Are cervical vertebrae suitable for age estimation?

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Disclosures of Conflicts of Interest: none declared

ACKNOWLEDGEMENT
The authors wish to express their gratitude to the anonymous reviewers whose comments were very helpful in improving the clarity of this paper and avoiding misunderstandings.

KEYWORDS

age estimation, cervical vertebrae, hand bones, third molars, method evaluation

J Forensic Odontostomatol 2017. Dec; (35): 66-78
ISSN :2219-6749

ABSTRACT

Background: The ability of cervical vertebrae (CV) staging to contribute in forensic age estimation is being discussed controversially. The large variability of CV geometries in the end stage of development might be the reason for not reaching a performance competitive to hand or third molar methods. Here we study the geometry of adult CV and demonstrate that the description of their "typical" appearance is often not met.

Materials and methods: Lateral cephalograms from clinical routine of 320 subjects aged 20 years or above (median 24 years, 52% female) were evaluated. The criteria for the end stage of CV development (Hassel-Farman, Baccetti) were examined by assessing them in terms of metric measurements: (1) rectangular shape of C3/C4, (2) at least one of the height-width ratios of C3/C4 >1 (both not <1), (3) significant concavities at the inferior margin of C2, C3 and C4. Metric data of the adults were also compared to those of 100 children aged 8-10 years (50% female).

Results: Adult CV often violated the criteria of rectangular shape (44% C3, 36% C4), of height-width ratio (16% C3, 35% C4) and inferior concavity (10% C2, 10% C3, 19% C4). All of the criteria for adult CV were fulfilled in only 24% of the subjects (95%CI 19-28%). The variability of measures of the CV shapes was large; e.g., the 95% reference ranges for the height-width ratios were 0.81-1.19 (C3) and 0.77-1.14 (C4). There was a material overlap of ranges of CV measures of adults and children.

Conclusion: While hand bones and teeth have well-defined appearances in the end stage of development, adult CV have a large biological variance of shapes; it is hard to define their "typical" appearance. Moreover, measures of CV geometry do not strictly separate adults from children. These facts might reason the limited usefulness of CV in age estimation.

INTRODUCTION

Dental age derived from the mineralization stages of teeth (for example Demirjian's classification[1]) and skeletal age assessed from the hand[2,3] are well established in forensic age estimation. In the recent time, the development of cervical vertebrae has been proposed to be used for the assessment of skeletal maturation and age estimation.[4-6] Part of the sources report clinical usefulness of cervical vertebrae.[7-9] Some authors are more careful and state it might be possible to use them,[10] whilst others are more brave and claim lateral cephalograms could replace hand radiograms.[11,12] However, the current discussion is controversial. There are a considerable number of reports of no or modest gain in using cervical vertebrae[13-15] and criticism of poor performance[16] and serious methodological flaws of such methods[17] and the suggestion to use other techniques for the assessment of skeletal development.[18] Thevissen and colleagues[19] as well as an Italian research group[20] proposed combined age estimation from teeth and cervical vertebrae instead of using cervical vertebrae alone.

The goal of this paper is to approach the question why age estimation from cervical vertebrae might be inferior to well established methods. This question is relevant for forensic odonto-stomatologists as well since age estimation should incorporate the combination several methods.[21] Optimal choice of the estimation method for dental age is hence not an isolated problem of odonto-stomatology. The point is rather which combination of dental and skeletal age estimation methods works best.

The development of cervical vertebrae is characterized by the change from a trapezoid towards a rectangular shape, by an increase of the height-width ratio, and the by the formation of the concavity at the inferior margin (see Figure 1). These characteristics are used by several staging systems.[4-6] However, this development is not as linear with a well-defined end stage as it is for the teeth[1] and the hand.[2,3] In our work with lateral cephalograms, we observed an apparently large variance in the geometry of adult cervical vertebrae. Therefore, we aimed at expressing the morphological description of cervical vertebrae having reached the end stage of development in terms of metric data, in order to replace subjective assessment with objective measurements. Here we present a quantitative analysis of the variability of the shapes of adult cervical vertebrae and a comparison with cervical vertebrae of children. Based on this examination, we propose an explanation why the use of cervical vertebrae might be not competitive to other methods in forensic age estimation.

Figure 1: Development of the cervical vertebrae in a sample individual. Lateral cephalograms were obtained at the ages of 9, 12, 15, 17 and 20 years. C3 and C4 change from trapezoids to a nearly rectangular shape, their height reaches and eventually exceeds their width, and the inferior margins of C2 through C4 which are initially flat develop a marked concavity.

MATERIALS AND METHODS

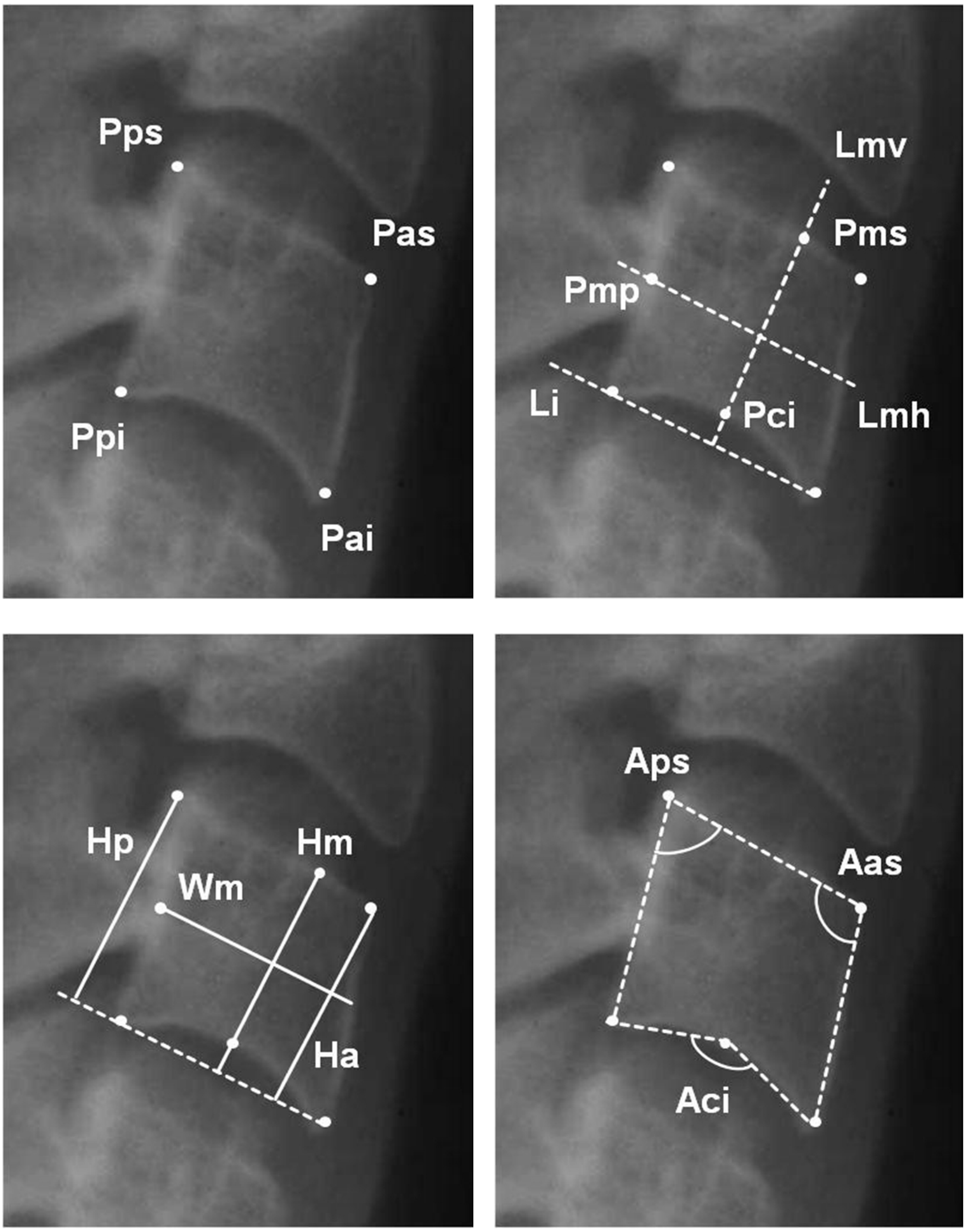
We analysed lateral cephalograms obtained in the clinical routine at the orthodontic department of the University Hospital Würzburg, Germany. In order to study the variability of adult cervical vertebrae shapes, we included a cross-sectional sample 320 adult subjects who were aged 20 years or older to ensure that the development of cervical vertebrae was finished and the end stage was reached. In order to examine the separation of adult cervical vertebrae shapes from those of children, we included a cross-sectional sample of 100 children aged from 8 to 10 years (before the pubertal growth spurt) for comparison. Patients

with syndromes that might affect skeletal development were not eligible. Of 442 selected radiographs, 22 were excluded due to image quality or superposition that might lead to difficulties in the precise evaluation.
The metric evaluation of the radiographs was carried out with the software OnyxCeph³ ™ (Image Instruments GmbH, Chemnitz, Germany). Landmarks on the images were set according to the definitions Table 1. Illustrations are provided in Figure 2. From the landmarks, the software calculated the following quantities that were then exported for statistical processing: inferior concavity angles of C2, C3, and C4; posterior, anterior and median height, median width, and posterior and anterior angle at the superior side of C3 and C4.

Table 1: Steps of the metric evaluation of cervical vertebrae (CV). The items with an asterisk (*) apply to all CV, the others only to C3 and C4

Symbol	Description	Construction
Pps	posterior superior vertex	free selection by observer
Pas	anterior superior vertex	free selection by observer
Ppi *	posterior inferior vertex	free selection by observer
Pai *	anterior inferior vertex	free selection by observer
Li *	inferior line	computed line through Ppi and Pai
Lmv	vertical median line	computed line perpendicular to Li through the midpoint of Ppi–Pai
Lmh	horizontal median line	computed line parallel to Li through the midpoint of Pas–Pai
Pci *	inferior concavity vertex	constraint selection by observer on Lmv and the inferior margin of the CV
Pms	superior median point	constraint selection by observer on Lmv and the superior margin of the CV
Pmp	posterior median point	constraint selection by observer on Lmh and the posterior margin of the CV
Ha	anterior height	perpendicular distance of Pas from Li
Hp	posterior height	perpendicular distance of Pps from Li
Hm	median height	perpendicular distance of Pms from Li
Wm	median width	distance of Pmp from midpoint of Pas–Pai
Ha/Hp	anterior-posterior ratio	computed from Ha and Hp
Hm/Wm	height-width ratio	computed from Hm and Wm
Aci *	inferior concavity angle	angle with arms Pci–Ppi and Pci–Pai
Aps	posterior superior angle	angle with arms Pps–Ppi and Pps–Pas
Aas	anterior superior angle	angle with arms Pas–Pps and Pas–Pai

Fig.2: Landmarks of the metric evaluation of cervical vertebrae



Inferior concavity angles of C2, C3, and C4, and median height-width ratio, anterior-posterior height ratio and superior side angles C3 and C4 were analysed. Criteria for the highest stage of development of cervical vertebrae as described, for example, by Hassel and Farman[4] or Baccetti and colleagues[5] were translated into terms of metric quantities as follows:

1. rectangular shape of C3 and C4 – considered fulfilled if the anterior-posterior height ratio was ≥0.9 (the anterior side of the trapezoid of children's C3/C4 is shorter than the posterior side), the posterior superior angle was ≥70 degrees and the anterior superior angle was ≤110 degrees (i.e. both angles differ from a right angle by no more than 20 degrees)

2. at least one of C3/C4 is rectangular in vertical shape (if not both, the second is squared) – considered fulfilled if the height-width ratio was ≥0.9 (this is quite liberal as ratios <1 indicate a horizontal rectangle)
3. significant concavities at the inferior margin of C2, C3 and C4 – considered fulfilled if the angles of the concavities were ≤160 degrees (note that reference images for established staging schemes suggest <150 degrees)

The statistical software SPSS 23 (IBM Corp., Armonk, NY, USA) was used for analysis. Summary data were presented by means and standard deviations. Comparison of male and female subjects was carried out by t-tests for adults and children separately. Frequencies and percentages of men, women and all subjects fulfilling the criteria for adult shapes of cervical vertebrae were computed, and rates of matching were displayed at the level of single measures, all measures of each of the cervical vertebrae, and perfect match (all measures of C2, C3 and C4). To characterize the variability of the geometry of cervical vertebrae and the overlap of adults and children, 95% ranges (2.5th to 97.5th percentile) of all measures were presented for male, female and all subjects. In addition, cumulative distribution functions were computed and displayed in diagrams.

For quality control, repeat measurements with a time-lag of two weeks were carried out on 50 lateral cephalograms to assess the intra-observer agreement (statistically expressed by the intra-class correlation coefficient, ICC).

The local ethics committee at the Medical Faculty of the University of Würzburg has confirmed that, according to the applicable legal and regulatory requirements in Germany, no ethical approval is needed for this research in the given setting (reference number 20170317-01).

RESULTS

Regarding data quality, reproducibility was excellent for height-width ratios, anterior-posterior height ratios, and inferior concavity angles (ICC from 0.95 to 0.99), good for the posterior superior angle of C3 and the superior angles of C4 (ICC from 0.88 to 0.92), and acceptable for the anterior superior angle of C3 (ICC 0.83).

Table 2 displays the averages of the metric data in our sample. In adults, the mean angles of the inferior concavities were ranging from 149 to 156 degrees which is close to the amount suggested by reference images in the literature. The mean height-width ratios were slightly (C3) or materially (C4) below 1, indicating that the "average" adult C3 and C4 is not a rectangle in vertical shape. Regarding the measures of rectangularity, the mean anterior-posterior height ratios of C3 and C4 were about one standard deviation below 1 in both men and women, and the mean deviations from a right angle of the angles at the superior vertices were ranging from 12 to 18 degrees.

Women had on average more pronounced inferior concavities than men. This was the only difference between sexes in adults with high significance (P<0.001 for C2, C3 and C4). With the exception of the anterior superior angle of C4, there were no other significant differences between adult men and women. In children, the means of the height-width ratios and part of the measures of rectangularity were closer to the adult values in girls than in boys, probably due to their earlier development. Of note, no significant differences between boys and girls were found for the inferior concavities of all three cervical vertebrae.

Table 3 lists the percentages of adults who fulfilled the metric criteria for adult cervical vertebrae. The inferior concavity angle is 160 degrees or below in 90% of the subjects for C2 and C3 and in 81% for C4. This means that 10% of the adult C2 and C3 and 19% of C4 might possibly not be considered to be typically adult with respect to their inferior concavity. The height-width ratio does still more often not match the description of adult cervical vertebrae. Every sixth C3 and every third C4 did not reach a ratio of 0.9 or above. When more strictly applying Baccetti's[5] description of the highest stage of development (at least one of C3/C4 is a vertical rectangle, the other is at least a square), we might require that both height-width ratios are ≥0.9 and at least one is >1. These criteria would be met by only 147 subjects (46%; 42% of men, 49% of women). The criteria for rectangular shape were fulfilled in less than two-thirds of the C3 and C4, which was most frequently attributable to a ratio of anterior and posterior height below 0.9.

Only one out of four subjects fulfilled all criteria (18% of men, 29% of women, P=0.02 for difference between sexes). The criteria for the inferior concavity were more frequently met by women (P=0.02 for C2, P=0.01 for C3, P=0.03 for C4), while men and women did not significantly differ with respect to the height-width and anterior-posterior height ratios (P-values from 0.29 to 0.82).

Table 2: Subject characteristics

ADULTS	**Men**	**Women**	**P-value**
Number of subjects	153	167	–
Age [y]	26.8 (7.8)	27.0 (7.4)	–
20 to <25 years – number (%)	87 (57)	92 (55)	–
≥25 years – number (%)	66 (43)	75 (45)	–
C2: inferior concavity angle [°]	153.2 (6.5)	149.5 (6.7)	<0.001
C3: height-width ratio	0.992 (0.100)	0.987 (0.093)	0.64
C3: anterior-posterior height ratio	0.933 (0.066)	0.945 (0.067)	0.12
C3: posterior superior angle [°]	76.4 (4.0)	75.6 (4.1)	0.11
C3: anterior superior angle [°]	107.2 (4.2)	107.8 (3.1)	0.17
C3: inferior concavity angle [°]	152.4 (7.0)	149.4 (6.6)	<0.001
C4: height-width ratio	0.934 (0.091)	0.943 (0.093)	0.38
C4: anterior-posterior height ratio	0.933 (0.070)	0.931 (0.063)	0.73
C4: posterior superior angle [°]	77.9 (4.8)	77.6 (4.1)	0.57
C4: anterior superior angle [°]	104.8 (4.4)	105.9 (3.8)	0.01
C4: inferior concavity angle [°]	156.0 (5.9)	151.8 (6.6)	<0.001
CHILDREN	**Boys**	**Girls**	**P-value**
Number of subjects	50	50	–
Age [y]	9.2 (0.5)	9.2 (0.6)	–
8 to <9 years – number (%)	19 (38)	14 (28)	–
9 to ≤10 years – number (%)	31 (62)	36 (72)	–
C2: inferior concavity angle [°]	173.5 (4.7)	172.6 (6.5)	0.43
C3: height-width ratio	0.614 (0.083)	0.679 (0.072)	<0.001
C3: anterior-posterior height ratio	0.672 (0.099)	0.756 (0.116)	<0.001
C3: posterior superior angle [°]	68.5 (5.7)	73.0 (4.4)	<0.001
C3: anterior superior angle [°]	117.2 (5.3)	113.1 (4.6)	<0.001
C3: inferior concavity angle [°]	173.5 (4.6)	173.8 (4.6)	0.72
C4: height-width ratio	0.627 (0.088)	0.666 (0.077)	0.02
C4: anterior-posterior height ratio	0.693 (0.126)	0.719 (0.100)	0.25
C4: posterior superior angle [°]	69.2 (5.4)	74.1 (5.7)	<0.001
C4: anterior superior angle [°]	113.6 (5.4)	112.0 (5.0)	0.13
C4: inferior concavity angle [°]	174.3 (4.5)	174.0 (4.1)	0.73

Data are means and standard deviations except for age groups which are N and %.

Table 3: Numbers of adults matching the criteria for adult CV geometry

Criteria		Total	Men	Women
C2: inferior concavity angle	≤160°	289 (90%)	132 (86%)	157 (94%)
C3: height-width ratio	≥0.9	268 (84%)	126 (82%)	142 (85%)
C3: rectangular shape		178 (56%)	84 (55%)	94 (56%)
anterior-posterior height ratio	≥0.9	227 (71%)	105 (69%)	122 (73%)
posterior superior angle	≥70°	297 (93%)	143 (93%)	154 (92%)
anterior superior angle	≤110°	234 (73%)	113 (74%)	121 (72%)
C3: inferior concavity angle	≤160°	287 (90%)	130 (85%)	157 (94%)
All criteria for C3 fulfilled		146 (46%)	63 (41%)	83 (50%)
C4: height-width ratio	≥0.9	209 (65%)	95 (62%)	114 (68%)
C4: rectangular shape		204 (64%)	102 (67%)	102 (61%)
anterior-posterior height ratio	≥0.9	220 (69%)	110 (72%)	110 (66%)
posterior superior angle	≥70°	308 (96%)	143 (93%)	165 (99%)
anterior superior angle	≤110°	283 (88%)	139 (91%)	144 (86%)
C4: inferior concavity angle	≤160°	259 (81%)	116 (76%)	143 (86%)
All criteria for C4 fulfilled		119 (37%)	53 (35%)	66 (40%)
All criteria for C2, C3, C4 fulfilled		76 (24%)	27 (18%)	49 (29%)

Table 4 shows the 95% ranges (i.e. the intervals from the 2.5^{th} to the 97.5^{th} percentile) of each measure in adults compared to children for males, females and all subjects. With the exception of the height-width ratio of C3 in males and the inferior concavity angle of C3 in females, the intervals for adults and children do overlap. This means that there are values of these measures that can occur in adults as well as in children and, since 95% ranges are presented, this fact is not attributable to single outliers.

More comprehensive information on the amount of overlap can be read from the cumulative distribution functions in Figure 3. For each value x on the horizontal axis, the corresponding percentage on the vertical axis says which part of the population has a value ≤x. The vertical lines mark the most extreme values for adults and children, and the corresponding percentages marked by the horizontal lines allow concluding about the extent of overlap. For example, in the first diagram referring to the inferior concavity angle of C2, the maximum value of adults was 172 degrees, and 35% of the children had a value below this limit. The minimum value of children was 153 degrees, 59% of the adults had a value below this limit, and hence, 41% had a value below. Thus, 41% of the adults and 35% of the children had their values within the intersection interval of the adults' and children's ranges of measurements.

DISCUSSION

Our analyses revealed a large variance in the appearance of adult cervical vertebrae and a high percentage of adult individuals whose shapes of cervical vertebrae disagree with descriptions of the end stage of their development.[4,5] To illustrate this, Figure 4 shows four examples of different patterns of not matching the criteria. The first case (female, 22 years) has very small height-width ratios of 0.67 (C3) and 0.78 (C4), and the shape of C3 cannot really be called rectangular (anterior superior angle 112 degrees).

Table 4: 95% ranges (2.5th to 97.5th percentile) of metric characteristics of cervical vertebrae in adults and children

Characteristic		Adults	Children
C2: inferior concavity angle [°]	male	139.2-165.0	162.5-180.0
	female	136.4-163.1	153.5-180.0
	all	137.0-163.7	155.2-180.0
C3: height-width ratio	male	0.804-1.210	0.440-0.788
	female	0.796-1.177	0.545-0.851
	all	0.806-1.193	0.475-0.812
C3: anterior-posterior height ratio	male	0.810-1.073	0.460-0.869
	female	0.818-1.074	0.547-1.010
	all	0.817-1.073	0.509-0.971
C3: posterior superior angle [°]	male	69.0-85.3	53.5-79.2
	female	66.1-82.2	63.8-86.1
	all	67.4-84.5	57.6-82.4
C3: anterior superior angle [°]	male	99.1-115.3	106.3-129.6
	female	101.5-113.7	105.2-125.1
	all	100.2-114.8	106.1-126.8
C3: inferior concavity angle [°]	male	139.7-167.8	159.8-179.8
	female	138.2-162.5	163.5-179.8
	all	138.7-167.3	163.4-179.8
C4: height-width ratio	male	0.772-1.138	0.472-0.875
	female	0.769-1.150	0.499-0.860
	all	0.772-1.137	0.484-0.872
C4: anterior-posterior height ratio	male	0.760-1.073	0.464-1.032
	female	0.820-1.049	0.480-0.913
	all	0.813-1.058	0.467-0.912
C4: posterior superior angle [°]	male	67.4-87.1	59.6-80.0
	female	70.2-86.2	61.2-87.4
	all	68.5-86.5	60.5-84.0

C4: anterior superior angle [°]	male	96.3-114.7	103.9-128.2
	female	98.9-114.3	101.0-122.0
	all	97.2-114.4	103.7-122.8
C4: inferior concavity angle [°]	male	144.0-167.6	161.9-179.7
	female	140.1-164.3	163.0-179.4
	all	140.6-166.3	163.1-179.4

In the second case (male, 25 years), the shapes of C3 and C4 are quite nicely rectangular (which is seen rather in a minority of the individuals), but the depths of the concavities are less than one-half of those shown in reference images (angles about 167 degrees), and the height-width ratios are 0.87 and 0.83, respectively. The third individual has a bit more pronounced inferior concavities, but again, the height-width ratios are not adult (0.79 and 0.73), and the anterior-posterior height ratios of 0.75 and 0.81 do not support a rectangular shape. In the last example, the inferior concavity angles (144 to 148 degrees) and the height-width ratios (1.15 and 1.19) represent adult values, but C3 is a typical trapezoid rather than a rectangle (anterior-posterior height ratio 0.83).

The large variability of the shapes of adult cervical vertebrae is probably a major reason for their lower performance when compared to other methods of age estimation. For example, in the end stage of development, the epiphyses of hand bones are joined with the metaphyses and the epiphyseal line is eventually no longer recognizable.[2,3] For teeth, the end stage is characterized by the closed apex.[1] In both cases, there is no variability in the appearance of these anatomic structures. In contrast, cervical vertebrae have many possible end points of their development and, therefore, it is difficult to determine from a radiograph which part of the way to the end point has already been passed.

In general, there are many anatomic measures are highly correlated with age but not all of them are suitable for age estimation. For example, body height or the waist-hip circumference ratios do materially change with increasing age, and their mean values are different between the age groups and sexes with high statistical significance. However, these measures have a high variability at the individual level which makes them unsuitable for forensic age diagnostics. The same argument might possibly apply to cervical vertebrae.

A second disadvantage seems to be the considerable overlap of the ranges of metric characteristics of cervical vertebrae of adults and pre-pubertal children. This situation is not found in hand bones where the epiphyses and metaphyses are not connected in children aged 10 years or below, and the epiphyseal lines are completely closed and disappear in most cases in adults aged 20 years or above.[2,3] For third molars, Demirjian's stage[1] is usually A to C (or only a crypt is present) in children aged 10 years or below, and G or H (rarely lower) in young adults. Now consider a real-world situation of forensic age estimation where the challenge is not distinguishing between adults and pre-pubertal children, but the diagnosis whether a young violator who is apparently about 16 years old and who claims to be 13 years old has passed the age of criminal responsibility (which is 14 years in Germany and many other countries). It is not hard to imagine that this type of diagnosis is difficult with a measure that does not strictly separate individuals aged 10 and 20 years.

The limitations discussed above do not imply that cervical vertebrae should be abandoned in general. Cameriere and colleagues[22] investigated age estimation from the ratio of the lengths of the anterior and the posterior side of C4. The mean absolute errors they reported indicate inferiority compared to age estimation from hand atlas methods.[2,3] However, the data presented in their paper suggest that the ratio under consideration increases rapidly around the age of 10 years. Hence, this measure might be valuable in the diagnosis of criminal responsibility in countries were the age threshold is 10 years. We should not forget in our debates the possibility that certain tools might be not recommended in general, but are particularly useful in distinct situations.

Fig.3: Cumulative distribution functions (CDF) of metric characteristics of cervical vertebrae in adults (black curve) and children (grey curve). Dashed lines mark the overlap of children's and adults' ranges

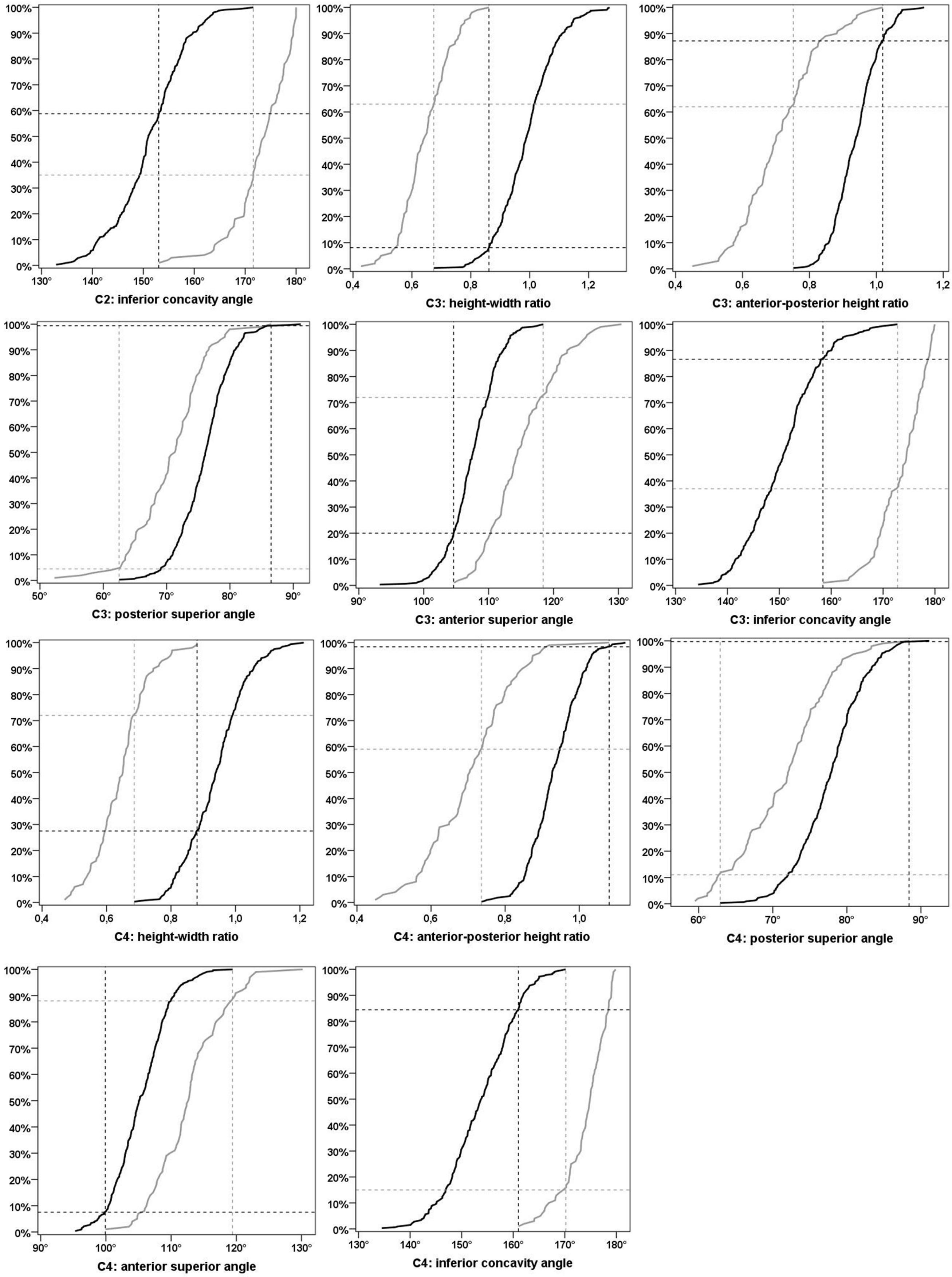
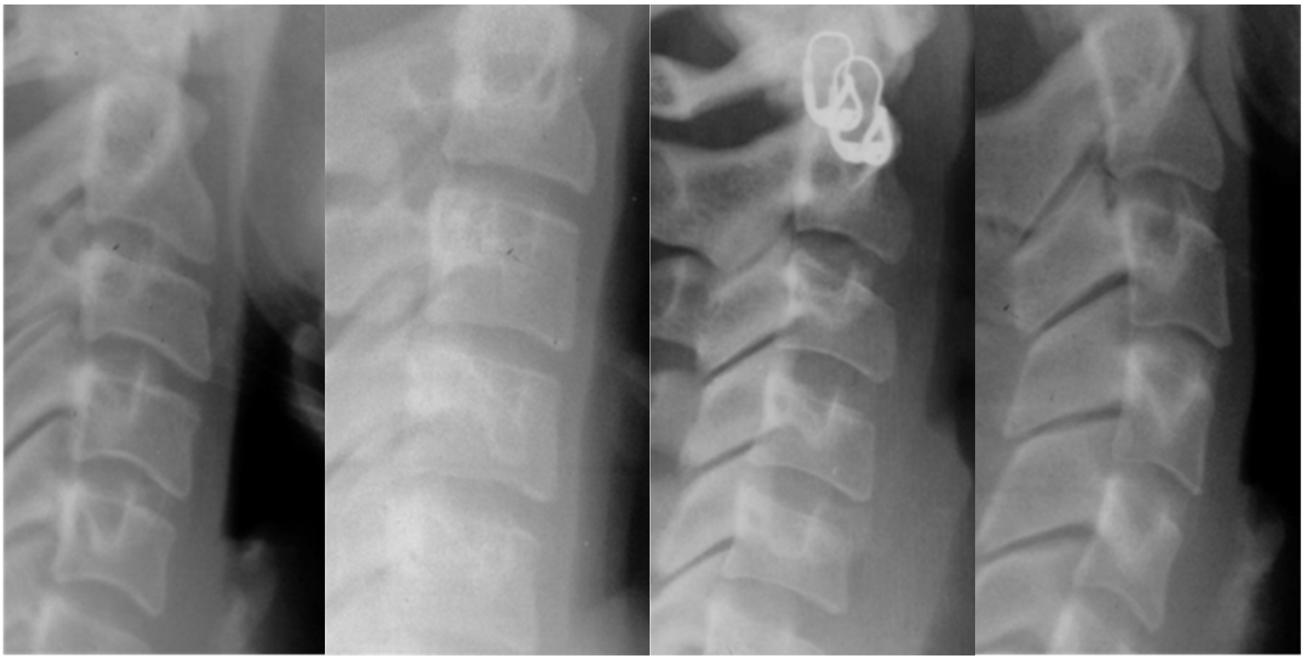


Fig.4: Sample cases of individuals aged 20 years or above illustrating different patterns of violation of the shape criteria for adult cervical vertebrae



Another way of metric evaluation of cervical vertebrae was proposed by Rhee and colleagues.[23] They used cone beam computed tomography which allows for a better exploration of C2, compared to lateral cephalograms. It is hard to conclude from this small exploratory study (35 boys, 45 girls) whether the formulas provide a gain competitive to, or additional to the information available from hand bones. The advantage for forensic age estimation (if there is any) of the additional dose of radiation associated with this approach needs to be demonstrated before it can be routinely used in living subjects.

It is well-known that, due to biological variability, each single method of age estimation is too imprecise in the forensic context. Several methods need to be combined to achieve satisfactory results. The key question is hence which combination of methods is the best one, and not which single method is superior to others. In a recent investigation[24] we have shown how to combine independent age estimates from hand bones and third molars in order to optimally explore the information gathered from each of these well-established methods. We suggest that evaluations of any new methods (using cervical vertebrae or other anatomic structures) should focus on the incremental information gained by the use of these methods on top of those that are routinely applied in forensic age estimation so far. In particular, comparison of dental age estimation methods should not focus solely on the question which of them performs best as a single method. It is rather necessary to examine which one is superior in combination with skeletal age estimation methods. The possibility to get dental and skeletal information from a lateral cephalogram, i.e. a single radiograph, is appealing. However, this combination is probably less promising due to the above studied handicap of the cervical vertebrae.

Nonetheless, we suggest that age estimation from cervical vertebrae should still be studied and be part of the forensic toolkit, even if they were inferior to methods using hand bones. We should have in mind that the hand might not be assessable (for example, if only parts of a body were found), and a second-line age estimation method will be useful in such situations.

CONCLUSION

In comparison to other methods of age estimation, the use of cervical vertebrae suffers from significant handicaps. First, the appearance of adult cervical vertebrae has a large biological variance, and many individuals do not match the descriptions of the "typical" shape that are currently being used. Second, there is a material overlap of the metric ranges of relevant measures between adults and pre-pubertal children which indicates an inferior performance in discriminating different ages. We propose that these handicaps explain the inferior performance of cervical vertebrae in age estimation. We suggest that enhancement of this performance, if possible at all, might require a revised staging of cervical vertebrae development taking into account the variance of metric characteristics described herein. Evaluation of the usefulness of cervical vertebrae for age estimation should demonstrate that their incorporation on top of teeth and hand radiographs provides a gain for the age prediction performance (i.e. more precise age estimates). Odontologists should be aware that forensic age estimation needs to combine dental and skeletal methods and, therefore, knowledge of both should be incorporated in the studies to enhance the precision of age estimation.

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