Validation of the Third Molar Maturation Index (I₃M) to assess the legal adult age in the Portuguese population

João Albernaz Neves¹, Nathalie Antunes-Ferreira²,³, Vanessa Machado¹,⁴, João Botelho¹,⁴, Luís Proença⁵, Alexandre Quintas²,³, Ana Sintra Delgado¹, José João Mendes¹,⁴ & Roberto Cameriere⁶,⁷

Age estimation is a major step in forensic and legal procedures. Its relevance has been increasing due to growing society issues, such as identification of missing people, crimes against minors or lack of valid identification papers from locals or foreigners. Evaluation of the cut-off value of the Third Molar Maturation Index (I₃M) = 0.08 for discriminating minors from adults in the Portuguese population. The left lower third molars were analysed by applying a specific cut-off value of 0.08 determined by Cameriere et al. in 2008. A sample of 778 digital panoramic radiographs of a representative Portuguese sample (442 females and 336 males), in the age range of 12–24 years (mean age 17.7 ± 2.98 years in females and 18.1 ± 3.0 years in males), was retrospectively evaluated. I₃M decreased as the real age gradually increased in both sexes. The 0.08 cut-off score was valuable in discriminating adults from minors. According to the pooled results, the accuracy, by means of area under the curve, was 92.8% (95% confidence interval (CI) 91.0–94.6%). The proportion of correctly classified subjects (sensitivity) was 90.7% (95% CI 88.7–92.8%) and the specificity was 94.9% (95% CI 93.3–96.4%). The results show that I₃M is a valuable method to differentiate minors from adults in the Portuguese population.

Age estimation is a key step in human identification, legal practice and clinical research. Some areas of interest are in the aid of identification of missing people, crimes against minors, adoption procedures, invalid identification documents and mass migration¹⁻⁴. For such reasons, a reliable standardized age estimation tool to differentiate minors from adults is of great interest¹⁵.

Based on research on the distinguishable features of skull bones, symphysis pubis, long bones, hand bones and permanent dentition²⁻¹⁰, the Study Group on Forensic Age Diagnostics of the German Society of Legal Medicine (AGFAD) presented the criteria for age estimation in lawsuits. Dental examination is highly reliable because dental development is less influenced by both internal and external factors¹⁴. With the exception of the third molars, permanent dentition completed its development between 12 and 14 years of age. Third molars are estimated to develop between the ages of 15.7 and 23.3 years, and because this timeframe intercrosses the legal age of 18, they can serve as a discriminant tool to distinguish adults from minors¹⁻³,⁵.

In Portugal, the age of criminal and legal responsibility is 14 years old. If a crime is committed by an individual between 12 and 16 years old, he will be tried in a juvenile court. In the event of an conviction, the individual may be condemned to serve time in a closed educational center. On the other hand, if the person who commits the crime has between 16 and 18 years of age, may be considered adult and will be judged under general criminal laws. For all purposes, the legal age of adulthood in Portugal is 18 years old¹¹,¹².

In 2008, Cameriere et al.¹³ proposed the Third Molar Maturation Index (I₃M) as a predictive tool to discriminate adult age. The I₃M relies on the relationship between chronological age and the measures of the open apices

¹Clinical Research Unit (CRU), Centro de Investigação Interdisciplinar Egas Moniz (CiEM), Egas Moniz, CRL, Monte de Caparica, Portugal. ²Laboratório de Ciências Forenses E Psicológicas Egas Moniz (LCFPEM), Centro de Investigação Interdisciplinar Egas Moniz (CiEM), Egas Moniz CRL, Monte de Caparica, Portugal. ³Laboratory of Biological Anthropology and Human Osteology (LABOH), CRIA/FCSH, Universidade NOVA de Lisboa, Lisbon, Portugal. ⁴Evidence-Based Hub, CiEM, Egas Moniz, CRL, Monte de Caparica, Portugal. ⁵Laboratory of Health Research (MQIS), CiEM, Egas Moniz, CRL, Monte de Caparica, Portugal. ⁶Department of Forensic Medicine, University of Sechenov, Moscow, Russia. ⁷AgEstimation Project, FOR.MED.LAB, University of Macerata, Macerata, Italy. *email: jalberamnezneves@gmail.com
of the lower left third molar. The bidimensional widths of the apical and tooth lengths are measured and used to calculate the $I_{3M}$. Then, a cutoff value (0.08) is set to differentiate minors from adults\(^\text{13}\). Over the last years, $I_{3M}$ was successfully validated worldwide, in Europe\(^{2,13-23}\), Africa\(^{1,24-27}\), Asia\(^{5,28-31}\), America\(^{3,32-36}\) and Oceania\(^\text{37}\). Overall, $I_{3M}$ had considerable reliability among all countries, however, to date, this method is not validated for a Portuguese sample. Therefore, investigating if the $I_{3M}$ is suitable for the Portuguese population would be of great interest.

Given the evidence on high population movements in the Portuguese population, contributing to a higher level of diversity than some neighboring populations\(^{38,39}\), we aimed to test the validity of the $I_{3M}$ in a Portuguese representative sample, by using panoramic radiographs (PRs). Secondly, we looked for the influence of sex on its validity. Our null hypothesis was that the $I_{3M}$ has no validity to discriminate adult age in this Portuguese sample.

Materials and methods

Source of data and sample size. This retrospective observational study has received approval from the Egas Moniz Ethics Committee (ID 887). Written informed consent was obtained for each participant, during the first appointment at the Egas Moniz Dental Clinic (EMDC), (Almada, Portugal). Regarding participants under 18, a parent and/or legal guardian and this study gave and signed the informed consent. This research was conducted in accordance with the Declaration of Helsinki, as revised in 2013.

The present study follows the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) reporting guidelines\(^\text{40}\) for validation of prediction models. This study was conducted on a triple-blind basis with respect to: (1) diagnosis and clinical outcome; (2) data collection; and (3) analysis.

Participants. A consecutive sample of 1267 digital PRs, taken between September 2017 and January 2020, were considered for this study.

The inclusion criteria were: the presence of the lower third molar; known chronological age between 12 and 24 years old\(^{5,13,36}\); and absence of evident bone pathologies or systemic diseases that may affect tooth development. Exclusion criteria included spatial orientation of the third molar that prevents correct measuring, endodontic treatment and/or coronary restoration on the third molar, extensive cavities or abnormal dental anatomy, congenital anomalies and poor-quality or distorted X-rays\(^\text{41}\).

Outcome, predictors and measurement reproducibility. All digital PRs were converted to JPEG documents for the examination with ImageJ image processing software (Graphics Suite X7, Ottawa, Canada) by one trained and calibrated observer (JAN). The $I_{3M}$ index of each evaluated third molar was performed according to the Cameriere et al. method\(^\text{13}\). If the root development of the third molar is complete, then $I_{3M} = 0.0$. If not, $I_{3M}$ was calculated as the distance between the inner sides of the open apex (A and B) divided by the tooth length (C) (Fig. 1). In case of $I_{3M} < 0.08$, the individual is classified as 18 years old or older and if $I_{3M} \geq 0.08$, the individual as considered a minor.

The Kappa correlation index was used to test the agreement of classification of individuals younger than 18 years, aged 18 years or older. The Intraclass Correlation Coefficient (ICC) was used to analyze the measurements of open apices. Previously, twelve PRs were randomly chosen from the total sample, measured and
remeasured one week later by the same researcher (JAN). Kappa index indicated a perfect agreement for the inter-examiner analysis ($\kappa = 1.00$). Intraclass correlation coefficient (ICC) showed absolute agreement (ICC = 0.96), according to Landis and Koch42.

**Statistical analysis.** Data analysis was performed using SPSS Statistics v. 25.0 for Windows (IBM; Armonk, New York, USA). The estimated $I_{3M}$ index result was compared against the actual chronological age to determine the method’s performance in global and according to sex (females and males). For this purpose, contingency tables were used to calculate true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values (Table 1). Then, several performance indicators were determined and detailed in Table 143. Performance measurement was assessed through binary and multiclass area under the curve (AUC), through receiver operating characteristics (ROC) analysis. The correspondent 95% confidence intervals (95% CI) were also determined. Bayes post-test probability ($p$) of being 18 years or older is computed to discriminate between those who are or are not aged 18 years or more. According to Bayes’ theorem, $p$ may be written as:

$$
 p = \frac{Se \times po}{Se \times po + (1 - Sp)(1 - po)}
$$

In the post-test probability $p$, $po$ defines the probability that a participant is 18 years or older given that he or she is aged between 12 and 24 years, in the target population. In this study, we calculated the probability $po$ as the proportion of participants between 18 and 24 years of age who live in Portugal and those who are aged between 12 and 24 years. This value ($po$) was considered to be 0.55 (global, males and females) according to the Portuguese National Statistics Institute - Instituto Nacional de Estatística (INE) (https://www.ine.pt/xportal).

**Results**

**Participants.** A total of 778 digital PRs (336 males and 442 females) met the inclusion criteria, with 489 being excluded due to absence of third molars. The distribution of age and sex is depicted in Table 2. The mean ages of the males and females, aged between 12 and 24 years, were 18.1 ± 3.0 years old and 17.7 ± 3.0, respectively, without statistically significant difference ($p = 0.078$).

**Model performance.** The estimated age of majority was correlated with the chronological age ($p < 0.001$). The $I_{3M}$ values decreased as age increased across all age groups in both sexes, showing that the lower third molar mineralization occurred earlier in females than in males (Fig. 2).

Then, pooled data of both sexes as well as separately was analysed (Table 3). The overall results were 92.8% (95% CI 91.9–94.6%), 90.7% (95% CI 88.7–92.8%) and 94.9% (95% CI 93.3–96.4%) for accuracy, sensitivity and specificity, respectively. Concerning the LR+ and the LR−, their values were as follows: 17.7 (95% CI 12.5–20.3) and 0.10 (95% CI 0.08–0.12). Global post-test probability was 95.6% (95% CI 94.1–97.0%).

Males were better classified into adults or minors (94%; 95% CI 92.4–95.7%) than females (91.2%; 95% CI 89.9–93.8%). Specificity was better for females (95.7%; 95% CI 94.3–97.1%) compared to males (93.5%; 95% CI 91.8–95.3%) while sensitivity was better for males (94.9%; 95% CI 93.3–96.4%) compared to females (87.5%; 95% CI 85.2–89.8%). Both estimating post-test probability results were excellent; in males was 94.7% (95% CI 93.1–96.3%) and 96.2% (95% CI 94.2–97.5%) in females. The LR+ and LR− were 20.5 (95% CI 15.0–23.3) and 0.13 (95% CI 0.11–0.15), respectively, in females and 14.6 (95% CI 9.9–17.1) and 0.06 (95% CI 0.04–0.08), respectively, in males.

**Data from articles showing results with cutoff value 0.08 in various European samples.** Also, we compared the results of the present study with previous European studies (Tables 4 and 5 present).

| Table 1. Diagnostic performance indicators used in the comparative analysis. FN False Negative, FP False Positive; TN True Negative; TP True Positive; PPV Positive Predictive Values; MCC Matthews Correlation Coefficient. Adapted from Glas et al. (2003). |
| --- |
| **Sensitivity** | TP/(TP + FN) | Proportion positive test results among diseased |
| **Specificity** | TN/(TN + FP) | Proportion negative test results among the “healthy” |
| **Accuracy** | (TP + TN)/(TP + TN + FP + FN) | Proportion of correctly identified subjects |
| **PPV** | TP/(TP + FP) | - |
| **LR+** | Sensitivity/(1 – Specificity) | Ratio of the probability a true negative is classified as a true negative |
| **LR−** | (1 – Sensitivity)/Specificity | Ratio of the probability a false negative is classified as a true negative |
| **Youden’s index** | Sensitivity + Specificity – 1 | Measures the performance of a dichotomous diagnostic test |
| **F1 Score** | 2TP/(2TP + FP + FN) | Harmonic mean of precision and sensitivity |
| **MCC** | (TP × TN – FP × FN)/\[\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}\] | Measure of quality of binary classifications |
Discussion

The present study is the first to test the validity of I3M in a Portuguese sample. Overall, the null hypothesis was rejected, that is, the I3M is a reliable tool to discriminate adult age in this Portuguese population. Further, I3M was more accurate in male participants. These results have important implications because this tool has potential to be used in legal and criminal settings.

Discriminating minors from adults is important to prevent legal wrongful procedures, specially in cases where valid identification documents are lacking, in order to prevent legal wrongful procedures\(^4,32\). Notwithstanding, age estimation is challenging, particularly in the differentiation teens from young adults (aged 15 years old to early 20 s), as the physical appearance and characterization are not clearly related of being an adult\(^1,4\). From 15 years of age, the third molars are only teeth not fully developed, as this clinical circumstance is useful in forensic sciences\(^44\).

Table 2. Participants age and sex distribution (n = 778).

| Age (years) | Males | Females | Total |
|------------|-------|---------|-------|
| 12         | 13    | 16      | 29    |
| 13         | 11    | 22      | 33    |
| 14         | 10    | 21      | 31    |
| 15         | 29    | 36      | 65    |
| 16         | 41    | 64      | 105   |
| 17         | 51    | 75      | 126   |
| 18         | 36    | 43      | 79    |
| 19         | 43    | 38      | 81    |
| 20         | 28    | 44      | 72    |
| 21         | 15    | 28      | 43    |
| 22         | 20    | 22      | 42    |
| 23         | 27    | 16      | 43    |
| 24         | 12    | 17      | 29    |
| Total      | 336   | 442     | 778   |

Figure 2. Boxplot of relationship between chronological age and I3M of open apices of lower left third molar, according to females and males.
Table 3. Performance values (in percentage), derived from $2 \times 2$ contingency tables (with correspondent 95% confidence intervals), of test of age of majority in the Portuguese population.

| Global | Female | Male |
|--------|--------|------|
| Sensitivity | 90.7 (88.7–92.8) | 87.5 (85.2–89.8) | 94.5 (92.9–96.1) |
| Specificity | 94.9 (93.3–96.4) | 95.7 (94.3–97.1) | 93.5 (91.8–95.5) |
| Accuracy | 92.8 (91.0–94.6) | 91.9 (89.9–93.8) | 94.0 (92.4–95.7) |
| Precision | 94.6 (93.1–96.2) | 94.8 (93.2–96.4) | 94.5 (92.9–96.1) |
| LR+ | 17.7 (12.5–20.3) | 20.5 (15.0–23.3) | 14.6 (9.9–17.1) |
| LR− | 0.10 (0.08–0.12) | 0.13 (0.11–0.15) | 0.06 (0.04–0.08) |
| AUC | 85.6 (83.1–88.1) | 83.2 (80.6–85.9) | 88.0 (85.7–90.3) |
| Bayes | 95.6 (94.1–97.0) | 96.2 (94.8–97.5) | 94.7 (93.1–96.3) |
| Youden’s index | 85.6 (83.1–88.1) | 83.2 (80.6–85.9) | 88.0 (85.7–90.3) |
| F1 Score | 92.7 (90.8–94.5) | 91.0 (89.0–93.0) | 94.5 (92.9–96.1) |
| MCC | 85.7 (83.2–88.1) | 83.8 (81.2–86.4) | 88.0 (85.7–90.3) |

Table 4. Global performance values (in percentage, with correspondent 95% confidence intervals), of test of age of majority in several European samples. *In studies reporting validation of I₃M method not reporting 95% Confidence Interval (CI) we calculated 95% CI following Higgins et al. (2011).

| Author | Country/Region | n | Accuracy | Sensitivity | Specificity |
|--------|----------------|---|----------|-------------|-------------|
| Cameriere et al. (2008)* | Macerata, Italy | 906 | 83.0 (80.6–84.5) | 70.0 (67.0–73.0) | 98.0 (97.1–98.9) |
| De Luca et al. (2014)* | Milan, Italy | 397 | 91.4 (82.8–99.9) | 86.6 (88.8–91.1) | 95.7 (92.1–98.0) |
| Cameriere et al. (2014)* | Rome, Italy | 287 | 88.5 (84.8–92.2) | 84.1 (76.7–89.9) | 92.5 (87.0–96.2) |
| Rozyło-Kalinowska et al. (2017) | Poland | 982 | 86.6 (84.4–88.7) | 84.6 (81.9–87.2) | 92.0 (88.7–95.3) |
| Spinas et al. (2018) | Sardinia, Italy | 336 | 86.0 (82.0–89.0) | 82.0 (76.0–86.0) | 95.0 (89.0–97.0) |

Table 5. Performance values (in percentage, with correspondent 95% confidence intervals), discriminated by sex, of test of age of majority in several European samples. *In studies reporting validation of I₃M method not reporting 95% Confidence Interval (CI) we calculated 95% CI following Higgins et al. (2011).

| Author | Country/Region | n | Accuracy | Sensitivity | Specificity | Post-test probability |
|--------|----------------|---|----------|-------------|-------------|-----------------------|
| Male | Female | Male | Female | Male | Female | Male | Female |
| Galic et al. (2014) | Croatia | 1416 | 91.5 (89–93.5) | 88.8 (86.3–90.9) | 91.2 (88.7–93.1) | 84.3 (80.6–87.5) | 91.9 (88.8–94.3) | 95.4 (92.5–97.5) | 94.5 (94.3–94.7) | 96.5 (95.9–97) |
| Cameriere et al. (2014) | Albania | 298 | 92.5 (89.9–96.2) | 87.5 (81.2–90.4) | 94.1 (87.6–97.8) | 75.4 (68.1–78.8) | 90.9 (84.2–94.7) | 96.6 (91.1–99.1) | 94.4 (88.7–97.3) | 97.2 (91.9–99.1) |
| Zelic et al. (2016) | Serbia | 589 | 95.0 (92.0–98.0) | 91.0 (87.0–92.0) | 96.0 (93.0–98.0) | 86.0 (83.0–97.0) | 94.0 (90.0–98.0) | 98.0 (94.0–99.0) | 96.0 (91.0–100) | 99.0 (93.0–100) |
| Gulsahi et al. (2016)* | Turkey | 293 | 97.6 (94.9–100) | 92.7 (88.7–96.6) | 94.6 (88.1–99.8) | 85.9 (71.7–92.8) | 100 | 100 | 100 | 100 |
| Rozyło-Kalinowska et al. (2017) | Poland | 982 | 87.6 (84.8–90.3) | 85.3 (82–88.6) | 86.2 (82.8–89.6) | 82.6 (78.4–86.7) | 91.2 (86.7–95.8) | 93 (88.3–97.7) | 96.3 (92.3–100) | 97.0 (92.4–100) |
| Kelmendi et al. (2017) | Kosovo | 1221 | 96.8 (92.6–98.5) | 90.9 (87–91.7) | 96.2 (92.5–97.8) | 82.6 (78.7–83.4) | 97.6 (92.9–99.5) | 99.1 (95.3–100) | 97.5 (90.5–100) | 98.9 (92.6–100) |
| Dogru et al. (2018) | Netherlands | 360 | 88.9 (83.8–91.8) | 83.8 (77.7–85.8) | 84.0 (78.9–86.6) | 72.7 (67.6–75) | 95.0 (88.7–98.3) | 96.3 (90.0–99.0) | 95.7 (88.4–100) | 96.3 (89–1–100) |
| Spinas et al. (2018) | Sardinia, Italy | 336 | 87.0 (82.0–91.0) | 84.0 (78.0–89.0) | 85.0 (78.0–91.0) | 79.0 (71.0–85.0) | 91.0 (82.0–96.0) | 100 (92.0–100) | - | - |
| Tafrount et al. (2018) | France | 339 | 91.6 (87.1–92.5) | 89.7 (84.2–93.4) | 87.1 (80.4–90.3) | 81.3 (75–84.5) | 95.3 (89.8–98.3) | 96.2 (91.3–97) | 95.5 (87.7–96.1) | 96.1 (89.1–100) |
| Antunović et al. (2018) | Montenegro | 683 | 93.0 (90.0–96.0) | 89.0 (85.0–91.0) | 92.0 (88.0–96.0) | 82.0 (79.0–94.0) | 94.0 (90.0–98.0) | 96.0 (93.0–98.0) | 96.0 (90.0–100) | 97.0 (92.0–100) |
In the Portuguese context, age estimation methods involving dental measures were reported in previous studies\(^4\text{5}\text{}-\text{50}\). Caldas et al. (2011)\(^4\text{6}\) have evaluated third molars, however the method and the age range of these
participants were not comparable. Thus, to the best of our knowledge, this is the first study that validates the use of the \( I_{3M} \) cutoff value of 0.08 in the Portuguese population.

Cameriere et al. (2008)\cite{Cameriere2008} introduced a new methodology for age estimation based on the ratio between measures of the open apices and height of the third molar. Since then, the \( I_{3M} \) has been validated in every continent with very strong results, showing only slight variations among different ethnicities.

**Figure 4.** Discriminated results by sex, from different European populations.
The sensibility indicates the I3M ability to correctly identify individuals who are 18 years or older (I3M ≥ 0.08).

On the other hand, specificity is the ability to discriminate individuals younger than 18 years old (I3M < 0.08).

Santiago et al. (2018)41 reported that I3M outperforms in individuals younger than 18 years due to the higher specificity of this tool. This is key in forensic science because if a minor is wrongly processed as an adult it would violate its rights24,41.

To better integrate the results of our study within the European scenario, we analyzed every European study that have analyzed the validity of I3M (Fig. 3). Our pooled overall results on accuracy (92.8%; 95% CI 91.0–94.6%) outperformed all previous studies that presented pooled data23,27,22,23. Both sensitivity and specificity pooled results also surpassed most previous European studies2,22,23 except for Cameriere et al.13 (98%) and De Luca et al.17 (95.7%; 95% CI 92.1–98.0%). The estimated post-test probability of the pooled data (95.6%; 95% CI 94.1–97.0%) showed similar results with previous studies.

When analysing our data regarding males and females (Fig. 4), we demonstrated alike maturation of third molars in both males and females, which is in line with similar studies14–16,19–23,44,51. Previous European studies present slightly better accuracy and sensibility in males than in females14–16,19–23,44,51. Contrary, females presented better specificity than males14–16,19–23,44,51. Therefore, I3M seems to be slightly more accurate in males, although it is equivalent to the female participants in this Portuguese population. The effect of age on I3M performance is proposed to rely on the maturation development according to sex as women tend to develop at a younger ages than men22. This maturation difference is suggested to explain the I3M results discrepancies between both sexes, however more studies are needed to fully understand the biological reasons upon this subject19,41.

Estimated post-test probability of both male (94.7%; 95% CI 93.1–96.3%) and female (96.2%; 95% CI 94.8–97.5%) demonstrated results are useful to compare with other European studies6,14,15,19–23,44,51. It is important to refer that Zelic et al.20 and Kelmendi et al.21 reported results very close to 100% in females, and Gulsahi et al.19 reported results of 100% in both sexes, although the sample size was one of the smallest in all European studies (n = 293), which may influence these results.

The likelihood ratio is a practical measure of diagnostic accuracy. The higher the value of LR +, the more the test has the capability of establishing the tested condition; an LR + value greater than 10 defines it as a good diagnostic test. In our study, balanced values of LR + and LR − have been achieved. Although I3M presented higher LR + in males (20.5; 95% CI 15–23.3) then males (14.6; 95% CI 9.9–17.1), I3M is an excellent prediction of the probability of majority; and lower LR − (0.06; 95% CI 0.04–0.08) in males than (0.13; 95% CI 0.11–0.15) in females, prove that the test is also an excellent tool at classifying individuals younger than 18 years of age, despite the sex of the individuals19,23.

In conclusion, I3M is a suitable method in legal and forensic purposes to identify minors from adults in the Portuguese population. Further, the cut-off value used (0.08) is predictable and useful to discriminating individuals younger than 18 years of age (high specificity in both sexes).

Received: 11 July 2020; Accepted: 13 October 2020

Published online: 28 October 2020

References

1. Angelakopoulos, N. et al. Third molar maturity index (I3M) for assessing age of majority: study of a black South African sample. Int. J. Legal Med. 132, 1457–1464 (2018).
2. Cameriere, R. et al. Assessment of legal adult age of 18 by measurement of open apices of the third molars: Study on the Albanian sample. Forensic Sci. Int. 245(205), e1-205.e5 (2014).
3. da Nóbrega, J. B. M. et al. Validation of the Third Molar Maturation Index to estimate the age of criminal responsibility in Northeastern Brazil. Forensic Sci. Int. 304, 109917 (2019).
4. Cunha, E. et al. The problem of aging human remains and living individuals: a review. Forensic Sci. Int. 193, 1–13 (2009).
5. Chu, G. et al. Third molar maturity index (I3M) for assessing age of majority in northern Chinese population. Int. J. Legal Med. 132, 1759–1768 (2018).
6. Ozveren, N., Serindere, G., Mercic, P. & Cameriere, R. A comparison of the accuracy of Willems' and Cameriere's methods based on panoramic radiography. Forensic Sci. Int. 302, 109912 (2019).
7. Cameriere, R., Cingolani, M., Giuliodori, A., De Luca, S. & Ferrante, L. Radiographic analysis of epiphyseal fusion at knee joint to assess likelihood of having attained 18 years of age. Int. J. Legal Med. 126, 889–899 (2012).
8. Liverisidge, H. M., Speechly, T. & Hector, M. P. Dental maturation in British children: are Demirjian's standards applicable? Int. J. Paediatr. Dent. 9, 263–269 (1999).
9. Garamendi, P. M., Landa, M. I., Botella, M. C. & Aleman, I. Forensic age estimation on digital X-ray images: medial epiphyses of the clavicle and first rib ossification in relation to chronological age. J. Forensic Sci. 56, 3–12 (2011).
10. Cipriani, D. Children's rights and the minimum age of criminal responsibility: a global perspective. Br. J. Criminol. 50, 990–991 (2010).
11. Justiça, M. da. Diário da República nº 273. Decreto de Lei nº 496/77 (1977).
12. Cameriere, R., Ferrante, L., De Angelis, D., Scarpino, F. & Galli, F. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. Int. J. Legal Med. 122, 493–497 (2008).
13. Boyacioglu Dogru, H. et al. Age of majority assessment in Dutch individuals based on Cameriere's third molar maturity index. Forensic Sci. Int. 282, 231.e1-231.e6 (2018).
14. Ferrante, L., Cameriere, R. et al. Adult or not? Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age for legal purposes. Minerva Stomatol. 63, 283–294 (2014).
15. De Luca, S. et al. Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age. Forensic Sci. Int. 235(102), e1-102.e6 (2014).
16. Galic, I. et al. Cameriere's third molar maturity index in assessing age of majority. Forensic Sci. Int. 252, 191 e1-191.e5 (2015).
19. Gulsohi, A., De Luca, S., Cehreli, S. B., Tizali, R. E. & Cameriere, R. Accuracy of the third molar index for assessing the legal majority of 18 years in Turkish population. Forensic Sci. Int. 266, 584 e1-584.e6 (2016).
20. Zelic, K. et al. Accuracy of Cameriere’s third molar maturity index in assessing legal adulthood on Serbian population. Forensic Sci. Int. 259, 127–132 (2016).
21. Kelmendi, J. et al. The third molar maturity index in indicating the legal adult age in Kosovo population. Int. J. Legal Med. 132, 1151–1159 (2018).
22. Różyło-Kalinowska, I., Kalinowski, P., Kozek, M., Galić, I. & Cameriere, R. Validity of the third molar maturity index I3M for indicating the adult age in the Polish population. Forensic Sci. Int. 290(352), e1-352.e6 (2018).
23. Spinias, E., De Luca, S., Lampis, L., Velandia Palacio, L. A. & Cameriere, R. Is the third molar maturity index (I3M) useful for a genetic isolate population? Study of a Sardinian sample of children and young adults. Int. J. Legal Med. 132, 1787–1794 (2018).
24. Cavrić, J. et al. Third molar maturity index (I3M) for assessing age of majority in a black African population in Botswana. Int. J. Legal Med. 130, 1109–1120 (2016).
25. Dardouri, A. A. K., Cameriere, R., De Luca, S. & Vanin, S. Third molar maturity index by measurements of open apices in a Libyan sample of living subjects. Forensic Sci. Int. 267, 230 e1-230.e6 (2016).
26. AlQahtani, S., Kawthar, A., AlAraik, A. & AlShalan, A. Third molar cut-off value in assessing the legal age of 18 in Saudi population. Forensic Sci. Int. 272, 64–67 (2017).
27. El-Bakary, A. A., El-Azab, S. M., Abou El Atta, H. M., Palacio, L. A. V. & Cameriere, R. Accuracy of the cutoff value of the third molar maturity index: an Egyptian study. Egypt. J. Forensic Sci. 9, 52 (2019).
28. Balla, S. B. et al. Validation of third molar maturity index (I3M) for discrimination of juvenile/adult status in South Indian population. J. Forensic Leg. Med. 49, 2–7 (2017).
29. Sharma, P., Wadhwan, V., Ravi Prakash, S., Aggarwal, P. & Sharma, N. Assessment of age of majority by measurement of open apices of the third molars using Cameriere’s third molar maturity index. J. Forensic Dent. Sci. 9, 96 (2017).
30. Kumagai, A. et al. Accuracy of the third molar index cut-off value for estimating 18 years of age: validation in a Japanese samples. Leg. Med. 38, 5–9 (2019).
31. Jiménez, L. G. et al. Validation of the third molar maturity index (I3M): study of a Dominican Republic sample. J. Forensic Odontostomatol. 37, 27–33 (2019).
32. Deitos, A. R. et al. Age estimation among Brazilians: Younger or older than 187. J. Forensic Leg. Med. 33, 111–115 (2015).
33. De Luca, S. et al. Accuracy of cut-off value by measurement of third molar index: study of a Colombian sample. Forensic Sci. Int. 261(160), e1-160.e5 (2016).
34. Quispe Lizarbe, R. J., Solís Adrianzáñ, C., Quezada-Márquez, M. M., Galić, I. & Cameriere, R. Demirjian’s stages and Cameriere’s third molar maturity index to estimate legal adult age in Peruvian population. Leg. Med. 25, 59–65 (2017).
35. Khare, P. et al. Validation of the third molar maturity index cut-off value of <0.08 for indicating legal age of 18 years in Eastern Chinese region. Leg. Med. 42, 101645 (2020).
36. Cameriere, R. et al. Assessment of second (I2M) and third (I3M) molar indices for establishing 14 and 16 legal ages and validation of the Cameriere’s I3M cut-off for 18 years old in Chilean population. Forensic Sci. Int. 285(205), e1-205.e5 (2018).
37. Franklin, D. et al. Accuracy of a cut-off value based on the third molar index: validation in an Australian population. Forensic Sci. Int. 266(575), e1-575.e6 (2016).
38. Pereira, L., Prata, M. J. & Amorim, A. Diversity of mtDNA lineages in Portugal: Not a genetic edge of European variation. Ann. Hum. Genet. 64, 491–506 (2000).
39. Pimenta, J. et al. Spatially explicit analysis reveals complex human genetic gradients in the Iberian Peninsula. Sci. Rep. 9, 1–9 (2019).
40. Collins, G. S. et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann. Intern. Med. 162, W1–W73 (2015).
41. Cañeto, B. M., Almeida, L., Cavalcanti, Y. W., Magno, M. R. & Maia, L. C. Accuracy of the third molar maturity index in assessing the legal age of 18 years: a systematic review and meta-analysis. Int. J. Legal Med. 132, 1167–1184 (2018).
42. Landis, J. R. & Koch, G. G. The measurement of observer agreement for categorical data. Biometrics 33, 159 (1977).
43. Glas, A. S., Lijmer, J. G., Prins, M. H., Bonsel, G. J. & Bossuyt, P. M. M. The diagnostic odds ratio: a single indicator of test performance. J. Clin. Epidemiol. 56, 1129–1135 (2003).
44. Galić, I. et al. Dental age estimation on Bosnian–Herzegovinian children aged 6–14 years: evaluation of Chaillet’s international maturity standards. J. Forensic Leg. Med. 20, 40–45 (2013).
45. Tomás, L. F., Mónico, L. S. M., Tomás, I., Varela-Patiño, P. & Martin-Biedma, B. The accuracy of estimating chronological age from Demirjian and Nolla methods in a Portuguese and Spanish sample. BMC Oral Health 14, 1–12 (2014).
46. Caldas, I. M. et al. Chronological age estimation based on third molar development in a Portuguese population. Int. J. Legal Med. 125, 235–243 (2011).
47. Cameriere, R., Cunha, E., Sassaroli, E., Nuzzolese, E. & Ferrante, L. Age estimation by pulp/tooth area ratio in canines: study of a Portuguese sample to test Cameriere’s method. Forensic Sci. Int. 235, 97–103 (2017).
48. Anastacio, A. C., Serras, C., Vargas Sousa de Santos, R. F. & Palmela Pereira, C. Validation of Cameriere’s medical-legal age estimation method using seconds premolars in a Portuguese population. J. Forensic Leg. Med. 60, 30–34 (2019).
49. Vlahajic, S., Palmela Pereira, C. & Vargas Sousa de Santos, R. F. Age estimation in Portuguese population: the application of the London atlas of tooth development and eruption. Forensic Sci. Int. 272, 97–103 (2017).
50. Pereira, C. P., Caldas, R. & Pestana, D. Legal medical age estimation in Portuguese adult cadavers: evaluation of the accuracy of forensic dental invasive and non-invasive methods. J. Forensic Sci. Criminal. 1, 1–6 (2013).
51. Antunovic, M. et al. The third molars for indicating legal adult age in Montenegro. Leg. Med. 33, 55–61 (2018).
52. Vlahajic, S., Rosenmich, J. N. & Rogol, A. D. Gender and sexual maturity dimorphism in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females: a general clinical research center-based study. J. Clin. Endocrinol. Metab. 85, 2385–2394 (2000).

Author contributions
Conceptualization, V.M, J.B and R.C.; methodology, J.N.; validation, J.B., V.M. and L.P.; formal analysis, L.P.; investigation, J.N.; resources, A.D. and J.J.M.; writing—original draft preparation, J.N.; writing—review and editing, J.J.M., N.A.F. A.Q. and R.C.; supervision, J.J.M, A.D. N.A.F and A.Q.; project administration, J.J.M, A.D. and R.C. - All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-75324-x.

Correspondence and requests for materials should be addressed to J.A.N.
Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020