Is the cervical bone a biological marker for bone age assessment?

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KEYWORDS

Bone Age Assessment, Cervical Vertebrae, Cephalometric, Baccetti's morphological staging system, Cameriere's morphometric method

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ABSTRACT

Age assessment is essential in forensic practice when the chronological age of living individuals is unknown, particularly in legal, civil, migration, and asylum contexts requiring the judicial determination of age-related rights and responsibilities. This study aimed to evaluate the performance of two cervical vertebral methods in a Portuguese reference subadult population (6-23 years), ethnically homogeneous, using the analysis of the fourth cervical vertebra (C4) on lateral cephalometric radiographs. Two approaches were applied: Baccetti's qualitative morphological staging of C2–C4 and Cameriere's morphometric ratio of C4. A total of 528 lateral cephalometric radiographs, balanced by age and sex, were analysed independently by trained observers. Intra- and inter-observer reliability were evaluated using Intraclass Correlation Coefficient (ICC) and weighted Cohen's kappa (κ). Statistical procedures included correlation analyses, multiple linear regression, Classification Trees (CT), and Receiver Operating Characteristic (ROC) curves to assess performance at legally relevant thresholds (12, 14, 16, 18 years).

Baccetti's method explained 75.3% (coefficient of determination R^2=0.753) of chronological age variability, with a mean error of approximately two years and high accuracy for thresholds of 12–16 years. Cameriere's ratio showed lower explanatory capacity (R^2=0.568) and a mean error of 2.7 years, although its ROC curves demonstrated good discriminatory performance across all age cut-offs. The combination of both methods showed a slight increase in predictive performance (R^2=0.784), although this combined approach was not formally evaluated as an independent model. Females reached skeletal maturity earlier, although error rates were similar between sexes. Reliability was excellent across methods.

Both approaches proved applicable for forensic age assessment, offering complementary advantages: Baccetti's method provides broader morphological interpretation, whereas Cameriere's morphometric ratio offers objectivity and reproducibility. These population-specific reference data support the forensic use of cervical vertebral analysis as part of a multimodal age estimation framework.

Highlights

•Cervical vertebrae (C4) analysis provides reliable forensic age assessment

- Baccetti's staging shows higher accuracy than Cameriere's morphometric ratio
- Combined use of both methods may enhance predictive performance
- Excellent intra- and inter-observer agreement supports reproducibility
- Useful reference data for multimodal forensic age assessment

INTRODUCTION

Forensic odontology plays an essential role in the medico-legal sciences through the examination of dental and craniofacial evidence for purposes of human identification, trauma evaluation, and biological profiling. One of its most critical applications concerns age assessment in living individuals, a procedure with ethical implications in criminal, civil, asylum and immigration contexts.[1-3] In cases where reliable documentation is lacking, age assessment contributes to determining whether a person has reached legally relevant thresholds, such as 12, 14, 16, or 18 years of age.[4,5]

In forensic odontology, biological markers-including dental and skeletal indicators-are the most accurate of age assessment methodologies. These markers reflect biological maturation rather than chronological age and are influenced by genetic, environmental, and socioeconomic factors.[6,7] Dental and skeletal development have long been recognized as the two most reliable systems for assessing biological age.[1,8] In the Portuguese context, multiple studies have validated dental atlases[9-12] and dental quantitative models derived from morphometric radiological parameters.[13-16] These works collectively established the population-specific reference studies in dental age assessment.

Over the last decade, Portuguese forensic odontology has expanded from single-modality assessments toward integrated, multimodal approaches, combining dental and skeletal parameters to improve diagnostic precision. Studies by Pereira and collaborators have contributed substantially to this evolution-ranging from ROC-based model comparisons[17], pulp/tooth ratio analysis via CBCT[18], and combined dental scoring systems[19], to multidisciplinary research on bone and dental age assessment in judicial contexts.[20] Moreover, recent investigations have incorporated machine learning and deep learning models for automated dental evaluation[21,22], reinforcing the transition toward AI-assisted forensic decision-support systems.[22]

Despite these technological advances, manual multimodal assessment-based on measurable and morphological criteria-remains essential to establish validated reference datasets and ensure methodological transparency before AI implementation.

Two manual methods for assessing skeletal maturation in lateral cephalometric radiographs are Cameriere's morphometric approach, which quantifies dimensional ratios of the fourth cervical vertebra (C4), and Baccetti's morphological Cervical Vertebral Maturation (CVM) method, which classifies the developmental stages of the second to fourth cervical vertebrae (C2–C4) based on shape and contour characteristics. [23-25]

Originally developed for orthodontic growth assessment, both methods have since been adapted and validated for forensic age assessment, offering possible alternatives to hand–wrist radiographs while minimizing radiation exposure-an ethical requirement when evaluating living individuals.[20]

In this context, the present study applies these cervical vertebral indicators within a manual multimodal biological marker framework, exploring their medico-legal applicability in a Portuguese subadult population aged 6 to 23 years. The study aims to establish population-specific reference data and to assess the accuracy of the cervical vertebral biological indicator for age assessment in the judicial context, integrating its performance with established dental and hand–bone methods within a multimodal assessment framework.

OBJECTIVES

Main Objectives

The primary objective of this study was to evaluate the performance of the fourth cervical vertebra (C4) in a Portuguese child and subadult population aged 6–23 years, using two independent methods applied to lateral cephalometric radiographs:

(1) Baccetti's morphological staging system, based on qualitative assessment of vertebral shape, and

(2) Cameriere's morphometric method, which quantifies the ratio between the anterior and posterior heights of C4.

Specifically, the study aimed to:

1. Evaluate the correlation between chronological age and bone maturation estimated by each method.
2. Assess the accuracy of both methods in classifying individuals below legally relevant age thresholds (12, 14, 16, and 18 years).

Secondary Objectives
Secondary objectives included examining sex-related differences in the performance of both methods, in order to determine whether male and female developmental patterns significantly influence vertebral maturation scores and bone age assessment.

Final Objective
Finally, this study sought to verify the accuracy and forensic applicability of Baccetti's morphological and Cameriere's morphometric methods for assessing biological age from the fourth cervical vertebra (C4), contributing population-specific reference data to support their integration into forensic odontology practice.

MATERIALS AND METHODS

Experimental Design
This study was conducted jointly at the Department of Stomatology of the Local Health Unit of Santa Maria Hospital (ULS Santa Maria) and the Faculty of Dental Medicine, University of Lisbon (FMDUL). The target population comprised patients who received dental or stomatological care at these institutions and whose clinical records included a lateral cephalometric radiograph as part of routine diagnostic documentation.
Ethical approval for the study was obtained from the Ethics Committee of ULS Santa Maria (approval no. 217/23). The study protocol and all supporting documents were reviewed and approved, including authorization from the Director of the Department of Stomatology and the Clinic Director of FMDUL. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Sample and Data Collection
The dataset consisted of 528 lateral cephalometric radiographs from healthy Portuguese individuals aged 6-23 years.
Radiographs were sourced from the archives of the ULS Santa Maria Stomatology Department and FMDUL. These radiographs were originally acquired for clinical diagnostic purposes (orthodontic assessment), and not for forensic use. Only individuals without known growth disorders or craniofacial abnormalities were included, representing a generally healthy population. Only images of diagnostic quality, free from distortions or artifacts, were included to ensure measurement precision.
To maintain statistical validity, radiographs were selected to achieve balanced distribution by age and sex across the sample. All data were retrieved from existing clinical records with no direct patient contact, and full anonymisation was ensured. Case identifiers were removed after linkage to project codes, and all variables (sex, birth date, examination date and age at the date of examination) were stored in a structured Excel® file designed to maintain data traceability and confidentiality.
For intra- and inter-observer reliability, a randomly selected subsample of 114 radiographs (more than 20%) was re-evaluated.

Baccetti's Method
Baccetti's method is a non-metric, morphological approach that evaluates the maturation of the second, third, and fourth cervical vertebrae (C2-C4) using lateral cephalometric radiographs.
It assesses two main features:
1. The presence or absence of concavity along the inferior border of each vertebral body.
2. The shape transformation of the vertebral bodies, which progress from trapezoidal to rectangular forms as skeletal maturation advances.

Skeletal development follows a predictable pattern characterized by increasing concavity of the inferior margins and a gradual change in the vertebral height-to-width ratio.[26] Baccetti's classification defines six cervical maturation stages (CVM I–VI), ranging from flat inferior borders and trapezoidal vertebrae (CVM I) to pronounced concavities and vertically rectangular shapes (CVM VI). These stages provide a morphological scale of skeletal maturity that can be consistently identified on cephalometric radiographs.

Cameriere's Method
Cameriere's method adopts a morphometric quantitative approach applied specifically to the fourth cervical vertebra (C4). The method

involves measuring the anterior (A) and posterior (B) heights of the C4 vertebral body and computing the Vba ratio (A/B). This ratio reflects the degree of vertebral body development: younger individuals typically show trapezoidal shapes (B > A and, consequently, Vba < 1), while progressive skeletal maturation leads to a rectangular configuration (A ≈ B and, therefore, Vba ≈ 1)[2]. Hence, Vba increases towards 1 with age. Measurements were obtained using ImageJ® software (National Institutes of Health, Bethesda, MD, USA) for accuracy and reproducibility.
Special attention was given to ensure that the anterior height was measured along the straight segment of the anterior surface, excluding any curvature near the superior border to avoid measurement bias. Only radiographs showing clear visualization of the C4 vertebral body were analyzed. This method is indicated for subadult individuals without developmental or traumatic anomalies affecting the cervical region.

Statistical Analysis
All statistical analyses were performed using IBM SPSS Statistics, version 30.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $\alpha = 0.05$, with results considered significant at $p < 0.05$. Confidence intervals (CI) will be displayed using 95% confidence.

Reliability Analysis
Intra- and inter-observer reliability were evaluated using the Intraclass Correlation Coefficient (ICC) (two-way mixed-effects, absolute-agreement model) and weighted Cohen's kappa (κ). A random subsample of at least 20% was re-measured after two weeks for intra-observer consistency.

Descriptive and Inferential Statistics
Descriptive statistics (mean, standard deviation (SD), range) were calculated for all variables.
Correlation and multiple linear regression models were developed to assess the relationship between chronological age and morphological (Baccetti) and morphometric (Cameriere) parameters of the C4 vertebra. Predictive performance was expressed as the coefficient of determination (R^2), the mean error of estimate (MEE), the standard error of estimate (SEE), and the mean absolute error of estimate (MAE).

Classification Accuracy
To test the discriminatory ability of both methods for legally relevant age thresholds (12, 14, 16, and 18 years):

1. Classification Tree (CT) analyses were performed with 10-fold cross-validation.
2. Receiver Operating Characteristic (ROC) curves were generated, and the Area Under the Curve (AUC) was computed to quantify classification accuracy and diagnostic performance.

RESULTS

Reliability Analysis

Baccetti's Method
To assess reliability of vertebral staging, a random subsample of at least 20% of the dataset (114 cephalometric radiographs) was re-evaluated:

- Intra-observer agreement: weighted Cohen's κ = 0.914 → almost perfect concordance.
- Inter-observer agreement: weighted Cohen's κ = 0.804 → substantial/good concordance.

Although the two-week interval may be considered relatively short, it was deemed sufficient to minimise recall bias given the sample size and randomisation of cases.
These findings indicate high repeatability, particularly within-observer (Supplementary Table S1).

Cameriere's Method
Using the same n = 114 subsample, replicability of Vba was quantified with ICC (two-way mixed, absolute agreement) Table 1:

- Intra-observer ICC = 0.999 → excellent.
- Inter-observer ICC = 0.946 → excellent.

Table 1. Intra- and inter-observer concordance coefficients for Cameriere's Method

Type of validation	ICC	Interpretation
Intra-observer	0.999	Excellent
Inter-observer	0.946	Excellent

Thus, Cameriere's linear measurements and the derived Vba (A/B) ratio show very high measurement reliability).

Sample Description

All 528 radiographs from subjects aged 6–23 years were classifiable: 267 males (50.6%), 261 females

(49.4%) (Supplementary Table S2; Figure S1). The sampling strategy achieved an approximately uniform distribution per age and sex, with small shortfalls at 6, 22, and 23 years due to limited archives.

Baccetti's Method

Distribution of Cervical Maturation Stages

Stage frequencies were: CS5 = 31.4% (n = 166), CS4 = 18.4% (n = 97); least frequent CS1 (n = 56) and CS6 (n = 55). Mean chronological age increased monotonically from CS1: 8.05 ± 1.66 years (throughout the article, ± will be used to represent mean ± standard deviation) to CS6: 20.32 ± 2.56 years (range minima/ maxima detailed in Supplementary Table S3). This pattern confirms the expected temporal association between vertebral morphology and maturation.

Linear Regression Model for Age Estimation

A dummy-coded regression using CS2–CS6 as predictors yielded $R^2 = 0.753$ and SEE = 2.55 years ($p < 0.001$ for model and for each coefficient):

Estimated Age (years) = 8.05 + 1.80 × CS2 + 3.37 × CS3 + 7.75 × CS4 + 11.19 × CS5 + 12.26 × CS6.

A statistically significant linear relationship between chronological age and CVM stages was observed ($p < 0.001$).

The model was internally validated using cross-validation procedures applied in classification analyses.

Sex-specific models showed strong fits (Males: $R^2 = 0.785$, SEE ≈ 3.19 years; Females: $R^2 = 0.726$, SEE ≈ 2.68 years) (Supplementary Tables S4–S5).

Estimation Error

For the full sample (N = 528): MEE = 0.00 years (median error of 0.06 years; SD = 2.54 years) and MAE = 2.04 ± 1.51 years (median absolute error of 1.78 years).

The mean estimation error (MEE) of 0.00 years reflects the symmetrical distribution of positive and negative residuals rather than perfect prediction accuracy.

By sex:

- Males: MEE = -0.20 years (SD = 2.41); MAE = 1.92 ± 1.46 years.
- Females: MEE = +0.20 years (SD = 2.66); MAE = 2.17 ± 1.56 years.

No systematic over/under-estimation was observed (Supplementary Tables S4–S5).

Classification at Legal Age Thresholds

Performance at thresholds 12, 14, 16, and 18 years is summarized below Table 2 (full CT/ ROC in Supplementary Tables S6–S9; Supplementary Figures S3–S4):

Table 2. Legal Age Threshods 12, 14, 16 and 18 years old by Baccetti's Method

Legal age (years)	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC (95% CI)
12 years	88.8%	86.5%	93.6%	0.949 (0.932-0.966)
14 years	93.0%	97.6%	87.1%	0.979 (0.970-0.988)
16 years	87.5%	83.0%	91.1%	0.934 (0.913-0.955)
18 years	83.0%	87.4%	80.7%	0.889 (0.862-0.916)

Sex-stratified results remained high (e.g., 14 years males: accuracy 94.8%, AUC 0.985; 12 years females: accuracy 89.7%, AUC 0.951), with a modest drop at 18 years, more pronounced in females (Supplementary Table S7). However, it should be noted that the classification is carried out in the same way for both sexes in all age groups (DT presented in Supplementary Figures S3–S4).

Cameriere's Method

Sample Vba Distribution

In the N = 528 dataset, Vba (A/B) ratio for C4 ranged 0.54–1.09; mean 0.83 ± 0.09 overall (Males 0.82 ± 0.09; Females 0.83 ± 0.08). The ratio increased with age (children < adolescents < at least 16 years), reflecting the transition from trapezoidal to rectangular vertebral body morphology (Supplementary Table S8).

Linear Regression for Age Estimation

A model including A, B, and Vba showed $R^2 = 0.568$; SEE = 3.37 years ($p < 0.001$). The simplified model (A + Vba) performed identically ($R^2 = 0.568$; SEE = 3.37 years), confirming Vba as the dominant predictor.

Pooled equation: Age (years) = -18.70 + 0.040 × A + 39.04 × Vba (*both coefficients p* < 0.001).
Sex-specific fits: R^2 = 0.621 (males) vs 0.533 (females); SEE ≈ 3.37 years in both (Supplementary Tables S9–S10).

Estimation Error
Overall: MEE = 0.00 years (median error of -0.005 years; SD = 3.36 years) and MAE = 2.67 ± 2.05 years (median absolute error of 2.33 years).
By sex:

- Males: EE = +0.29 years; MAE = 2.84 ± 2.16 years.
- Females: EE = +0.09 years; MAE = 2.72 ± 2.20 years (Supplementary Tables S9–S10).

Classification at Legal Age Thresholds
Performance at thresholds 12, 14, 16, and 18 years is summarized below (table 3). CT and ROC analyses (Supplementary Tables S11–S14; Supplementary Figures S5–S8):

Table 3. Legal Age Thresholds 12, 14, 16 and 18 years old by Cameriere's Method.

Threshold	Accuracy	Sensitivity	Specificity	AUC (95% CI)
12 years	82.6%	89.3%	68.8%	0.886 (0.857–0.915)
14 years	85.6%	90.8%	78.9%	0.921 (0.899–0.944)
16 years	80.5%	89.8%	73.0%	0.904 (0.879–0.928)
18 years	83.5%	73.1%	88.7%	0.899 (0.874–0.925)

Performance peaked at 14 years (AUC 0.921). At 18 years, specificity was high (88.7%) but sensitivity declined (73.1%), indicating a conservative classification above majority.
Sex-stratified results clustered accuracy 80.5-85.6% with AUC ≥ 0.889 up to 16 years; at 18 years, specificity stayed very high (>92%) while sensitivity dropped (notably males 60.9%) (Supplementary Table S15). On the other hand, it should be observed that the classification is carried out using different classification thresholds in the two sexes (DT presented in Supplementary Figures S9–S12).

Comparative Performance of Baccetti's and Cameriere's Methods at Legal Age Thresholds

At 12 years, Baccetti outperformed Cameriere on overall accuracy (88.8% vs 82.6%) and specificity (93.6% vs 68.8%), while Cameriere showed slightly higher sensitivity (89.3% vs 86.5%).
At 14 years, Baccetti achieved 93.0% accuracy and AUC 0.979 vs 85.6% and 0.921 for Cameriere.
At 16 years, Cameriere had higher sensitivity (89.8% vs 83.0%), but Baccetti retained higher specificity (91.1% vs 73.0%) and overall accuracy (87.5% vs 80.5%).
At 18 years, overall accuracy was comparable (83.0% vs 83.5%). Baccetti favored sensitivity (87.4%), whereas Cameriere favored specificity (88.7%). AUCs were similar (0.889 vs 0.899).
(Full comparison in Supplementary Table S16)
Resume from the main results:

- Reliability was high/excellent for both methods (κ up to 0.914; ICC up to 0.999).
- Predictive strength for age was higher with Baccetti (R^2 ≈ 0.75; SEE ≈ 2.55 years) than with Cameriere (R^2 ≈ 0.57; SEE ≈ 3.37 years).
- Classification near legal thresholds was excellent at 12–16 years for both methods; Baccetti consistently delivered higher accuracy/AUC up to 16 years.
- At 18 years, methods were comparable overall, with complementary profiles (Baccetti exhibits greater sensitivity; Cameriere exhibits greater specificity).
- Sex effects were modest; patterns were broadly consistent between males and females.

DISCUSSION

Reliability analysis

Both approaches showed high reproducibility. For Baccetti's CVM staging, weighted Cohen's *κ* reached 0.914 (intra-observer; almost perfect) and 0.804 (inter-observer; substantial), in line with reports that CVM can be reliably scored after calibration and with standardized viewing conditions[10,25,27]. For Cameriere's morphometric method, ICCs were 0.999 (intra-observer) and 0.946 (inter-observer), indicating excellent repeatability and consistent with published recommendations from AGFAD (Schmeling et al., 2008) and IOFOS (International Organization for Forensic Odonto-Stomatology) guidelines for forensic age estimation in living individuals.[1,28,29] As expected, metric measurements (A, B, A/B) tend to attenuate subjective variability relative to visual staging, a

trend also observed in national validations using vertebral and dental indices. [5,13,19]
Importantly, all radiographs used in this study were originally acquired for clinical diagnostic purposes, avoiding additional radiation exposure. This is particularly relevant in forensic age estimation of living individuals, where ethical guidelines recommend the use of existing medical images whenever possible.[1,4]

Baccetti's method

Sample characteristics
The dataset (N = 528; 267 males, 261 females; 6–23 years) was broadly balanced by age and sex, with only modest under-representation at 6, 22 and 23 years—sufficient for robust modelling and sex-stratified analyses. This mirrors prior Portuguese cohorts and facilitates comparability across studies. [9,10,20]

Stage distribution and biological plausibility
Chronological age increased monotonically from CS1 (8.05 ± 1.66 years) to CS6 (20.32 ± 2.56 years), reproducing the expected maturation trajectory described in classical skeletal/dental research[32,33,34] and recent CVM literature[25,35]. Wider age dispersion at advanced stages (CS5–CS6) reflects known interindividual variability around and after the pubertal growth spurt. Females presented lower mean ages per stage, consistent with sexual dimorphism in skeletal timing. [19,36]

Regression performance
CVM stages predicted age with R^2 = 0.753; SEE = 2.55 years (p < 0.001), aligning with Portuguese/European validations that report strong effect sizes for CVM.[10,15,22,25] Sex-specific models preserved high fit (males R^2 = 0.785, females R^2 = 0.726). These findings support CVM as a dependable predictor for forensic purposes when scored under standardized criteria and with examiner training, as advocated by AGFAD.[1]
The predictive performance observed in this study ($R^2 \approx 0.75$) is consistent with previous studies in European populations, which report values ranging between 0.70 and 0.80, supporting the robustness of this method across different cohorts. [25,35]

Classification at legal thresholds
CVM showed excellent discrimination between 12–16 years (accuracy ≥ 87%; AUC 0.934–0.979), peaking at 14 years (accuracy 93.0%, sensitivity 97.6%, AUC 0.979). As widely reported, performance declined modestly at 18 years (accuracy 83.0%, AUC 0.889) due to increased biological heterogeneity near adulthood[32,33]. Sex-stratified metrics remained high, with slightly different sensitivity/specificity balances across sexes-patterns consistent with earlier maturation in females.

Cameriere's method

Morphometric trends
The C4 Vba ratio rose progressively with age (overall mean 0.83 ± 0.09; males 0.82 ± 0.09, females 0.83 ± 0.08), corroborating Cameriere's original observations and subsequent validations that the vertebral body evolves from trapezoidal to rectangular geometry during adolescence.[2,26]

Regression performance
Models including A, B, and Vba achieved R^2 = 0.568; SEE = 3.37 years (p < 0.001). A reduced model (A + Vba) performed equivalently, confirming Vba as the dominant predictor. Sex-specific fits were R^2 = 0.621 (males) and 0.533 (females). Although precision was lower than CVM, residuals and MAE = 2.67 ± 2.05 years remained within accepted forensic ranges for radiographic indicators14,18,37.
Similar levels of performance ($R^2 \approx$ 0.50–0.60; SEE ≈ 3–4 years) have been reported in previous validations of Cameriere's method, indicating that the present results fall within the expected range for morphometric skeletal indicators[2,4,13].

Classification at legal thresholds
Accuracy ranged 80.5–85.6% with AUC 0.886–0.921 for 12–16 years, with highest performance at 14 years (AUC 0.921). At 18 years, overall accuracy was 83.5%, with high specificity (88.7%) and lower sensitivity (73.1%), mirroring the general decline in discriminative power near majority.[26-33] Sex-stratified analyses confirmed consistent patterns, with females showing very high specificity at 18 years but reduced sensitivity.

Comparative appraisal of methods

Across models, adjusted R^2 spanned ~0.53–0.75. CVM provided higher predictive precision (R^2 = 0.753; SEE = 2.55 years; MAE ≈ 2.0 years) and stronger AUC/accuracy at 12–16 years. Cameriere offered greater measurement reproducibility and transparent quantification, with MAE ≈ 2.7 years, making it a robust, objective complement. At 18 years, both methods converged in overall accuracy (approximately 83%) but with distinct profiles: CVM favored sensitivity, Cameriere favored specificity. Such complementarity supports combined use to balance false-positive/false-negative risks at legally critical thresholds.[4,22,34-37]

Where ethical considerations apply-e.g., protection of minors in asylum/justice contexts-approaches that minimize over-classification as adult may be preferred, consistent with EU guidance.[38,39]

Reference value of the study

From a practical perspective, lateral cephalometric radiographs offer an important advantage over hand–wrist radiographs, as they involve relatively low radiation doses and are frequently available in routine orthodontic records. This supports their use in forensic contexts without the need for additional imaging, in line with radiation protection principles.[1,20]

This work provides population-specific reference data for Portugal (6–23 years; N = 528) for both CVM stages and C4 morphometrics, extending earlier national studies.[9,10,13,15,22] The dataset contributes to the development of regression and classification models that are broadly consistent with AGFAD and IOFOS recommendations, although further external validation is required[40,,41], emphasizing transparency, proportionality, and accountability in medico-legal evaluations. Methodological limitations inherent to 2D cephalometry (e.g., projection geometry) underline the value of standardized acquisition and calibration (e.g., ISO/IEC 23053 frameworks) to reduce geometric bias.

CONCLUSION

Conclusions related to the main objectives

Group I – Baccetti's Method

The application of Baccetti's Cervical Vertebral Maturation (CVM) method yielded consistent and statistically robust results:

- There was no significant difference between chronological and estimated biological ages, confirming the method's accuracy for the studied population.
- The regression model explained approximately 75% of the variability in chronological age (Adjusted $R^2 \approx 0.75$) with a mean absolute error of almost 2.0 years, demonstrating high predictive precision.
- The model achieved excellent classification performance below the 12-, 14-, and 16-year legal thresholds (AUC > 0.93), indicating strong discriminative power for forensic purposes.
- At the 18-year threshold, classification accuracy and AUC decreased, reflecting the reduced sensitivity of morphological indicators once skeletal maturation nears completion. Consequently, the method should not be used as a stand-alone criterion for adulthood determination.

Group II – Cameriere's Method

For the Cameriere's morphometric model, the following conclusions were established:

- A statistically significant correlation was observed between chronological and estimated biological ages. The regression model explained approximately 56% of the chronological age variability (Adjusted $R^2 \approx 0.56$) with a mean absolute error of approximately 2.7 years, indicating good, but lower predictive accuracy compared to Baccetti's method.
- The method showed satisfactory classification capacity below the 12-, 14-, and 16-year thresholds (AUC range: 0.886–0.921).
- Performance declined at the 18-year threshold, consistent with the increased biological variability typical of late adolescence and the transition to skeletal maturity.

Conclusions related to the secondary objectives

Group I – Baccetti's Method

- No statistically significant sex differences were detected in biological age estimation using the CVM method.
- Nonetheless, the analysis confirmed the biological pattern of earlier skeletal maturation in females, a well-documented form of sexual dimorphism.

Group II – Cameriere's Method

- Similarly, no statistically significant sex differences were observed in Cameriere's method.
- The regression model explained a slightly higher proportion of chronological age variability in males, whereas females reached

skeletal maturity earlier, confirming known developmental trends.

Conclusions related to the final objective

Both Baccetti's morphological staging and Cameriere's morphometric model exhibited strong correlations with chronological age and demonstrated practical applicability in forensic age estimation.

When directly compared, Baccetti's method provided greater precision and predictive power, while Cameriere's method offered enhanced objectivity and reproducibility, reducing observer-related variability.

From a forensic standpoint, the two techniques are complementary rather than competing:

- CVM captures the morphological progression of skeletal maturation;
- Cameriere's ratio quantifies it objectively.

Their combined use may improve diagnostic confidence, minimize estimation bias, and align forensic evaluations with AGFAD and IOFOS recommendations for multi-parameter age estimation in living individuals.

This study also provides population-specific reference standards for Portuguese adolescents and young adults, contributing to the establishment of nationally validated scoring systems and supporting the international effort to harmonize forensic age assessment protocols under ethical, transparent, and standardized frameworks.

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SUPPLEMENTARY MATERIAL

Table S1. Intra- and inter-observer concordance coefficients for Baccetti's method

Type of validation	Cohen's Kappa (κ)	Interpretation
Intra-observer	0.914	Almost perfect
Inter-observer	0.804	Substantial/Good

Table S2: Intra- and inter-observer ICC coefficients for Vba of Cameriere's method

Type of validation	ICC	Interpretation
Intra-observer	0.999	Excellent
Inter-observer	0.946	Excellent

Table S3. Table of the sample distribution by age and sex

		Sex		
		Male	**Femal**	**Tota**
Age	6	15	7	22
	7	15	16	31
	8	15	15	30
	9	15	15	30
	10	15	15	30
	11	15	15	30
	12	15	15	30
	13	15	15	30
	14	16	15	31
	15	14	15	29
	16	15	15	30
	17	15	15	30
	18	15	15	30
	19	15	14	29
	20	15	16	31
	21	15	15	30
	22	15	13	28
	23	12	15	27
Total		267	261	528

Table S4. Minimum, maximum, mean, and standard deviation of age for each Baccetti stage

Baccetti stage	N subjects	Minimum age (years)	Mean (years)	Standard deviation	Maximum age (years)
1	56	6.15	8.05	1.66	13.94
2	80	6.24	9.85	2.04	13.32
3	74	7.26	11.42	2.14	18.36
4	97	9.09	15.81	3.36	23.75
5	166	14.16	19.25	2.64	23.95
6	55	14.23	20.32	2.56	23.87

Table S5. Regression model summary and ANOVA for chronological age prediction based on Baccetti's cervical vertebral maturation stages

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.868[a]	0.753	0.751	2.55400

a. Predictors: (Constant), Baccetti_classification=Stage 2 Baccetti's method, Baccetti_classification=Stage 3 Baccetti's method, Baccetti_classification=Stage 4 Baccetti's method, Baccetti_classification=Stage 5 Baccetti's method, Baccetti_classification=Stage 6 Baccetti's method

b. Dependent Variable: Age

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.	
1	Regression	10386.260	5	2077.252	318.455	<.001[b]	
	Residual	3404.956	522	6.523			
	Total	13791.216	527				

a. Dependent Variable: Age
b. Predictors: (Constant),
Baccetti_classification=Stage 2 Baccetti's method,
Baccetti_classification=Stage 3 Baccetti's method,
Baccetti_classification=Stage 4 Baccetti's method,
Baccetti_classification=Stage 5 Baccetti's method,
Baccetti_classification=Stage 6 Baccetti's method

Table S6. Model summary and Statistic by sex

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Male	.885[a]	0.785	0.780	3.19
Female	.852[a]	0.726	0.721	2.68

a. Predictors: (Constant),
Baccetti_classification=Stage 2 Baccetti's method,
Baccetti_classification=Stage 3 Baccetti's method,
Baccetti_classification=Stage 4 Baccetti's method,
Baccetti_classification=Stage 5 Baccetti's method,
Baccetti_classification=Stage 6 Baccetti's method
b. Dependent Variable: Age

Table S7. Classification results (confusion matrix and AUC) of the CT model for the 12-year threshold based on Baccetti's CVM

	Observed	Predicted			Area Under the ROC Curve			
		No	Yes	Percent Correct	Area	Std. Error[a]	Asymptotic Sig.b	Asymptotic 95% Confidence Interval
12 years	No	162	11	93.6%	0.949	0.009	0.000	**Lower bound** 0.932
	Yes	48	307	86.5%				
	Overall Percentage	39.8%	60.2%	88.8%				**Upper Bound** 0.966
14 years	No	203	30	87.1%	0.979	0.005	0.000	**Lower bound** 0.970
	Yes	7	288	97.6%				
	Overall Percentage	39.8%	60.2%	93.0%				**Upper Bound** 0.988
16 years	No	267	26	91.1%	0.934	0.011	0.000	**Lower bound** 0.913
	Yes	40	195	83.0%				
	Overall Percentage	58.1%	41.9%	87.5%				**Upper Bound** 0.955
18 years	No	285	68	80.7%	0.889	0.014	0.000	**Lower bound** 0.862
	Yes	22	153	87.4%				
	Overall Percentage	58.1%	41.9%	83.0%				**Upper Bound** 0.916

Table S8. Accuracy, sensitivity, specificity, and AUC for legal age thresholds by sex (Baccetti's method)

Legal age (years)	Sex	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12	F	89.7	89.3	90.4	0.95
	M	88.0	83.6	96.7	0.94
14	F	91.2	98.6	81.4	0.97
	M	94.8	96.6	92.5	0.98
16	F	85.1	80.5	88.8	0.91
	M	87.3	100.0	77.3	0.95
18	F	80.5	84.1	78.6	0.86
	M	85.4	90.8	82.8	0.91

Table S9. Mean, standard deviation, minimum and maximum of the Vba by sex (Cameriere's method)

	N	Mean	SD	Min	Max
Males	267	0.82	0.09	0.54	1.00
Females	261	0.83	0.08	0.60	1.09
Total sample	528	0.83	0.08	0.54	1.09

Table S10. Regression results of Cameriere's method for age estimation.

Variables Entered/Removed[a]			
Model	Variables Entered	Variables Removed	Method
1	Ratio Vba, Posterior		Enter
2		Posterior side	Backward (criterion: Probability of F-to-remove >= ,100).
a. Dependent Variable: Age			
b. All requested variables entered.			

Model Summary[c]				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.754[a]	0.568	0.566	3.37193
2	.753[b]	0.568	0.566	3.36966
a. Predictors: (Constant), Ratio Vba, Posterior side, Anterior side				
b. Predictors: (Constant), Ratio Vba, Anterior side				
c. Dependent Variable: Age				

Table S11. Regression results of Cameriere's method for age estimation by sex (Cameriere's method) Model Summary[b] by sex

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Male	.788[a]	0.621	0.617	3.370
Female	.730[a]	0.533	0.528	3.370

a. Predictors: (Constant), Ratio Vba, Posterior side, Anterior side b. Dependent Variable: Age

Table S12. Classification results (confusion matrix and AUC) of the CT model for the 12-year threshold based on Cameriere's Vba ratio

Observed	Predicted		
	No	Yes	Percent Correct
No	119	54	68.8%
Yes	38	317	89.3%
Overall Percentage	29.7%	70.3%	82.6%

Area Under the ROC Curve

			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig	Lower Bound	Upper Bound
0.886	0.015	0.000	0.857	0.915

Table S13. Classification results (confusion matrix and AUC) of the CT model for the 14-year threshold based on Cameriere's Vba ratio

Observed	Predicted		
	No	Yes	Percent Correct
No	184	49	78.9%
Yes	27	268	90.8%
Overall Percentage	40.0%	60.0%	85.6%

Area Under the ROC Curve

			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig.	Lower Bound	Upper Bound
0.92	0.012	0.000	0.899	0.944

Table S14. Classification results (confusion matrix and AUC) of the CT model for the 16-year threshold based on Cameriere's Vba ratio

Observed	Predicted		
	No	Yes	Percent Correct
No	214	79	73.0%
Yes	24	211	89.8%
Overall Percentage	45.1%	54.9%	80.5%

Area Under the ROC Curve

			Asymptotic 95% Confidence	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
0.904	0.012	0.000	0.000	0.000

Table S15. Classification results (confusion matrix and AUC) of the CT model for the 18-year threshold based on Cameriere's Vba ratio

Observed	Predicted		
	No	Yes	Percent Correct
No	313	40	88.7%
Yes	47	128	73.1%
Overall Percentage	68.2%	31.8%	83.5%

Area Under the ROC Curve

			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig.	Lower Bound	Upper Bound
0.89	0.013	0.013		

Table S16. Accuracy, sensitivity, specificity, and AUC for legal age thresholds by sex (Cameriere's method)

Legal age	Sex	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12 years	M	82.6	89.3	68.8	0.88
	F	85.6	90.8	78.9	0.912
14 years	M	80.5	89.8	73.0	0.93
	F	83.5	73.1	88.7	0.928
16 years	M	82.4	76.9	86.7	0.927
	F	82.4	75.4	88.1	0.90
18 years	M	82.0	60.9	92.2	0.919
	F	84.3	62.5	95.4	0.90

Table S17. Comparison of Baccetti's and Cameriere's methods at legal age thresholds (12, 14, 16, and 18 years)

Legal age	Method	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12 years	Baccetti	88.8	86.5	93.6	0.949
	Cameriere	82.6	89.3	68.8	0.886
14 years	Baccetti	93.0	97.6	87.1	0.979
	Cameriere	85.6	90.8	78.9	0.921
16 years	Baccetti	87.5	83.0	91.1	0.934
	Cameriere	80.5	89.8	73.0	0.904
18 years	Baccetti	83.0	87.4	80.7	0.889
	Cameriere	83.5	73.1	88.7	0.899

Figure S1. Histogram of the sample distribution by age and sex

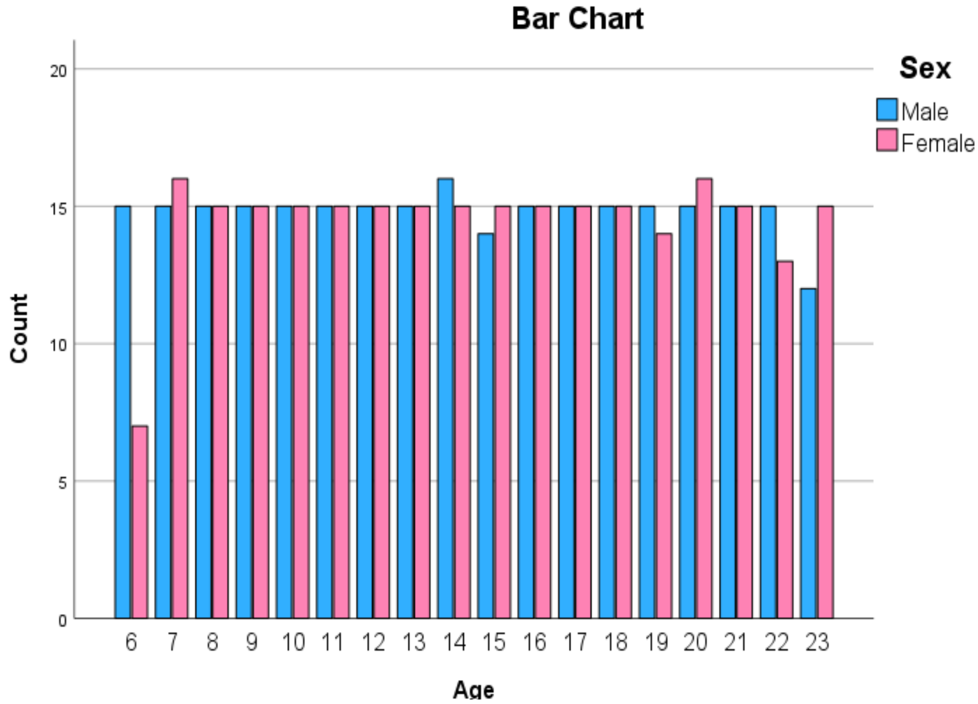

Figure S2. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 12 years old

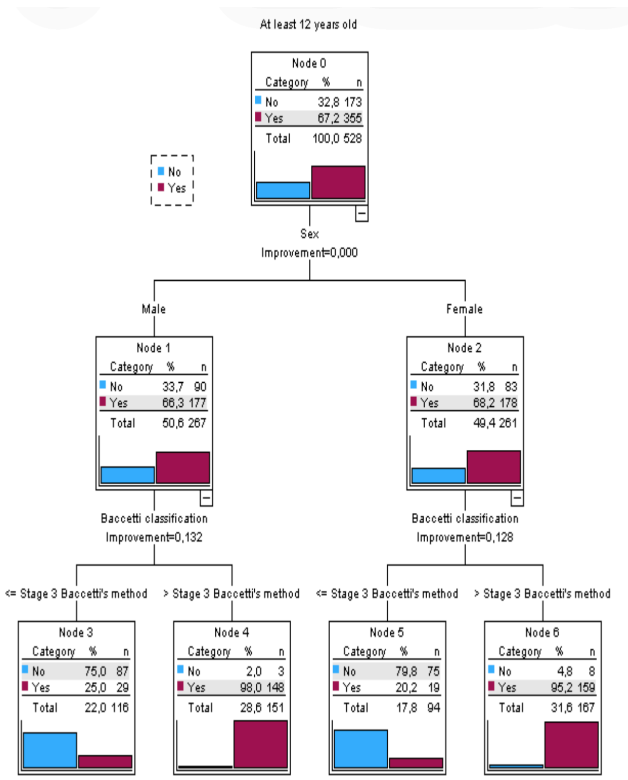

Figure S3. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 14 years old

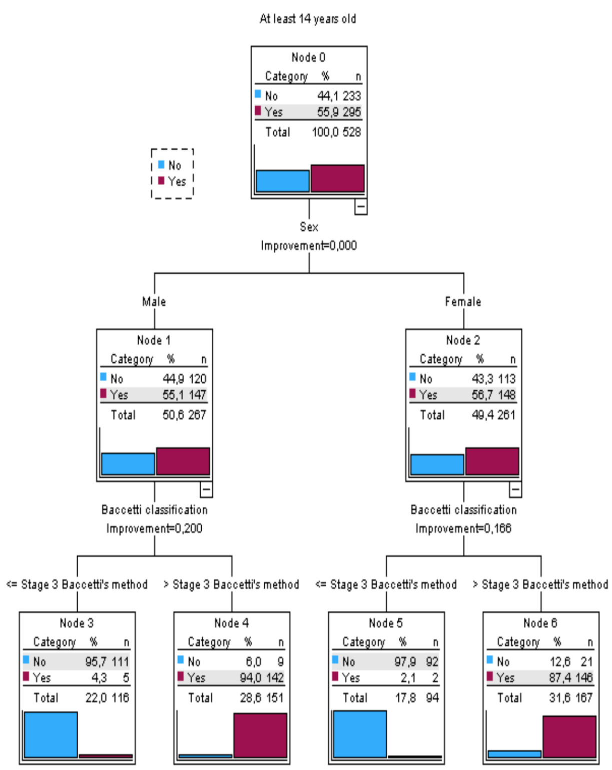

Figure S4. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 16 years old

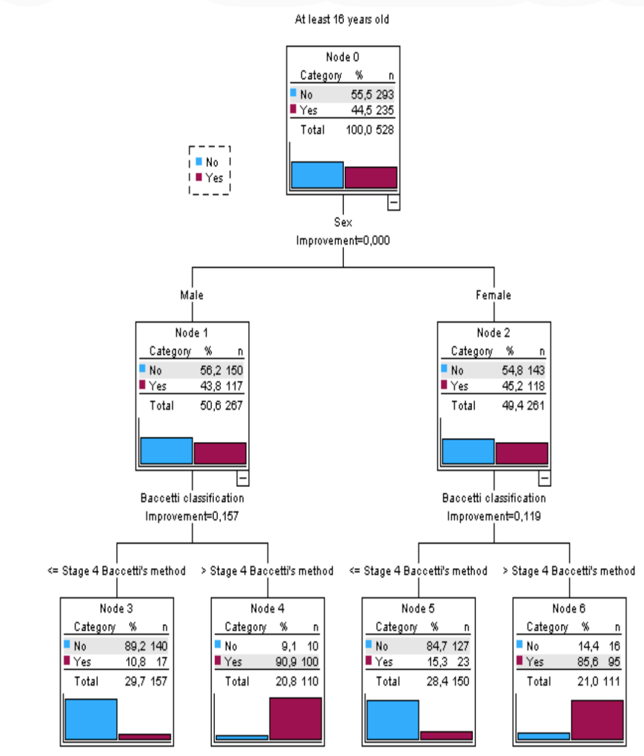

Figure S5. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 18 years old

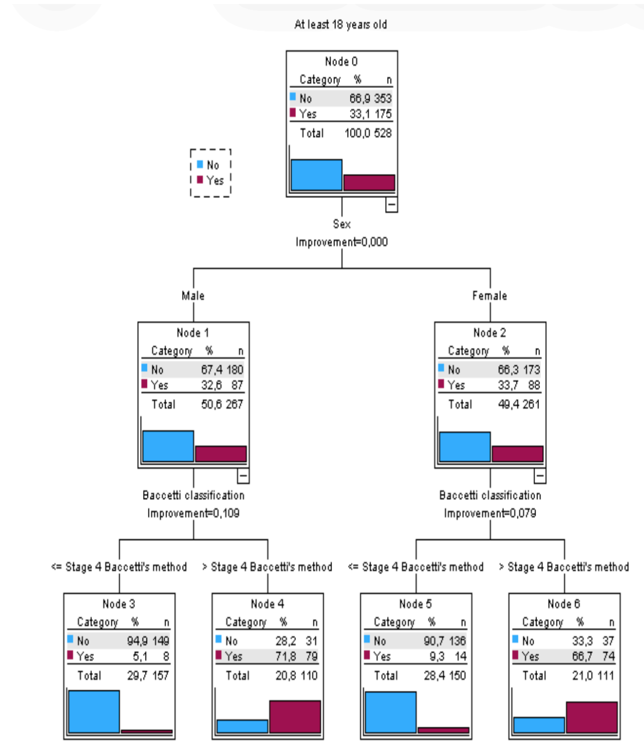

Figure S6. Classification tree (CT) for predicting whether subjects are at least 12 years old based on Ratio Vba

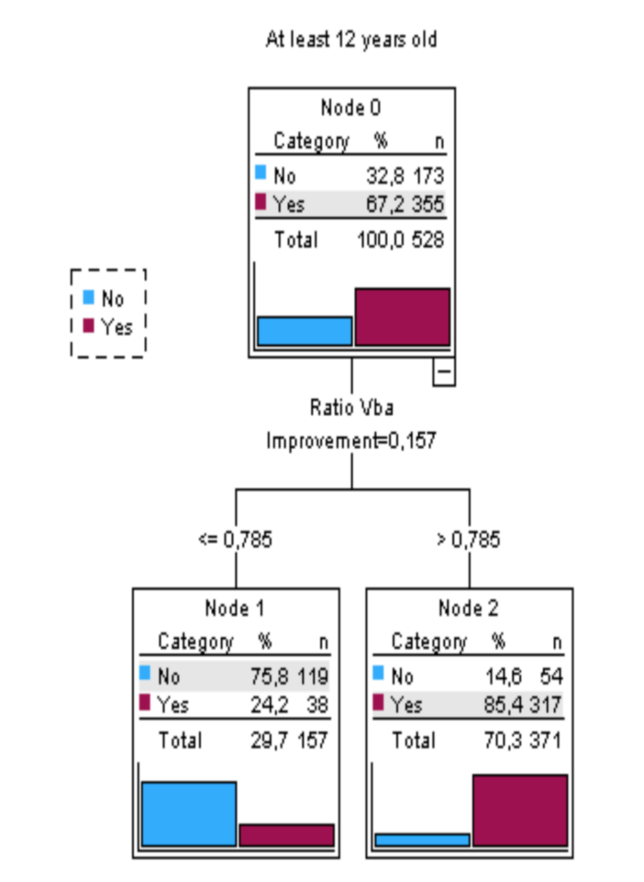

Figure S7. Classification tree (CT) for predicting whether subjects are at least 14 years old based on Ratio Vba

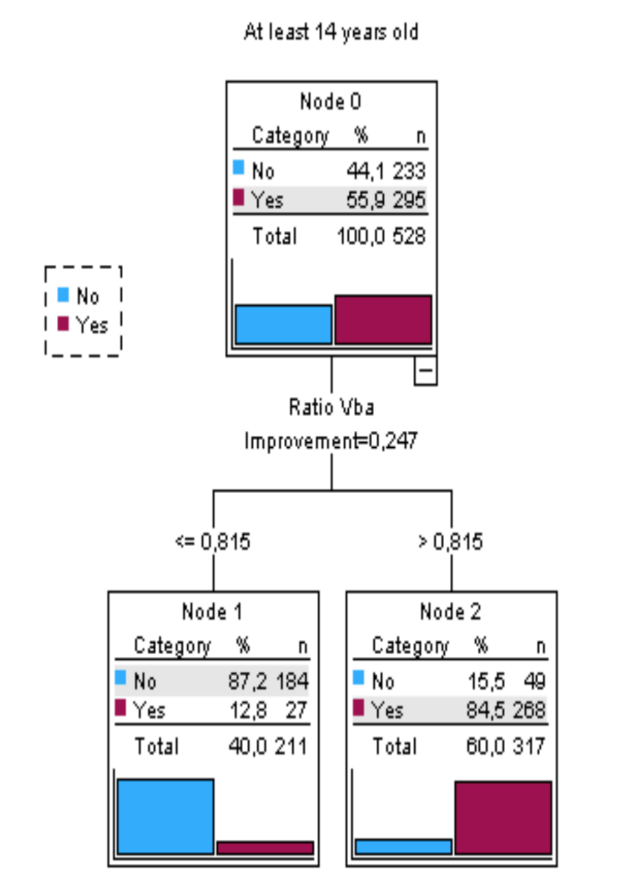

Figure S8. Classification tree (CT) for predicting whether subjects are at least 16 years old based on Ratio Vba

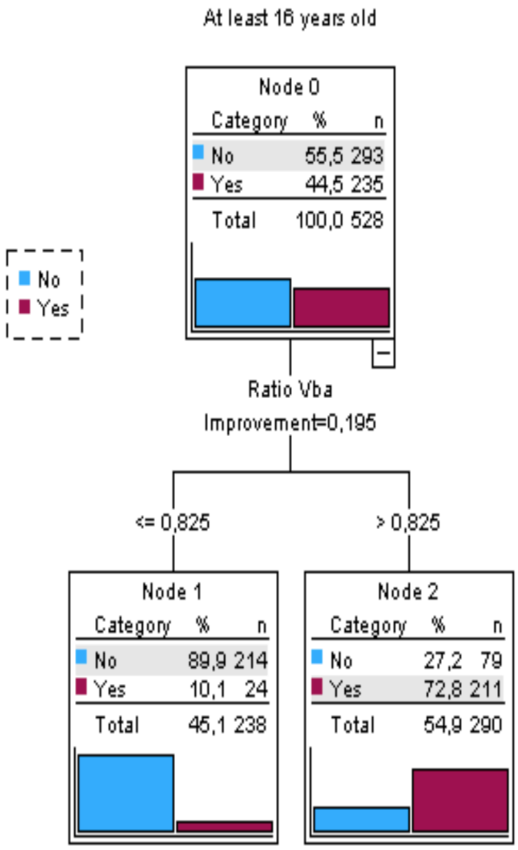

Figure S9. Classification tree (CT) for predicting whether subjects are at least 18 years old based on Ratio Vba

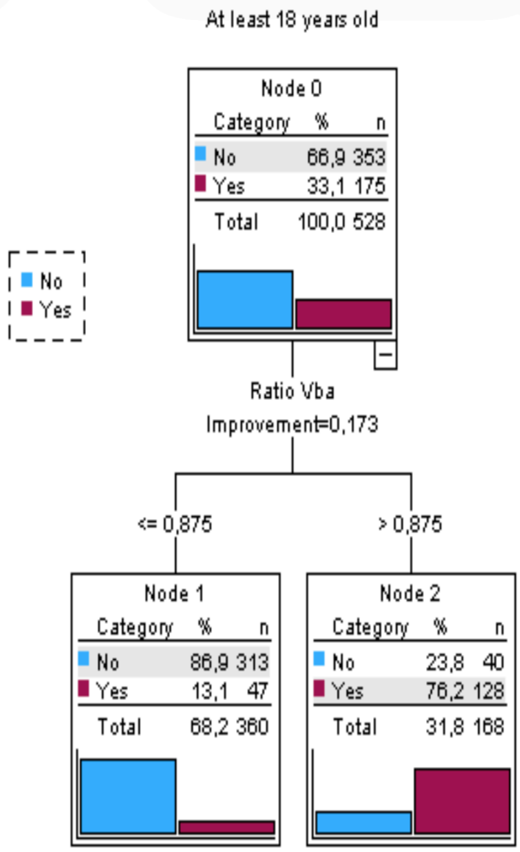

Figure S10. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 12 years old

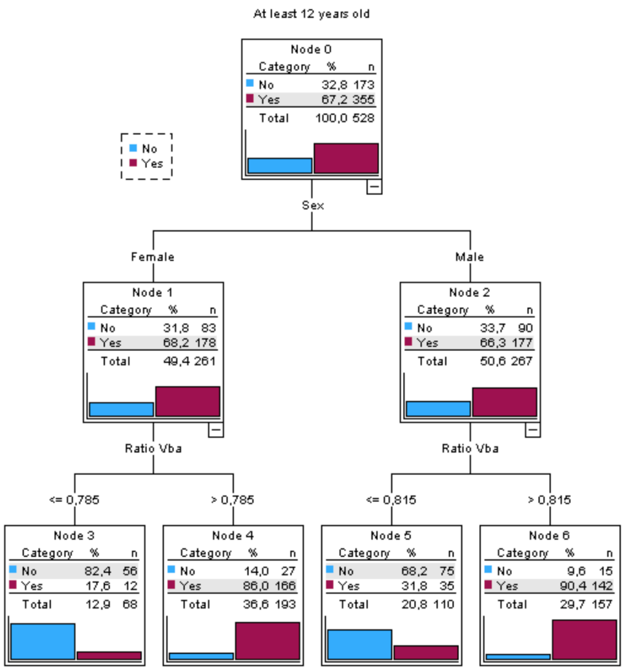

Figure S11. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 14 years old

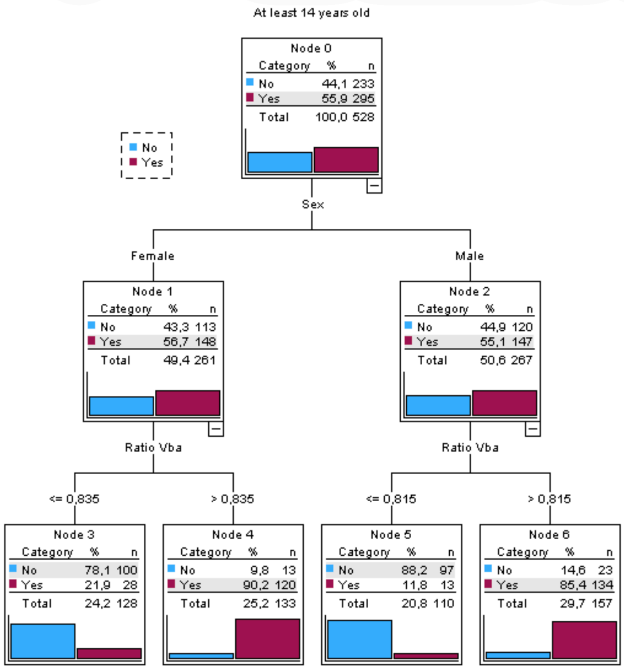

Figure S12. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 16 years old

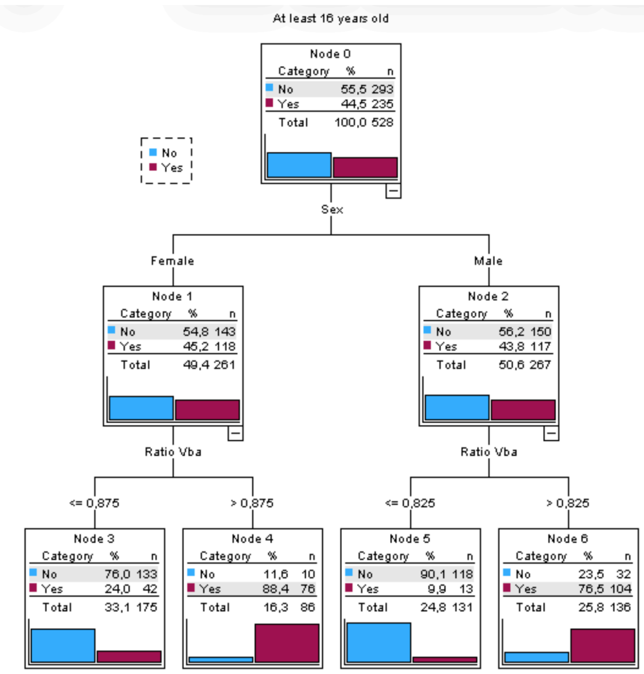

Figure S13. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 18 years old