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Case Report

Estimation of the time since death—Even methods with a low precision may be helpful in forensic casework

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ABSTRACT

Gold standard for the estimation of the time since death in the early postmortem period is the temperature based nomogram method together with time of death dependent criteria of postmortem lividity, rigor mortis and supravital reactions. There is also a huge literature on chemical methods proposed for estimating the time since death which however play obviously no role in forensic practice. Especially the rise of vitreous potassium has been studied intensively. Also immunohistochemical methods have been proposed for estimating the time since death but obviously not yet applied in casework. We present a case where a woman was found murdered 8 days after having been seen last alive. Due to lack of putrefactive changes postmortem interval was thought to be not more than 2 days. However, immunohistochemical stainings and vitreous potassium concentration revealed that time since death was more than 6 days and the woman was obviously murdered immediately after she was seen lastly alive.

1. Introduction

Estimation of the time since death is a daily task in forensic medicine. The gold standard in the early postmortem interval (PMI) is the temperature method developed by Henssge based on the two exponential formula of body cooling by Marshall and Hoare together with data on supravital reactions, postmortem lividity and rigor mortis (so called compound method) [14,15,31,32]. However, dependent on the ambient temperature temperature-based methods and the compound method work only within the first 2–3 days. There is also a huge literature on chemical methods proposed for estimating the time since death which however play obviously no role in forensic practice [42,43]. However, the application of chemical methods differs from country to country.

One of the most investigated analytes is the potassium concentration in vitreous humor (VH) [1–13,16–25,27,28,30–37,41,43–49,55,56]. The linear rise of vitreous potassium over the postmortem interval is as well-known as the different statements concerning the slope and intercept of the regression line. The precision of death time estimation by vitreous potassium was recently compared on a random sample of 600 cases using 5 different formulas [37]. The best results were obtained using equations with "mean" slopes of about 0.17 or 0.19 mmol/l/h. Equations with a steeper or flatter slope reveal already great mean deviations between real and extrapolated time since death and do not allow a reliable death time estimation [37].

Besides vitreous potassium many other analytes have been investigated in VH, for instance hypoxanthine (Hx) [26,34,40,41] and encouraging results have been obtained by combining VH Hx, potassium and ambient temperature [40,41].

Zilg et al. [56] were also successful in developing a new model for estimation of the time since death from vitreous potassium levels corrected for age and temperature [56]. In contrast to other authors they investigated a much longer postmortem interval (pmi) although after a certain amount of time (about 100–120 h) the vitreous gets saturated with potassium and potassium levels ceases to increase. This means that potassium levels around 30 mmol/l can only be used for an estimation of the minimum time of death. According to Zilg et al. [56] also other investigators used the environmental temperature for estimating the time since death [9,30,41,55] which is really an important step in VH and pmi research.

Further studies in the last years investigated variations in VH chemical values as a result of pre-analytical treatment [4,7,49], to detect contaminations of VH samples [8] or used new statistical approaches for estimating pmi [35,36] and new methodology [5,48].

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Beside pmi estimation VH is also used for determining the cause of death and proposals have been made for the postmortem diagnosis of hypertonic dehydration [27] and the interpretation of sodium and chloride levels in VH [57].

A further developing research project, which continues elder research is the protein based approach for death time estimation [39].

Beside vitreous potassium immunohistochemical stainings were also investigated as a suitable marker for estimating the postmortem interval [50–54]. F.i. a systematic analysis 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 thyreoglobulin can no longer be stained, glucagon after 14 days and insulin after 29 days. A recent follow-up study revealed that immunohistochemistry may also be useful as an additional method for estimating the time since death in forensic cases [38]. Again, as for vitreous potassium we know in our country of no case where this method has been applied in forensic casework.

2. Case history

A 36 year old woman was found dead in bushes near a railway line on 20th of October. She was last seen alive 8 days prior at 8.30 pm and was missing since this time. Near the place of discovery her car was found which was standing there the whole time. Ambient temperature at the day of discovery was low (9 °C), rectal temperature 8.4 °C.

The autopsy revealed the following: 36 year old woman. Cyanosis and petechiae of the face and the eyelids. Ligature strangulation mark. Above the ligature strangulation mark a deep incised wound of 12 cm length of the anterior neck (Fig. 1a + b).

No hemorrhages within the deep incised wound.

Rigor mortis in the jaw already resolved, very weak rigor mortis in the elbow, no reestablishment of rigor mortis in the elbow after breaking, persistent rigor mortis in the knee joint.

Postmortem lividity of the back. No disappearance on thumb pressure, incomplete disappearance on sharp pressure.

No signs of putrefaction especially no green discoloration of the abdomen.

Cause of death was ligature strangulation. The incised wound of the neck was produced postmortem. The pathologist attending the scene made a first estimation of the postmortem interval: there are no hints that the PMI is 8 days. After the autopsy the pathologist emphasized again that the signs of death especially the absence of putrefactive changes speak against a time of death already on October 12th. The question of the police was of course: is an earlier time of death possible? A time since death after the October 12th would mean a completely different criminalistic approach: the murderer had the woman in his power for 2 or more days, killed her than and brought her to the place of discovery. The other alternative would be that the murderer killed her immediately and hide her in the bushes.

Two days after the autposy vitreous humor was withdrawn for chemical analysis:

Sodium 128 mmol/l.
Potassium 33.8 mmol/l.
Chloride 96 mmol/l.
Creatinine 0,8 mg/dl.
Urea 35 mg/dl.

3. Immunohistochemical stainings

Immunohistochemical stainings were carried out on thyroid gland and pancreas using routine methods and technology [38,50–54] and these stainings were positive for insulin, glucagon, thyreoglobulin and negative for calcitonin (Table 1,Fig. 2).

Fig. 1. a) Place of discovery, b) ligature strangulation mark an postmortem incised wound.

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Table 1
Immunohistochemical investigations.

Case no.	LS XXX/XX			
	Found dead October 20th 2015		Date of autopsy October 21th 2015	
	Negative		Positive	
Insulin		- ≥13 days	X	≤29 days +
Glucagon		- ≥7 days	X	≤13 days +
Thyreoglobulin		- ≥6 days	X	≤12 days +
Calcitonin	X	- ≥5 days		≤12 days +
Death between minimum		5	and maximum	12 days

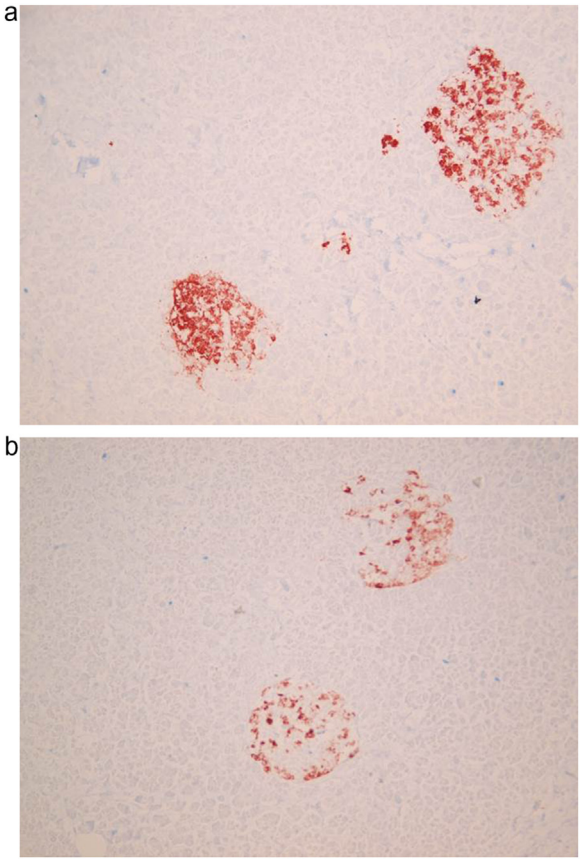

Fig. 2. Positive stainings for insulin and glucagon.
a) Pancreas anti-human insulin 200×. b) Pancreas anti-human glucagon 200×.

From the formulas in Table 2 three were used for estimating the time since death from vitreous potassium. According to the formula of Madea et al. time since death is over 6 days with 95% limits of confidence of ±24 h.

Although Sturner has reported the flattest rise of vitreous potassium in the literature his formula can be used in this case quite well because the ambient temperature was over the whole time very low. According to the temperature profile between 12th and 20th of October the mean ambient temperature was below 8 °C, in some days even below 5 °C. Using the formula from Sturner the time since death is over 7 days.

Zilg et al. developed a formula which takes into account also the ambient temperature for estimating the time since death. With a mean ambient temperature of 5 °C the resulting time since death is between 5.84 days and 67 days, with a mean ambient temperature of 6 °C the time since death is between 8.7 days and 63.5 days. According to these calculations a postmortem interval between time of missing and time of discovery of 8 days is possible. A shorter time since death (for instance 2 days) can even be ruled out.

All equations in Table 2 except the one by Zilg et al. [56] are based on the assumption that the postmortem potassium increase is linear, this may be true for the early postmortem interval (about 100–120 hpm) but as already mentioned with increasing pmi the vitreous gets saturated with potassium. This means that potassium levels about 30 mmol/l can only be used for an estimation of the minimum time since death and this is why the Zilg formula gives a time of death between 6 and 67 days.

When using vitreous potassium for extrapolating the time since death for Central Europe equations with a slope of about 0.19 mmol/l × h and an intercept of 5.88 mmol/l reveal an estimation with no systematic deviations. If there was a deep ambient temperature like in this case, even equations with a very flat slope may be advantageous. As in the present case different

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Table 2
Equations for the rise of potassium in potassium vitreous (from Zilg et al. [56]).

Authors (year)	Equation (h)	n	Max PMI (h)	Comments
Adelson et al. (1963)	PMI = 5.88 [K+] – 31.53	209	21	–
Sturner & Gantner	PMI = 7.14 [K+] – 39.1	125	104	–
Hanson et al. (1966)	PMI = 5,88 [K+] – 47.1	203	310	–
Coe (1969)	PMI = 6.15 [K+] – 38.1	145	100	A separate equation was provided for a PMI < 6 h.
Stephens & Richards (1987)	PMI = 4.20 [K+] – 26.65	1427	35	Outliers, drownings, SIDS, electrolyte imbalances, and temperature extremes were excluded.
Madea et al. (1989)	PMI = 5.26 [K+] – 30.9	107	130	Cases involving elevated urea and prolonged agony were excluded.
James et al. (1997)	PMI = 4.32 [K+] – 18.35	100	80	Also included hypoxanthine.
Munoz et al. (2001)	PMI = 3.92 [K+] – 19.04	133	40	Only non-hospital cases were examined, there was a change in variables.
Zhou et al. (2007)	PMI = 5.88 [K+] – 32.71	62	27	–
Jashnani et al. (2010)	PMI = 1.076 [K+] – 2.81	120	50	Mostly included cases involving sepsis or tuberculosis.
Bortolotti et al. (2011)	PMI = 5.77[K+] – 13.28	164	110	–
Mihailovic et al. (2012)	PMI = 2.749 [K+] – 11.98	32	30	Repetitive sampling.
Siddamsetty et al. (2013)	PMI = 4.701 [K+] – 29.06	210	170	–
Zilg et al. (2015)	$PMI - \frac{\ln\left(\frac{M-C_0}{M-[K^+]}\right)}{L_0 m_A A + m_T T}$	462	409	No cases were excluded. The proposed equation includes temperature and patient age.

formulas should be used, especially the formula by Zilg et al. [56] taking into account the ambient temperature.

Unfortunatelly Hx was not determined and skeletal muscle was not available for protein based death time estimation [39]. It would have been interesting to have these results also for comparison.

The immunohistochemical detection of antigens allows only a very rough estimation of the time since death which may however be helpful in single cases. The philosophy behind these investigations is that with an increasing postmortem interval, the tertiary structure of the antigen undergoes postmortem changes and as a result of protein denaturation, stainings become negative. However, the time limits may change in different environmental conditions and further control studies on independent case material in different ambient temperatures are necessary [29,30,32,38]. Nevertheless, together with vitreous potassium in the present case a short postmortem interval could be ruled out.

4. Conclusions

In this case even methods with a low precision of death time estimation were helpful to avoid a miscarriage of justice.

The husband of the victim was identified as perpetrator and accused for homicide. He committed suicide in jail during the trial.

CRediT authorship contribution statement

Burkhard Madea: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Jan Ortmann:** Formal analysis. **Elke Doberentz:** Formal analysis.

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