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Research Paper

Transitioning from conventional to digital methods for estimating time since death: a multi-parameter forensic software

Roberto Scendoni [a,*], Luca Tomassini [b,**], Giovanni Bianchini [c], Laura Baldelli [d], Piergiorgio Fedeli [e], Mariano Cingolani [a]

[a] Department of Law, Institute of Legal Medicine, University of Macerata, Macerata, Italy
[b] School of Advanced Studies, University of Camerino, Camerino, Italy
[c] External Consultant Forensic Medicine Laboratory, Spin-off University of Macerata, Macerata, Italy
[d] University of Granada, Granada, Spain
[e] School of Law, Legal Medicine, University of Camerino, Camerino, Italy

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ABSTRACT

Introduction: In forensic pathology, estimating the postmortem interval (PMI) is crucial for legal investigations, particularly in homicide cases. Traditional methods for determining the time since death offer only estimations, and formulas related to solute concentrations in vitreous humor vary, complicating the process.
To present a novel software solution that integrates body temperature data and solute concentrations in the vitreous humor (potassium, albumin, hypoxanthine, and urea).
Methods: The software combines the Henssge method with current knowledge of solute concentration evolution in the vitreous humor post-death. Formulas derived from the scientific literature were implemented in a Java-based program. The program features a intuitive interface and is compatible with Windows and MacOS. Functional and non-functional requirements were addressed, ensuring quick data input, low resource consumption, and easy interpretation of results.
Results: The software provides more precise PMI estimations by unifying the Henssge method with regression equations from existing literature. This integration enhances usability for practical applications and academic research.
Conclusion: This study introduces a cohesive software solution that integrates traditional and modern methodologies for PMI estimation. The program's comprehensive approach and intuitive design promise to improve accuracy and facilitate broader application in forensic investigations.

1. Introduction

It is common practice in forensic pathology for an examiner to be called upon to estimate a postmortem interval (PMI), as this data has significant implications in legal investigations. Consider, for example, homicide cases where it is important to determine when the crime occurred.

It should be emphasized that classic parameters for determining the time since death – postmortem changes such as rigor mortis, livor mortis, algor mortis, and putrefactive phenomena – can only provide approximate estimates of the time since death.[1]

These classical parameters are subject to considerable variability: rigor mortis may appear in less than an hour or only after many hours, and in some cases may fail to develop altogether (e.g., in infants or cachectic individuals). Environmental temperature markedly alters the timing: severe cold can suspend rigidity for several days, whereas heat may compress the entire cycle to less than 24 h. Comparable uncertainties apply to livor mortis and algor mortis, which are likewise influenced by environmental and individual factors.[2]

Besides traditional techniques for estimating the time since death, research in the field of thanatocronology has explored new approaches, but these have been found to involve numerous intrinsic variables that

* Corresponding author.
** Corresponding author.
E-mail addresses: r.scendoni@unimc.it (R. Scendoni), luca.tomassini@unicam.it (L. Tomassini), giovanni.biankini@gmail.com (G. Bianchini), laura.baldelli94@gmail.com (L. Baldelli), piergiorgio.fedeli@unicam.it (P. Fedeli), mariano.cingolani@unimc.it (M. Cingolani).

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are beyond the examiner's control; indeed, the time since death should be represented as an interval between two values, which can currently only be estimated.[3]

Regarding the specific tools employed, the use of cadaveric temperature for PMI estimation has been studied extensively. This method is based on the use of mathematical calculations to estimate the time since death, building upon the well-known and extensive research conducted by Claus Henssge. However, this temperature-based methodology still presents unresolved limitations, resulting in only approximate reconstructions of the time since death due to the multitude of variables involved[4–7]

In addition to cadaveric temperature, biochemical parameters have been studied over the years with the aim of achieving more precise PMI estimation. Extensive efforts in the specialized literature have focused on the measurement of solutes within the vitreous humor, which has been proposed as a promising method for estimating the time since death because of the postmortem processes that lead to increased permeability of the blood-retinal barrier.[8,9]

Biochemical parameters also present intrinsic limitations: potassium concentration, although it generally shows a linear increase, is influenced by the analytical method, temperature, pre-mortem clinical conditions, and differences between the two eyes. Reported error ranges vary from a few hours to more than a day, thereby reducing the reliability of this method as a routine tool.[2]

Despite thorough investigations of this method, particularly concerning the temporal variations in potassium (K+) concentrations, limitations persist, mainly due to the high variability of results, influenced by concentration estimation techniques and individual factors.

These challenges have prevented the scientific community from widely accepting the method as a routine tool; it therefore remains a controversial procedure despite the publication of numerous reports on its efficacy.

The real conditions of forensic cases further complicate the application of existing methods: bodies found outdoors in extreme climates, submerged in water, or affected by severe terminal metabolic disturbances render any PMI estimate highly uncertain if based on a single parameter. In such scenarios, the postmortem interval must be expressed as a range rather than as a single value.[2]

Another crucial element to consider is the cause of death itself, which significantly affects postmortem changes and thus the accuracy of PMI estimation. Deaths from asphyxia, poisoning, drowning, hypothermia, or severe infections may modify the onset and progression of rigor mortis, lividity, and temperature loss, as well as biochemical parameters such as vitreous humor electrolyte concentrations. Similarly, sudden cardiac deaths or massive trauma can alter metabolic status at the time of death, producing deviations from expected postmortem patterns. Forensic literature emphasizes that any attempt to estimate the time since death must therefore be interpreted in light of the specific cause of death and its pathophysiological consequences, as ignoring this factor may lead to serious miscalculations.[2,3,10]

Given the limitations of current methodologies, modern digital systems appear to give fresh impetus to the study of techniques for estimating the time since death. Their ability to automatically process large amounts of data make it possible to tackle the significant variability in both new and existing methodologies, including vitreous humor analysis.[11,12]

The need for an innovative approach arises from the increasing complexity and variability in causes of death, as well as the demand for more precise and reliable results. While traditional methods remain valid, they can be limited by subjective factors or by a lack of complete data. New computer systems that can relate to existing methodologies must be implemented and validated before a hypothetical court.

The aim of this study is to integrate the Henssge method with regression formulas describing the relationship between vitreous solutes and postmortem interval (PMI), as reported in the scientific literature, into a single computational tool. By combining these independent approaches, the software not only facilitates use in both practical and research settings but also enhances the reliability of PMI estimation. The convergence between temperature-based and biochemical models provides an internal form of validation within each calculation, strengthening the robustness of the inferred PMI compared with single-parameter methods.

2. Materials and methods

The application is based on formulas (linear regressions) derived from the scientific literature regarding the use of the vitreous humor for estimating the time since death, using the search string " (postmortem interval) OR (thanatochemistry) OR (time since death) OR (time of death) AND (vitreous humor) AND (potassium) OR (ions) OR (postmortem biomarkers) OR (hypoxanthine) OR (albumin) OR (urea) OR (ammonium) AND (regression analysis)" on the PubMed platform.

The references of the selected documents were checked to identify any additional relevant works that were not included in the results of the search string. The search yielded a total of 28 formulas, including research conducted on major systematic reviews[13,14]

For each formula, the following information was recorded: author; year; affiliated journal; geographical area of the subjects; temporal range of the formula; the analytes taken into account (K+, Hx, U, and Alb), with potassium being considered as the primary analyte; the method of chemical analysis; and a summary of the exclusion criteria for each study. The formulas with their respective specifications are summarized in Table 1 [15–41].

Regarding the use of temperature, we mainly referred to the scientific work of Henssge, which forms the basis of the Henssge nomogram, and the various correction factors associated with it.[1,3,5,43,6,42]

For the extraction of data relevant to the development of the software, we referred to the graph related to the Henssge nomogram for both ambient temperatures up to 23 °C and ambient temperatures above 23 °C.

The graph was extrapolated from the website address http://www.rechtsmedizin.uni-bonn.de/dienstleistungen/for_Med/todeszeit (Fig. 1).

The Henssge formulas, where PMI is denoted by the variable t, are as follows and the choice is related to the value of T_e with respect to $23^{\circ}C$. In particular, if $T_e \leq 23^{\circ}C$, then

$$\frac{T_b - T_e}{37.2 - T_e} = 1.25e^{Bt} - 0.25e^{5Bt},$$

where $B = \left(-1.2815\left(Kg^{-0.625}\right) + 0.0284\right)$. While, if $T_e > 23^{\circ}C$

$$\frac{T_b - T_e}{37.2 - T_e} = 1.11e^{Bt} - 0.11e^{10Bt},$$

in which T_e is ambient temperature and T_b denotes rectal temperature.

In order to extrapolate the PMI value, we used an iterative procedure. In particular, let

$$f_1(t) = \frac{T_b - T_e}{37.2 - T_e} - 1.25e^{Bt} + 0.25e^{5Bt}$$

$$f_2(t) = \frac{T_b - T_e}{37.2 - T_e} - 1.11e^{Bt} + 0.11e^{10Bt}$$

and fix the starting time t_0 and the acceptable error ε. If $f_1(t_0) < \varepsilon$ (or $f_2(t) < \varepsilon$, according to the value of T_e with respect to $23^{\circ}C$) then the algorithm will give t_0 as output since the Henssge formula is very close to be satisfied. While, if $f_1(t_0) \geq \varepsilon$ (or $f_2(t) \geq \varepsilon$), then the cycle should be repeated reducing or enlarging t_0, according to the sign of $f_1(t_0)$, until founding an acceptable time. For the detailed steps of the algorithm see Subsection ***Functioning Logic***. Note that the calculations are mathematically correct since f_1, f_2 are both monotone.

Regarding the formulas related to PMI estimation shown in Table 1, the difference between the expressions should be noted; in most cases,

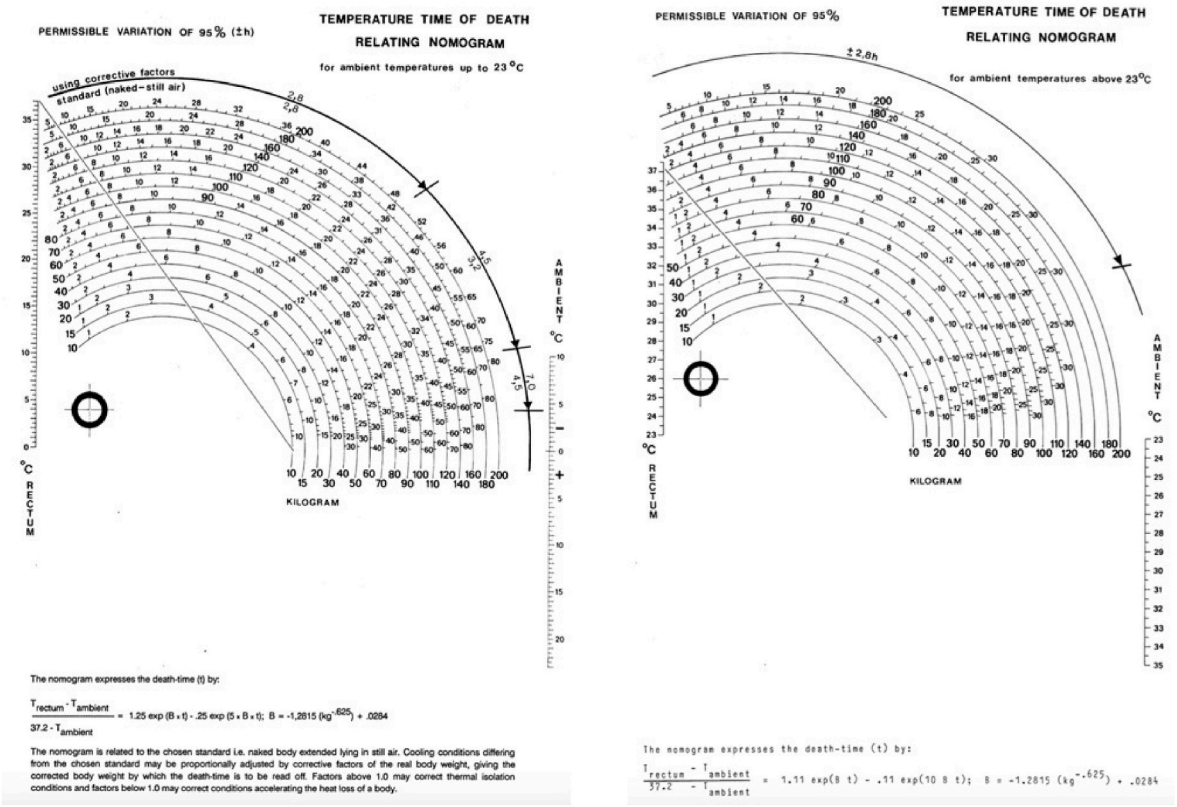

Fig. 1. Specifically, two graphs extracted from the website of the University of Bonn depict the two nomograms along with their associated formulas. Both the provided formulas and the graphical representation have been utilized for programming purposes.

Table 1
Time to reach thermal equilibrium of the cadaver according to weight ranges, based on the graphical formulas of Henssge's monogram.

Ambient temperature up to 23 °C (included)		Ambient temperature above 23 °C	
Weight	Maximum time to reach cadaveric thermal equilibrium.	**Weight**	Maximum time to reach cadaveric thermal equilibrium.
10 - 15 Kg	10 h	**10 Kg**	6 h
20 Kg	15 h	**15 Kg**	8 h
30 Kg	20 h	**20 Kg**	10 h
40–50 Kg	30 h	**30 Kg**	12 h
60–70 Kg	40 h	**40 Kg**	16 h
80–90 Kg	50 h	**50 Kg**	18 h
100–110 Kg	60 h	**70–80 Kg**	25 h
120 Kg	70 h	**90–200 Kg**	30 h
140–200 Kg	80 h		

these have been included without the need for specific procedures. However, the situation is different when it comes to the expressions extrapolated from the studies by Cordeiro (2018) and Palacio et al. (2020).[15,16]

Palacio et al. described the correlation between PMI and ammonium NH_4^+ and potassium K^+ ions with the following polynomial equations:

$$NH_4^+ = 0.000002t^2 + 0.0127t + 0.1461$$

$$K^+ = -0.0005t^2 + 0.2018t + 6.173.$$

In both equations t denotes the PMI.

Since we were looking for PMI by using the concentration of both ions, we had to invert the functions described above. By using the general formula $y = at^2 + bt + c$, we found the following two inverse functions according to the value of y, namely

$$t = \frac{-b + \sqrt{b^2 - 4a(c-y)}}{2a} \text{ or } t = \frac{-b - \sqrt{b^2 - 4a(c-y)}}{2a}.$$

First, we considered the concentration of ammonium, for which $a = 0.000002, b = 0.0127, c = 0.1461$. If $NH_4^+ \geq 0.1461$, then PMI could be found as follows:

$$t = \frac{-0.0127 + \sqrt{0.00016129 - 0.000008(0.1461 - NH)}}{0.000004}.$$

However, if $0 \leq NH_4^+ \leq 0.1461$ the formula lost its significance.

With regard to the concentration of potassium, for which $a = -0.0005, b = 0.2018, c = 6.173$, if $0 \leq K^+ \leq 6.173$, then

$$t = \frac{0.2018 + \sqrt{0.04072324 + 0.002(6.173 - K^+)}}{0.001}$$

and we know that $403.6 \leq t \leq 432.16762$. If $6.173 \leq K^+ \leq 26.53462$ and $0 \leq t \leq 201.8$, we obtained

$$t = \frac{0.2018 - \sqrt{0.04072324 + 0.002(6.173 - K^+)}}{0.001}.$$

If $6.173 \leq K^+ \leq 26.53462$ and $201.8 \leq t \leq 403.6$, we obtained

$$t = \frac{0.2018 + \sqrt{0.04072324 + 0.002(6.173 - K^+)}}{0.001}.$$

Finally, if $K^+ \geq 26.53462$ the formula lost its significance.

In what follows, we report Model 3 in Cordeiro et al., precisely:

$$log(PMI) = \alpha + f_1(T_{rectal}) + f_2(T_{ambient}) + f_3(H_x) + f_4(K^+) + f_5(U) + f_6(W)$$

where α is the usual Y-intercept, the predicted value of Y, which is *log* (*PMI*) in our case, when the sum of all f_i is 0. Thus, considering the value in Table S2 in Cordeiro et al., we obtained

$$\propto = log_e(mean(PMI)) = log_e(6.81) = 1.92.$$

The functions $f_1, .., f_6$ are the partial effect of the i-variable and have zero mean since they are translated with respect to the means, by Cordeiro et al., of T_{rectal}, $T_{ambient}$, H_x, K^+, U and U respectively. So, we could define them as follows:

$$f_1(T_{rectal}) = (T_{rectal} - mean(T_{rectal})) \,/\, (T_{rectal}) = (T_{rectal} - 32.93) \,/\, T_{rectal}$$

$$f_2(T_{ambient}) = (T_{ambient} - mean(T_{ambien})) \,/\, (T_{ambien}) = (T_{ambien} - 18.54) \,/\, T_{ambient}$$

$$f_3(H_x) = (H_x - mean(H_x)) \,/\, (H_x) = (H_x - 70.53) \,/\, H_x$$

$$f_4(K^+) = (K^+ - mean(K^+)) \,/\, (K^+) = (K^+ - 6.86) \,/\, K^+$$

$$f_5(U) = (U - mean(U)) / (U) = (U - 42.39) / U$$

$$f_6(W) = (W - mean(W)) / (W) = (W - 75.20) / W$$

In view of the discussion above, we rewrote the formula of *PMI* as

$$PMI = e^{\alpha + f_1(T_{rectal}) + f_2(T_{ambient}) + f_3(H_x) + f_4(K^+) + f_5(U) + f_6(W)}$$

and the prediction interval for PMI could be found by adding and subtracting ε, the standard error defined by $\varepsilon = 0.2381$:

$$\left[e^{\alpha + f_1(T_{rectal}) + f_2(T_{ambient}) + f_3(H_x) + f_4(K^+) + f_5(U) + f_6(W) - \varepsilon}, e^{\alpha + f_1(T_{rectal}) + f_2(T_{ambient}) + f_3(H_x) + f_4(K^+) + f_5(U) + f_6(W) + \varepsilon}\right]$$

In terms of programming, it should be noted that our PMI calculator uses a set of parameters provided by the user (who interacts with the system via a graphical interface) to estimate the PMI, using the mentioned formulas extrapolated from the literature (Table 1).

3. Results

3.1. Overview and technologies

The PMI Calculator software uses a set of parameters provided by the operator (who interacts with the system through a graphical interface) to estimate the PMI, using formulas extracted from the literature (see Excel spreadsheet).

The software was implemented using the Java 15 programming language and the Java Swing framework for the graphical component, all within the Eclipse IDE development environment. It can be executed in both Windows and MacOS environments.

3.2. Functional requirements

The software, given a series of input data (analyzed in the following paragraphs), uses formulas provided by the scientific literature to provide a numerical estimate of the PMI expressed in hours and minutes, within a margin of error. This estimate is calculated as the mathematical average of the results from all the formulas used. Each formula's validity criteria are based on the values of the input data; therefore, not all available formulas may be used in the calculation, for the estimation related to that specific case, but only those selected based on the specific characteristics entered for the deceased. In the worst-case scenario, if the input data does not satisfy any of the criteria for using such formulas, no result may be returned. The accuracy of the provided results is linked to the accuracy of the formulas extracted from the literature.

3.3. Non-functional requirements

The software is intuitive. Inputting the required data is quick, and the output results are easily interpretable. The software has low resource consumption, including memory, CPU, and disk space. However, specific requirements regarding security and accessibility have not yet been defined, as it is not based on an artificial intelligence (AI) system. The security aspect will be considered in a potential future implementation of AI.

In future implementations, AI can be leveraged to bolster privacy through advanced data anonymization techniques, ensuring that sensitive information is effectively protected from unauthorized access. Machine learning algorithms could be trained to identify and mitigate potential security threats in real-time, enhancing the overall security landscape. Additionally, employing decentralized data storage solutions can further safeguard user information, reducing the risks associated with centralized data breaches.

3.4. Interface and usage

The program interface is divided into two tabs, the main one being the first, which can be logically divided into three sections.

The first section contains fields where the user can enter data collected from the body and the surrounding environment, as well as additional parameters necessary for estimation. In particular, it is possible to input:

- Rectal temperature (Tb), within a range of 0–37 °C
- Ambient temperature (Te), within a range of −10 to 45 °C
- Body weight (W), within a range of 10–200 kg
- Henssge correction factor, selectable from a list of values
- Potassium concentration detected in the vitreous humor (K+), expressed in mmol/L
- Potassium detection technique, selectable from a list of values
- Hypoxanthine concentration detected in the vitreous humor (Hx), expressed in mmol/L
- Hypoxanthine detection technique, selectable from a list of values
- Albumin concentration detected in the vitreous humor (Alb), expressed in g/L
- Subject's age (Age)
- Urea concentration detected in the vitreous humor (U), expressed in mg/dL
- Time interval between temperature measurement and the measurement of other parameters, expressed in hours and minutes
- Geographic area to which the subject belongs, selectable from a list of values

The input of values for Tb, Te, and W is mandatory for the proper functioning of the program (Fig. 2).

All fields accept both integer and non-integer values, as long as the decimal separator used period.

In the second section, users can define exclusion criteria through a series of checkboxes, which are then taken into consideration when the

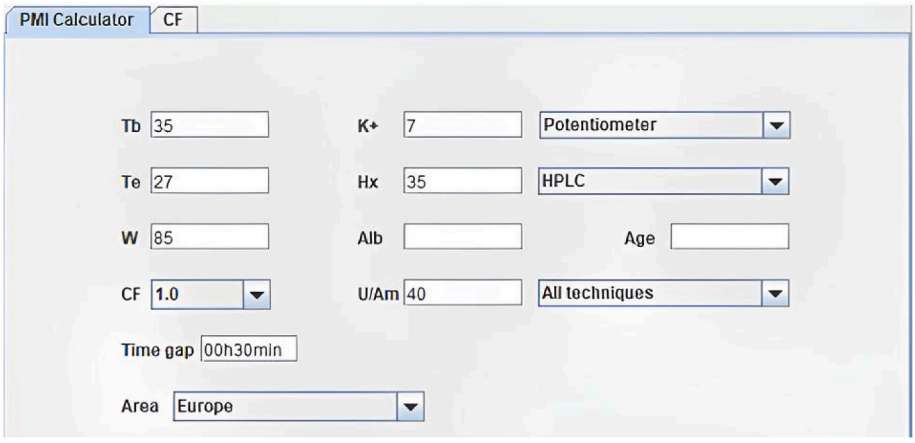

Fig. 2. Graphical user interface (GUI) of the software where the parameters mentioned in the text can be entered.

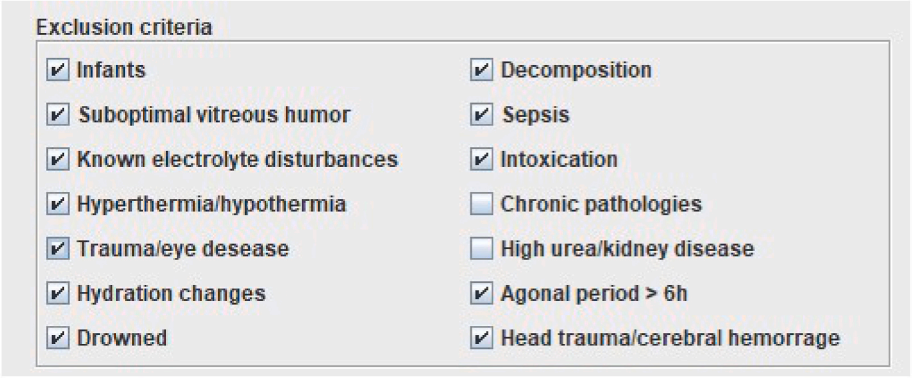

Fig. 3. Main variables related to the application of each formula indicated by the individual authors.

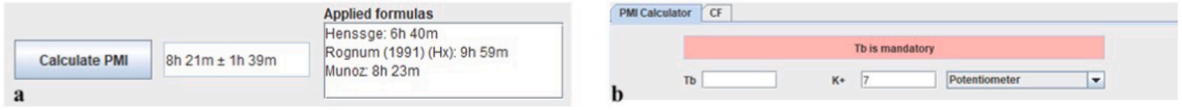

Fig. 4. *ab* Detail of the output windows. Window displaying all the formulas used for estimating PMI with their respective results (a). In case of errors, a red-colored message indicating a data processing error (b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

formulas are used for PMI estimation. It is highlighted that the inclusion criteria presented are those adopted by various studies related to the estimation of PMI through the concentration of vitreous solutes, and therefore they can also be extrapolated from the literature cited in the text. (Fig. 3).

Finally, the third section contains fields related to the result. Clicking

PMI Calculator | CF

CF	dry clothing or covering	in air	wet	in air	water
0.35			naked		moving
0.5			naked		still
0.7			naked	moving	
0.7			1-2 thin layers	moving	
0.75	naked	moving			
0.9	1-2 thin layers	moving	2 or more thick layers	moving	
1	naked	still			
1.1	1-2 thin layers	still	2 thicker layers	still	
1.2	2-3 thin layers		more than 2 thicker layers	still	
1.2	1-2 thicker layers	still or moving			
1.3	3-4 thin layers	still or moving			
1.4	more thin or thicker layers	without influence			
1.8	more thin or thicker layers	without influence			
1.8	more thin or thicker layers				
2.4	more thin or thicker layers				

Fig. 5. Support table for selecting the correct value of the Henssge correction factor.

on the "Calculate PMI" icon initiates the processing of the entered data. One field displays the estimated PMI in hours and minutes within a margin of error, and another area shows all the formulas used for estimation along with their respective results (Fig. 4a).

If no formula is found that meets the criteria set by the user, an error message is displayed, and the text "N. A." (not available) appears in the results field.

Once processing is completed, users can simply enter new data and click "Calculate PMI" again to initiate a new process. In the event of any errors encountered during the processing, a message is displayed within a pink panel at the top of the first section. In normal functioning conditions, this panel remains hidden. The panel disappears if the subsequent program execution is successful (Fig. 4b).

The second tab only displays a support table for selecting the correct value of the Henssge correction factor (Fig. 5).

3.5. Functioning logic

Following the overview of the interface, this section describes the methods used for estimating the PMI calculation.

The computation occurs in two phases. The first phase involves calculating the estimation using the Henssge formulas. As these equations are not analytically solvable for the time variable t (PMI), we developed an algorithm to iteratively determine the value of t.

Algorithm for estimating t in the Henssge formula:

while $(err > \varepsilon)$ {

if $(\mathrm{err} < 0)$ {

$\mathrm{t_i} = \mathrm{t_{i-1}} + \mathrm{k}t_{i-1}$

} else {

$\mathrm{t_i} = \mathrm{t_{i-1}} - \mathrm{k}\mathrm{t_{i-1}}$

}

if $(\mathrm{T_e} \leq 23^{\circ}\mathrm{C})$ {

$$\mathrm{err} = \mathrm{f_c}\left(\frac{\mathrm{T_b} - \mathrm{T_e}}{37.2 - \mathrm{T_e}} - 1.25\mathrm{e}^{\mathrm{Bt}} + 0.25\mathrm{e}^{5\mathrm{Bt}}\right)$$

} else {

$$\mathrm{err} = \mathrm{f_c}\left(\frac{\mathrm{T_b} - \mathrm{T_e}}{37.2 - \mathrm{T_e}} - 1.11\mathrm{e}^{\mathrm{Bt}} + 0.11\mathrm{e}^{10\mathrm{Bt}}\right)$$

}

}

$\mathrm{t\_0} = 1$ from whom $err_0 = f_c\left(\frac{T_b - T_e}{37.2 - T_e} - 1.25e^{B} + 0.25e^{5B}\right)$

The formulas $t_i = t_{i-1} + kt_{i-1}$ and $t_i = t_{i-1} - kt_{i-1}$ were empirically derived with $k = 0.0001$ and $\varepsilon = 0.0001$. Moreover, f_c is the correction factor, a fixed parameter according to some conditions, such as the weather. It improves the Henssge algorithm which evaluates the error, that can be positive or negative, and refines the estimate until there is a negligible error so that the estimate can be considered acceptable.

For the proper functioning of the algorithm, rectal temperature must be equal to or higher than ambient temperature. If the input data does not meet this constraint, an error message is displayed at the start, and the process is halted.

Once the PMI is estimated using the Henssge formula, the result undergoes validation based on body weight. Specifically, this estimation is not discarded if the conditions summarized in Table 1 are satisfied.

After completing this initial processing phase – which, as seen, is based on only the parameters of temperature, weight, and correction factor – the second phase begins, which uses additional formulas extracted from the scientific literature (Table S2).

These formulas incorporate additional parameters that may have been entered by the user via the interface. Furthermore, if the PMI value according to the Henssge formula is deemed acceptable based on the criteria described above, it is used in combination with the value of the "Time gap" field as an additional filter for selecting the methods to be applied, specifically regarding the timing within which these methods are considered reliable.

For example, if the Henssge method provides an estimation of 30 h and the time gap entered is 40 h, only the formulas whose timing encompasses 70 h will be used for the second phase of estimation.

Two considerations need to be made when the value of the time gap is non-zero. Firstly, if a formula relies on both electrolyte values and temperature values, for example, in the case of Cordeiro et al. (2019) and Zilg et al. (2015), it cannot be used, since using temperature values measured at time t_0 and electrolyte values measured at time $t_0 + t_{gap}$ would yield an inaccurate estimation.[15,41]

Additionally, in the calculation of the mean and deviation, the estimated PMI value obtained from the Henssge formula is added to the time gap.

The additional criteria for selecting which formulas are suitable for application are as follows:

- The techniques used for the detection of substances.
- The geographic region to which the subject belongs (it should be noted that in selecting "Europe" the application will include all European subregions).
- The exclusion criteria.

Once the formulas are applied, the final estimation of the PMI is calculated as the mathematical average of all the results, while the deviation is calculated as $\frac{PMI_{max} - PMI_{min}}{n}$, where PMI_{max} and PMI_{min} are, respectively, the highest and lowest estimation values returned by the formulas, and n is the number of formulas used.

After completing a processing cycle, it is sufficient to enter new data and click "Calculate PMI" again to initiate a new process.

The summary software operation diagram is shown in Fig. 6.

4. Discussion

Traditional techniques, such as the Henssge method, have been widely used to estimate the postmortem interval (PMI) up to approximately two days based on body and ambient temperatures.[44–46] However, advances in computing power have created opportunities to enhance PMI estimation by integrating analytical data from vitreous humor analysis and by drawing on existing systems based on cadaver temperature.[44,45,47]

Previous studies have established a relationship between PMI and certain vitreous humor solutes found in the posterior segment of the eye, specifically focusing on potassium (K+) and hypoxanthine (Hx) levels.[8,13]

Other substances, including urea (U), have also been investigated and have shown promising results.[15,48]

This article presents a software program that outlines a new approach to forensic pathology: The program provides an integrated estimation of PMI based on measurements of both vitreous humor metabolites and cadaveric temperature. While repeatability alone does not imply precision, the integrated use of temperature and vitreous biochemical data increases the reliability of the estimate. When both sources of information yield consistent results, the PMI can be regarded as more robust, being supported by independent and physiologically distinct parameters. In parallel with the vitreous humor-based approach,

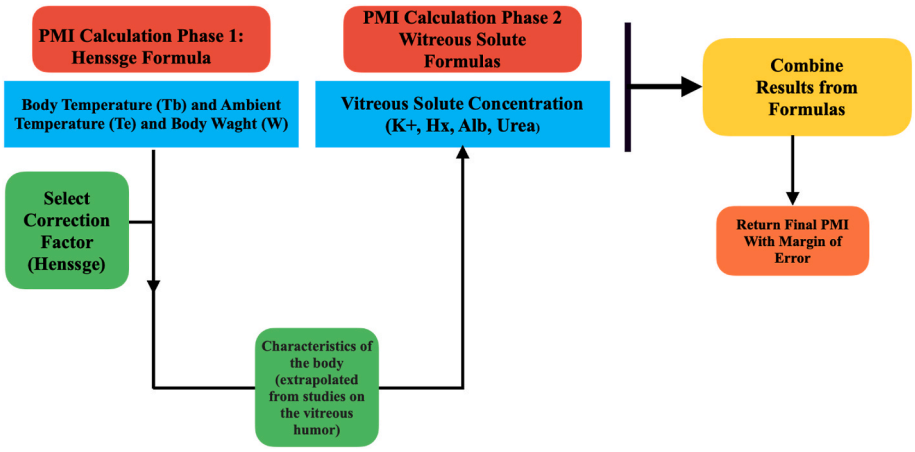

Fig. 6. Software operation diagram.

we preliminarily integrated the Henssge nomogram, originally developed by simplifying the Marshall and Hoare equation.[49]

Ultimately, our program provides an estimation of PMI by employing at least two models. We use the Henssge method, which requires body temperature, cadaveric temperature, and body weight, along with a model established from 28 expressions related to the correlation between vitreous humor solute concentrations and the estimated time since death extrapolated from the literature about vitreous biochemistry. It is important to note that the vitreous humor model is not necessarily unique, and each estimate may require multiple applicable expressions, depending on the specific case. The present software is an initial attempt to address the problem of there being numerous variables (related to solute concentrations in the vitreous humor) involved in the estimation of the time since death; our system leans on new digital technologies, in anticipation of the implementation of artificial intelligence software capable not only of processing vast amounts of data but also of incorporating self-learning functions not present in conventional software like the one presented here.

Furthermore, it should be highlighted that the expressions employed by the software were extrapolated from the international literature, and the sources were partly validated. Additionally, since these expressions represent known correlations, we were able to extract the temporal limits and population characteristics from which each technique was derived, enabling the programming of inclusion criteria in the software.

Although systems based on the application of regression trees and support vector machines using cadaveric temperature have already been proposed in some studies, the present work represents the authors' attempt to combine two very different PMI estimation techniques. In this sense, the software proposed here should be understood as a complementary and preliminary tool towards a future perspective of time of death estimation that integrates multiple techniques, possibly through the application of artificial intelligence.[50]

We recommend the simultaneous use of both approaches, as they are based on different but equally valid statistical models and offer different confidence levels.

It is worth noting that an estimate of time since death can be provided, in a general sense, by considering only the parameters relating to the Henssge formula (in the absence of parameters relating to solute concentrations), or in cases where not all the solutes have been quantified in the analyzed sample. Furthermore, the presence of multiple formulas allows for estimation even in the scenario where the cause of death is not known, or no information is available about the subject.

In practice, the software automatically provides a broad PMI interval, displaying the individual estimates derived from each parameter and equation. This output is not meant to replace, especially in this preliminary stage, the forensic pathologist's evaluation but to support it. Wide intervals should be interpreted as indicative boundaries within which the most plausible PMI can be defined by integrating contextual and investigative data. By making visible how each parameter contributes to the final estimate, the software enhances transparency and helps the operator understand the reasoning path behind the result.

5. Limitations

The limitations of the software should be taken into consideration when interpreting its results. One significant limitation is the potentially wide range of uncertainty, especially when multiple formulas are employed, which necessitates cautious use of the obtained results. Furthermore, the formulas extracted from the literature may also exhibit substantial variation, adding to the complexity of the system. Another limitation is that the software has only been tested on a limited set of cases. Therefore, further validation on a broader range of scenarios is necessary. Additionally, when multiple formulas are used, the software performs a simple averaging of the results from each formula, which may not capture the nuances of individual calculations.

Lastly, this is conventional software which should be paired with an innovative machine learning system.

Moreover, the software is affected by the peculiarities of every formula with its limits and potentialities. For instance, the results of Palacio et al. led to two formulas which were mathematically considered even if they are not used in all applications, since they hold for high PMI, i.e. above 150 h.

Note that if no specifications are provided regarding the characteristics of the corpse, the system includes multiple formulas, resulting in data that tends to be dispersed.

This aspect, extraneous to the software, and apparently framed as a limit, derives from the limitations of the studies relating to vitreous biochemistry for the estimation of the PMI which, if they do not indicate clear exclusion criteria, still provide a too large margin of error.

6. Validation and testing

The software was validated using a preliminary dataset of 15 real forensic autopsy cases, encompassing a range of environmental and individual conditions. Each case included recorded rectal temperature (Tb), ambient temperature (Te), body weight (W), and when available, biochemical parameters of the vitreous humor such as potassium (K^+), hypoxanthine (Hx), albumin (Alb), and urea or ammonium (U/Am). Postmortem intervals (PMI) were independently reconstructed based on investigative information (last-seen-alive data, discovery time, and circumstantial evidence).

The dataset covered PMI values between 4 and 72 h, ambient temperatures from 17 °C to 30 °C, and body weights ranging from 50 to 100 kg. Each case was analyzed through the integrated algorithm, which

automatically selected applicable formulas based on the entered parameters and exclusion criteria. When biochemical data were missing, the Henssge-based estimation module remained operative, allowing a valid temperature-only PMI computation.

Table S3 summarizes the main characteristics of the 15 forensic cases, including weight, ambient and body temperatures, detected biochemical values, and the PMI estimates returned by the software together with their respective margins of uncertainty. In most cases, multiple formulas were used simultaneously, and the software displayed both the individual estimates and the computed average PMI.

The findings indicate that the software provides consistent and repeatable performance under a variety of real forensic conditions. Nonetheless, the relatively broad uncertainty range observed in some cases underscores the influence of missing biochemical parameters and the heterogeneity of environmental conditions.

7. Practical forensic application and biochemical considerations

From a practical forensic perspective, the proposed software is designed to complement, rather than replace, the examiner's analytical and interpretive role. In real-case scenarios, its main advantage lies in providing a quantitative reference that integrates temperature- and chemistry-based information. This dual approach enables cross-verification of results and supports expert evaluation when the time since death must be estimated under complex or uncertain conditions.

Biochemical parameters of the vitreous humor (potassium, hypoxanthine, urea, and albumin) were measured using standard hospital laboratory methods routinely applied in postmortem biochemistry. Samples were collected during autopsy from the lateral canthus using a sterile syringe, avoiding ocular wall rupture, and immediately stored in sealed polypropylene tubes. Transport and storage were performed at 4 °C, and analyses were completed within 12 h to minimize post-collection degradation. These standard conditions align with published recommendations for postmortem biochemical analysis and ensure comparability with data from the literature used in the software.

Operator variability may influence both sampling and analytical outcomes. However, because the program accepts numerical input rather than raw samples, its output depends on the accuracy of the measurements rather than on subjective interpretation. In this sense, variability primarily affects laboratory precision rather than the software itself. The use of automated analyzers and predefined measurement protocols reduces this variability to an acceptable range for forensic purposes.

When biochemical sampling is not feasible—for instance, in decomposed bodies, severe ocular trauma, or cases where only thermal data are available—the software remains operational through the Henssge module. This feature allows the user to obtain a preliminary PMI estimation based on temperature data alone, while transparently indicating the reduced reliability of such estimates compared with multiparametric analysis.

Vitreous humor samples were collected during ecroscopic examination performed in a controlled environment from the lateral canthus using a sterile syringe, avoiding rupture of the ocular wall. Each sample was immediately sealed in polypropylene tubes, stored at 4 °C, and analyzed within 12 h to prevent degradation. Measurements of potassium (K^+), hypoxanthine (Hx), urea (U), and albumin (Alb) were conducted using standard hospital laboratory analyzers routinely applied in postmortem biochemistry. The analytical techniques (ion-selective electrode for K^+, enzymatic assays for Hx, spectrophotometric or immunochemical methods for Alb and U) followed validated protocols consistent with those reported in the literature used for formula derivation.

All assays were performed duplicate, and the mean value was entered into the software. These standardized procedures ensure comparability with published data and minimize analytical variability.

Overall, the system's flexibility reflects realistic forensic conditions, where not all parameters are always available. By allowing partial inputs and indicating their impact on reliability, the software maintains usability across a wide range of scenarios, supporting expert judgment rather than substituting it.

8. Resources and perspectives

The present software (named Scendoni-Tomassini) has been made available as a forensic tool and can be implemented with new resources. For instance, it can be used in future studies on measuring biochemical parameters of the vitreous humor for postmortem interval (PMI) estimation purposes. New formulas can be introduced from other groups or subgroups of populations or featuring other ions/electrolytes still being tested or not yet scientifically consolidated, such as K+.

It should be reiterated that we are introducing an initial multiparametric processing system for estimating the time since death, with the intention of eventually converting it into AI software through extensive data training. In this regard, the development of a next-generation system will aim to overcome the aforementioned challenges and provide a significantly narrower range of uncertainty. This approach has already been partially attempted by other machine learning software, which has explored the use of vitreous solute concentrations alone.

Thus, by offering the present software as a resource, with consideration for its potential implementation with new resources, we envision the creation of an advanced AI-based system. This transformation would involve training the software with large quantities of data, including comprehensive datasets on the vitreous humor and other relevant parameters. By doing so, we would expect to achieve improved accuracy and precision in PMI estimation.

The transition to an AI-based system would bring numerous advantages. Firstly, the ability to process and analyze vast amounts of data would enable a more comprehensive understanding of the complex relationships between input parameters and PMI. Secondly, the AI model should be able to continuously learn and adapt to new data, allowing for ongoing improvement and refinement of PMI estimation. Lastly, incorporating machine learning techniques would offer the potential for a significantly reduced range of uncertainty, surpassing the limitations of traditional methods.

In conclusion, the present software, available as a resource, holds promise for PMI estimation. By implementing it with new resources and advancing towards an AI-based system, we can overcome existing challenges and provide more accurate estimates. The development of a next-generation system would deliver a narrower range of uncertainty, building upon the efforts of previous machine learning software that has explored the use of vitreous solute concentrations alone.[12]

Data availability statement

Data are contained within the article.

Author contributions

R.S. and L.T.: conceptualization; L.B. and G.B.: data curation; L.B.: formal analysis; P.F.: investigation; P.F. and R.S.: methodology; all authors: project administration; M.C.: resources; G.B.: software; M.C.: supervision; M.C. and G.B.: validation; R.S. and L.T.: Roles/Writing - original draft.

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Conflict of interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jflm.2025.103009.

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