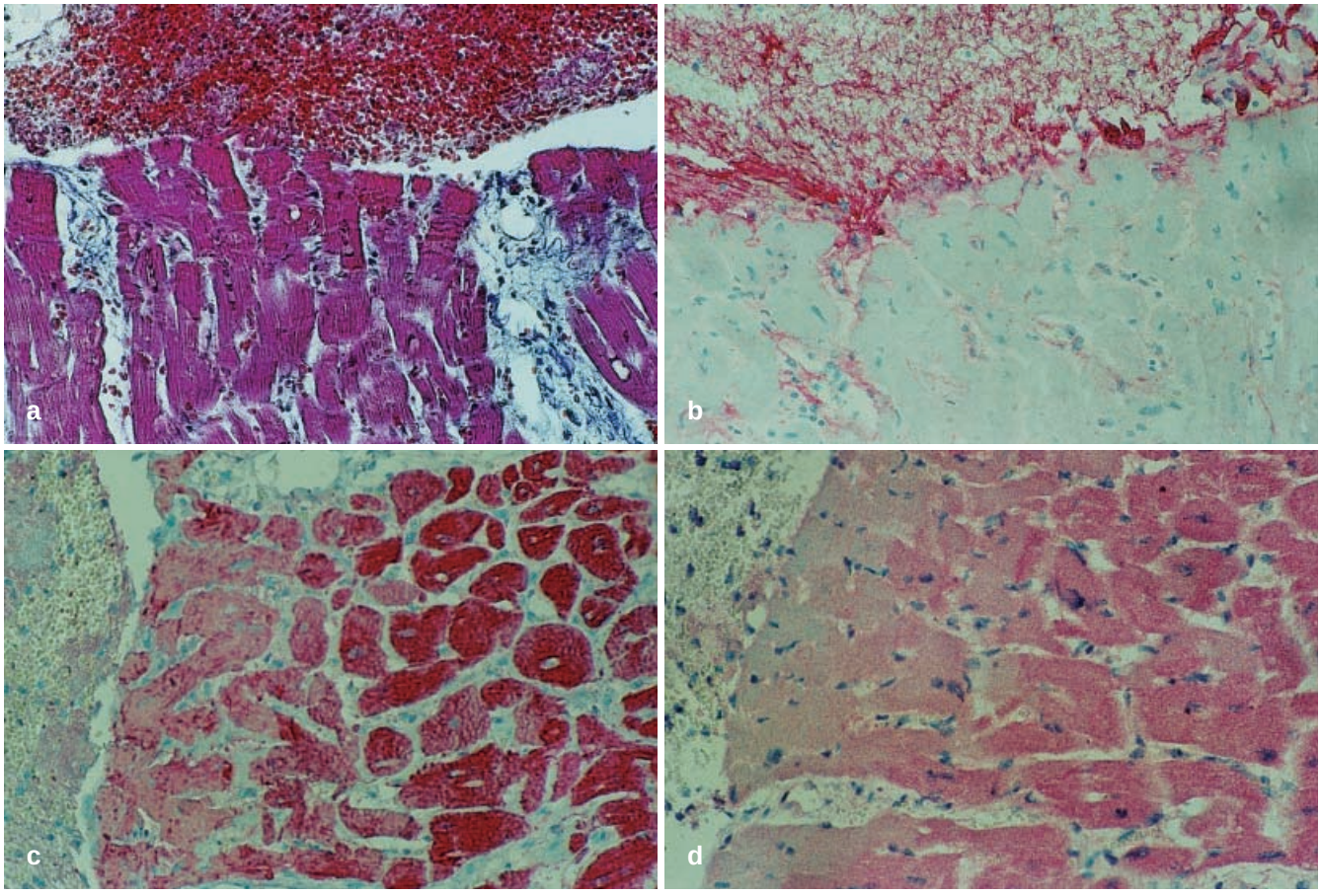

Fig. 1 **a** Stab wound (*top*) of a porcine heart at 10 min p. m. showing a layer 2–3 cells thick with contraction bands and blood in the stab canal (Ladewig, 200 × magnification). **b** Visualisation of fibronectin reveals adhesions on the surface of the myocytes of cut edges10 min p. m. and no intrasarcolemmal deposition. **c** Depletion and some clumped accumulation of troponin C within the zone of post-mortem contraction bands (*stab wound left*), 10 min p. m. **d** Contraction bands with distinct depletion of FABP (*stab wound left*) 10 min p. m.

Post-mortem infliction

The porcine control samples exhibited two types of tissue/cell damage, i.e. cut-related and death-related. The cut-related damage was a narrow zone surrounding the cut surface, 2–3 myocytes wide. This zone contained slight eosinophilia of the cut surface, discontinuities of myocytes and contraction bands (Fig. 1a). The structure proteins myoglobin, FABP, troponin C and desmin showed either depletion or, in contracted myocytes, accumulation. The process of depletion and accumulation of the four proteins showed a sequence in relation to time, i.e. myoglobin was the first to show depletion and the last to show accumulation and the sequence was: myoglobin – FABP – troponin C – desmin (Table 1, Fig. 1c,d). The aforementioned changes started to occur at the earliest post-mortem (p. m.) time interval tested (5 min p. m.) and did not occur in p. m. times of trauma infliction later than 40 min (Table 1). This direct zone of damage also showed accumulation of fibrinogen and fibronectin but only on the cut surface and between the myocytes and not in the sarcolemma.

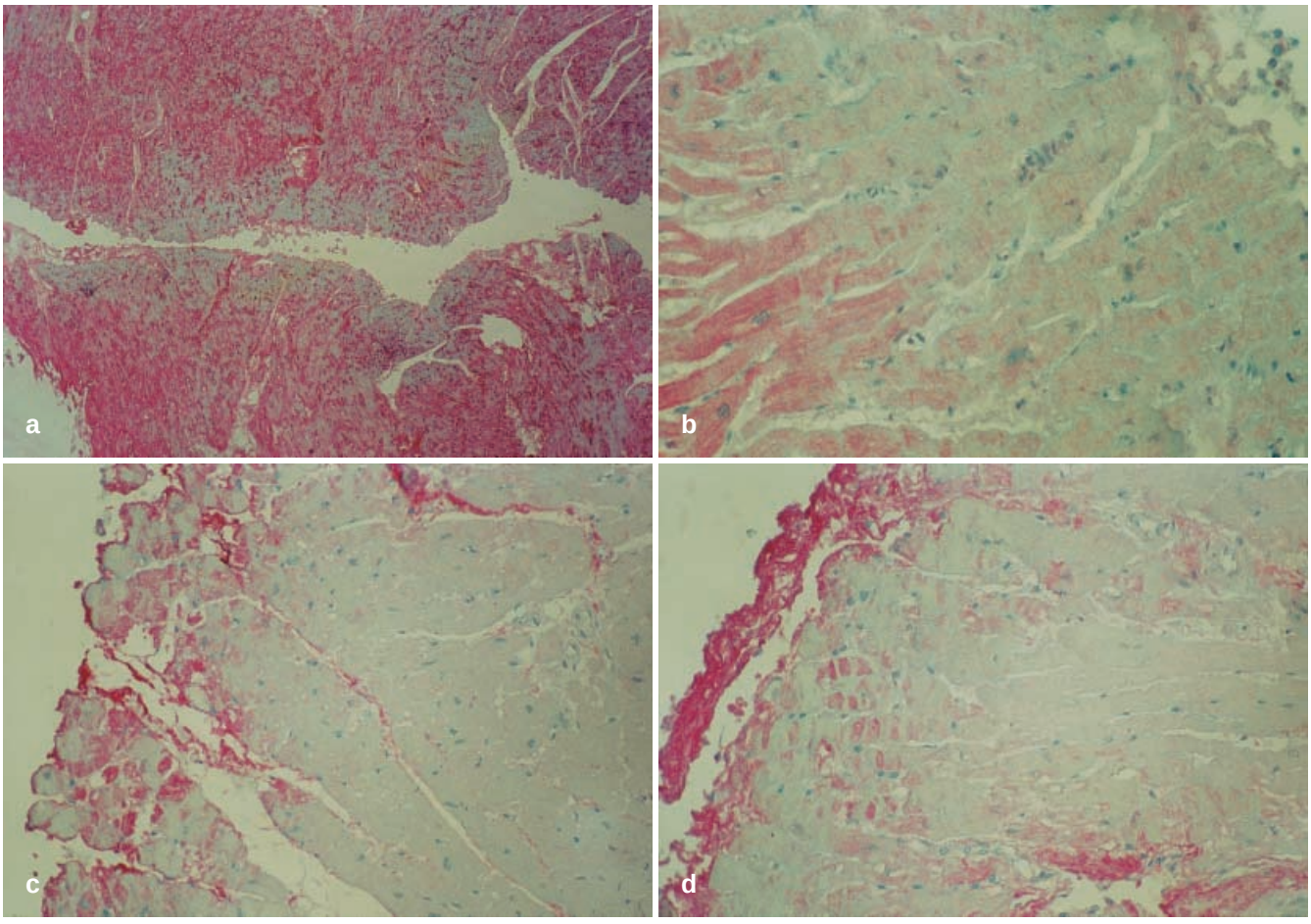

Fig. 2 **a** Case 16 (Table 2), vital infliction, heavy depletion of FABP of a wide irregular zone around the stab wound (25 × magnification, stab wound horizontal in the middle), 10 min a.m. **b** Case 16 (Table 2), heavy depletions of troponin C (*stab wound right*). **c** Case 16 (Table 2), accumulation of fibronectin on the surface and intrasarcolemmal of cardiomyocytes in the near surroundings of the stab canal, vesiculation. **d** Case 16, (Table 2) distinct accumulation of fibrin/fibrinogen on the surface of the stab canal and intrasarcolemmal of cardiomyocytes with contraction band necrosis

Also C5b-9 sometimes showed a weak reaction on the cut cell surfaces. Independent of the cut/stab wound there existed single cell damage scattered diffusely with weak accumulation of fibrinogen/fibronectin in isolated cells particularly in the subendocardial zone accompanied by focal depletion of structure antigens as death-related myocardial damage.

Vital infliction

In cases of vital mechanical heart damage the traumatised zones were much broader than in p. m. trauma infliction especially in the ruptures and the gunshot but also in the stab wounds (Fig. 2a versus Fig. 1d and Fig. 2b versus Fig. 1c). In the vital cut/stab wounds one could distinguish two afflicted zones. The direct zone which had the same relationship to the wound as in p. m. infliction but which was much broader, i.e. 8–10 cells thick. This zone was in continuity with an indirect zone which formed a three dimensional network of comparable changes in the form of a network of approximately the same width as the direct zone (Figs. 2a, 3). Contraction bands, tearing, fibre rupture and disintegration of myocytes were pronounced. In contrast to the p. m. infliction there occurred haemorrhages with paravasal erythrocyte extravasations. Furthermore, a leucocytic sticking was detected inconsistently and intravascularly after some minutes of vital wound infliction but a clear leucocytic in-

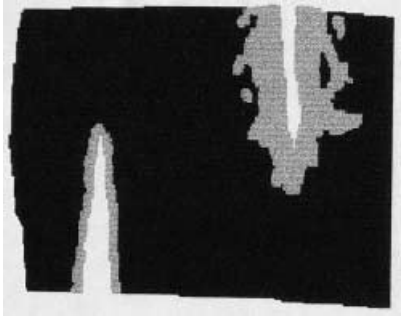

Fig. 3 Schematic comparison of depletion of troponin C. *Left* 10 min post-mortem and *right* 10 -min-old vital stab wound. The vital wound is characterised by a much broader direct zone and a indirect zone with a three-dimensional network of depletion

filtration was absent in all cases due to the short survival times of up to 30 min (Table 2).

Structural antigens, especially troponin C and desmin showed a gradually higher depletion in the traumatised regions and more aggregations in clumped cytoplasmatic segments (Fig. 2a,b). A tendency of the time-dependent depletion of structural antigens in the order myoglobin – FABP – troponin C – desmin was also observed (Table 2). In contrast to the p. m. infliction, fibrinogen and fibronectin showed positive intrasarcolemmal accumulation also in the altered myocytes starting some minutes after vital infliction (Fig. 2c,d). The visualisation of C5b-9 in the myocytes started about 15–20 min after vital infliction (Table 2). In the blunt types of cardiac lesions, the zonal arrangement was sometimes difficult to assess, but even there the same features seemed to exist although they were more irregular.

Discussion

A significant factor promoting tissue damage by bullets, blunt trauma or by stabbing is the velocity of the object striking it (Betz et al. 1996). Thus, different types of trauma are capable of delivering different amounts of energy to the myocardium and certain trauma can cause more extensive injury than would be expected. Therefore, the cases analysed showed great differences in the proportion of the myocardium afflicted, not only in size and course of trauma but also in the time for development.

In series 1 we investigated porcine hearts which were exenterated after electrocution and bleeding the pigs dry. This process and also a preceding phase of stress are held to be the underlying mechanisms for the generation of single cell damage particularly in the subendocardial zone and this would be in accordance with the literature (Keil et al. 1984; Ortmann et al. 2000a). This disseminated type of damage can be easily distinguished from trauma-related alterations. Structural alterations can be explained by direct effects of the mechanical trauma with disintegration of membranes. Since the width of the zone(s) affected and the degree of such alterations are much more expressed in vital injuries, there seem to exist additional factors promoting contraction and contracture of myocytes.

In the porcine controls, supravital contraction bands were produced up to 30–40 min p. m. within the direct zone of trauma. A similar time dependence for contraction band occurrence was reported by Abdomian et al. (1976) who analysed post-mortem biopsy samples from animals. In the early p. m. period clear depletions of structural antigens, especially myoglobin and FABP, occurred in the direct zone of trauma. After post-mortem clamping of skeletal muscles, depletion of myoglobin has not been observed (Fechner et al. 1991). This can be due to another type of trauma applied and to basic differences in the ability of the samples to react due to their origin.

The time-dependent depletion and accumulation of antigens may depend on their molecular weights and cellular distribution (myoglobin 17 kD and cytosol, FABP 15 kD and cytosol, troponin C 18 kD and contractile apparatus, desmin 51 kD and cytoskeleton). The process of depletion/accumulation was very much pronounced in vital wounds and included the indirect zone of trauma. Among all structural antigens analysed, troponin C showed the best discrimination power (Tables 1, 2).

Fibronectin and fibrinogen exhibited aggregation on the surface of myocytes in p. m. and vital wounds. In vital wounds, these proteins were also accumulated in the intrasarcolemma. This is a finding not observed in p. m. wounds but it was positive in the vital porcine control. Their appearance a few minutes after the trauma and their topology renders these proteins rather discriminative (Betz 1995). These results correlate with the findings in skeletal muscles (Fechner et al. 1993). C5b-9 gave positive results ca. 20 min after vital infliction and earlier than in ischaemic injury (Ortmann et al. 2000a). However an exact estimation of the time of infliction in series 2 was not always possible. Perfusion seems to be at least necessary for the deposition of fibronectin, fibrinogen and, for the appearance of C5b-9.

Conclusions

1. In the very early period after infliction of injury, myocyte proteins start to deplete in a regular order. This depletion process is overlapped and continued by the deposition of plasma proteins and the accumulation of structure proteins associated to structural changes.
2. In vital wounds there exists a zonal arrangement of all changes observed which is much less expressed in p. m. wounds. Also fibrinogen and fibronectin accumulate in the intrasarcolemma. The formation of contraction bands is more expressed than in p. m. wounds and the whole pattern of pathological changes is much more variegated and pronounced.
3. Certain IH methods are capable of more clearly discriminating between intravital and p. m. injuries. In addition to classical methods such as H&E we recommend the following antigens: troponin C, fibronectin or fibrinogen and C5b-9.

Acknowledgements The authors are grateful for the support of the Institute for Biochemistry at the University of Münster for supplying the h-FABP antibody.

References

Abdomian GE, Laks MM, Gardner D, Swan HJC (1976) The determinants for the production of contraction bands in normal rats and dogs. Fed Proc 35:229

Amberg R (1995) Immunmorphologische Reaktionsmuster des Myokards bei kardialen und nicht-kardialen Perfusionsstörungen. In: Bratzke H, Schröter A (eds). Immunhistochemie in der Rechtsmedizin: Ein Workshop. Hänsel, Hohenhausen, pp 101–116

Betz P (1995) Immunohistochemical parameters for the age estimation of human skin wounds. A review. Am J Forensic Med Pathol 16:203–209

Betz P, Stiefel D, Eisenmenger W (1996) Cranial fractures and direction of fire in low velocity gunshots. Int J Legal Med 109: 58–61

Brinkmann B, Sepulchre MA, Fechner G (1993) The application of selected histochemical and immunohistochemical markers and procedures to the diagnosis of early myocardial damage. Int J Legal Med 106:135–141

Fechner G, Hauser R, Sepulchre MA, Brinkmann B (1991) Immunohistochemical investigations to demonstrate vital direct traumatic damage of skeletal muscle. Int J Legal Med 104:215–219

Fechner G, Bajanowski T, Brinkmann B (1993) Immunohistochemical alterations after muscle trauma. Int J Legal Med 105: 203–207

Glatz JFC, Kleine AH, Nieuwenhoven FA van, Hermens WT, Dieijen-Visser MP van, Vusse GJ van der (1994) Fatty-acid-binding protein as a plasma marker for the estimation of myocardial infarct size in humans. Br Heart J 71:135–140

Keil W, Yoshida H, Ishiyama I (1984) Untersuchungen zur Wirkung von Elektrizität auf den Myoglobingehalt humaner Herz- und Skelettmuskulatur. Z Rechtsmed 91:185–193

Kleine AH, Glatz JFC, Havenith MG, Nieuwenhoven FA van, Vusse GJ van der, Bosman FT (1993) Immunohistochemical detection of very recent myocardial infarctions in humans with antibodies against heart-type fatty acid-binding protein. Cardiovasc Pathol 2:63–69

Leadbetter S, Wawman HM, Jasani B (1989) Immunocytochemical diagnosis of early myocardial ischaemic/hypoxic damage. Forensic Sci Int 40:171–180

Ortmann C, Brinkmann B (1997) The expression of P-selectin in inflammatory and non-inflammatory lung tissue. Int J Legal Med 110:155–158

Ortmann C, Pfeiffer H, Brinkmann B (2000a) A comparative study on the immunohistochemical detection of early myocardial damage. Int J Legal Med 113:215–220

Ortmann C, Pfeiffer H, Brinkmann B (2000b) Demonstration of myocardial necrosis in advanced putrefaction. Int J Legal Med 114:50–55

Osuna E, Perez-Carceles MD, Alvarez MV, Noguera J, Luna A (1998) Cardiac troponin I (cTn I) and the postmortem diagnosis of myocardial infarction. Int J Legal Med 111:173–176

Thomsen H, Held H (1994) Susceptibility of C5b-$9_{(m)}$ to postmortem changes. Int J Legal Med 106: 291–293

Thomsen H, Held H (1995) Immunohistochemical detection of C5b-$9_{(m)}$ in myocardium: an aid in distinguishing infarction-induced ischemic heart muscle necrosis from other forms of lethal myocardial injury. Forensic Sci 71:87–95