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The time of death in Dutch court; using the Daubert criteria to evaluate methods to estimate the PMI used in court

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ABSTRACT

When a capital crime is committed the post-mortem interval (PMI) is of particular importance in investigating a suspect's alibi in court. A forensic expert can use different methods to estimate the PMI. This research focuses on who is considered an expert in court and whether the methods used to estimate the PMI are reliable. In this study, the methods used to estimate the PMI and the experts consulted, available in Dutch jurisprudence, in the period 2010–2019 were investigated. Ninety-four judicial cases were included and multiple experts and methods of estimating the PMI were found. As part of this study, the methods that were used to estimate the PMI in court were subjected to the Daubert criteria. Of these methods, only the Henssge nomogram and entomological methods met the Daubert criteria. However, the methods are only useful when applied by the right forensic expert and in the right manner. Unfortunately, this was not always the case.

1. Introduction

In the Netherlands there have been between 95 and 202 murder cases per year over the last twenty years [1]. Murder cases are investigated by the Dutch criminal law system, which is an inquisitorial system. In the Netherlands there is no jury trial, the judges solely assess the evidence presented and then decide on the charges against the suspect. After the trial, the verdict can be published on rechtspraak.nl, a databank for judicial decisions, if the verdict is regarded as being of interest to the public.

In a great number of murder and manslaughter cases, the exact time of death (TOD) is known because the violent incident was witnessed and the victim died immediately after the incident or later in the hospital. In these cases the TOD, which is necessary to place a suspect at the crime scene, was hardly a point of discussion or of interest in the verdict. Determining the TOD becomes of legal importance when the crime was not witnessed or when there are doubts about the testimony.

After death, physiological, biological and chemical processes alter the state of the human remains [2]. These processes are analysed in a variety of methods used to estimate the post-mortem interval (PMI), which are divided into body temperature-based methods and methods which are not directly based on changes of body temperature. The former group includes the Henssge nomogram method and the body-cooling simulation method. The latter group of methods focuses on livor and rigor mortis, electrical and mechanical stimulation of muscles, biomarkers, decomposition and entomology. The algor, livor and rigor mortis are usable in the early post-mortem interval [3], as well as the body-cooling simulation, electrical and mechanical stimulation of muscles and the use of biomarkers. Decomposition is usable in the late post-mortem interval [3]. Entomology is usable in both the early and late post-mortem interval. The term TOD is used by judges and is defined as a point in time. The term PMI is used by scientists and is a time span in which the death has most likely occurred. An expert can estimate the PMI based on one or more of the aforementioned methods. Before a trial starts, the judges need to verify the knowledge and education of the expert in order to deem him an expert in the trial. The judges themselves do not have the knowledge to determine whether the right method was used or if it was used correctly, which means the expert should clarify which method was used and its limitations.

For this study, cases were analysed in which death was not witnessed

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and the TOD was of importance to determine whether a suspect could have been at the crime scene at that time. The main question to be answered in this article is if the methods, used to estimate the PMI in Dutch criminal court, meet the Daubert criteria.

2. Material and methodology

Two studies were performed, one concerning the Dutch jurisprudence of criminal case law and one literature study. The jurisprudence study focused on criminal case law between 2010 and 2020 where the TOD was mentioned. Within the Dutch databank 'rechtspraak.nl' criminal law cases were searched using the following term: 'Tijdstip van overlijden' (Time of death) for the years 2010–2019. In cases in which a court decision and a high court decision was published, only the high court decision was used for this research. Cases in which only the time of death was mentioned were excluded. Cases in which more than one suspect was on trial (doubles), only one case was included in this study. Interim decisions and revision requests were excluded.

The court rulings were systematically investigated to collect information in order to answer the following questions: 1) Which method was used to determine the TOD? 2) Which expert was consulted to determine the TOD? 3) Was the TOD contested and, if so, by whom? 4) Was there a discussion about the TOD and what was the point of discussion? 5) How did the judge come to his decision about the TOD?

The result of the search in the court rulings was then used to answer the following questions: 1) Who was considered to be an expert in Dutch criminal court? and 2) Which methods for estimating the PMI were accepted as evidence in court by Dutch judges in criminal law?

The literature study was conducted between 2019 and 2020 and focused on the following methods: livor, rigor and algor mortis, the body-cooling simulation, electrical and mechanical stimulation of muscles, biomarkers (specifically potassium), decomposition and entomology. There was no limitation regarding the timeframe of the published articles. The literature search was used to investigate whether the used PMI estimation methods met the Daubert criteria. The Daubert criteria are as follows: 1) Is the theory or technique in question tested? 2) Has the method been subjected to peer review and publication? 3) What is the known or potential rate of error? 4) The existence and maintenance of standards controlling its operation. 5) Is the method widely accepted within a relevant scientific community? Databanks PubMed, Science Direct, Cochrane and Scholar Google were searched using multiple terms appropriate for the methods concerned in combination with either post-mortem interval or time of death (supplementary data 1). By using the 'snowball effect' the bibliographies of the articles were used to search for additional literature. Lastly, bibliographies of multiple textbooks were searched, such as the Handbook of Forensic Medicine (2014) and The Estimation of the Time Since Death (2016) to verify if articles were missed in the databank search. Further, these textbooks were used to assess contents of articles unavailable to the authors [4-6]. The reliability and relevance of scientific articles were classified using the ABC method, where level A articles are randomized, double blind control trials; level B randomized control trials of moderate quality or non-randomized control trials and level C on case studies [7]. Scientific articles of level A and B were included in this research. Theses from graduate students were excluded. The found articles were reviewed to establish if one or more of the Daubert criteria were met. The criteria were assessed on a 3-point Likert scale; positive (+), inconclusive (±), and negative (−). Positive means that the theory was tested by at least by two independent research groups. Inconclusive means at least one research group tested the theory and negative means no research group tested the theory.

Finally, the results of the literature search were used to investigate whether the methods that were used were applied by the right expert and in the right way.

Descriptive statistics were performed by using Microsoft Excel© v. 2010.

3. Results

3.1. Court rulings

Ninety-four cases were included after applying the selection criteria (Fig. 1). Fig. 2 shows the experts who gave their opinion in court (report or testimony), for example the forensic physician, the forensic pathologist and the entomologist. In 12 cases, police officers and non-forensic medical professionals, such as ambulance nurses, gave an expert opinion in court on the TOD. The methods used by the experts are shown in Fig. 3. The methods used by the experts were provided in the documentation of 43 out of the 94 cases. The most frequently used method was Henssge's algorithm based on algor. Livor was never used as the sole method. In the combination of methods, the Henssge method was used in combination with livor and rigor. In two cases in which a combination of methods was used, the cooling-down simulation method was one of the methods.

In 37 out of 94 cases, there was a discussion in court about the PMI (Fig. 4). There were no discussions about which method was or should have been used. In five cases there was discussion about the way a method was used, in seven cases the results differed when experts used the same method and in 20 out of 94 cases the PMI could not be determined.

The judge's decision on the time of death was made by using the expert opinion in 60 out of 94 cases (in 50 cases solely by using the expert opinion, in ten cases by using the expert opinion in combination with other evidence). In six cases the statements of witnesses were used and in one case the statement of the suspect was used. In two cases the decision of the judge on the time of death was based on the use of electronic devices. In three cases the judge used a combination of methods (but no expert opinion). In 22 out of 94 cases, the judge did not state what motivated his decision on the time of death.

3.2. Literature search

The complete literature list, categorized per method, is given in supplementary data 2. Using the bibliography, no new articles were

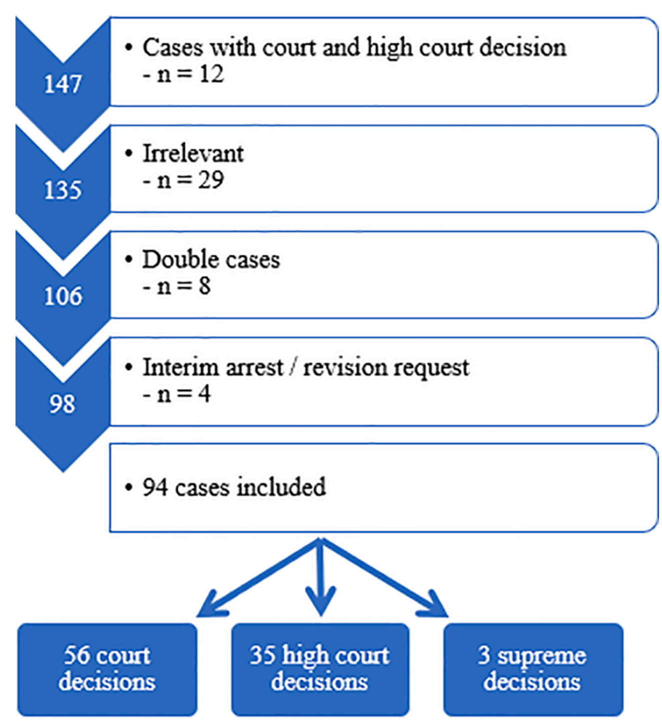

Fig. 1. Selection of judicial cases.

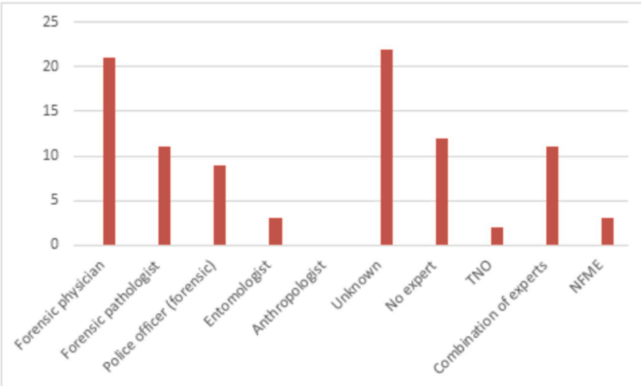

Fig. 2. Experts in court.
TNO: the Netherlands Organisation for applied scientific research.
NFME: non-forensic medical experts.

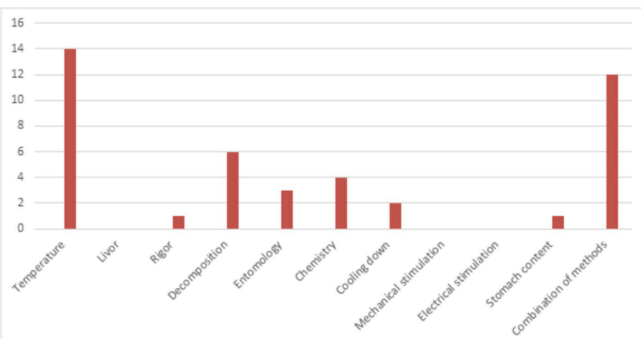

Fig. 3. Methods used by experts, N = 43.

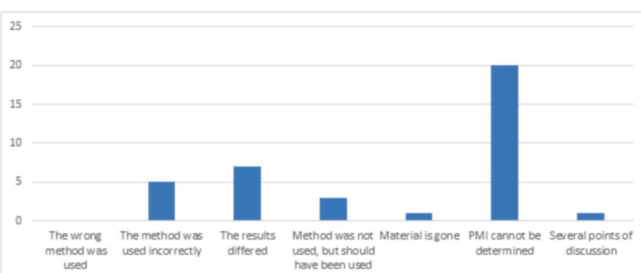

Fig. 4. Subject of discussion in court, N = 37.

found.

3.2.1. Livor mortis

Livor mortis develops after irreversible circulatory arrest due to initial hypostasis and subsequent increasing vascular permeability and autolysis as PMI increases. These post-mortem mechanisms are also responsible for the time lapse of the disappearance of livor mortis on blunt pressure and fixation of livor mortis. In the first few hours after death, the lividity disappears completely after applying pressure. Turning the body in the first six hours post-mortem results in complete shifting of the livor mortis into the lowest part of the body. As the PMI increases, livor mortis will shift incompletely until no relocation of the livor mortis takes place [8].

Mallach's (1964) data analysis of lividity from publications between 1905 and 1963 is considered the best available. Mallach calculated mean values, standard deviations and 95% confidence intervals based on the time lapse of livor mortis mentioned in textbook reports [8]. Several studies have been performed concerning the pressure needed to blanch lividity in connection to the PMI [4,9]. Romanelli et al. combined the intensity of pressure and degree of blanching with other factors such as the time needed for the blanched spot to regain colour. The best correlation with PMI was found to be a pressure intensity of 1.5 kg/cm^2 applied for three seconds [10].

In 1994, Kaatsch et al. tested a method using colourimetry on 50 human remains and correlated it to the PMI, which showed that a system of combined photometry and dynamometry to measure pressure-induced blanching of livor mortis is superior to the use of thumb pressure [11]. In 1996, Vanezis and Trujillo published a study concerning the intensity of livor mortis in relation to the PMI and they found a good correlation [12]. Usumoto et al. developed a new method to objectify the colour of livor mortis and combined the colour factors with autopsy findings for approximating the PMI. This research concluded that the PMI can be estimated within ±4.76 h [13].

Daubert criteria

The studies on livor are published in peer-reviewed journals, but none of them meet all of the Daubert criteria. Although Mallach's data analysis is the most accepted method, there is a large variation in results and the method is subjective [14]. Different methods are used to achieve blanching of livor mortis with the same goal, the measurement of the lividity colour and ultimately estimating the PMI. With the exception of Romanelli et al., objectifying the colour changes occurred with the L*a*b system developed by the Commission Internationale de l'Eclairage (CIE) which can be used to convert skin colour into a digital expression [15]. The methods are not validated, with the exception of the method of Kaatsch et al. [11]. The methods do not mention a standard error or 95% standard deviation concerning the PMI, with the exception of Usumoto et al., who found a root mean square error of ±4.76 h [13].

Mallach's analysis of lividity can provide a rough PMI estimation, but the subjectivity is an important limitation. To overcome the subjectivity, multiple studies used the L*a*b system to obtain an objective measurement of the colours and to be able to compare multiple studies. This method is limited by the fact that the development of livor mortis is different for each individual, for example when bleeding is the cause of death, the livor mortis will be less pronounced (if it is visible). Only one study mentions a recording of ethnicity of the deceased, but it does not mention the influence of the skin colour on the outcomes of the lividity intensity [12]. None of the studies mention using the livor mortis for estimating the PMI for children. Furthermore, a 95% standard deviation for estimating the PMI using colourimetry was not found in the current literature.

3.2.2. Rigor mortis

Immediately after death, full muscle relaxation occurs because adenosine triphosphate (ATP) separates the proteins actin and myosin. After a variable amount of time, the muscles run out of ATP and the separation of the proteins stops, resulting in rigor mortis. Rigor mortis remains until decomposition advances to the point of muscle break down. Rigor mortis occurs between 3- and 6-hours post-mortem but can also be detected earlier. Depending on the circumstances, it often reaches a maximum between 6 and 12 h. After 18–36 h it begins to fade [16].

Rigor mortis can be measured both objectively and subjectively. Mallach's (1964) literature search concerning the period 1811–1960 is considered the best review on this topic. The results, such as mean time in which complete rigidity or resolution occurs with standard deviations and 95.5% probability, are based on the degree of rigidity manually assessed. The method is not reproducible because of the manual assessment and therefore subjective and difficult to validate [17].

The course of rigor mortis and the factors influencing rigidity such as temperature and electrocution, have mainly been investigated by means of experimental studies on rats. It was shown that higher temperatures and electrocution resulted in a fast progress of the rigor mortis [18–20].

Measuring the rigor mortis using a device without breaking the rigor mortis has been validated as well as the re-establishment of rigor after breaking the rigor [21–23]. Varetto and Curto investigated rigor mortis on human bodies. They found persisting complete rigor mortis up to 16 days after death and partial rigor starting on the 11th day after death, when human bodies were kept at a constant temperature of four degrees Celsius [24]. Anders et al. tested the re-establishment of rigor mortis on 79 corpses of people who had died in a hospital. It was found that the rigor mortis re-established up to 19 h post-mortem in 38.5% of the muscles around the elbows and knees [23]. Four years later, the results were confirmed by the same research group [22].

Daubert criteria

Although the normal rigor mortis curve is understudied and based on subjective data from Mallach, the variables influencing rigor mortis and re-establishment of rigor mortis have been widely investigated [17]. Mallach's analysis can provide a rough PMI estimation, but also here, the subjectivity is an important limitation. Two studies of Krompecher describe the standard deviations of the intensity of rigor mortis but not a standard deviation of the PMI. Anders et al. and Crostack et al. describe the 95% confidence intervals for the intensity of re-establishment of rigor mortis [18,22,23,25]. Measuring the force needed to break the rigor using devices overcomes the subjectivity but is impractical to use in forensic casework. Moreover, most of the experiments were performed on rats and it is unknown if these results can be extrapolated to humans. Therefore, the data on which the timeline of rigor mortis is based is insufficient to be used in court.

3.2.3. Algor mortis, post-mortem body cooling

After death the body cools down until the ambient temperature is reached. Body temperature of a deceased can be determined in various ways, such as measuring the outer ear temperature [26]. Henssge developed an algorithm for estimating the PMI of a human (adult) body based on the rectal temperature, ambient temperature, body weight and a correction factor [27,28]. The method can be used as long as the body is cooling down to environmental temperature. The starting body temperature is assumed to be 37.2 degrees Celsius. Henssge determined the dependence of the correction factor on a very high or low body weight under conditions with a high degree of insulation [29]. Henssge et al. investigated the applicability of the nomogram method in forensic casework. Out of 60 cases with a known PMI based on police investigation in 50 cases the estimated PMI corresponded completely with the known PMI while in the remaining 10 cases there was a partial correspondence [30]. Honjyo et al. applied the nomogram of Henssge to 212 deceased. The included 212 cases had a known PMI within 24 h based on police investigation. Although estimated body weights were used and correction factors were not taken into account, Henssge's method showed the highest correlation coefficient between the estimated and known PMI compared to four other applied methods [31].

Demierre et al. performed a retrospective study on 744 cases of violent deaths to determine the incidence of hyperthermia and its consequences for forensic casework. In 80 cases (10.8%) of violent death, hyperthermia was found or postulated [32]. Muggenthaler et al. conducted cooling experiments on 84 recently and suddenly deceased humans with known PMI. The experiments were performed under strictly controlled environmental conditions using a climate chamber. Hubig et al. noted that in 48 cases (57%) the 95% confidence interval did not contain the true PMI. In addition, systematic overestimation of PMI by applying Henssge's nomogram in case of high BMI and body surface should be taken into account. Nonetheless, no error rate was mentioned in this research [33,34].

A limitation of Henssge's model is that it cannot be used in circumstances in which the measured body and ambient temperatures could have been influenced by exogenous factors like direct sunlight on the skin of the deceased, unusual cooling conditions and climatic influence due to possible transport of the body. It also does not take into account possible hyper- or hypothermia by setting the starting body temperature at 37.2 degrees Celsius.

Daubert criteria

Hubig et al. pointed to the influence of errors in measuring the initial rectal temperature, the environmental temperature and body mass [35,36]. When applying Henssge's nomogram, the usage of correction factors is crucial in case of non-standard cooling conditions. Different studies have focused on this aspect of Henssge's method and questioned the reliability for application of the nomogram method. Critical comments have been made regarding the practical usefulness, subjective bias in choosing an appropriate correction factor and availability of limited data on choosing correction factors [29,30,36–39]. Although the method has been criticised, the nomogram by Henssge is considered the most reliable and accepted method for estimating the PMI in forensic casework.

3.2.4. Post-mortem body-cooling simulation

In 2004, den Hartog and Lotens used a finite element model (FEM) to simulate a human torso and its clothing. The FEM subdivides the human body into geometrical segments. The human body was divided into 12 compartments and the clothes into another 4 compartments [40]. Mall and Eisenmenger used the FEM to make a three-dimensional model for a human body to simulate the post-mortem body cooling. This method divides the human body into 8328 segments and 10,154 nodes (the endpoints of the elements) and by using a formula the model height and weight can be adjusted to the actual height and weight so that the actual body mass is taken into account. Using a core temperature of 37 degrees and a body shell temperature of 27 degrees, the cooling of a human body could be calculated and predicted over time while taking into account conduction, convection, radiation or the heat gain by production of thermal energy within the body. Using this model, all sorts of simulations can be performed with differences in the shape of the body, the clothes and the initial temperature [41]. In a second study, Mall and Eisenmenger used the FEM to estimate the PMI in non-standard cooling situations and in practical casework. Here also, data such as ambient temperature, ground temperature, rectal temperature, body mass and height were collected. Five cases with a known time since death were used. The FEM produced 'very plausible results in all presented cases' according to the authors [42]. Bartgis et al. further developed the whole-body heat transfer model by not only simulating the time of death but also the temperature distribution of the body before death. Bartgis et al. used actual body measurements and determined an 'overall heat transfer coefficient' to model the thermal resistance of conduction, convection and radiation [43]. Muggenthaler et al. investigated both the influence of cooling of the substrate the body lies on [44] and the influence of hypo- and hyperthermia [45]. Both factors influence the estimation of PMI. Both these factors can be incorporated into the FEM.

Daubert criteria

Over the years, the finite element method has been validated for both body-cooling simulations [40–43] and in two studies [42,44] for casework in different contexts. These validations have not been tested by other research groups. Using Mall and Eisenmenger's three-dimensional model of a human body, many different simulations can be performed [41]. This model is criticised however, because according to Bartgis et al., the human body temperature was assumed to be a constant and uniform temperature of 37 °C or based on an inaccurate temperature distribution estimation [43]. Bartgis et al. proposed a further developed model. Since weight is an influencing factor in thermal cooling of a body [46], they found it unlikely that the generated cooling curves in the study can be readily applied to other body shapes and sizes [43]. Wilk et al. developed a simulation method where large ranges in age, weight, body length and fat percentages were overcome [47]. Thus, there are different heat-flow models that have not been extensively tested by other research groups. The models are not widely accepted and based on the literature review there are only two studies [42,44] that used forensic casework to validate the model.

In summary, the post-mortem body-cooling simulation where the body is divided into compartments and nodes and a computer simulation predicts the PMI, has made large developmental steps in the last two decades. The most recently published research using non-invasive thermometry found a maximum error of ±3.2 h and an average error ± 38 min [47]. This research could change the acceptance of this method in the Dutch legal system.

3.2.5. Electrical stimulation of skeletal and facial muscle

In the recently deceased, various cells like skeletal muscle cells are still active. This phenomenon is called supravitality. This activity is self-limiting and depends on the storage of glycogen and adenosine triphosphate (ATP) and irreversible changes in the internal environment of these cells due to anaerobic glycolysis (drop in pH due to cumulation of lactate) [48].

Post-mortem electrical excitability of the skeletal muscles of animals was first mentioned in the late eighteenth century by Galvani (1780). During the nineteenth century, many experiments on the electrical excitability of skeletal muscle were performed by inserting needles into the muscles. The results indicated that analysis of the electrical excitability of skeletal muscle could be a suitable method for estimating the PMI.

Prokop initiated succeeding investigations on this topic, focusing on facial muscle. Different degrees of facial muscle excitation were published, including the six gradations by Klein and Klein and the four gradations by Krause [49–52]. Klein and Klein derived six degrees of muscular contraction for which Henssge calculated the 95% confidence limits [53]. Madea and Henssge re-examined those 95% confidence limits established by investigating the corpses of 30 people who had suffered traumatic or sudden natural death. Although differing modes of excitation were used (type and duration of impulse, current intensity), the 95% confidence limits were confirmed [54].

Madea examined muscular contraction (thenar and hypothenar) by electrical stimulation of skeletal muscle; all cadavers were cases of sudden natural or traumatic death (n = 50). The results pointed to an increasing force-related relaxation time of muscle after stimulation with increasing PMI. The 95% confidence limits were confirmed on a random control group of 21 bodies [55]. Madea and Rödig confirmed these results in 56 cases by remodelling and matching data extracted from the same study in 1992, such as the beginning of the investigation, duration of electrical excitability and age [56].

Poposka et al. examined 50 cases with known PMI between 2 and 24 h. The authors concluded that electrical excitability of the facial muscle is of major importance in estimating PMI up to 10 h after death [57]. However, an error rate was not mentioned and no information was given about the cause of death [56].

Although Henssge et al. demonstrated the span of PMI could be narrowed by applying electrical stimulation of muscle, it must be emphasized that only facial muscles were used [30,58].

With the exception of Zink and Reinhardt who used surface electrodes, in all studies needle electrodes were applied [54].

Daubert criteria

The studies are published in peer-reviewed journals. Several research groups tested the method of electrical excitability of muscle for estimating PMI and demonstrated a connection with the PMI. The most important general limitation of the studies performed is that the results can hardly be compared due to subsequent modifications of various elements of methods used such as the position of electrodes, way of excitation and grading of muscular reactivity. Thus, no general conclusions can be drawn about the reliability of this method in estimating PMI.

3.2.6. Mechanical stimulation of skeletal muscle

Muscle excitation by mechanical stimulation of skeletal muscle is another way to demonstrate supravitality. Post-mortem mechanical excitability of skeletal muscle (idiomuscular contraction after applying a blow with a reflex hammer) had already been described in the nineteenth century, but it was first investigated in a population of deceased people by Näcke (1911) and Dotzauer (1958) [59,60]. Different muscles were used to examine this phenomenon and its duration; however, clear cut off points for use in estimation of the post-mortem interval (PMI) were not identified in previous studies [48]. Warther et al. examined the mechanical excitability of skeletal muscle in 270 cases; 208 of the people had died in hospital and 62 had died following unsuccessful resuscitation after observed collapse (sudden death). Time since death was known and varying between 7 and 15 h. In 45 cases (16.7%) a positive idiomuscular contraction was observed after mechanical stimulation of the musculus biceps brachii and musculus quadriceps femoris. It was thereby confirmed that there was an upper time limit of 13 h post-mortem in which an idiomuscular contraction could be observed [61]. Henssge et al. combined non-temperature-based methods such as mechanical excitability of skeletal muscle with the temperature-based nomogram method in order to improve or confirm the results of PMI estimations made using body cooling. In 49 of 69 cases examined at the scene of death, a reduction in the span of the PMI could be obtained using this combination of methods. In another 6 of the 69 cases, at least one of the limits obtained from the nomogram method was confirmed. However, as far as mechanical excitability is concerned, improvement of the estimated PMI could only be reached in one case and confirmation of the PMI in another case [58]. In the remaining 14 cases, non-temperature-based methods had no added value regarding the estimation of the PMI.

Warther et al. showed that in cases of death due to an infectious disease, mechanical excitability was rarely observed [61]. This observation seems to be consistent with the assumption that a substantial amount of energy loss (loss of ATP and glycogen) due to underlying diseases would result in a lower degree of idiomuscular excitability. This may indicate a possible limitation in the use of mechanical excitability for estimating PMI in cases dealing with a high level of metabolism antemortem due to hyperthermia (e.g. fever, drug intoxication) [61]. Nevertheless, because of limited information in the cases of sudden death, a possible influence of the cause of death on the degree of muscular excitability could not be analysed. Therefore, no unambiguous conclusion can be drawn from this study regarding possible restrictions in using mechanical excitability of skeletal muscle to estimate PMI.

Daubert criteria

Although Henssge et al. concluded that electrical and mechanical stimulation of muscle positively contributed to the estimation of the PMI in the majority of cases, in only two cases an improvement or confirmation of the PMI could be obtained based on idiomuscular contraction by mechanical stimulation of skeletal muscle [58]. Clear cut off points for estimating PMI by mechanical stimulation of skeletal muscle are not available. Moreover, Warther et al. showed a positive reaction of muscle up to 13 h post-mortem after mechanical stimulation in only a minority of cases. This implies that negative reactions can also occur in the timespan that a positive reaction is expected, depending on the storage glycogen and ATP level in muscle cells. Thus, no conclusions can be drawn about the PMI in the early phase of death in case of absence of muscle excitability after mechanical stimulation.

3.2.7. Biomarkers

The human body contains biological markers, or biomarkers. These biomarkers are under investigation to estimate the PMI [62–66]. A study by Meurs et al. (2018) showed there were 388 post-mortem biochemical markers related to PMI at that time. Considering the work already conducted on this topic, the focus here lies on the post-mortem biomarker which was the most investigated, namely potassium [67].

Body fluids such as cerebrospinal fluid, vitreous humour and synovial fluid are matrices used to measure PBM's. These fluids are contained in almost closed compartments, are therefore not easily redistributed or contaminated post-mortem, and thus able to reflect the degree of PM change best [62]. As with all methods to estimate the PMI, biomarkers are also influenced by, but not limited to, factors, such as ambient temperature, age, the period of time of being terminal, renal failure, alcohol level at the time of death [68]. Uemura et al. investigated eleven biomarkers and found significant differences in values per sample site for eight out of eleven biomarkers [69]. Blana et al. investigated pre-analytical treatments to liquify vitreous humour and recommend ultrasound or centrifugation to liquify the samples instead of heat treatment, hyaluronidase or enzymatic treatment [70]. Thierauff et al. investigated the pre-analytical treatments centrifugation and ultrasonic baths and found a tendency towards higher concentrations when the ultrasonic bath was used, without being statistically significant [71]. When using biomarkers, the investigator should hold in regard the following important factors: 1) a sample should be taken as early as possible in the investigative phase, 2) the outcome of the analysis is only relevant to correlate with PMI during the early post-mortem period, 3) the source of the sample and the manner of acquisition, and last 4) the analytical method [72].

Daubert criteria

The articles on potassium as PBM are published in peer-reviewed journals. There are multiple analytical methods to analyse the potassium value in vitreous humour, such as flame photometry [73–75], using ion selective electrodes [64,68,76,77], capillary ion analysis [78,79] or low pressure ion chromatography [80]. The different analytical methods used make it difficult to compare the results. Over the years, multiple equations to estimate the PMI by using potassium have been made and improved [68,79,81-85], but the intercept and slopes all differ, probably as a result of influencing factors [86,87]. Ortmann et al. [84] compared five equations developed to estimate the PMI using vitreous potassium and found an overestimation of the PMI using Sturner's equation [88] and an underestimation of the PMI using Jashnani's equation [63]. Using their own and the equation of Munoz [89] they found a minimal mean difference.

Multiple studies mention a standard error or a 95% confidence interval [68,75,77,79,82,87-93] in 1963, Sturner found a standard error of 4,7 h [88]. Zilg et al. developed a new equation to estimate the PMI, validated it test datasets and found an error rate of 4.05 $\pm$ 1.7% (mean $\pm$ SD) [68].

Of all the biomarkers, potassium is the most accepted to estimate the PMI, but there are still limitations in the form of the multiple analytical methods being used, multiple different equations, lack of consensus on the sample site and analytical pre-treatment, and the limited data on influencing factors.

3.2.8. Decomposition

Decomposition is caused by autolysis and bacterial activity, which is expressed in post-mortem changes like green discolouration, epidermolysis and bloating of the body. There are many internal and external factors which influence the rate of the decomposition process. Due to these factors, it is complex to use the decomposition process to estimate the PMI.

Over the years, multiple methods have been developed and published to estimate the PMI using decomposition [94-102]. Most of the decomposition scoring methods use temperature in the form of Accumulated Degree Days (ADD) to estimate the PMI [95-99,101]. The ADD is the sum of the average daily ambient temperatures between the date of death and the date of being found [96].

These methods involve using human remains but are further tested using pigs as human proxies. However, differences in the decomposition process between humans and pigs have been found [103]. Compared to human remains, pigs are easier to obtain and can be selected based on certain characteristics. Conducting research on a large homogeneous population is impossible with human remains. A recent review advises that the methods be validated with human remains before they are used in forensic practice [104].

It is thought that the developed decomposition models and equations can be used in comparable climates, but this is also criticised [105]. When these methods are applied to juvenile remains, a weaker correlation is found between TBS and both ADD and PMI, which suggests they cannot be directly applied to juvenile remains [106].

Daubert criteria

All of the articles are published in peer-reviewed journals. The Total Body Score (TBS), Total Decomposition Score (TDS), Total Aquatic Decomposition Score (TADS) and Vass's formula were validated. The TBS, TDS and TADS all have high interrater reliability [98,100,107–109]. Most of the methods involve using photographs of decomposing bodies and a study found significant differences when TBS is scored using photographs and when it is scored in the presence of the remains [110].

Moffatt et al. proposed an adjustment to the statistical approach used by Megyesi et al. due to multiple statistical errors [97]. Moffat's model was found to be more accurate in earlier decomposition stages while Megyesi's model was more accurate in later decomposition stages [105]. The latter results are both confirmed and contradicted by subsequent research [109,111,112].

Vass's Universal method was validated in Australia and was found to underestimate the PMI [113]. This is partially comparable with a Canadian study in which the formula for aerobic decomposition overestimated the PMI during warm temperatures and underestimated the PMI during cold temperatures [114]. On the other hand, the findings regarding underestimation are contradicted by those of a study conducted in Nebraska and Hawaii, which found Vass formula to be 100% and 60% accurate respectively [115].

Only some studies mention a standard error or a 95% confidence interval. The TBS has a large standard error of 388.16 ADD, which is therefore unusable in practice. The TDS has a standard error of 29.6 and 52 ADD, which should be tested in practice. Moffat's model comes with 95% prediction intervals while using different total body scores.

Of all the methods, the TBS method correlated with the ADD is the most accepted and at the same time the most criticised internationally [97,98,101,105,109,112,116].

3.2.9. Entomology

Forensic entomology studies insects in a legal context and the clarification of estimation of the PMI is the most important task. This is done by estimating the age of the juvenile stages of necrophagous insects found on the body and its surroundings and by evaluating the species diversity of the necrophagous fauna [117,118]. The resulting entomological report indicates a period of time in which the body has been infested with insects but this period does not necessarily indicate the TOD [119]. Due to e.g. bad climatic conditions, low or no insect activity or limited accessibility of the body for insects, the infestation may not have taken place immediately after death but days or even weeks later [120]. This fact leads to the scientific term minimum post-mortem interval (PMI_{min}), the minimum amount of time a person has been dead [121].

Identification of the insects is the first and most important task, as growth and aging of arthropods as well as their occurrence on the body is species specific. Identification can be done morphologically and on a molecular level, which should be seen as mutually supportive. While molecular identification was implemented in forensic entomology by Sperling et al. [122] in 1994 and has developed rapidly since then [123–126], the progress in the morphological identification of necrophagous insects varies depending on the zoogeographical region due to the local faunal composition. In Europe, for necrophagous flies (the most important insects in forensic entomology) the works of Szpila and colleagues are particularly noteworthy [127–129], as they are illustrated photographically and not graphically and contain only forensically relevant species. Photographic illustration supports correct identification as in Central Europe for example, more than 100 species of blow flies can occur of which only about 15 are forensically relevant.

After identifying the species, one must then establish the temperature conditions of the past days or weeks (i.e. the period during which the deceased person was missing) under which the specimens have developed. Insects develop faster at higher temperatures and slower at lower temperatures. This reconstruction is a difficult task due to different habitats of the crime scene and the weather station, but recently there have been several promising approaches to identify e.g. the correct period and timeline of measurements on site as well as the most appropriate model for reconstruction [130–132].

The development of an insect on the body can take two to six weeks on average depending on the species and temperature, and in this time window it is possible to limit the development to the day. Moreover, necrophagous insects can show different preferences for e.g. fresh or advanced stages of decay. Describing this process, the occurrence timeline of insect species during the decomposition of a cadaver enables the indication of a period of several weeks or even months post-mortem [133–135].

Daubert criteria

Forensic entomology benefits from a large number of publications and ongoing research projects. Nevertheless, there can be several restrictions which make it difficult to use forensic entomology in routine case work. These limitations include poor sampling of evidence, lack of developmental and ecological data of the identified insect species (mostly flies and beetles), and an unknown thermal history of the body on site [130]. While the latter is important to know for calculating the species-specific development of the ectotherm animals and estimating their age (i.e. the PMI_{min}), such calculations can be made using reference data collected in the laboratory on the temperature-dependent development of the insects. Due to geographic differences and population adaptations caused by e.g. different climatic conditions, there might be a need for local data [136–140]. If such data are missing, there is at least always the possibility of obtaining reference data specifically for the local environmental and temperature conditions of the case, preferably with insects from the population colonizing the body. However, such a procedure requires several weeks to months. Normally, forensic entomology can provide answers and time data after a few days or weeks and this meets the needs of a crime investigation.

One important limitation of especially earlier papers [136,137] is the fact that they neither present standard deviations nor confidence intervals around their estimates but only accurate data points to calculate the age. More recent studies implemented confidence intervals and also introduced efficient and important models like inverse prediction [141], quantile mixed effects models [142] and generalized additive models [143]. Another limitation is the key indicators referred to regarding age estimation of larvae or pupae. These are mainly size (of the larvae) and ADD (see “3.2.8 Decomposition”) or accumulated degree hours (ADH) needed to reach certain developmental landmarks like pupariation.

Finally, there is a rather limited amount of reference data for insect occurrence on dead bodies – there are many experiments but just a few based on a sufficient number of cadavers and replications to justify the establishment of patterns. All these studies used pig cadavers as analogues for human bodies [104], as such experiments are easier to repeat and more practical for the control of disruptive factors than studies based only on humans. But there are also arguments which indicate a need for reference data based on human bodies. Most recently Dawson et al. showed for Australia that there was a delay in the colonization of humans by both flies and beetles. Moreover, species richness of these taxa was between two and five times higher during the first two weeks of decomposition on pigs compared to humans during both summer and winter [144].

3.3. Daubert criteria

Nine methods were assessed according to the Daubert criteria. Table 1 shows the results. Of all the methods, only algor mortis and entomology meet all of the Daubert criteria and are both nationally and internationally accepted.

4. Discussion

For this research the rulings of criminal cases were used which were published on rechtspraak.nl. The verdicts which are published, are the verdicts which are regarded as being of interest to the judges and the public. The other verdicts are not freely available for research purposes. Scientific literature in English and German was used which was available in PubMed, Science Direct, Cochrane and Scholar Google. There might be literature of interest which has not been found or found in a foreign language and relevant casework might not have been available. Some articles are, due to their age, practically unavailable. In such cases, books describing these articles were used [4–6]. These limitations are existent but unfortunately unavoidable.

In court, the judge is interested in the TOD. The time of death can determine who could have been at the crime scene and therefore who is a suspect. For this reason, the method by which the TOD is determined should be clear and should be clearly stated in the court ruling. To make a decision on the TOD, the judge is free to choose which evidence to use. The judge may even decide to determine the TOD himself [145]. The judge can also use statements of suspects and witnesses and information from devices like telephones and computers. In 22 cases, the judge did not clarify how the decision on the TOD was made. Verdicts in criminal courts have to be made public in order to uphold the transparency and testability of the law and its progress. If a verdict is reached, it should be known which arguments substantiate the verdict. Since there are multiple methods and experts that can be used to come to an estimated PMI, it should be published how the judge comes to the TOD that is used. In our research, in 60 of the verdicts the judge used the expert opinion to conclude on the TOD. When using the expert opinion, three main questions need to be answered.

The first question is whether the expert is really an expert. In 9 out of 94 cases, a police officer was the alleged expert on the PMI. In three cases other professionals, like ambulance nurses, were the alleged expert and in 22 cases the profession of the alleged expert was unclear (Fig. 2). A forensic anthropologist has not been consulted as an expert in court in any of these 94 cases since the cases did not involve skeletonized remains. In the Netherlands, forensic police officers receive limited training with regard to determining the time of death. Ambulance nurses do not receive any such training. The question ‘who is an expert in

Table 1
PMI methods subjected to the Daubert criteria.

	Theory tested	Peer reviewed	Known error rate	Accepted in international community	Accepted in Dutch community
Livor	+	+	–	+	+
Rigor	+	+	–	+	+
Algor	+	+	+	+	+
Body-cooling simulation	+	+	+/-	–	–
Electrical stimulation	+	+	+	–	–
Mechanical stimulation	+	+	+	+	–
Post-mortem biochemical marker - potassium	+/-	+	+/-	+/-	–
Decomposition	+	+	+/-	+	–
Entomology	+	+	+[a]	+	+

a: quality of the data is highly species and regions specific.

criminal court?' has been puzzling judges for a long time. In the Netherlands, the NRGD (Nederlands Register Gerechtelijk Deskundigen, The Dutch register of judicial experts) was established in 2010 [146]. Forensic pathologists are registered with the NRGD. Forensic physicians are not registered yet, however this is expected to occur in 2021. No forensic entomologists are registered. This raises the question of how judges should determine whether an expert has sufficient expertise to testify in court if they are not registered as an expert. By introducing this register, the quality of the experts is guaranteed. The expert needs to be independent, qualified in the registered profession, and only testify within the scope of his area of expertise.

The second question is whether the methods which are used by the experts are reliable. Although a method may be accepted in the scientific community, this does not mean it is reliable enough under the given circumstances of a forensic case. The judge is the one who has to decide if the method is reliable. To do this, judges can use the Frye and the Daubert criteria. The Frye standard was used in the American Courts between 1923 and 1993, stating that an expert testimony could only be accepted if it was based on a generally accepted method in the scientific community [147]. In 1993, the Supreme Court incorporated the Frye standard into the Daubert criteria [148]. Due to the fact that the Daubert criteria are more extensive (and now include the Frye standard), only the Daubert criteria were used in this research. To determine whether a method is reliable the following questions have to be answered. The first question is if the theory or technique in question has been tested. Having just a theory is insufficient. The theory should be tested by, preferably, multiple research groups using a scientific approach [149]. The second question, has the method been subjected to peer review and publication, ensures that scientific colleagues agree with the work before publication [150]. The third question considers the known or potential error rate of the theory or technique. The methods to estimate the PMI are never 100% accurate due to the influencing factors and a reduced accuracy and reliability of the method should be mentioned. The fourth question considers the existence and maintenance of standards controlling its operation, i.e. best practises or standards not just for sampling but for measuring and evaluating certain parameters in e.g. body samples. Lastly, is the method widely accepted within a relevant scientific community? If there is minimal support for the method, the admission of the testimony should be doubted [149].

The usage of the Daubert criteria is being discussed in literature. Although multiple articles conclude the Daubert criteria should be used in court [151–153], it seems to be difficult for judges to actual use the criteria. Partially because judges do not always understand the criteria and apply them in the right manner [153] and partially because underlying research to completely fulfill the Daubert criteria are missing. The expert can provide information on the question whether a method is generally accepted in the scientific community, in other words, if the method is accepted for use in criminal court law. Whether it is accepted to some extent depends on their own opinion. The problem is how the judge can determine whether a method meets the Daubert criteria or whether a method is used correctly. The judge can decide that a method is scientifically reliable without knowledge of the method [154]. However, to reach the correct conclusion, the judge needs expert knowledge which he often does not have and therefore the judge has to depend on the opinion of the expert [155]. The role of the expert is to provide the judge with understandable evidence [156]. What is understandable depends on the capacities of the expert to translate scientific information into words which have meaning in court. The evidence has to be presented in such a way the judge can follow the line of reasoning. This makes the system susceptible for mistakes, especially when an expert is not doing his job well or when experts have contradicting opinions. In our research, in one case this led to the use of both (contradicting) statements [157]. Which was, in this case, clearly a sign of not understanding what the experts were stating. If the judge uses the Daubert criteria, the judge needs to know whether the method meets (all) of the Daubert criteria. To decide whether the method meets the criteria, the judge has to depend on the opinion of the expert. The paradox of the judge needing expert knowledge to decide whether methods and experts are reliable has been discussed in literature [155,158]. In the Netherlands, judges are free to use the Daubert criteria to decide if the method used is reliable, but it is not a requirement. Another option is to hear a second expert about the first expert's report [155].

There is a difference between methods which are internationally accepted and which are accepted in the Netherlands. For instance, using potassium in vitreous humour is internationally accepted, but seldom used in the Netherlands to estimate the PMI (chemistry is used in 4 cases out of 94). The same counts for decomposition. If decomposition is used to estimate the PMI, it is often without using one of the methods mentioned in 3.2.8 (decomposition). Estimating the PMI solely based on the forensic physician's experience with the decomposition process is very unreliable and can therefore not be regarded as a scientific method [144]. Another problem is the usage of the terms 'Time of Death' and 'Post-mortem Interval'. Using the methods discussed in this study, a scientist is only able to give a time span in which death has most probably occurred. There still is a 5% chance of being incorrect when using the 95% confidence interval. This time span, or PMI, is often incorrectly translated as TOD by the judge. The judge may therefore misinterpret the information given by the expert which could lead to incorrectly concluding that the suspect was or was not at the crime scene at the TOD.

Equipment has been developed to measure the strength needed to either blanch lividity or overcome rigor mortis, but no error rate or 95% confidence interval of the PMI is mentioned. With regard to post-mortem body cooling various methods have been developed, but not all of these methods have been tested by other researchers. Although Henssge's nomogram is considered the most reliable method for determining PMI, there are practical problems with applying the nomogram in forensic casework. Subjective bias of the expert has to be taken into account concerning estimation of weight of the deceased and choosing the right correction factor. In addition, inadequate determination of body temperature, for instance by measuring the temperature in the outer ear instead of measuring the rectal temperature, should also be considered.

Moreover, the algor mortis method does not take into account the fact that during the criminal investigation the temperature is measured after several hours. During these hours, the circumstances may have changed. For example, doors have been opened, air movement has changed, there is movement of individuals or the body has been moved. The compound method described by Henssge and Madea, which combines temperature-based methods with non-temperature-based methods such as supravital muscle reactions, has reliably shown to narrow the PMI [28,30,46,58]. However, supravitality is not mentioned as a method for estimating PMI in any of the 94 court cases. Apparently, measuring supravitality is not part of the standard toolbox of forensic physicians in the Netherlands and above all systematic population studies are lacking. For decomposition, Megyesi's method is the most accepted and tested but has also been criticized and is not usable in different climates. In order for all the methods to meet the Daubert criteria research is needed, preferably on human remains since it is mostly unknown whether results acquired through research on animals can be extrapolated to humans. Post-mortem research on human remains is not generally accepted in the Netherlands which makes it difficult to obtain a suitable location for taphonomic research. Furthermore, even if human remains are obtained, they will not be in sufficient numbers for rigorous scientific research which requires homogeneity of the remains.

One decomposition study mentions that the method is not usable for juvenile remains [106]. It is known that Henssge's nomogram was developed for adult remains. The other methods do not mention juvenile remains which raises the question of whether the methods are reliable for juvenile remains.

As with all equipment used in medicine and research, instruments should be calibrated regularly. There is no standard control for livor, rigor or algor mortis or for electrical and mechanical stimulation, although temperature metres should be calibrated. For methods involving biomarkers, there should be standard controls for calibrating the instruments.

The third question is whether the experts use the methods and interpret the results correctly. Incorrect use of a method, e.g. using the Henssge nomogram after measuring the temperature with an ear thermometer or measuring the temperature of a deceased child [157], is hard for a judge to detect. In 37 cases, there was discussion in court about PMI. The discussions focused mainly on whether the PMI was correctly estimated using a method. Inter-individual differences in outcome between experts using the same method was observed. This raises the question if the method was used correctly, since the data needed to use the method, were the same. Notably, in 20 cases the PMI could not be determined. There were no discussions about which method should be used to estimate PMI. This outcome is remarkable given the known limitations of the different methods available from a scientific point of view.

5. Conclusion

In this study an effort was made to make judges more aware of the limitations and implications of application of methods that are used to estimate the post-mortem interval for TOD determination. Using the Daubert criteria, the methods currently used to estimate PMI were assessed. This study shows that these methods, with the exception of Henssge's nomogram and entomology, do not meet all of the Daubert criteria both nationally and internationally with the exception of mechanical stimulation which is accepted internationally. A method must meet all the Daubert criteria before it can be deemed scientifically reliable and only scientifically reliable methods should be permissible in court. Nevertheless, these methods are being used in Dutch courts and possibly elsewhere. The judges do not seem to be aware of or informed about the fact that, even though these methods are currently the best available, they have significant limitations which should be addressed before or during the trial. A legal system is only as strong as its weakest link and these links should be addressed and thoroughly discussed in court.

CRediT authorship contribution statement

Tamara Gelderman: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Erik Stigter:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Tristan Krap:** Conceptualization, Methodology, Writing – review & editing. **Jens Amendt:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Wilma Duijst:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2021.101970.

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