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Immunohistochemical methods as an aid in estimating the time since death

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ABSTRACT

For the estimation of time since death in the early postmortem interval, a large repertoire of valid methods is available. With increasing time since death the estimation of the postmortem interval becomes more imprecise and unreliable because validated methods are missing. Wehner et al. developed a method to estimate the time since death using immunohistochemical staining. Proteins undergo degradation after death and this reduces their stainability. Previous studies have explored the development of a method to estimate the time since death using immunohistochemical staining. A systematic analysis (Wehner et al., 1999–2002) demonstrated that calcitonin can still be stained 4 days, thyreoglobulin 5 days, glucagon 6 days, and insulin 12 days after death. After 12 days, calcitonin and thyroglobulin can no longer be stained, glucagon after 14 days, and insulin after 29 days. The aim of the present study was to test this original data on independent case material. Included in this control study were 105 cases with known time since death (between several hours and 22 days). Pancreatic tissue was stained for insulin and glucagon, and the thyroid gland for thyreoglobulin and calcitonin. The original findings could be generally confirmed, however for calcitonin and thyreoglobulin we observed earlier negative stainings. Altogether immunohistochemistry may still be useful as an additional method for estimating time since death in forensic cases.

1. Introduction

Morphological methods for time since death estimation are thought to be of no practical value in forensic practice. Although a comprehensive evaluation of all histologically, histochemically and electron-microscopically detectable postmortem changes shows that human tissues and organs decompose and break down in a certain sequence, in practice this sequence can be difficult to recognize, or not be seen at all in some cases. Laboratory experiments with artificial autolysis have shown that under precise conditions it is possible to histologically and histochemically establish the course of the first 48 h of this process [1]. Therefore, under laboratory conditions and after a suitable series of tests and controls, the postmortem interval could be determined from the degree of decomposition found in an organism [1].

In forensic practice the influencing factors are extremely diverse and cannot all be taken into account. According to Janssen [1], a determination of histological–histochemical autolysis and time of death estimation for forensic purposes can only be attempted when all possible influential factors are known. Valid evidence indicating the time of death can perhaps be taken from the cardiac musculature, skeletal muscles, kidneys, and liver, but none of this is conclusive. An attempt to estimate the time of death without knowledge of the environmental conditions is entirely futile and forensically unjustifiable, regardless of the combination of histological, histochemical, and biochemical methods being applied [1]. However, despite this assertion being a long-standing doctrine in forensic medicine [1–4], there is some evidence that the application of immunohistochemistry methods may still be useful, even without all the available background data.

Wehner et al. [5–9] tested whether positive immunoreactions to various antigens like insulin, glucagon, thyreoglobulin, or calcitonin correlated with the time since death. The premise is that the tertiary structure of the antigen undergoes postmortem changes, and with increasing postmortem interval the protein denaturation staining efficacy decreases. For example, the colloid and the follicular cells of the thyroid gave a positive immunoreaction for thyreoglobulin until 5 days postmortem, whereas none

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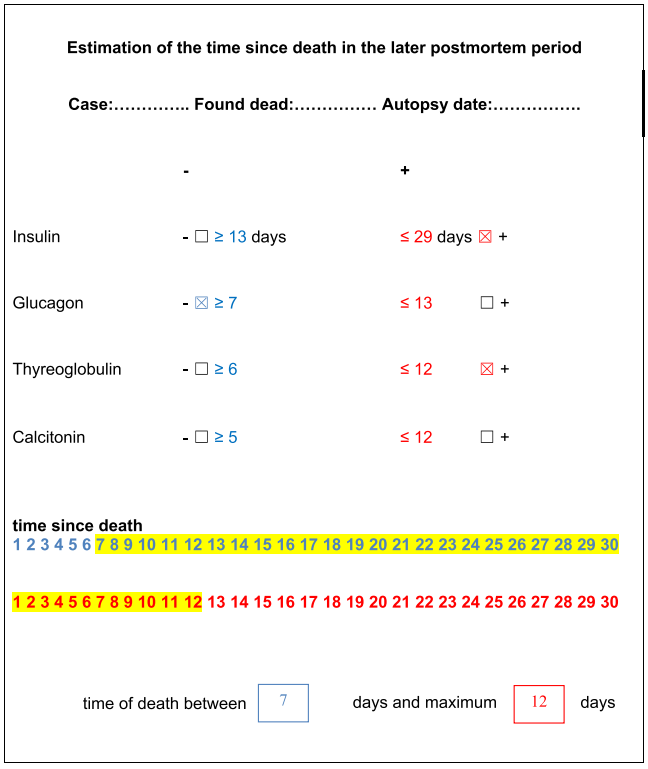

Fig. 1. Positive immunoreaction of insulin: time since death maximal 29 days. Additional investigation of Glucagon with a negative immunoreaction: delimitation of the time since death between 7 and 29 days. Additional investigation of Thyreoglobulin with a positive immunoreaction: further delimitation of the time since death between 7 and 12 days.

of the cases older than 13 days showed such a reaction. This indicates that a negative reaction means more than 6 days postmortem, and a positive reaction means the postmortem interval is less than 12 days.

Likewise, pancreatic β-cells from up to 12-day-old corpses produced a positive immunoreaction towards insulin in all cases, whereas none of the corpses older than 30 days showed such a reaction [5–9]. This means that in a case of negative immunoreaction the time since death can be assumed to be more than 12 days before the autopsy, but a positive stain means that death occurred a maximum of 29 days earlier.

Calcitonin was always detectable in c-cells of the thyroid up to 4 days postmortem, in bodies older than 13 days the staining was always negative.

Meanwhile a chart was developed to give a rough estimation of time since death when using immunohistochemical methods [5] (Fig. 1).

Until now, only the original investigations by Wehner et al. [5–9] have been published. A controlled investigation of the validity of their findings, using independent case material, has not yet been carried out. The aim of the present study was to establish a time course of the immunohistochemical staining of insulin, thyreoglobulin, calcitonin and glucagon with independent case material, and compare this with the original data.

2. Material and methods

One hundred and five bodies were included in the examination, and the case information is summarized in Table 1. The time since death ranged from several hours up to 22 days. Cases with diabetes mellitus or diseases of the thyroid gland were excluded, as were cases with macroscopic pathologies of the pancreas and thyroid

Table 1
Case material and results of the immunohistochemical investigation for 4 antigenes.

Time since death	Place found	Clothing/cover	Time of year/month	Immunoreaction			
				Ins.	Glu.	Thyr.	Cal.
1 Day	Bathtub	Unclothed	May	+	+	+	–
	Home	Light clothing	May	+	+	+	+
	Home	Light clothing	May	+	+	+	+
	Hospital	Clothed	April	+	+	+	+
	Hospital	Light clothed	March	+	+	+	+
	Home	Light clothed	December	+	+	+	–
2 Days	Home	Clothed	July	+	+	+	+
	Hospital	Clothed	June	+	+	+	+
	Meadow	Clothed	June	+	+	+	+
	Carpark	Clothed	March	+	+	+	+
	Bicycle lane	Clothed	March	+	+	+	+
	Street	Clothed	March	+	+	+	+
	Automobile	Clothed	February	+	+	+	+
	Hospital	Clothed	February	+	+	+	–
	Home	Light clothed	February	+	+	+	+
	Home/hospital	Light clothed	February	+	+	+	+
	Street/automobile	Clothed	January	+	+	–	–
	Home/bed	Clothed	January	+	+	+	–
	Home/bed	Blanket	January	+	+	–	–
	Home	Clothed	November	+	+	+	+
	Home	Clothed	November	+	+	+	+
	Hospital	Light clothed	November	+	+	+	+
3 Days	Home	Clothed	July	+	+	+	+
	Hospital	Light clothed	June	+	+	+	+
	Home/garden	Clothed	June	+	+	+	+
	Home	Clothed	June	+	+	+	+
	Home	Clothed	May	+	+	+	+
	Hospital	Light clothed	May	+	+	+	+
	Hospital	Light clothed	May	+	+	+	+
	Hospital	Clothed	May	+	+	+	+
	Hospital	Light clothed	May	+	+	+	+