



Study of the ethnicity's influence on the third molar maturity index (I_{3M}) for estimating age of majority in living juveniles and young adults

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Abstract

Estimation of age of majority has important applications in the forensic daily practice because of the increasing demand for age estimates of unaccompanied minors. Diagnostic accuracy of I_{3M} to assess legal adult age of 18 years has been already tested in several specific population samples. The aim of this work was to compare the available data about sensitivity and specificity of the third molar maturity index (I_{3M}) from five different regional groups' radiographic samples to study possible ethnic difference. For this purpose, a sample of 6157 orthopantomograms (OPGs), coming from 15 countries, was analysed. Data about sensitivity and specificity were pooled using a bivariate modelling approach. The one-way MANOVA analysis was applied to assess the likelihood that sensitivity and specificity of the five regional groups are sampled from the same population. The result of the one-way MANOVA showed that both sensitivity and specificity did not depend from the regional groups. The obtained sensitivity was 84% (95% CI: 82% and 85%), and its specificity was 94% (95% CI: 93% and 95%). Based on these results, the I_{3M} was a useful statistical tool to identify whether a subject has reached the legal age of 18 years old, regardless of his or her ethnicity.

Keywords Forensic sciences · Age estimation · Unaccompanied minors · Third molar maturity index (I_{3M}) · Bivariate approach · Ethnicity

Introduction

Age of majority is the threshold at which an individual is legally considered to have attained adulthood and, in most countries, it is set at 18 years [1–3]. Several exceptions exist and, before the age of majority, a minor may acquire partial or full legal emancipation through the consent of parents, legal guardian, or the court approval. In criminal proceedings, this does not mean that emancipated subjects under the age of 18 can no longer benefit from protection by the specific national law. In terms of the best interest and rights

of minors, they cannot be detained with adults until at least 18 years and, in cases of uncertainty about the real age, the individual must be given the benefit of the doubt until proven otherwise; this means that, in such cases, a minor must be treated as such [3, 4].

In the European Union (EU), the best interest of the child, in particular of unaccompanied minors, is protected in the Dublin Regulation (Article 6) [5]. Increasing cross-border migration has resulted in numerous unaccompanied minors who cannot prove their chronological age with valid identification documents or who deliberately provide false age information [3–7]. Being unable to prove age, or incorrectly assessing a minor as an adult, can restrict the minor from having access to some basic human rights such as healthcare and education. This fact puts into focus age assessment: the judicial authorities often request a medical age assessment solid report issued by a forensic expert in which the most likely age and/or the minimum age is indicated [8, 9].

The European Asylum Support Office (EASO) has contributed intensively to the debate with its recently updated “Practical Guide on age assessment”, in which a distinction between “radiation-free” techniques and those involving

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radiation is made. In addition, priority is given to the least intrusive methods [10]. The EASO established that, within the EU, no agreement has been reached so far about the possible ethical and legal justification of methods [10].

The Study Group on Forensic Age Diagnostics (AGFAD: *Arbeitsgemeinschaft für Forensische Altersdiagnostik*), of the German Society of Legal Medicine, created and updated the guidelines for age estimation and institutionalisation of quality control, with special attention to legal and ethical implications [3, 11]. These researchers stated that age assessment reports should indicate data on the reference population regarding genetic and geographic origin, and variations of age-related parameters. That is important in order to allow the decision-making institutions to consider any doubt such that it leads to the more favourable legal outcome for the undocumented minors [3, 11, 12].

Along with the analysis of hand-wrist bones, the examination of lower third molar mineralisation, performed by an orthopantomogram of the mandibular region, is one of the most employed methods for the age periods towards 18 years [13–16]. In the last few years, many works have focused on the third molar because it is still in development around the age of 18 years old [17–21]. The most important one according to Demirjian [22] uses eight stages. However, it has been demonstrated that ethnicity plays an important role when a certain phase is reached [23–26]. Recently, Rolseth et al. [27] highlighted that, although such variations have been interpreted as ethnic differences, this can very likely be attributable to inadequate sample size in each stage of classification and age mimicry bias.

Cameriere et al. [28] developed a quantitative method for assessing if an individual is younger than 18 years. Its accuracy has been already tested on several populations but the outcomes of these studies have not been mutually compared to detect country-specific or ethnical differences in a pooled international group [29, 30].

The aim of this research was to compare the validity of third molar maturity index (I_{3M}) to assess age of majority ($I_{3M} < 0.08$ suggests that the subject is older than 18 years; instead $I_{3M} \geq 0.08$ suggests that the subject is younger than 18 years) between various countries based on the available data about sensitivity and specificity from five different regional groups' radiographic samples.

Materials and methods

The sample consisted of 6157 radiologically scored orthopantomograms (OPGs), coming from 15 countries and analysed in detail in previous studies (AL = Albania, AUS = Australia, CHN = China, CO = Colombia, DOM = Dominican Republic, ET = Egypt, F = France, I = Italy, IND = India, J = Japan, PL = Poland, RCH = Chile, SRB = Serbia, TR = Turkey, ZA = South Africa) [31–45] (Fig. 1).

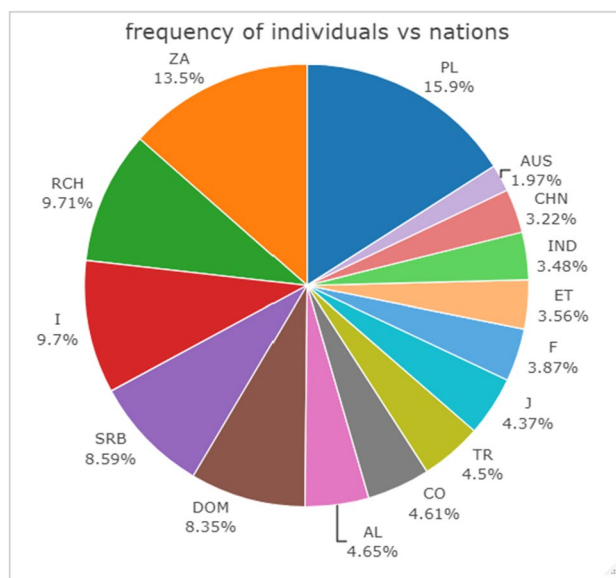


Fig. 1 Pie chart showing the distribution of the nationality of individuals in the sample

The following regional groups were considered and modified from Liversidge et al. [46], Hennessy and Stringer [47], and Cavalli-Sforza et al. [48]: Australian (AUS), African (AF), South American (SA), European (E), and Asian (A) (Fig. 2). It is not a racial categorisation but only a geographical representation of living human population [49].

The distribution of countries for different regional groups is reported in Table 1, and the age distribution according to

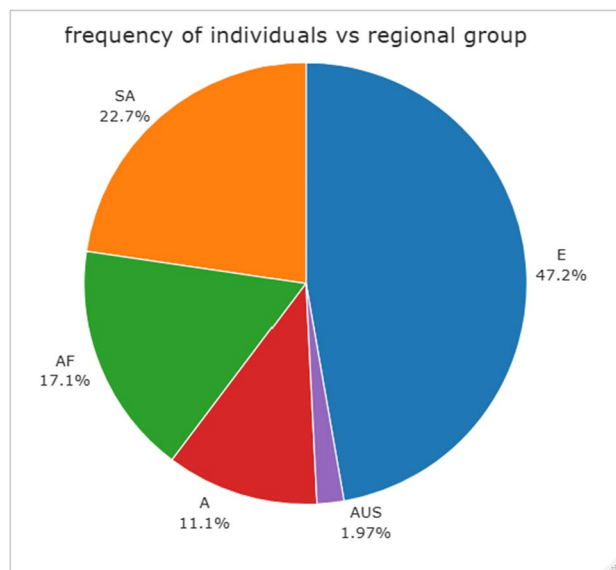


Fig. 2 Pie chart showing the distribution of the considered regional groups

Table 1 Sample distribution according to the country and regional group (abbreviations for countries: *AL*, Albania; *AUS*, Australia; *CHN*, China; *CO*, Colombia; *DOM*, Dominican Republic; *ET*, Egypt; *F*, France; *I*, Italy; *IND*, India; *J*, Japan; *PL*, Poland; *RCH*,

Chile; *SRB*, Serbia; *TR*, Turkey; *ZA*, South Africa. Abbreviations for regional groups: *AUS*, Australian; *AF*, African; *SA*, South American; *E*, European; *A*, Asian)

| Regional group | Country | | | | | | | | | | | | | | | total |
|----------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| | AL | AUS | CHN | CO | DOM | ET | F | I | IND | J | PL | RCH | SRB | TR | ZA | |
| A | 0 | 0 | 198 | 0 | 0 | 0 | 0 | 0 | 214 | 269 | 0 | 0 | 0 | 0 | 0 | 681 |
| AF | 0 | 0 | 0 | 0 | 0 | 219 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 831 | 1050 |
| AUS | 0 | 121 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 121 |
| E | 286 | 0 | 0 | 0 | 0 | 0 | 238 | 597 | 0 | 0 | 982 | 0 | 529 | 277 | 0 | 2909 |
| SA | 0 | 0 | 0 | 284 | 514 | 0 | 0 | 0 | 0 | 0 | 0 | 598 | 0 | 0 | 0 | 1396 |
| Total | 286 | 121 | 198 | 284 | 514 | 219 | 238 | 597 | 214 | 269 | 982 | 598 | 529 | 277 | 831 | 6157 |

sex and regional group is showed in Table 2. The following I_{3M} range categories were considered to analyse the relationship between index and real age (years) of each subject: [0, 0.08], [0.08, 0.22], [0.22, 0.4], [0.4, 0.7], [0.7, 1], [1, 2.4]. These categories were chosen according to the available published researches on the I_{3M} [29–44].

Regarding the selection criteria of the sample, they were as follows: clear OPGs and all permanent teeth present, including third molars. The presence of any visible dental and bone pathology (e.g., large carious lesions or endodontic treatments), children with any systemic diseases or endocrine anomalies (e.g., dysmorphology, abnormally short roots), and subjects with previous root canal treatment in the left lower permanent teeth were excluded from the study. Heavily rotated and impacted teeth with no visible roots were excluded also from the analysis.

Data was recorded in an excel file, whose columns contained individual’s identification number, sex, date of birth, and date of OPG.

Statistical analysis

A subject is positive in the test for the age of majority if his value of I_{3M} is less than 0.08. On the contrary, a subject is negative to the test if the value of his I_{3M} is greater than or

equal to 0.08. In this work, data about sensitivity (the proportion of individuals being 18 years of age or older whose test is positive) and specificity (the proportion of individuals younger than 18 whose test is negative) of five different regional groups were pooled together using a bivariate modelling approach.

Given the prevalence of adults/minors in the populations, we evaluated the positive/negative post-test probability, i.e., the proportion of subjects testing positive/negative who truly are adults/minors. These probabilities can help a court judge make an uncertain decision about an individual’s age of majority. Furthermore, we defined the positive likelihood ratio, $LR+$, and negative likelihood ratio, $LR-$, of the test which are the likelihood of a test result, positive or negative, in adult subjects divided by the likelihood of the test result in minor subjects:

$$LR+ = \frac{P(I_{3M} < 0.08 | \text{subject is adult})}{P(I_{3M} < 0.08 | \text{subject is minor})}$$

$$LR- = \frac{P(I_{3M} \geq 0.08 | \text{subject is adult})}{P(I_{3M} \geq 0.08 | \text{subject is minor})}$$

A likelihood ratio close to 1 means that the test result does not change the likelihood of adult age of a subject appreciably. The more the likelihood ratio for a positive test ($LR+$) is greater than 1, the more likely the subject is adult. The more a likelihood ratio for a negative test is less than 1, the less likely the subject is adult. Thus, LRs

Table 2 Age and sex distribution of the sample according to the different regional groups (2929 males, 3228 females. Abbreviations for regional groups: *AUS*, Australian; *AF*, African; *SA*, South American; *E*, European; *A*, Asian)

| Age (years) | regional group | | | | | | | | | | total | |
|-------------|----------------|-----|-----|-----|-----|---|----|------|------|-----|-------|------|
| | A | | AF | | AUS | | E | | SA | | | |
| | M | F | M | F | M | F | M | F | M | F | | |
| [14, 16) | 53 | 49 | 132 | 192 | 8 | | 9 | 238 | 224 | 181 | 215 | 1301 |
| [16, 18) | 86 | 75 | 97 | 160 | 20 | | 17 | 366 | 340 | 154 | 188 | 1503 |
| [18, 20) | 75 | 78 | 90 | 127 | 13 | | 13 | 283 | 280 | 135 | 159 | 1253 |
| [20, 22) | 71 | 87 | 60 | 98 | 15 | | 13 | 293 | 326 | 106 | 164 | 1233 |
| [22, 25) | 49 | 58 | 37 | 57 | 7 | | 6 | 316 | 243 | 44 | 50 | 867 |
| Total | 334 | 347 | 416 | 634 | 63 | | 58 | 1496 | 1413 | 620 | 776 | 6157 |

correspond nicely to the concepts of ruling in and ruling out adult age of a subject.

The results of each group can be summarised by a 2×2 contingency table (Table 3). It summarised the number of subjects who resulted positive to the test and are adult (true positive values, tp_i , $i = 1, \dots, 5$), then subjects who resulted positive to the test and are underage (false-positive values, fp_i , $i = 1, \dots, 5$), followed by those negative to the test who are adults (false-negative values, fn_i , $i = 1, \dots, 5$), and finally those negative to the test who are underage (true negative values, tn_i , $i = 1, \dots, 5$). The data model is binomial:

$$tp_i \sim \text{Bin}(n_{1i}, p_{1i}), i = 1, \dots, 5$$

$$fp_i \sim \text{Bin}(n_{2i}, p_{2i}), i = 1, \dots, 5$$

where $\text{Bin}(n, p)$ stays for binomial distribution with parameters n and p , and p_{ki} , $i = 1, \dots, 5$, is the probability that the test results positive in adults, $k = 1$, or in underage, $k = 2$ in the population $i = 1, \dots, 5$. In a more technical jargon, p_{1i} is the true positive rate or sensitivity, se_i , and p_{2i} is the false positive rate or complementary rate of the specificity, $1 - sp_i$, of the i th regional group, $i = 1, \dots, 5$. Before estimating sensitivity and specificity in the five regional groups and possibly their pooled estimates, their dependency from the regional group was tested transforming them as follows:

$$D_i = \text{logit}(p_{1i}) - \text{logit}(p_{2i})$$

$$S_i = \text{logit}(p_{1i}) + \text{logit}(p_{2i}) \tag{1}$$

where,

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right), 0 < p < 1$$

The transformations (1) were performed because the logit scale allows the practitioner, first, to define the parameters on the range $(-\infty, +\infty)$ and can plausibly fitted by the normal distribution and, second, the linear transformation should leave the pair (D_i, S_i) approximately independent.

Table 3 Contingency table showing the discriminating performance of the test

| | | Subject status | |
|--------------|-----|----------------|----------|
| | | Adult | Underage |
| Test outcome | + | tp_i | fp_i |
| | – | fn_i | tn_i |
| | Sum | n_{1i} | n_{2i} |

tp, true positives; *fp*, false positives; *fn*, false negatives; *tn*, true negatives

Since the main aim is to assess the likelihood that sensitivity and specificity of the five regional groups are sampled from the same population, the equality of mean vectors (D_i, S_i) $i = 1, \dots, 5$, for the five regional groups, was tested using the one-way multivariate analysis of variance (MANOVA) analysis. Considering the invertibility of relation (1), affirming that the bivariate distribution of vectors (D_i, S_i) $i = 1, \dots, 5$, does not depend on the regional groups is equivalent to affirm also the independence of both sensitivity and specificity from the regional groups.

Consequently, if the one-way MANOVA analysis and Wilks’ test point out that the hypothesis of equality of means cannot be rejected (the p -value is greater than the significant level of the test, $\alpha = 0.05$, then it is possible to assess the validity of I_{3M} by pooling all the data together). The result of the one-way MANOVA analysis is usually reported in a MANOVA table. Statistical analysis of data and related graphs was carried out by the R statistical program version 4.0.3 [50].

Results

Table 4 showed that only 6% of the individuals with $I_{3M} < 0.08$ were minor. In fact, if the number of minors in the first category ($(I_{3M}$ class $[0, 0.08) = (27 + 149)$) and the total number of individuals in the class ($[0, 0.08) = 2972$) are considered, the proportion of minors with $I_{3M} < 0.08 = (27 + 149)/2972 = 0.06$ or 6%. Similarly, when I_{3M} increased from the second class $[0.08, 0.22)$ to the last one $[1, 2.4]$, the percentage of adults wrongly classified as minor also decreased from 31.8% [calculation: $(28 + 72 + 23)/1015 = 0.318$ or 31.8%] to 6.4% $[(12 + 3 + 2)/263 = 0.0646$ or 6.46%] with an overall percentage of 17.4% $= (\text{number of adult individuals with } I_{3M} \geq 0.08) / (\text{number of subjects with } I_{3M} \geq 0.08) = (228 + 72 + 23 + 87 + 33 + 6 + 47 + 7 + 1 + 29 + 7 + 0 + 12 + 3 + 2) / (6157 - 2972) = 0.17488 = 17.488\%$.

The result of the one-way MANOVA, reported in Table 5, showed that the Wilks’ Lambda was equal to 0.762 with a p -value of 0.944. Therefore, both sensitivity and specificity did not depend from the regional groups and its validity can be tested pooling all the data together.

Table 6 displayed the strong association between adult age and positivity of the test (i.e., $I_{3M} < 0.08$). In fact, 5431 of 6157 individuals were correctly classified. According to the results reported in Table 6, the sensitivity was 84% with a 95% confidence interval equal to (82%, 85%), and its specificity was 94%, with a 95% confidence interval equal to (93%, 95%). The proportion of correctly classified individuals was 88% with a 95% confidence interval equal to (87%, 89%).

Table 4 I_{3M} and age distribution of the overall sample. Both age and I_{3M} classes are closed on the left

| I_{3M} | Age (years) | | | | | Total |
|--------------|-------------|----------|----------|----------|----------|-------|
| | [14, 16) | [16, 18) | [18, 20) | [20, 22) | [22, 25) | |
| [0, 0.08) | 27 | 149 | 850 | 1111 | 835 | 2972 |
| [0.08, 0.22) | 171 | 521 | 228 | 72 | 23 | 1015 |
| [0.22, 0.4) | 305 | 341 | 87 | 33 | 6 | 772 |
| [0.4, 0.7) | 334 | 239 | 47 | 7 | 1 | 628 |
| [0.7, 1) | 291 | 180 | 29 | 7 | 0 | 507 |
| [1, 2.4] | 173 | 73 | 12 | 3 | 2 | 263 |
| Total | 1301 | 1503 | 1253 | 1233 | 867 | 6157 |

Considering the probability that an individual in the sample of the considered regional groups is 18 or older was equal to 54%, the estimated positive post-test probability (Table 7) was 94%, with a 95% confidence interval of 93% and 95%. Hence, the probability that a subject positive on the test (i.e., $I_{3M} < 0.08$) is 18 years of age or older resulted 94%. In addition, the high LR+ and low LR− values showed that minors and adults were clearly discriminated.

Discussion

Due to the increasing rise in the number of people attempting to reach Europe from different countries, unaccompanied minors have become a recognised part of today's global and mixed migration flows [10, 23]. Therefore, when the age estimation procedures are performed, it should be fundamental to take into account their geographic origin or nationality [10, 23].

Since the original study was conducted for the first time in 2008 in a Caucasian sample [28], one of the main concerns has been its validation in several populations. Previous studies have already demonstrated the validity of I_{3M} to estimate age of majority in numerous samples coming from different countries [30, 51].

In this study, the aim was two-fold: to address a specific target issue more related to the daily forensic routine, whether a subject is below or above the legal age of 18, and to assess the influence of ethnicity in the application of I_{3M} . The I_{3M} was studied on a pooled collection of OPGs from several world regions. The obtained results showed that both sensitivity and specificity did not depend on the geographic origin and that this age marker could be applied without considering ethnicity

as a statistical variable for discriminating between child and adult.

In the daily forensic practice, when verifying the status of the unaccompanied minors and implementing the assisted return procedures become very complex, the scientific best age estimation procedures should be considered for a uniform and optimal ethical age estimation practice, being thus a relevant task for the forensic experts [23, 51]. In those cases, the main aim is to achieve “the best interest” of the minor, to avoid ethically and technically unacceptable misclassifications (minors wrongly classified as adults or the opposite), and to allow other experts to reproduce all performed examinations, to check the results and to evaluate the clinical interpretations [52]. A more precise indication about a particular age (or age range) becomes important not only for discriminating between child and adult but also to answer to the question of how long a subject, assigned to the status “child”, remains in that status [23].

In a recent systematic review and meta-analysis published by Santiago et al. [30], the slight tendency of higher specificity over sensitivity-pooled values has been highlighted, thus meaning that the I_{3M} is better in classifying the subjects younger than 18 years of age when compared with its accuracy of detecting the subjects older than this age. In this study, the sensitivity and specificity were 84% and 94%, respectively, with a 95% confidence interval. This supports the fact that this method is usually more precise at identifying minors and discriminating between the two age categories at the extremes of the root maturation (i.e., early and apical stages) [20, 47, 53].

Several works on third molar development and maturity stages in a specific population have been already published

Table 5 One-way MANOVA

| Variable | df | Wilks | F value | num df | den df | p-value |
|----------------|----|-------|---------|--------|--------|---------|
| Regional group | 4 | 0.762 | 0.328 | 8 | 18 | 0.944 |
| Residuals | 10 | | | | | |

Df, degree of freedom

Table 6 Contingency table describing performance of the I_{3M}

| Test | Age | | Total |
|--------------------|------|------|-------|
| | < 18 | > 18 | |
| $I_{3M} \geq 0.08$ | 2804 | 177 | 2981 |
| $I_{3M} < 0.08$ | 549 | 2627 | 3176 |
| Total | 3353 | 2804 | 6157 |

[54]. Whilst sex dependence was always taken into account, the impact of ethnicity and nutrition or socio-economic status on these procedures has not been analysed properly [23].

On the other side, only few studies have been carried out so far to analyse the problem of ethnicity and compare the influence of this variable on a pooled sample coming from different continents. The obtained outcomes were dissimilar according to the characteristics of each used sample and the performed method [20, 54].

Thevissen et al. [54] showed that, in spite of some changes in speed and onset of development, differences in value were small and not constant over the considered age range. Conversely, Liversidge et al. [47] indicated that some group differences were evident in third molar timing, but this had some impact on the confidence interval of estimated age in females and little impact in males because of the large variation in age. Olze et al. [55], in their study about the application of Demirjian method on three population samples (one German, one Japanese, and one South African), concluded that population-specific standards would enhance the accuracy of forensic age estimates based on wisdom tooth mineralisation in living minors involved in criminal proceedings.

All these studies indicated that some discrepancies eventually observed are largely due to inter-individual variability in mineralisation time of third molar teeth more than to the population-specific criteria [3, 38, 49, 56].

Although the OPGs were used in this study, age estimation could be performed also by taking one periapical dental radiograph. In certain forensic cases, where an orthopantomogram is available, or patient safety and maximum reduction of radiation doses have to be accomplished, this method could be useful. Anyway,

Table 7 Validity of I_{3M} test for pooled regional groups

| Parameter | Estimate | 95% CI |
|--------------------------------|----------|----------------|
| True prevalence | 0.54 | (0.53, 0.56) |
| Sensitivity | 0.84 | (0.82, 0.85) |
| Specificity | 0.94 | (0.93, 0.95) |
| Positive post-test probability | 0.94 | (0.93, 0.95) |
| Negative post-test probability | 0.83 | (0.81, 0.84) |
| Positive likelihood ratio | 13.25 | (11.48, 15.29) |
| Negative likelihood ratio | 0.17 | (0.16, 0.19) |

consent protocols should be followed, and informed consent must be obtained every time for these procedures in accordance with international paediatric guidelines and the specific jurisdictional regulation [10, 23].

The main strength of this research was the possibility to gather reliable data from the world regions that are represented in these samples of ascertained ethnic background, general health, and chronological age. On the other side, limitations were the relatively uneven age distribution of the sample and the difficulty in separating ethnicity from socioeconomic status. In fact, since the OPGs were selected retrospectively from data obtained for dental care, a limited number of radiographs were available in Asia and Australia, where no further information was available from the whole regions. In addition, nearly half (47.2%) of the population sample is from Europe. Finally, no sample information was reported about the socioeconomic status.

As regards the future challenges and research objectives, a new large sample is being currently gathered in order to provide new data on the I_{3M} in a better-balanced sample distribution, in which both the right and left lower molars, and the mesial and distal root, are considered for the analysis. To date, only few studies have showed differences in the relative development of each root within the same third molar [57].

Conclusions

Based on the results of this study, further population-specific reference data for the I_{3M} are not required. The I_{3M} is useful to identify whether a subject has reached the legal age of 18 years, regardless of his or her ethnicity.

In order to accomplish the legal certainty, the welfare of the minor, and the fair distribution of resources, the I_{3M} could be included in a new catalogue of age markers with available references, associated data, and sound statistical support. In fact, since different age markers cover different age ranges, if a specific age marker may not provide a useful result to decide whether a subject's chronological age is below or above the threshold of 18 years, the usage of more than one age marker should be recommended [23].

Regarding the socioeconomic status, further databases should be collected in order to investigate possible differences in the I_{3M} application between samples coming from diverse regional groups.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00414-021-02622-y>.

Declarations

The study was carried out in accordance with the ethical standards laid down by the Declaration of Helsinki (Finland).

Conflict of interest The authors declare no competing interests.

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