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PRECISION OF ESTIMATING TIME OF DEATH BY VITREOUS POTASSIUM — COMPARISON OF VARIOUS EQUATIONS

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Summary

This paper is a study of the precision of estimating the time since death comparing the equations developed by different authors. Our aim is to determine with the maximum degree of accuracy the exact time of death of the individual. We consider that the study has been fully justified by the observed differences in the results obtained from the different equations under study when the concentration of potassium in the vitreous humour was identical.

Key words: Vitreous potassium; Time of death; Mathematical equations; Postmortem interval

Introduction

The increase in the concentration of vitreous potassium during the postmortem period has been one of the most widely studied biochemical parameters in recent years, for its role in estimating the time since death. Nevertheless, the conclusions reached by different authors concerning the accuracy of this parameter in estimating the postmortem interval do not totally coincide.

It would seem likely that such differences arise in part from the influence of certain factors such as method of sampling, instrumentation employed [1], environmental temperature [1–4], duration of terminal episode [3–5] and age of the individual [1]. The efficiency of the method under study will largely depend on the correct assessment of these factors.

Materials and Methods

This paper is a comparative study to assess the accuracy of estimating the time since death, using equations proposed by different authors (Table 1). Its aim is to establish as closely as possible the moment when death took place.

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TABLE 1

RELATION OF STUDIED EQUATIONS

Authors	*Proposed equations*
1. Sturner [6]	$y = 0.14x + 5.6$
2. Adelson et al. [5]	$y = 0.17x + 5.36$
3. Hansson et al. [7]	$y = 0.17x + 8$
4. Coe [8]	$y = 0.1625x + 6.19$
5. Adjutantis and Coutselinis. [9]	$y = 0.55x + 3.4$
6. Cespedes et al. [10]	$y = 0.2065x + 5.8973$
7. Umani et al. [11]	$y = 0.2x + 4.3$
8. Stephens and Richards. [12]	$y = 0.238x + 6.342$
9. Montalto et al. [13]	$y = 0.1652x + 6.805$
10. Madea et al. [2]	$y = 0.19x + 5.88$
11. Gamero et al. [14]	$y = 0.205x + 5.592$

In order to undertake the above-mentioned comparative study we have employed a pilot sample made up of 56 vitreous humours withdrawn from 56 human corpses obtained from the city morgue. These 56 vitreous humours were withdrawn from different deceased persons to those used to obtain the equation formulated by us in an earlier work. The postmortem times in which they were withdrawn were also different.

The samples used in this present study were withdrawn from deceased persons in whom the duration of the terminal episode was brief, who were exposed to environmental temperatures generally less than 10°C and with urea below 100 mg/dl [2]. The vitreous humour was withdrawn at postmortem times of less than 24 h in all cases. Extraction was carried out via perforation of the conjunctivae with sterile disposable syringes. Duly labelled, the samples were stored in polypropylene tubes, sealed hermetically and frozen at −25°C until studied.

Initial preparation of the sample, once defrosted, consisted of centrifugation of the vitreous humour, using for this purpose the liquid portion of the supernatant [9], whose potassium concentration was determined by flame photometry (Instrumentation Laboratory IL243), measurements being carried out at a wavelength of 766 nm after calibrating according to manufacturer's standards.

To give more weight to the asepsis of our approach we carried out a parallel study using an unrelated sample (Umani's sample) [11]. From this sample we thoroughly selected all those samples whose vitreous humours were extracted in a postmortem time of less than 24 h and withdrawn from corpses of persons in whom the duration of terminal episode was brief. Special attention was paid to the cause of death in each case. The subsample chosen in this way consisted of 20 cases.

From the two groups we obtained sample parameters that enabled us to establish comparative criteria for the accuracy in estimating the time of death obtained from equations developed by different authors.

Specifically, for each of the two sample groups, we calculated differences: $d_i = x_i - \hat{x}_i$, where x_i is real time postmortem (sampling observation) and $\hat{x}_i$ is extrapolated time since death calculated from regression lines formulated by different authors. Namely, if the formula was $y = ax + b$ ($y = [K^+]$ at mEquiv./l; x = time postmortem in hours), for each sample observed (x_i,y_i) the value for the extrapolated time postmortem: $\hat{x}_i = (y_i - b)/a$.

As a measure of the degree of adjustment between the different equations, we used Σd_i^2, summatory, which in the first sampling reached a total of 56 and in the second, 20. The values for this parameter were obviously lower when the slope was closer to the data from the samples used.

We also calculated mean differences ($\bar{d} = \Sigma d_i/n$), as well as standard deviations S.D. = $[\Sigma(d_i - \bar{d})^2/n]^{1/2}$; these parameters allowing us a more detailed analysis of the results obtained.

Results and Discussion

It must be made clear that the fact that we limit ourselves to those cases whose postmortem time is less than 24 h, as far as our pilot sample and Umani's sample [11] are concerned, is justified because when the corresponding residual plots are analyzed we observe a fan shaped structure. This indicates a tendency for the variance of the $[K^+]$ to increase as the postmortem time increases. This would cast doubts on the validity of the lineal model used by all the authors as regards the homocedasticity hypothesis.

The difficulties involved in statistically contrasting the hypothesis of homocedasticity lie in the fact that the independent variant has not been considered fixed. This is due to the problems that would arise in the taking of the

TABLE 2

STATISTICAL ANALYSIS OF THE DIFFERENCES BETWEEN ACTUAL TIME POSTMORTEM AND THE EXTRAPOLATED TIME USING THE SUBSAMPLE OF UMANI ET AL. [11] AND THE PROPOSED EQUATIONS OF DIFFERENTS AUTHORS

Authors	*Parameters*		
	Σdi^2	$\bar{d}$	*S.D.*
Sturner [6]	2423.922	-6.374143	8.975879
Adelson et al. [5]	1335.679	-4.105233	7.066189
Hansson et al. [7]	3608.857	11.42418	7.066186
Coe [8]	1112.014	0.144539	7.455186
Adjutantis and Coutselinis [9]	1089.157	5.173909	5.261987
Cespedes et al. [10]	725.919	1.782272	5.754951
Umani et al. [11]	1579.155	-6.616999	5.930691
Stephens Richards [12]	1099.865	5.331741	5.154201
Montalto et al. [13]	1404.997	4.101644	7.309334
Madea et al. [2]	786.8871	0.5882641	6.244863
Gamero et al. [14]	672.1311	0.200073	5.793662

sample. The extractions are not carried out by fixed values of the independent variable x = postmortem time, as advised by the statistical theory, but the values of $[K^+]$ have been observed for random values of x.

This methodology casts no doubts on the validity of the model but it does obstruct the carrying out of a statistical contrast of the hypothesis of homocedasticity. In order not to weaken this hypothesis, we have limited our studies to a postmortem period of less than 24 h and we consider it risky to draw conclusions for longer intervals.

Based on the completed comparative study, Table 2 shows the results obtained from the subsample selected from the work of Umani et al. [11], while Table 3 reflects the results corresponding to the 56 coroners' cases chosen by us.

The results observed with our samples and the samples of Umani et al. [11], demonstrate that the equations developed by Gamero et al. [14]; Cespedes et al. [10]; Madea et al. [2] and Adjutantis and Coutselinis [9], are, in the order stated, those that reveal a straight line closest to the values obtained in both samples. The formulas developed by Sturner [6] and Hansson et al. [7], reveal straight lines that were the furthest from the values obtained from these samples.

With respect to the first four equations mentioned in the preceding paragraph, it should be pointed out that in the case of the equation formulated by Cespedes et al. [10], there is a certain tendency toward underestimation in the extrapolated times (Fig. 1), while with the Adjutantis and Coutselinis equation [9] (Fig. 2), underestimation becomes systematic, as is seen by the fact that the mean differences ($\bar{d}$) give positive values (1.78 and 5.17 in Table 2 and 1.34 and 5.42 in Table 3, respectively). This is not shown by the equations of Madea et al. [2] (Fig. 4) and Gamero et al. [14] (Fig. 5).

TABLE 3

STATISTICAL ANALYSIS OF THE DIFFERENCES BETWEEN ACTUAL TIME POSTMORTEM AND THE EXTRAPOLATED TIME USING OUR SAMPLE AND THE PROPOSED EQUATIONS OF DIFFERENTS AUTHORS

Authors	*Parameters*		
	Σdi^2	$\bar{d}$	*S.D.*
Sturner [6]	7488.152	−7.339925	8.935465
Adelson et al. [5]	4303.807	−4.78335	7.346648
Hansson et al. [7]	9489.261	10.74606	7.346648
Coe [8]	3321.769	−0.5955361	7.678712
Adjutantis and Coutselinis [9]	2627.558	5.423295	4.184322
Cespedes et al. [10]	2218.853	1.341439	6.150034
Umani et al. [11]	5056.106	−7.09375	6.321893
Stephens and Richards [12]	3112.32	5.037177	5.495815
Montalto et al. [13]	3837.79	3.384522	7.55493
Madea et al. [2]	2452.409	0.05145696	6.617429
Gamero et al. [14]	2148.05	−0.2488461	6.617429

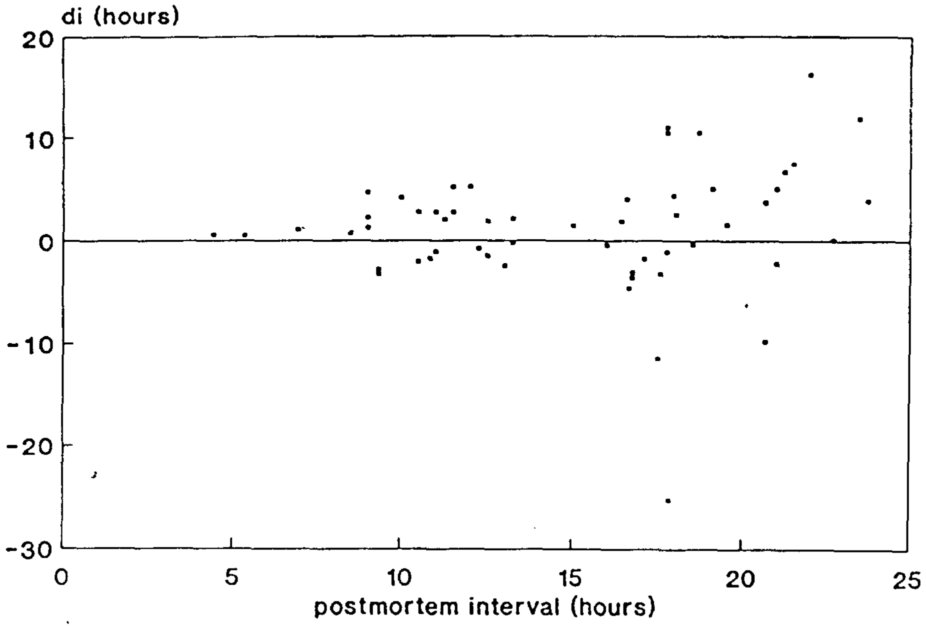

Fig. 1. Deviations between real and extrapolated time since death (di in hours). Deviations using the Cespedes et al.-equation [10].

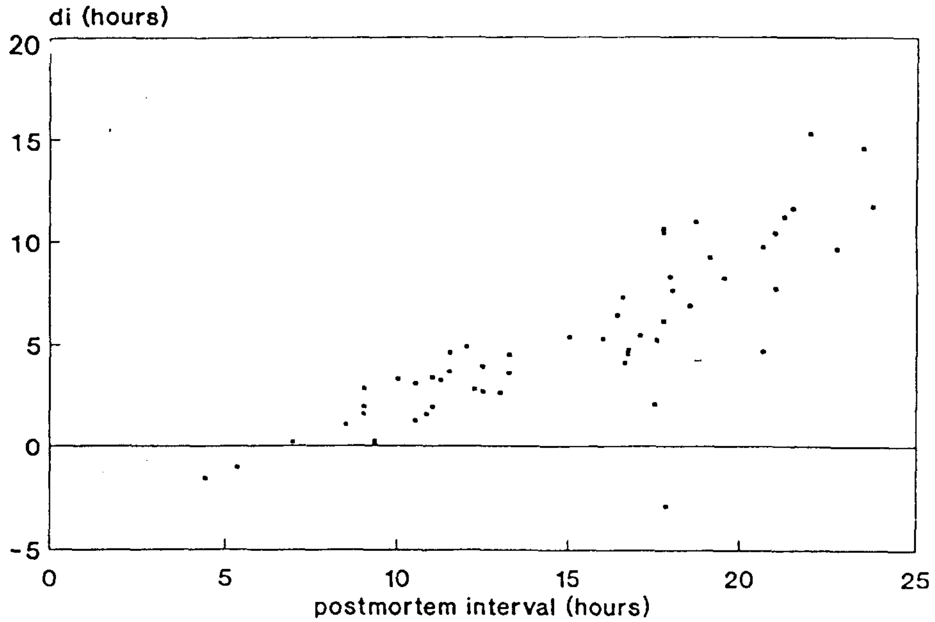

Fig. 2. Deviations between real and extrapolated time since death (di in hours). Deviations using the Adjutantis and Coutselinis-equation [9].

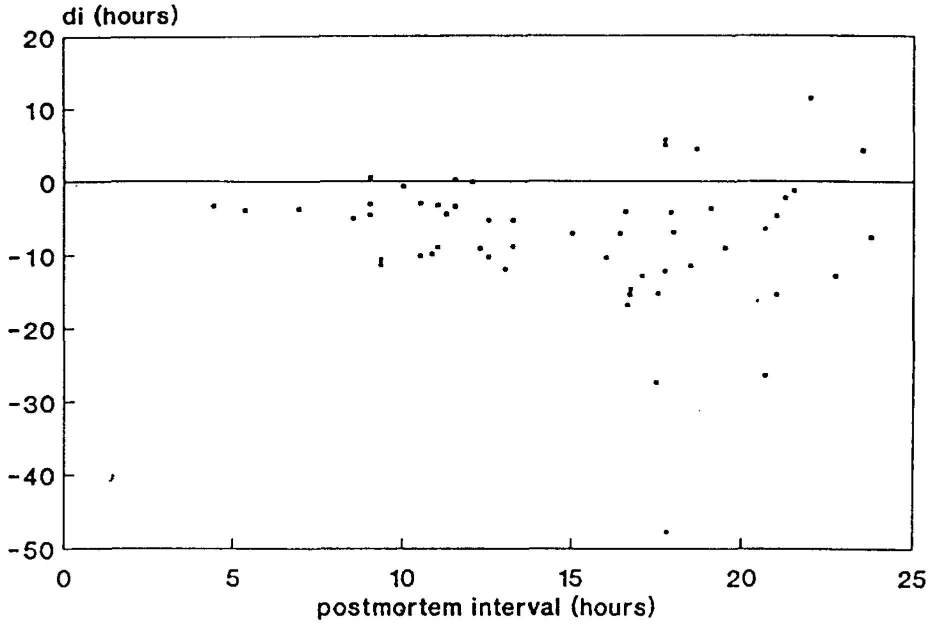

Fig. 3. Deviations between real and extrapolated time since death (di in hours). Deviations using the Sturner-equation [6].

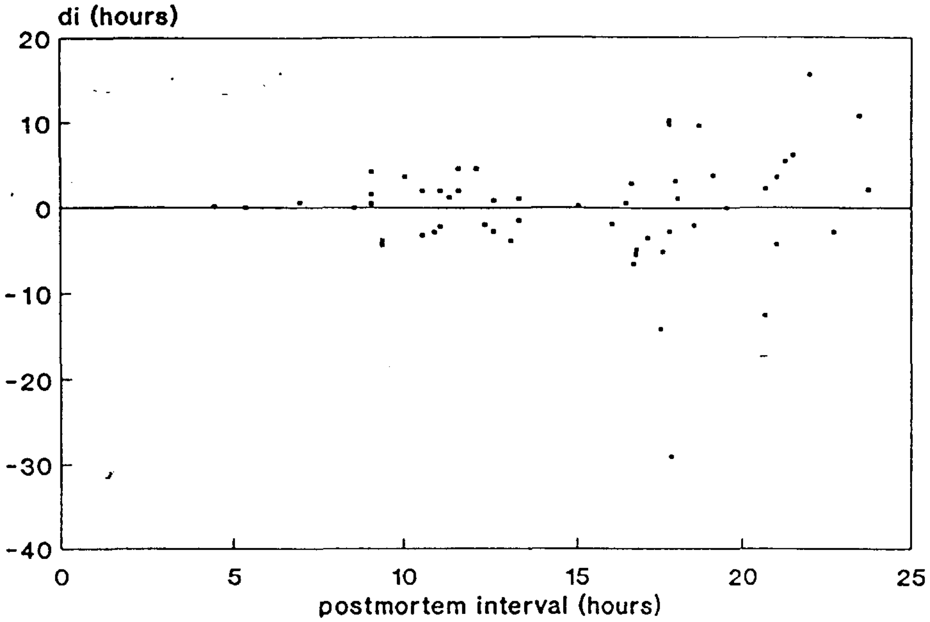

Fig. 4. Deviations between real and extrapolated time since death (di in hours). Deviations using the Madea et al.-equation [2].

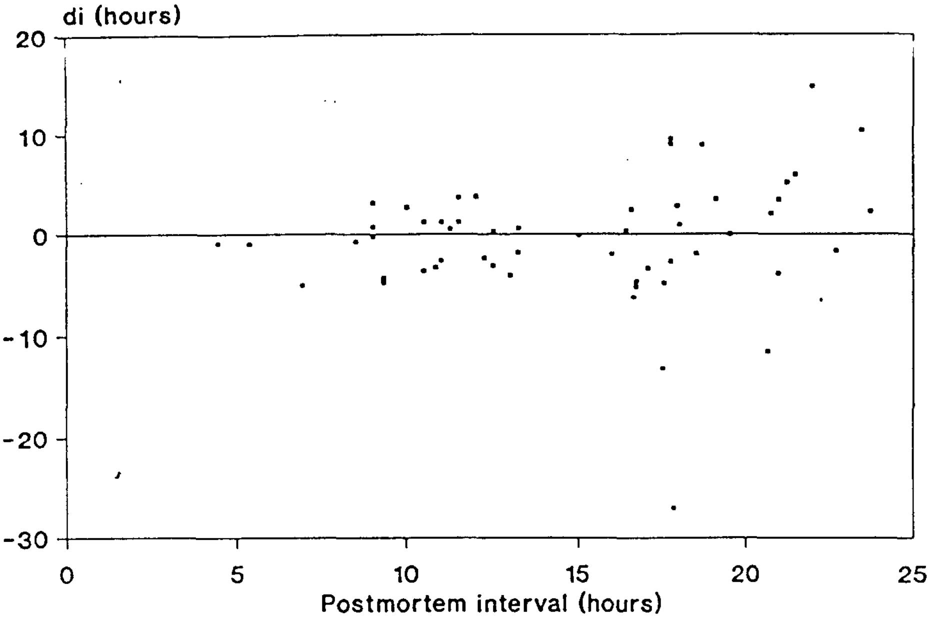

Fig. 5. Deviations between real and extrapolated time since death (di in hours). Deviations using the Gamero et al.-equation [14].

Conversely, of the four equations quoted, the one developed by Adjutantis and Coutselinis [9] which shows the lowest values for the standard deviation (S.D.) confirms the fact mentioned earlier, namely that it causes systematic underestimation of the postmortem interval. As is seen in Fig. 2, the differences (d_i) are small and systematically positive. However, in their favour it should be mentioned that these authors [9] were working with a range of values of only up to 12 h, while the samples used in this study have longer time intervals.

Likewise, with respect to the 'unfavorable' results in this comparative study from the equation developed by Umani et al. [11], it should be pointed out that such a circumstance — also observed when working with the subsample group used by the same authors — could be justified by the fact that the range of values of postmortem intervals in Umani et al.'s sample [11] is much greater than 24 h and also that some cases of prolonged terminal episode are present.

With respect to the regression lines that showed the least accuracy, our study shows that the Sturner formula gave rise to systematic overestimation of time since death (Fig. 3). This has already been pointed out by Madea et al. [3]. The equation developed by Hansson et al. [7] causes systematic underestimation, as can seen from the mean differences (Table 3).

Finally, we would point out that if a further study of a more inferential nature were undertaken, it would show that many of the different regression lines obtained are all estimations of the same population regression line and are therefore equivalent from a statistical viewpoint. As it is not possible to carry out an inferential statistical study (as we don't know the sample data that gave rise to the equations of the respective authors), we will use a heuristic method that will allow us to reach similar conclusions. To do this, confidence intervals of 95% for the parameters α and β of the population regression line will be obtained from our pilot sample

$y = \alpha x + \beta$, with $y = [K^+]$ in mEquiv./l

x = postmortem interval in hours

Since the coefficients a and b in the regression lines given by the different authors are the respective estimates of the parameters α and β in each equation, it would then seem reasonable to consider equivalent all those equations whose coefficients a and b belong to the confidence interval given for α and β, respectively.

The 95% confidence intervals are expressed as follows [15]:

$$\text{for } \alpha : a \pm t_{0.975;\,n-2} \cdot \frac{S_R}{n^{1/2} \cdot S_x}$$

$$\text{for } \beta : b \pm t_{0.975;\,n-2} \cdot \frac{S_R}{n^{1/2}} \cdot [1 + (x/S_x)^2]^{1/2}$$

where:

*a and b are the coefficients of the regression lines that we obtain with our samplings.

*$t_{0.975;n-2}$ is the 97.5 percentile of the t distribution with n−2 grades of freedom, that for n = 56 is 2.005.

$$*\bar{x} = \sum_i x_i / n$$

$$*S_x = \left[\sum_i (x_i - x)^2 / n \right]^{1/2}$$

$$*S_R = \left[\sum_i (y_i - ax_i - b)^2 / (n-2) \right]^{1/2}$$

We obtain the confidence interval of 95% for the parameters α and β from the sample statistics that correspond to our pilot sample (Table 4).

If we compare the corresponding values for a and b obtained by the different authors (Table 1), we can see that the equations of Sturner [6], Adelson et al. [5], Coe [8], Cespedes et al. [10], Montalto et al. [13], Madea et al. [2] and Gamero et al. [14] all show that the coefficient 'a' belongs to the confident interval obtain-

TABLE 4

STATISTICAL DATA OBTAINED FOR OUR SAMPLE

Confidence Interval for α (95%) = (0.0902, 0.2268); confidence interval for β (95%) = (5.2585,7.4367)

n	56
$\bar{x}$	15.1477
S_x	4.9844
S_R	1.2705
a	0.1585
b	6.3476

ed for 'α' and that the coefficient 'b' belongs to the confidence interval obtained for 'β'. As a result, we may consider all these equations to be equivalent from a statistical point of view; therefore it seems reasonable to state that they may all be used indistinguishably.

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