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ORIGINAL ARTICLE

The off-season of dental cementum investigations. A critical appraisal of season-of-death prediction in medico-legal investigations

Benoit Bertrand [a,b,*], **Ahmad Kaddoura** [a], **Eugenia Cunha** [c,d], **Anne Bécart** [a], **Didier Gosset** [a], **Valery Hédouin** [a], **Carina Marques** [d,e]

[a] *ULR 7367—UTML&A — unité de taphonomie médico-légale et anatomie, CHU de Lille, univeristé de Lille, 59000 Lille, France*
[b] *Muséum national d'histoire naturelle, département homme et environnement, UMR 7194 — HNHP, institut de paléontologie humaine, 75013 Paris, France*
[c] *National Institute of Legal Medicine and Forensic Sciences, South Delegation, Lisbon, Portugal*
[d] *Department of Life Sciences, Research Centre for Anthropology and Health (CIAS), University of Coimbra, Coimbra, Portugal*
[e] *Department of Anthropology and School of Integrative Biological and Chemical Sciences, The University of Texas Rio Grande Valley, Texas, USA*

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Summary It has been suggested that death seasonality could be estimated based on the dark/bright appearance of the outermost dental cementum deposit. The few existing studies show variable accuracy rates. The question whether estimating season-of-death based on dental cementum represents a realistic and achievable goal can arise. This work, based on a software-assisted procedure and direct human observation, aims to critically evaluate the applicability of dental cementum to estimate the season-of-death in forensic anthropology. The sample consists of 20 canines from individuals belonging to the 21st century Identified Skeletal Collection, University of Coimbra, Portugal, and with documented biodemographic data. Fifty percent of the individuals died during the summer and 50% during the winter. Five non-decalcified 100 µm cross-sections per tooth were prepared, using a standardized protocol. Images of the region of interest, with the acellular cementum, were captured, and imported into FIJI/ImageJ software. Performance of the season-of-death predictions was very low, with 50% accuracy, score of 0.0000 for Matthews Correlation Coefficient, and F1-score of 61.5% for Summer and 28.6% for Winter, were obtained for the observer assessment. For the software model, performance was

* Corresponding author: Institut de médecine légale, université de Lille, rue A.-Verhaeghe, 59000 Lille, France.
E-mail address: benoit.bertrand@univ-lille.fr (B. Bertrand).

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equally poor, with 52.6% accuracy, score of 0.0272 for Matthews Correlation Coefficient, and F1-score of 30.8% for Summer and 64.0% for Winter. This work is of relevance to the forensic science community by demonstrating the prospects and limitations of dental cementum as a method for estimating seasonality of death.

Introduction

Pioneer studies emphasized the considerable interest of dental cementum layers in forensic medicine, forensic dentistry, and forensic anthropology [1,2]. Dental cementum refers to the mineralized connective tissue covering the root and shows a layered structure under light microscopy [3]. It has been increasingly considered a reliable age-at-death indicator [4—6] and progressively adopted to broaden the age-at-death methods available in forensic anthropology, contributing, therefore, to the medico-legal identification of unidentified deceased individuals [7—9]. Among histological methods, the dental cementum histology (cementochronology) has opened the door to a marker considered to be the closest to a chronometric measure of age-at-death [2,4,6,9]. The principle of cementochronology in age-at-death estimation relies on the empirical evidence of yearly apposition of the acellular extrinsic fiber cementum (AEFC), which shows little to no remodeling [10]. Despite the unclear underlying mechanisms driving AEFC apposition, the dark incremental line is supposed to grow from late autumn to early spring while the brighter band grows from spring to autumn [11]. Based on this assumption, it has been suggested that, beyond age-at-death estimation, death seasonality can be estimated based on the classification of the outermost cementum deposit by examining whether the final cementum is deposited in the bright zone or within the dark annuli sequence [12,13]. The potential for the use of cementochronology for season-of-death estimation is well established in non-human mammalian species. In mammals, the nature of outmost cementum deposition varies through the year [14]. In regions with temperate climates, it is accepted that the dark annuli in mammalian cementum, which are often referred to as the ''winter line'' [15], typically form during the winter season and vice-versa for Summer.

Notwithstanding, the potential of cementochronology for seasonal variation of death it has been understudied in humans. A pioneer study on humans reported that the season-of-death can be determined to be between spring/summer (April-September) or fall/winter (October—March) with 99% accuracy [13]. In 2013, the same author confirmed the dental cementum's value in estimating season-of-death of a 37-year-old cold-case homicide [7]. The same approach was used to determine the season-of-death of individuals who died during the mid to late 1800s [16]. Interestingly, a re-examination of the teeth from 18—25-year-old individuals collected in Wedel (2007) original study was done by Wedel and Gocha (2019) [17]. The authors could not replicate the original accuracy rates for this subsample, reaching only 78%. Since Wedel (2007) original study, other researchers have attempted to reproduce the protocol and results, but failed to achieve a high accuracy rate. Results from Meckel (2016) reported 60% accuracy in a first observation but correct identification decreased to 18% in a second observation of the same slides, based on teeth extracted from the Texas State University Donated Skeletal Collection. Ralston (2016) study on teeth extracted from living people and cadavers from the Boston University and Antioquia Modern Skeletal Reference Collection, suggest a range between 61.5 and 71.2% of successful identification of the season-of-death. Wilson et al. (2017 and 2022) obtained a 72 to 76% correct assessment of the season-of-death from teeth clinically extracted from a US military cohort. Also, analyzing teeth from living US military personnel, Thomas and Corby (2020) estimates of seasonality ranged from 58.7 to 73.9% (with different microscopic techniques). The application of non-standardized procedures for histological preparation or micrographs analysis could partially explain variability in the outcomes across studies, but these cannot fully justify the discrepancies.

Considering that estimation of season-of-death has the potential to narrow down the list of potential missing persons, and thus contribute to the process of medico-legal identification of unidentified deceased individuals, it becomes crucial to test the potential of dental cementum techniques for season-of-death estimates. Another advantage of cementochronology is that teeth are likely to be preserved and recovered in forensic cases, contrary to several other anatomical structures, which means that this method could be applied even in highly fragmentary remains, thus, the use of cementochronology for the season-of-death has an enormous potential for the criminal justice system. This paper aims to demonstrate and discuss the prospects and limitations of dental cementum as a method for estimating seasonality of death, by evaluating its accuracy in a documented sample with a standardized protocol. It is also our goal to evaluate the differences between manual and software-assisted procedures on the accuracy of the season-of-death prediction.

Material and methods

The sample used in this study comprises well-preserved permanent canine teeth, without visible signs of pathological conditions or noticeable taphonomic changes, extracted from individuals belonging to the *Coleção de Esqueletos Identificados Século XXI* (CEI/XXI), curated at the Laboratory of Forensic Anthropology, Department of Life Sciences at the University of Coimbra [18,19]. The collection comprises 302 individuals with known age-at-death, sex, date of death and exhumation, with deaths occurring between 1982 and 2012. The individuals are of Portuguese nationality [18,19].

Our sample selection criteria included individuals with at least one canine tooth present, information on postmortem

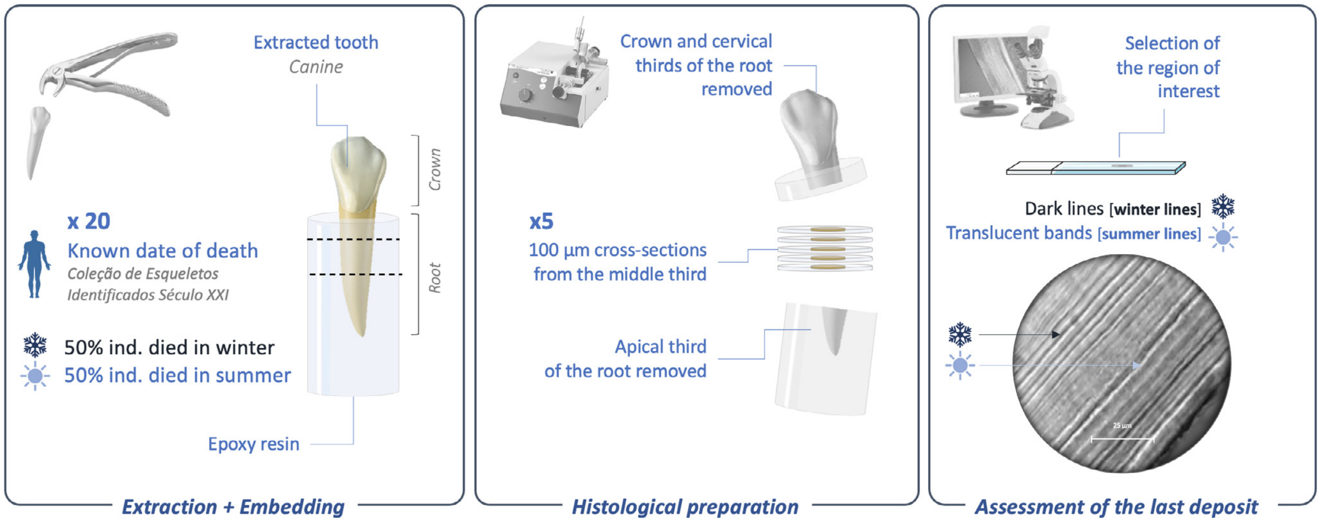

Figure 1 Season-of-death estimation preparation and premises.

interval, known age-at-death, sex, and date/season-of-death. We also selected individuals who died at the end of the summer and end of the winter. This approach ensured that there was sufficient time for the full development of dental cementum during the seasons under investigation. The sample in our study consisted of 20 canines — i.e., one per individual — (four upper canines (right [$n = 2$]; left [$n = 2$]); and sixteen lower canines (right [$n = 7$]; left [n = 9])), from persons of both sexes (65% [$n = 13$] males, 35% [$n = 7$] females; $P = 0.263$). Ages ranged from 46 to 89 years ($\bar{x} = 75.3$ years; $\sigma = 10.8$ years; $n = 20$). Individuals died between 1997 and 2009 ($\bar{x} = 2004$; $\sigma = 4.4$ years; $n = 20$). Time between death and exhumation for most individuals was six years, with exception of two individuals with 15 years and 7 years of interval ($\bar{x} = 6.6$ years; $\sigma = 2.0$ years; $n = 20$). The sample consists of 50% of the individuals died at the end of the summer and 50% at the end of the winter.

The dental sample underwent processing in accordance with our established histological protocol (Fig. 1) aiming to minimize subjective variations, as described in Bertrand et al. (2019). Each tooth was cleaned and dried before embedding the root in epoxy resin (Araldite® 2020). Subsequently, the crown and cervical third of the root were removed, and cross-sections were prepared in the middle third of the root. The rationale behind this choice is primarily related to the exposure of the apex and the cervical third to pathological conditions. Additionally, the apical third of the root is prone to hypercementosis, and this region is subject to various mechanical stresses that can lead to the production of reactive cellular intrinsic fiber cementum. Therefore, to maximize the chances of identifying AEFC in our preparations, we focus our sectioning on the middle third of the root. To ensure reproducibility of observations, we performed five standardized 100 micrometers cross-sections, as variations in thickness across studies have been shown to introduce bias and affect the interpretation of results. This process involved using a Buehler® IsoMet Low Speed Saw equipped with a diamond-coated blade, and the resulting sections were mounted on slides. Observations were carried out using a Leica® DMEP microscope at $\times$ 400 magnifications. Region of interest (ROI), defined when a well-preserved AEFC and appropriate layering structure for the intended analysis was identified, was captured in Leica Application Suite X (LAS X) as JPEG images using a Leica® K3C camera.

Two approaches for identifying the optical nature of the outermost deposit were compared. The first consisted of a single observer performing a visual assessment of the last cementum deposit on the micrograph, to determine if it corresponds to a dark incremental line (rest line also called winter line) or a bright zone (growth line also called summer line). A second approach was devised based on software-assisted analysis (Fig. 2). The software FIJI/ImageJ [20,21] was used to objectively categorize the outermost deposits into dark or bright categories. Digital micrographs were imported into the FIJI/ImageJ software. The microscope settings applied to highlight cementum alternating microstructure often leads to a decentering of the light path to provide optimum contrast of cementum structure, but it also provides uneven illumination across the field of view. Thus, to correct defects caused by uneven illumination, each image was adjusted using the FIJI/ImageJ plugin ''Subtract background''. To eliminate dental tissue coloration, we used the plugin ''Split color channels'' to work on the green channel, which contributes more than other RGB channels to the luminance signal. With the tool ''Plot profile'', a two-dimensional graph of intensities across clearly perceptible cementum microstructure was constructed for each digital micrograph. The tool ''Find peaks'' allowed the detection of maximum and minimum intensity values across the cementum, which correspond to bright bands and dark incremental lines. These steps were repeated for each tooth with the same parameters for the width line when plotting the profile (linewidth = 10) and the same minimum peak amplitude (min. peak amplitude = 04) when detecting the peaks. Then ten plots were positioned on the outermost deposit, using the ''Point tool'', and intensity values of the plots were computed on FIJI/ImageJ to be compared with maximum and minimum intensities of the peaks (Fig. 2). The intensity values of the outermost deposit were then compared with the mean intensity values of the bright and dark intensities of

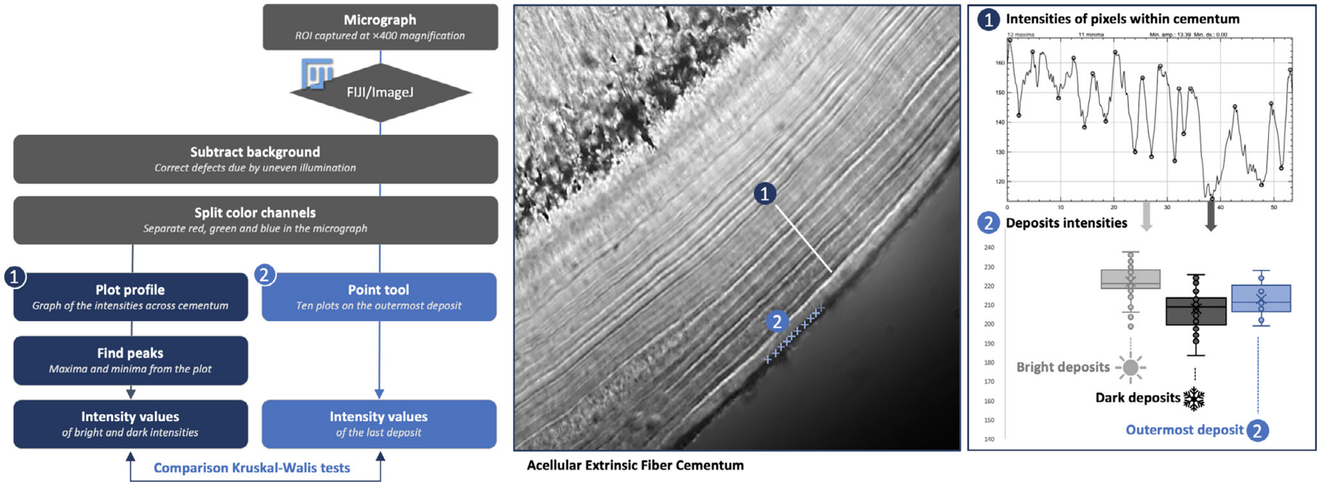

Figure 2 Procedure of the software-assisted analysis FIJI/ImageJ.

the cementum, by examining box and whisker plots. Kruskal-Wallis test was used to determine if the intensity of the outmost deposit was statistically different from intensity values of the bright or dark deposits obtained across the cementum (Fig. 2). When investigating box and whisker plots, we considered that a higher intensity of the last increment relative to the maximum values of the overall cementum, would correspond to a bright increment, and a lower intensity relative to the minimum values would correspond to a dark increment. These were the parameters used to categorize an outmost layer into bright or dark classification. The intensity values of the alternating deposits detected across the profiles and on the outermost deposits were saved as a Microsoft® Excel file.

A confusion matrix, also known as error matrix, was computed to measure the performance of the classification models [22] for both approaches: visual observation and software-assisted. The output matrix cells allow the calculation of the following overall performance metrics: accuracy, error rate, and Matthews correlation coefficient (MCC), which this summarizes the classifier performance in a single value, if both classes cases are of equal importance [23]. Precision (i.e., predicted value), specificity, recall (i.e., sensitivity) and F1-score (the harmonic mean of the precision and recall scores) were metrics obtained for both the Summer and Winter cohorts.

Statistical analysis was performed using the analyzed using XLSTAT software (Addinsoft, Paris, France 2017) and IBM SPSS Statistics (Version 27). Fisher's exact test, Mann Whitney U, and Kruskal-Wallis were used for inferential statistics. Significance was accepted for $P \leq 0.05$ for all tests except for Kruskal-Wallis where significance was set at P-value < 0.0001. Cohen's Kappa was the metric used to assess agreement and to compare between the operator and software performances.

Results

Since there was no significant difference between the group of individuals that died in the summer and the ones that died in the winter, regarding the variables sex ($P = 1.000$), age-at-death ($\bar{x}$Summer = 77.2 years, $\sigma = 7.5$ years; $\bar{x}$Winter = 73.3 years, $\sigma = 13.5$ years; $U = 43.500$, $P = 0.622$) or time since death and exhumation ($\bar{x}$Summer = 6.0 years, $\sigma = 0$ years; $\bar{x}$Winter = 7.2 years, $\sigma = 2.8$ years; $U = 65.000$, $P = 0.068$), these variables do not seem to function as biasing factors in the performance of the estimation.

Visual assessment of the season-of-death

The observer was able to visually categorize the outermost deposits into dark or bright categories on all the cross-sections considered ($n = 20$). In 80.0% (8/10) of the individuals who died at the end of the Summer ($n = 10$), the observer identified a bright outermost deposit in the teeth, and these individuals were rightly classified as dying in the Summer (Table 1). On the individuals who died in the Summer, two (20.0%) were classified by the observer as having dark outermost deposits and wrongly classified as dying in the Winter (Table 1).

Interestingly, only 20.0% (2/10) of individuals who died in the Winter had an outermost deposit that was classified by the observer as dark. The eight (80%) other teeth were classified as having a bright outermost deposit, thus incorrectly classified as individuals that died in the Summer (Table 1).

Table 1 depicts the observer's classification model and the overall model metrics. The overall classification has insufficient performance. Low accuracy scores (50.0%) coupled with a 0.000 score for the MCC coefficient (varies between −1 and 1) corresponds to a method whose overall predictive value is similar to one made by chance, i.e., coin toss. Even if F1-scores (61.5%) and recall (80.0%) suggest that the method works slightly better for estimating Summer deaths (i.e. bright outmost layers) than Winter deaths (with lower F1-scores [28.6%] and recall [20.0%]), it does have overall very low specificity (20.0%), i.e., produces a high number of false Summer classifications. Whereas the low recall (20.0%) scores in the Winter cohort, shows that the method fails to identify many individuals that actually died in the Winter.

Table 1 Confusion matrix and metrics of performance for the observer (visual assessment) model.

		Predicted		Group	Precision (predicted value)	Specificity	Recall (sensitivity)	F1-score
		Summer	Winter					
Actual	Summer	8	2	Summer	50.0%	20.0%	80.0%	61.5%
				Winter	50.0%	80.0%	20.0%	28.6%
	Winter	8	2	Accuracy	Error rate	MCC		
				50.0%	50.0%	0.0000		

MCC: Matthews correlation coefficient.

Software assessment of the season-of-death

Prior to analyzing the optical nature of the last cementum deposit, we ensured that there was a significant difference in intensity values between the light and dark increments across the acellular cementum. This step involved the computation of intensity profiles across 20 micrographs and generated a total of 953 maximum peaks and 974 minimum peaks (with an average of 48 maximum and 49 minimum peaks per micrograph). The mean intensity of the bright increments was 223.4 ($\sigma = 8.5$) and the mean intensities of dark increments was 208.7 ($\sigma = 9.9$). These intensity values are dimensionless measure ranging from 0 to 255 where 0 represents black and 255 represents white. A Kruskal-Wallis test validated the significant difference between the intensity values of bright and dark increments (P-value < 0.0001) for all the samples. The software categorized 95% (19/20) of outermost deposits into dark or bright categories in comparison with 100% noted in the human observer.

Results on ten teeth from individuals who died in the Summer, indicate that one classification was inconclusive, whereas in 20% (2/10) the outmost deposit was classified as bright and correctly classified as belonging to individuals who died in Summer, whereas 70% (7/10) of the outmost deposits were classified by the software as dark and thus wrongly classified as Winter deaths (Table 2).

In the group of individuals that died in the Winter ($n = 10$), 80% (8/10) were classified by the software as having a dark outmost deposit, thus being correctly classified as Winter deaths. Only 20% (2/10) were classified as having a bright outermost deposit and therefore incorrectly assigned to Summer deaths (Table 2).

Table 2 depicts the software classification and the overall model metrics. As noticed previously with the human observer, the software also has a poor performance. Low accuracy scores (52.6%) coupled with a 0.0272 for the MCC coefficient (varies between −1 and 1) corresponds to a method whose overall predictive value is very low. Even if F1-scores (64.0%) and recall (80.0%) suggest that the method works slightly better for estimating Winter deaths (i.e., outmost dark layers) than Summer deaths (with lower F1-scores [30.8%]) it does have very low specificity (22.2%), i.e., produces a high number of false Summer classification. Whereas the low recall (22.2%) for the Summer cohort, shows that the method fails to identify many individuals that actually died in the Summer.

Software and human observer agreement on the classification was merely 30.0% (6/20). As expected, the inter-rater (observer vs. software) reliability was very low, with Cohen's Kappa having values ≤ 0, indicating no agreement (value = −0.365).

In our study, season-of-death estimation based on the outmost deposit had a very low performance. The overall accuracy of the software is just slightly better than the one from the human observer but for both approaches, season-of-death predictions based on the assessment of the outermost cementum layer has an overall predictive value that is similar to one made by chance, i.e., coin toss. The software correctly identified 20.0% of the individual who died at the end of the summer while the human operator ability to correctly identify individuals who died in Summer reaches 80.0%. Conversely, the software correctly identified 80.0% of the individual who died at the end of the Winter, but the human observer only correctly identifies 20.0%. It seems the human observer has a higher capacity to detect bright deposits while the software performs better in dark deposits.

Discussion

The underlying cause of the seasonal pattern in cementum deposition remains unclear. A genetically controlled physio-

Table 2 Confusion matrix and metrics of performance for the software model.

		Predicted		Group	Precision (predicted value)	Specificity	Recall (sensitivity)	F1-score
		Summer	Winter					
Actual	Summer	2	7	Summer	50.0%	80.0%	22.2%	30.8%
				Winter	53.3%	22.2%	80.0%	64.0%
	Winter	2	8	Accuracy	Error rate	MCC		
				52.6%	51.6%	0.0272		

MCC: Matthews correlation coefficient.

logical process is supposed to be involved in the deposition of cementum but hypotheses in non-human mammalian species propose a potential connection to seasonal variations in diet composition impacting tooth wear and consequently compensatory deposition of cementum [24]. However, this explanation may not be universally applicable, particularly to domestic species or to modern humans given their lower susceptibility to dietary changes. This could partly explain that in non-human mammalian species assessment of the season-of-death has been successful [14,25–27], while in the human cohorts the accuracy levels have been surprisingly low and variable, as seen in our study and in previous studies as well [13,28–31].

Other factors that might explain the accuracy inconsistencies in assessing the season-of-death across studies are the sampling and source of the dental material. Due to sample availability, studies evaluating seasonality of death are mostly based on teeth collected from therapeutic avulsions for pathological, orthodontics, or prosthetic reasons [13,28–31]. Fewer on teeth collected for skeletal collection [32] or mixed sources such as therapeutic avulsions, skeletal and cadaver collections [33]. We hypothesize that the differential preservation of soft tissues could impact the examination of the outermost deposit of dental cementum. In clinical samples, if still attached to the mineralized tissue, the periodontal ligament adjoining the outermost cementum deposits could lead to confusion. As indicated by Lieberman (1990) [34], this connective tissue could be misinterpreted as a dark deposit or hide the latest deposit. Furthermore, on avulsed teeth, the periodontal ligament fibers are torn and this could result in cementum superficial damages. Certainly, the superior preservation of both the mineral and organic matrix undeniably constitutes favorable factors contributing to the observed enhanced performance. Further studies are required to elucidate the potential biasing influence resulting from sample sources.

Several studies exploring dental cementum highlighted that taphonomic alteration limits the applicability of cementochronology in age-at-death estimation [6,35–37]. Dental cementum undergoes various postmortem modification through processes like bioerosion, microfractures and infiltrations [6]. Regardless of the type of postmortem alterations, these changes likely primarily affect the outermost increment, which are particularly exposed to the environment. Ralston (2016) [33] study suggests that teeth extracted from skeletal remains/cadavers versus living individuals may influence the accuracy of the season-of-death estimation. The authors observed a slightly lower success rate (67.5%) of the season-of-death identification in the modern skeletal reference collection from Colombia and in teeth extracted from cadavers (63.0%) than on the teeth extracted from the living (71.2%). This may indicate that visibility of the cementum increment is influenced by taphonomic changes of the cementum tissues. The subjects included in our study were exhumed from a cemetery and time between death and exhumation was six years (with the exception of two individuals with 15 and 7 years). Thus, taphonomic alteration could be responsible for the low success rate. The evaluation of taphonomic alteration is of relevance but no studies have been conducted to date on the impact of taphonomy on the estimation of the season-of-death estimation.

Furthermore, the sampling of different tooth types and multiple teeth from the same individual can have an impact on the season-of-death estimation. Biological properties of human acellular cementum also may vary between dental types. Yet, cementum-based studies for season-of-death estimation deposits involve single rooted teeth [38] or involve material without differentiation between single and multi-rooted teeth [13,28–30,33]. Our sampling strategy, focusing exclusively on one dental type, the canines, did not result in high accuracy rates in comparison with previous studies. Paradoxically, our predictions exhibit one of the lowest accuracy rates (50% accuracy for the observer and 52.6% for the software-based assessment). Multiple dental extractions in the same individual (46 teeth/17 individuals [30]; 58 teeth/50 individual [29]; 132 teeth/100 individuals [32]) could possibly cause an unintentionally artificially improved accuracy. Another sample strategy that could impact the variability of season-of death accuracy is the uneven distribution of the sample in terms of Summer/Winter cohorts. A marked unbalance of seasons (29.1% Spring/Summer and 70.9% Fall/Winter [38]; 42% Spring/Summer and 58% Fall/Winter [28,29]; 67.4% Summer and 32.6% Winter [30]), can be a source of bias. We detected that the observer tends to detect the optical nature of the bright deposits, thus an uneven distribution of seasons of death in the sample could be detrimental to the assessment, unless statistical models for unbalanced categories are applied.

The identification of the outermost cementum deposit is challenging due to physical properties of the tissue. Even if the ultrastructure is still an open question, the main component of dental cementum is hydroxyapatite crystal and collagen type I. The alternating pattern of acellular cementum is related to the alignment of collagen fibrils and mineral density [39]. The fine details of the cementum ultrastructure make it difficult the optical investigations, since they are dependent on the numerical aperture and the resolution of microscope objectives, and on microscopy techniques/settings. Thomas and Corby (2020) [30] showed varied accuracy of season prediction using different microscopic approaches – brightfield, polarized, and phase-contrast techniques, showing the important influence of the techniques on the season-of-death estimation. Based in our experience and corroborated by Thomas and Corby (2020), we suggest a standardization of the microscopy techniques to adopt, and researchers should avoid adapting the equipment available in their laboratory for the use in season-of-death estimation. We do not recommend the use of reflected light microscopy, since Lieberman et al. (1990) raised a crucial problem in link with these technical aspects. While observed under transmitted light, increments associated with growth lines are translucent and broader than those linked to the rest lines which tend to appear opaque and narrower, in reflected light, growth bands are characterized by their dark appearance, whereas rest lines appear lighter. This may lead to inappropriate interpretation.

The identification of the outermost increment is also challenging due to the cross-section thickness. Consistency in processing histological slices is fundamental across studies so we can achieve comparability. Manual grinding and polishing phases to reach the recommend 100-μm thickness in cementochronology [6] should be avoided as it can produce the beveling of the cross-section (and consequently unequal thickness). The illumination used to enhance contrast of transparent samples may cast a shadow at the outer edge of the dental tissue that can mimic a dark increment. All the studies have been performed visually by human observers, making the identification of the outermost deposit subjective. This subjectivity was already mentioned by Ralston (2016). Thus, a wide variety of technical settings can account for the variability in results across published studies.

In a previous study dedicated to age-at-death estimation, we demonstrated a significant relationship between chronological age and cementum microstructure distinctness, indicating that cementum annuli could be hardly distinguishable in elderly individuals. This statement is aligned with observations in large mammals [40]. Meckel (2016) suggests that season-of-death estimation could be more accurately applied to young adults. The average age of individuals included in our sample ($\bar{x}$ = 75.3 years; σ = 10.8 years) could, therefore, partially explain our poor accuracy. However, Wedel and Gocha (2019) [17] reassessment of a younger subset of Wedel (2007) [13] sample did not find a particularly high accuracy rate (78%) when compared with Wedel (2007) assessment of a wider age-at-death sample. The question of the influence of chronological age on the accuracy of the season-of-death prediction remains open as well.

Finally, the fundamental difference for different climate zones was never addressed. A convenient assumption is that cementochronology does not require reference populations and its performance is independent of variables such as climate or latitude variation [8,41]. Yet, in non-human mammals a decrease in the distinctness of layers is documented in animals of different populations of the same species to the gradient of seasonality of climate between regions where they lived [12]. Condon et al. (1986) [42] suggested that the rates of the formation of dental cementum in human, and therefore its correlation coefficient, could potentially vary between populations, thus requiring population-specific equations to estimate age-at-death. In regard to season-of-death estimation, Grue and Jensen (1979), documenting incremental growth in 52 animal species, have discovered that annuli may be formed at other times during the year, in summer for instance [43]. The low accuracy of the season-of-death estimations on our sample derived from Southern European population could be explained to a certain extent by some factors such as latitude and climate. This is another potentially biasing factor that requires further research.

Our results suggest that the prediction works slightly better for estimating Summer deaths than Winter deaths for the human observer. This suggests an observer's tendency to more readily identify a bright outermost deposit and/or to fail to appreciate the dark last deposit. To get rid of subjective appreciation, we evaluated the feasibility of using the software-assisted tool to objectively quantify the luminance of the last cementum deposit. However, the utilization of the analytical tool developed on FIJI/ImageJ transfers subjectivity from identifying the optical nature the outermost deposit to positioning plots on the outermost deposit. In this regard, the adoption of this analytical tool system is not entirely objective since an operator is still required to target the outermost deposit. The overall accuracy of the software is just slightly better than the human but remains very low. The software-assisted approach correctly identified 80.0% of the individual who died in the Winter. It seems the human observer has a higher capacity to detect bright deposits while the software performs better in dark deposits. As we previously stated [44], subjectivity is often associated with bias, but it can also be viewed as a valuable asset. It is possible that the human observer can more readily distinguish the artifacts, however, as most of the abovementioned factors, further research is required to assess inter-observer error in order to evaluate whether human observers produce consistent estimates. Additionally, further research is necessary to properly understand the usefulness of cementum data for season-of-death estimation. This line of research has been much less investigated than other components of dental analysis in forensic anthropology. We believe that there is an enormous potential for season-of-death estimation and its usefulness in forensic anthropology. Yet larger scale studies on documented collections are needed to identify and evaluate intrinsic and extrinsic factors that may influence the accuracy of season-of-death estimations. These factors include age-at-death, date-of-death, sex, population affinity, pathological conditions, as well as environmental variables such as latitude, climate, and taphonomy. Understanding the impact of these variables is essential for refining the methodology and improving the reliability of season-of-death estimations in forensic contexts.

Conclusions

The present work highlights the importance of critically evaluate dental cementum-based methods for season-of-death estimation. Even with the use of a standardized protocol for preparation and a software-assisted analysis of luminance, this study reveals a performance that is not better than chance of correctly correlating the outermost deposits with a spring/summer or fall/winter season for both the human observer and for the software-assisted analysis. This study shows that identification of the outermost deposit is highly challenging due to physical properties of dental cementum, the optical properties of the microscope's objective lenses, and possibly taphonomic or intrinsic factors, which need to be further evaluated in future research.

Cementochronology has emerged as a versatile and reliable tool for age-at-death estimation in forensic contexts. By analyzing the incremental layers in acellular dental cementum, this method provides a valuable and precise means of estimating age-at-death, even in cases where other skeletal indicators may be compromised. However, even if cementochronology for the estimation of the season-of-death was tested and subjected to peer review publications [7,13,16], partially meeting Daubert standards [45], it does not yet fulfill additional and crucial requirements by the judicial system that requires the application of non-subjective, reliable, standardized, and reproducible methods in forensic science [46].

In the absence of further tests taking into account the impact of taphonomy, population and climate zones and publications validating this approach in human, the question whether estimating season-of-death based on dental cementum represents a realistic and achievable remains open.

Disclosure of interest

The authors declare that they have no competing interest.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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