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# Accuracy of Three Age Estimation Methods in Children by Measurements of Developing Teeth and Carpals and Epiphyses of the Ulna and Radius 


#### Abstract

The aim of this study was to compare the accuracy of three methods for age estimation in children: the measurements of open apices in tooth roots ( T ), the ratio between the total area of carpal bones and epiphyses of the ulna and radius (HW), and the combined method (THW). The sample consisted of 288 Caucasian Italian children ( 152 boys and 136 girls) aged between 5 and 15 years. Accuracy was determined as the difference between estimated age and chronological age, and accuracy was assessed by analyzing individuals' orthopantomograms and hand-wrist radiographs. Accuracies were 0.41 years for girls and 0.54 years for boys with the THW method; for the HW method, 1.00 years for girls and 0.92 years for boys; and for the T method, 0.62 years for girls and 0.71 years for boys. THW is the most accurate technique for age estimation in these children.


KEYWORDS: forensic science, age estimation, children, accuracy, tooth development, hand-wrist bones

Several techniques are available for age estimation in children. In the past, the accuracy and reliability of these methods have been subjected to constant evaluation by skeletal biologists and forensic scientists (1-8). Within clinical medicine, age assessment assists in diagnosis and treatment planning. It is also a fundamental issue in pediatric endocrinology and in orthodontic treatment (9). In forensic sciences and bio-archeology, age-at-death estimation can aid the identification of a dead child and also give important information with regard to past populations. Age estimation is also proving valuable when birth data are lacking or doubted, for instance, in supervising immigrants without documents and for identifying presumed under-aged juvenile perpetrators or victims of pedo-pornography ( $3,4,10-12$ ).

In the last few years, great attention has been given to child pornography, which has increased particularly because of the development of web technology. The misuse of the Internet as a criminal tool is a serious problem, particularly with regard to the rising issue of this type of child abuse (11). In Italy, for example, the criminal

[^0]code has precisely defined juvenile pornography as "the abuse of juveniles younger than 18 years of age in order to perform pornographic exhibitions or produce pornographic material" (Art. 600 cpp). Furthermore, there is a specific article in the criminal code (Art. 14 of law no. 269) concerning "rules against exploitation of prostitution, pornography, sexual tourism against minors, as new types of slavery" and a law (no. 38, 2006), which establishes the institution of a "National Centre against pedo-pornography on the web," to collect all reports on websites with pedo-pornographic content, even from foreign countries, and public and private associations. Interpol has established a database so that victims and suspects, who are already known to the authorities, can be identified rapidly. Europol also has set forth successful operations (e.g., Icebreaker), which have resulted in the arrest of suspects across 13 countries who were involved in child pornography. In the European Union, a "Council Framework Decision on Combating the Sexual Exploitation of Children and Child Pornography" has also been founded (11). Therefore, an accurate diagnosis of age is becoming increasingly important in verifying the existence of a crime punishable by law (11-13).

In European countries, the age threshold for criminal responsibility (the age from which the child is judged capable of contravening the criminal law) ranges between the 13th and 21st years of life, although in some places, individuals can be held accountable for their crimes from the age of $7(4,9,14)$. Under Italian criminal law, the minimum age of criminal responsibility is set at 14 years (4).

When examining living subadults or the remains of infants, children, and adolescents, the most suitable age indicators are the different stages of dental mineralization $(13,15,16)$, the length of long bone diaphyses, the appearance of ossification centers, and the fusion of epiphyses (6,17-19). Hands, especially their carpal bones,
have been used as age indicators in several studies $(9,14)$. The skeletal development of hand-wrist bones is evaluated by X-ray examination and comparisons with the Greulich and Pyle atlas (20), the Tanner-Whitehouse scores (21), and the FELS method (22). The quickest and most sufficiently accurate method for this purpose is the Greulich and Pyle atlas (20). Examination is facilitated by the absence of other hard tissues, the low level of radiation exposure, and the large number of bones, making this area preferential for age evaluation $(9,14)$.

The literature has indicated that dental methods are more reliable than skeletal analyses because they are presumed to be less influenced by racial and environmental factors $(11,12)$. However, a particular limitation of dental development standards is that the reliability of age estimation is not uniform from birth to adulthood. At around 14 years, most teeth are fully developed and age estimation becomes increasingly difficult (11). Generally, at this stage, the third molars are the only teeth still developing; this particular tooth, however, is not always useful as it is not only characterized by considerable variation in the timing of formation and growth, but also frequently congenitally absent. As Cunha et al. (11) noted, a comparison between dental and skeletal age is always necessary to corroborate results, to verify possible discrepancies, and also to look for indications of sex differences, because of the fact that males have a skeletal delay in growth with respect to females.

Since 2000, the researchers at the Institute of Legal Medicine of the University of Macerata (Italy) have been extensively studying new methods for age estimation in both living and deceased subadults. They have developed regression formulae for age estimation using measurements of teeth (T), hand-wrist bones (HW), and/or both areas (THW) $(2,9,23)$.

Accuracy is the degree of error in a measurement as calculated from the true value (24). For skeletal and dental age estimation, this is the ability of a method to continually and consistently provide age intervals that encompass the true age-at-death of individuals. Lovejoy et al. (25) describe the difference between estimated and known ages as biased as it measures the amount of over- and underestimation. An accurate method has no bias, that is, the mean difference between dental age and known age is zero or close to zero (26). Reliability is the degree to which a method produces the same results when it is used at different times, either by multiple observers or by the same observer. It can be tested by conducting inter-observer or intra-observer variation studies to determine error rates. Low inter-observer variation (or standard error) indicates high reliability (27). The standard deviation (SD) of the mean difference between dental age and real age, also known as the standard error of the estimate (12), refers to the precision or reliability of estimated age.

The aim of this study is twofold: first, to test the accuracy of three techniques in a new Italian sample; and second, to elaborate on a standardized procedure for using a combination of these methods. Last, it also aims at finding a method suitable in all cases of crimes involving children, particularly child pornography and child prostitution.

## Materials and Methods

## Sample

This work is a retrospective cross-sectional study of radiographs. X-rays of hand-wrist bones and orthopantomograms (OPGs), taken from 288 Caucasian Italian children ( 152 boys and 136 girls), aged between 5 and 15 years, were analyzed. Subjects' identification number, sex, date of birth, and date of X-rays were recorded.

Chronological age for each case was calculated from the date of birth to the date of the X-ray. All selected individuals were orthodontic patients and did not display any growth disorders. Studies of children with endocrine disorders have demonstrated that dental development and skeletal growth are not under identical hormonal control $(28,29)$. OPGs of patients with hypodontia or hyperdontia, as well as those with bilaterally extracted mandibular first molars, were excluded from the study because it was impossible to obtain complete data from them. Protocols to collect radiographs for human subjects were approved by the Ethics Committee for Research Involving Human Subjects of the University of Macerata (Italy), and the study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki (Finland).
The OPGs were taken as part of routine treatment between 2004 and 2008. To evaluate the dental maturity of each individual, the apical end of the roots of the seven left developing permanent mandibular teeth of each individual were analyzed $(2,5)$. Conventional radiographs of left hands and wrists were taken. Radiographic examination was carried out by Trophy with green-sensitive, $18 \times 24$, $24 \times 30$, and $30 \times 40$ films for the left hand and wrist (Kodak ${ }^{\circledR}$; Eastman Kodak Company, Rochester, NY). Exposure doses were calculated according to age, zone of exposure, and tissue thickness. The exposed doses ranged from 46 to 50 kV and 6.5 to 25 mA . Xrays were digitized with a scanner (HP G4050 scanjet, at least 300 dpi resolution; Hewlett-Packard Development Company, L.P., Houston, TX), and images were recorded on computer files, and then processed with a computer-aided drafting program (Adobe ${ }^{\circledR}$ Photoshop ${ }^{\circledR}$ CS4; Adobe Systems, Inc., San Jose, CA).

With regard to the hand-wrist bones, mineralization of this area begins at birth and lasts until approximately 13 years for girls and 15 for boys for carpals, and 16-17 years for epiphyses of the ulna and radius. Thus, a 5 -year-old girl has seven carpal centers, while the last carpal center (pisiform) appears only at 1213 years. In the majority of healthy children, there is an established sequence of ossification for the carpal, which is remarkably constant for both sexes. Overall, the first ossification center to appear in hand-wrist radiographs is the capitate, and the last is, most often, the sesamoid of the adductor pollicis of the thumb. The first epiphyseal center to appear is that of the distal radius, followed by those of the proximal phalanges, the metacarpals, the middle phalanges, the distal phalanges, and, finally, the ulna (30).

Following Cameriere and Ferrante (9) for hand-wrist bones, X-rays of the left hand were taken in the postero-anterior projection, with fingers slightly splayed. X-ray images were processed by a computer-aided drafting (Adobe ${ }^{\circledR}$ Photoshop ${ }^{\circledR}$ CS4). The mathematical area of the carpal bones (Ca) and epiphyses of ulna and radius were identified and defined by the polygonal lasso instrument of Adobe ${ }^{\circledR}$ Photoshop ${ }^{\circledR}$ CS4 software. The pixels of these areas were computed and presented as a histogram (Fig. 1). The mathematical area of each carpal bone was selected by the polygonal lasso, and the pixel areas were calculated and added together to yield the global values of bone areas (Bo). If two bones overlapped, the common area was calculated only once (Fig. 2). Last, to normalize measurements, the $\mathrm{Bo} / \mathrm{Ca}$ ratio between total area of bones and carpal area was calculated. With the $\mathrm{Bo} / \mathrm{Ca}$ ratio, the age was estimated as follows:

$$
\text { Age HW }=-3.253+0.719 \mathrm{~g}+20.610 \mathrm{Bo} / \mathrm{Ca}
$$

As regards teeth, the seven left developing permanent lower teeth were evaluated by the same observer. X-rays were digitized using a scanner (HP G4050 scanjet, at least 300 dpi resolution), and images were recorded on computer files, which were processed using a computer-aided drafting program (Adobe ${ }^{\circledR}$ Photoshop ${ }^{\circledR}$


FIG. 1-An example of the carpal area selected using the polygonal lasso instrument of Adobe ${ }^{\circledR}$ Photoshop ${ }^{\circledR}$ CS4 software.


FIG. 2-An example of correct selection of each carpal bone.

CS4). The number of teeth with complete root development, that is, apical ends of roots completely closed (N0), was counted. Teeth with incomplete root development, that is, open apices, were also examined and the distance ( $\mathrm{Ai}, \mathrm{i}=1, \ldots, 7$ ) between the inner side of the open apex was measured (Fig. 3). To take into account the effect of possible differences among X-rays in magnification and angulations, measurements were normalized by dividing by tooth length $(\mathrm{Li}, \mathrm{i}=1, \ldots, 7)$. Dental maturity was evaluated according to the normalized measurements of the seven left permanent mandibular teeth $(x i=A i / L i, i=1, \ldots, 7)$, the sum of normalized open apices (s), and number (N0) of teeth with complete root development. Last, age was calculated according to the formula (31):

$$
\begin{aligned}
\text { Age } \mathrm{T}= & 8.387+0.282 \mathrm{~g}-1.692 \times 5+0.835 \mathrm{~N} 0-0.116 \mathrm{~s} \\
& -0.139 \mathrm{~s} \mathrm{~N} 0
\end{aligned}
$$

This is the European linear regression formula, also available as an MS Excel template at the website of the Istituto di Medicina Legale, Universitá degli Studi di Macerata (Italy), AgeEstimation project (http://agestimation.unimc.it).

When both areas of the teeth and hand-wrist bones were considered, age was estimated by the following linear regression formula (9):


FIG. 3-(A) An example of tooth measurement. Ai, $i=1, \ldots, 5$ (teeth with one root), is the distance between the inner sides of the open apex; Ai, $i=6,7$ (teeth with two roots), is the sum of the distances between the inner sides of the two open apices; and Li, $i=1, \ldots, 7$, is the length of the seven teeth. (B) An example of measurement of a tooth with two roots. $A_{6}$ is the sum of the distances $\left(A_{6}=A_{61}+A_{62}\right)$ between the inner sides of the two open apices, and $L_{6}$ is the length of the second molar.

$$
\begin{aligned}
& \text { Age THW }=4.619+0.401 \mathrm{~g}+0.551 \mathrm{~N} 0-0.647 \mathrm{~s}+7.163 \\
& \mathrm{Bo} / \mathrm{Ca}-0.123 \mathrm{~N} 0 \mathrm{~s}
\end{aligned}
$$

where g is a variable with assigned value of 1 for boys and 0 for girls. All measurements were carried out by the same observer with ample experience of this technique. The intra-observer repeatability of this study was tested by re-examining $10 \%(N=30)$ of OPGs and hand-wrist X-rays after an interval of 2 weeks.

## Statistical Analysis

Each OPG and hand-wrist radiograph was labeled with a number to blind observers as to the name, sex, date of birth, and date of the radiograph. Intra-observer reproducibility of measurements was studied by Pearson's correlation coefficient.

The accuracy of age estimation was defined as how closely chronological age could be predicted. To evaluate the accuracy of these age estimation methods, the median absolute difference between estimated age and real age was calculated (MdE) as well as several other measures of accuracy (mean/median difference). Accuracy was determined separately by means of the absolute differences in real age - chronological age for girls and boys and age cohorts. The median prediction error (MdE) is one of many ways to quantify the difference between an estimator and the true value of the quantity being estimated. It also represents the mean and median of the difference between chronological and dental ages, $\delta$ (residual).
To detect differences of residuals in sex, the generalized Friedman rank sum test with replicated blocked data was applied.
To handle outliers and/or skewed distributions, differences between groups of individuals were analyzed by the Kruskal-Wallis nonparametric statistical test (32). Exact versions of the test were applied to handle major differences in sample sizes. Statistical analysis was carried out by R version 2.8 (33). The significance threshold was set at 5\%.

## Results

The age and sex distribution of the Italian individuals are listed in Table 1. There were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined panoramic and hand-wrist radiographs. The

TABLE 1—Age and sex distribution of Italian sample.

| Age (years) | Girls | Boys | Total |
| :---: | ---: | :---: | ---: |
| 5 | 4 | 6 | 10 |
| 6 | 8 | 5 | 13 |
| 7 | 7 | 6 | 13 |
| 8 | 13 | 12 | 25 |
| 9 | 14 | 12 | 26 |
| 10 | 24 | 14 | 38 |
| 11 | 17 | 20 | 37 |
| 12 | 14 | 12 | 26 |
| 13 | 15 | 24 | 39 |
| 14 | 14 | 27 | 41 |
| 15 | 6 | 14 | 20 |
| Total | 136 | 152 | 288 |

Friedman test showed that the residuals between boys and girls were not statistically significant ( $\delta=0.3148$, df $=1, p=0.5748$ ).

The distribution of results into age cohorts for girls and boys separately are shown in Fig. 4, illustrating median and interquartile ranges. Results comparing accuracy by all three methods for girls and boys are shown in Table 2 and Fig. 5. It is clear that younger individuals tend to show a negative difference and older individuals a positive one.

The differences between real and estimated ages were evaluated for each method and for all ages. When predicted age was obtained
only with teeth (T), it showed an underestimation with a median of residuals of 0.12 year (mean $=0.11$ year); in younger individuals, for both sexes, predicted age tended to be underestimated and overestimated in older ones. This age trend matches numerous previous studies in which the reliability of Cameriere's method was tested. In an Indian sample of children, this method (2) yielded a mean overestimation of 0.05 year for boys and 0.04 year for girls. This therefore led Rai et al. (34) to propose a specific formula for the Indian population. In Peruvian school children, Cameriere et al. (35) showed more accurate estimates compared with Demirjian's method (36). The mean error in age estimation was 0.75 year for Cameriere's method and 1.31 for Demirjian's method. In the same study, data broken down into age cohorts showed that Cameriere's method tended slightly to underestimate children's chronological age, whereas Demirjian's method overestimated it by more than 1 year, with higher variability of error. The accuracy of both methods was lower for the central age groups (11-13 years) and best for the oldest age group. In the study of El-Bakary et al. (37), Cameriere's method (2) underestimated the mean age by 0.26 year for girls and 0.49 year for boys. Similarly, Cameriere's method underestimated the mean age in various European populations $(30,38)$.
The hand-wrist bones method (HW) yielded an underestimation for boys by a median of residuals of -0.10 year, with a residual standard error of 0.08 year. Only for boys at ages 11 and 16 was


FIG. 4-Boxplots of differences between real and estimated ages according to teeth (T), hand-wrist (HW). and both areas (THW) (upper panel; girls; lower panel; boys). Solid circles; means of error distributions, to one standard deviation (arrows).

TABLE 2—Median of residuals (years), interquartile range (IQR), mean, and standard error (SE) of differences between chronological and dental ages for each method tested, for children aged 5-15 years. Last two columns list median prediction error (MdE) and its IOR range.

| Method | Sex | N | Summary Statistics for $\delta$ |  |  |  | MdE | IQR |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Median | IQR | Mean | SE |  |  |
| HW | Girls | 136 | -0.024 | 2.106 | -0.135 | 0.130 | 1.00 | 1.41 |
|  | Boys | 152 | -0.130 | 1.879 | -0.052 | 0.107 | 0.92 | 1.08 |
|  | Both | 288 | -0.096 | 1.936 | -0.092 | 0.084 | 0.94 | 1.22 |
| T | Girls | 136 | 0.092 | 1.081 | 0.150 | 0.103 | 0.62 | 0.80 |
|  | Boys | 152 | 0.120 | 1.318 | 0.073 | 0.097 | 0.71 | 0.92 |
|  | Both | 288 | 0.117 | 1.208 | 0.110 | 0.070 | 0.66 | 0.91 |
| HW + T | Girls | 136 | 0.098 | 1.044 | 0.074 | 0.079 | 0.41 | 0.75 |
|  | Boys | 152 | -0.037 | 1.088 | 0.057 | 0.079 | 0.54 | 0.66 |
|  | Both | 288 | 0.082 | 1.063 | 0.065 | 0.056 | 0.52 | 0.71 |



FIG. 5-Proportion of absolute value of residuals greater than settled values, according to methods T, HW, and THW.

HW slightly overestimated. For girls aged between 8 and 11 years, the HW was found to be considerably underestimated. There was a clear age trend in differences between chronological and estimated ages with HW. Although differences were greater in the first age group than in the second, from this group onward differences increased steadily. The previous study of Cameriere et al. (23) showed a median of the absolute values of residuals of 0.08 year, with an interquartile deviation of 1.59 year and a standard error of estimate of 1.19 years. In a sample of Slovenian children aged between 6 and 16 years, a new regression formula developed by Cameriere et al. (14) yielded the following results: the median of the absolute values of residuals (observed age minus predicted age) was 0.09 years, with an interquartile deviation of 0.79 year, and a standard error of estimate of 0.66 year.

Last, when the THW method was used, accuracy increased in all the age cohorts. When predicted age was obtained by a combined method (THW), involving both teeth and hand-wrist bones, the median of residuals was 0.08 year, with a residual standard error of 0.06 year. In the work of Cameriere and Ferrante (9), the median of the absolute values of residuals (observed age minus predicted age) was 0.465 year, with an interquartile range of 0.529 year. The MdE was 0.553 year and the standard error of estimate 0.73 year.

The accuracy of the three methods, in the different age groups, is showed in the Table 3. The accuracy of estimated ages from the HW method turned out to be worse ( $\mathrm{MdE}=0.94$ year) than that obtained from the method based on tooth development ( T ), which showed an MdE of 0.66 year. The THW method showed the lowest bias, with an MdE of 0.52 year (Table 2). This combined

TABLE 3-Accuracy of the three methods by sex and age cohort.

| Age | THW |  | T |  | HW |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Boys | Girls | Boys | Girls | Boys | Girls |
| 5-<8 | 0.51 | 0.36 | 0.69 | 0.65 | 0.91 | 0.99 |
| $8-<11$ | 0.63 | 0.39 | 0.73 | 0.6 | 0.98 | 0.95 |
| $11-<14$ | 0.53 | 0.47 | 0.75 | 0.63 | 0.88 | 0.99 |
| $14-<16$ | 0.49 | 0.43 | 0.68 | 0.61 | 0.91 | 1.2 |

technique therefore yielded better results than those achieved from teeth or hand-wrist bones, independently. The THW method yielded $51.9 \%$ of absolute residuals (differences between chronological and dental ages) $>0.50$ year and only $22.7 \%$ of absolute residuals exceeded 1.00 year. Conversely, the percentage of absolute residuals was $>1.00$ year and increased to $32.3 \%$ and $48.1 \%$, when the T and HW methods were employed separately (Fig. 2).

The Kruskal-Wallis test showed that the residuals were not statistically different among age classes for either boys (Kruskal-Wallis $\chi^{2}=5.9136, \mathrm{df}=3, \quad p=0.1159$ ) or girls (Kruskal-Wallis $\chi^{2}=6.6802, \mathrm{df}=3, p=0.08282$ ) groups.

## Discussion

In the last few years, the cases of age estimation in the living children and adolescents have become more and more frequent $(11,12)$. The main issues of age estimation concern adoption and imputability ( $14,16,18,21$ years, depending on the country). For forensic purposes, age assessment plays an important judicial role because of the classification of a crime that may have been committed by a juvenile who is $<14$ years of age or who is between 14 and 18 years of age. This determines how they will be penalized, the place of reclusion, and restoration of rights. In addition, in cases of victimizers, these will be aggravated if the victims are $<14$ years of age.

With respect to the dead and the relative requirements for a biological profile, aging the living requires the use of noninvasive methods and a higher accuracy and precision because of specific legal requests $(11,12)$. In line with recommendations issued by the Study Group on Forensic Age Diagnostics, and with special attention to sensitive legal and ethical implications, a forensic age estimate should combine the results of a physical examination and anthropometrical analysis, sexual development assessment, the X-ray of the left hand, and a dental examination by OPG $(11,39)$. In addition, one particular issue that should be kept in mind for court purposes is whether an individual has reached a specific threshold. In fact, most of the used methods give standard errors or standard deviations: in the main cases, the forensic response to the judge will be, for example, 13-14.5 years. This may put the judge in a "difficult" situation, in which it would be helpful to know the probability of that person actually having reached the threshold (e.g., 14 years). The explanation of the error is therefore crucial (11).

Because of the above-mentioned reason, several methods for age estimation, in both forensic and clinical settings, have been developed $(19,32,34,40)$. However, as there are considerable variations in the rate of bone and tooth development among populations and among statistical procedures, caution must be exercised in interpreting the results of various age assessment methods $(11,41)$. The accuracy of age estimation indicates how well chronological age can be predicted and greater accuracy can be obtained by choosing the method, which shows the least variability with age (42).

Hand-wrist bones and teeth are two of the most reliable parts of the body for age estimation in children (41,43-46). Certain skeletal developmental stages of the hand and wrist have been shown to be closely associated with the pubertal growth spurt, and hand radiographs have been used as an indirect method to assess the maturity stage $(43,47)$. In addition, the ease of recognition of dental development stages, together with the availability of periapical or OPGs in most orthodontic or pediatric dental practices are useful reasons for attempting to assess the physiologic maturity of children and adolescents $(48,49)$. Normally, the teeth and hand-wrist bones are evaluated separately, and only a few studies have analyzed these
regions together (9). Previous papers have reported the importance of using bones and teeth combined for age evaluation and have promoted new studies $(29,46,50)$. In one of the most recent studies, Cameriere and Ferrante (9) used a combination of both teeth and hand-wrist bones for age estimation in children. The results obtained were interesting and highlighted better accuracy compared with results achieved with teeth or hand-wrist bones separately.

In the present study, a new Italian sample of 288 individuals was examined. Results indicated that, although the combined method slightly underestimates age, a combination of tooth development (T) and hand-wrist bones (HW) is useful as a maturity indicator of the pubertal growth period. These results match those of Cameriere and Ferrante (9).

The previous finding that age in younger children can be more accurately predicted than in older ones was not observed in the present study. An age trend in differences between chronological and estimated ages in T, HW, and THW was observed, particularly in boys and less so in girls (Fig. 1). The origin of errors may be found in variations both between and within studied populations, in observer variations, and in the methods employed. The precision is mainly related to factors influenced by chance, that is, random errors (50). Besides inherent methodological errors, the biological variation should also be considered (51).

As regards hand-wrist bones, variations in the appearance of the center of ossification at the wrist joint show the influences of race, climate, diet, and regional factors $(10,11,32)$. As regards the T method, although regional differences in the timing of dental development have been recognized, their significance and cause are uncertain ( $4,8,40,49,51,52$ ). Differences in the timing of developmental events between these studies may be marked, and nonbiological factors (e.g., methodology, choice of morphological standard, differences in analytic approach, sample age distribution) have been identified as potential sources of variation $(50,53)$. In fact, although the quality of dental age assessments seems to depend predominantly on the use of specific geographic standards, other possible sources of variation are method of age calculation and age distribution of the sample $(42,54,55)$. The underestimation for younger individuals and overestimation for older ones may be an artifact of the regression equations used in the original studies. It may be due to the relatively small sample used in this study with respect to the larger sample used to obtain the regression formulae and, consequently, to low variability in terms of sequences and timing of dental mineralization within each category. A second plausible explanation is that individuals who mature early, or at an average rate, reach the various developmental stages earlier than those who mature late. Another possible bias in this analysis lies in the degree of resolution of the OPGs. Although the radiographs for dental diagnosis were recent (2004-2008) and high resolution film was used, it was sometimes difficult to analyze many structures, such as poorly mineralized root apices or tooth crowns. In practice, crown apices and root growth fronts may be poorly mineralized, and radiographic observations will always underestimate their maturity $(56,57)$. However, none of these factors can explain the poor accuracy for the older age groups, and this is compounded by the very small sample size of children over 13 years of age.

Few works have been published on comparing accuracy of quantitative dental methods by measuring developing teeth. Some have small sample sizes or uneven age distributions, or present results in a way that make comparison difficult. Liversidge (42) studied data from Maber et al. (58) and investigated the absolute difference of various radiographic methods with the addition of several other methods in 145 Caucasian children aged between 8 and 13 years. Age was underestimated in boys and girls using all the methods.

Accuracy was better for younger children compared with older ones and decreased with age. The median of accuracy for Willems' method for both sexes was 0.52 year, which is consistent with the results of the present work. Galić et al. $(59,60)$ compared the accuracy of Cameriere's European formula, and Willems' and Haavikko's methods on 1089 OPGs of Bosnian-Herzegovian children aged between 6 and 13 years. Cameriere's method turned out to be the most accurate for both sexes, followed by Haavikko's method; Willems' method was the least accurate. For girls, mean dental age was overestimated by 0.10 year according to Cameriere's method by a range of differences of -0.80 to 0.60 year in all age groups. For boys, mean dental age was underestimated by -0.02 according to Cameriere's method by the mean of differences of -0.60 to 0.09 year for the 10 -, 11 -, 12 - and 13 -year-old groups, but was overestimated by the mean differences of $0.09-0.45$ year for the age groups of children 6, 7, 8 and 9 years old. Staaf et al. (61) compared three radiographic methods, including Haavikko's method on 541 Swedish children, and found that underestimation was 0.38 and 0.55 year for girls under and over 10 years of age and 0.28 and 0.53 year for boys under and over 10 years of age.

## Conclusions

As regards the first aim of this study, these results highlight the great accuracy and significance of teeth and the hand-wrist bones as age indicators and, when the data enable it, the importance of using a combination of bones and teeth for more accurate estimation of age. The accuracy of age assessing for the Italian children was slightly better when the combined method was applied, rather than teeth and hand-wrist bones separately. It therefore seems reasonable to recommend the use of the combined method for these age groups ( $5-15$ years), for both boys and girls.

On the other hand, the greater accuracy of the combined technique to study hand-wrist and teeth can be applied in all cases of crimes involving children. In Europe, while nearly all countries have adopted an upper age of 18, remarkable differences still obtain on the minimum ages of criminal responsibility. A brief overview of the criminal responsibility and penalties across this continent allows to distinguish between worst offenders (notably the Anglophone countries) and those generally recognized as the most liberal, sometimes even referred to as "indulgent" nations such as Finland and Italy. Italy adopted in 1988/89 a new legislation that sets the rules for penal procedures concerning children in conflict with the law, introducing very important changes within the Italian judiciary system (DPR 448/88). According to this organization, the minimum age of criminal responsibility is 14 years, and that of complete criminal responsibility is 18 . Children of the age group between 14 and 18 can be charged if they are capable of understanding and willing. When there is uncertainty over the age of the accused child, the judge will have to call for an expert's report. When the expert's report still leaves doubts, the age will have to be guessed. Children below the age of 14 having infringed the criminal law receive care and supervision by the social service of the local community (municipalities, provinces). If children cannot remain within their family environment, the juvenile court may order a placement in a foster family, a family-type community, or in an institution, under the responsibility of the local social services (62). However, ascertainment of the minimum age is not only useful when an individual breaks the law, but also in all cases in which that individual is the victim, both in living and fresh cadavers. The combined technique can be applied in cases of child adoption, on which the difference in age between the child and the adoptive parents is important, or in the case of child pornography
or child prostitution, also of children, but with very different sentences depending on the age of the minor. In the last few years, for example, websites with pedo-pornographic contents are increasing in number: between 2002 and 2004, an increase of $92.7 \%$ was pointed out by different institutions and nonprofit associations in Italy (62). For this reason, more and more judges, magistrates, or the police, call experts such as forensic pathologists, pediatricians, or anthropologists to evaluate such material and verify with great precision the age of the possible victims and offenders.

Possible future developments must involve additional anthropometric measurements and statistic factors leading to better age estimation (63). It would be useful to check the precision and reliability of the combined method for age estimation in children of other populations. Although tooth mineralization is largely independent of environmental, mechanical, and nutritional factors, the growth rate of bones depends on genetic and environmental factors and may vary between sexes, between individuals of the same population, and between the populations themselves $(63,64)$. In addition, new studies are needed to verify whether the regional background, sex, and chronological age distribution of the sample, and statistical procedure represent major factors controlling accuracy and reliability in subadult age assessment.

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