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ORIGINAL ARTICLE

The role of CT analyses of the sternal end of the clavicle and the first costal cartilage in age estimation

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Abstract The ossification patterns of medial clavicle and the first costal cartilage represent interesting features of the same anatomical region which are accessible for conventional radiographic and computed tomography (CT) examinations in the same field of view. This study encompassed Serbian population and was carried out to examine whether CT analyses of medial clavicle and the first costal cartilage could be successfully applied, either individually or conjointly, in the age assessment. The study was based on CT examinations of thoracic region of 154 patients, aged between 15 and 35 years. Besides radiodensity and stages of epiphyseal cartilage ossification of medial clavicle, the study detected other features that expressed significant correlation with age, such as calculated anterior to posterior cortical thickness ratio, medullar canal diameter, and clavicular shaft diameter. However, although calculated ossified and calcified linear projections' (OCP) stages correlated to age, the distinction between stages was not satisfying. The interaction between the ossification status of medial clavicle and OCP was not significantly influenced by age. The results of our study outlined interesting new age predictors with mutual relationship: acquired radio density of the sternal epiphyseal-metaphyseal region and radio density of the first costal cartilage. Intersex variability was observed in several age-related features: calculated anterior to posterior cortical thickness ratio, diameter of medullar canal, and diameter of the clavicular shaft. Altogether, our study identified several radiological features of the first costal cartilage and medial clavicle that correlated with age and which could be applied as additional guidance for age estimation in each specific case.

Keywords Clavicle · First costal cartilage · Macroscopic · Histomorphometric · CT · Age assessment

Introduction

The sternal clavicular epiphysis is characterised by the longest period of growth among all long bones [1, 2]. Therefore, anatomical observations of the medial clavicle were introduced in the field of age analyses, as early as in the end of nineteenth century [3].

Independently from the anatomical approach to age estimation, radiological methodology was developed. For instance, the work of the *Study Group on Forensic Age Diagnostics of the German Association of Forensic Medicine—Arbeitsgemeinschaft für Forensische Altersdiagnostik* (*AGFAD*) suggested radiographic analyses of dentition and left hand as criteria for age estimation process in young individuals [4, 5]. Furthermore, the radiographic analysis of the medial clavicle was recommended as additional examination for age assessment in young adults [5].

Initially established conventional radiology methods and their impediments were gradually replaced by computed tomography (CT) analyses. The implementation of new methodology was initiated in 1998 by Kreitner et al. [6].

The ossification patterns of the first costal cartilage represent another interesting feature of the same body region that

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arise around puberty and develop mostly through the third and fourth decades of life [4, 7]. The anatomical position of the first costal cartilage makes it accessible for examinations in living individuals who undergo conventional radiographic and CT examinations in the same field of view as clavicle [4, 8]. However, while clavicles remain accessible in cadaveric material, the subtle changes of the first cartilage could be altered during preparation and would be certainly lost during deterioration process. According to the literature, the analyses of the ribs for the first time involved modern radiological techniques in 2010 when Moskovitch et al. studied the age-related changes of the first rib and its cartilage using computed tomography [8].

Since clavicles are spared of weight bearing and significant involvement in everyday locomotion of upper extremities, therefore avoiding influence of mechanical stress on their anatomical features, medial clavicular end could embrace full scientific focus. However, in contrast to the lower costal cartilages which are influenced by respiratory movements, it is considered that the first cartilage is only affected by extreme physical stress on the upper chest [7, 9].

Recent comprehensive studies dealt with age estimation in various populations [6, 10–19]. In fact, age assessment in different geographic regions emphasised the socioeconomic influence on skeletal epiphyseal union which was recently outlined as more important than interethnic variation [20–23]. Therefore, the importance of developing reliable population-specific age estimation methodologies is appreciated, including the methods appropriate for the population in the Balkan region [11, 15, 24].

The aim of this study was to evaluate whether introduction of new criteria and radiological features in analyses of the medial clavicle and/or the first costal cartilage can be useful for age estimation process. Furthermore, the already developed methods were tested for the first time in Serbian population.

Material and methods

The study data were derived from picture archiving and communication system (Carestream Health PACS) and medical archives of Institute for Oncology and Radiology of Serbia. The process of patient selection upon defined inclusion and exclusion criteria was based on the data provided in medical records of each patient. The inclusion criteria for this study represented CT examination of thoracic region and age of the patient in the range between 15 and 35 years in the moment of performed diagnostic procedure. The exclusion criteria encompassed involvement of hormone therapy, thoracic radiotherapy treatment, primary and secondary neoplastic lesions in the region of interest, presence of any visible deformity, or signs of trauma of the clavicle, first rib, or manubrium sterni.

All encompassed individuals belonged to contemporary Serbian population, and their socioeconomic status was considered in boundaries of upper-middle-income country with high human development index [25, 26].

The sample was selected from the group of patients who have undergone through diagnostic CT examination in the time period between 1 January 2011 and 31 December 2012 and therefore encompassed 181 patients.

The exclusion criteria disqualified from further analyses 27 (15 %) patients: due to thoracic radiotherapy treatment (12.2 %), due to primary and secondary neoplastic lesions in the region of interest (0.6 %), and lack of medical records due to different technical reasons (2.2 %). Therefore, the functional sample size for clinical study analyses comprised clavicles and the first costal cartilages of both sides derived from CT examinations of 154 patients, whereof 97 (62.99 %) were males and 57 (37.01 %) females.

Overall, due to image artefacts, clavicular analyses excluded one right-sided medial clavicle from radiodensity measurements while one per both sides were excluded from all other analyses except of estimation of progress of medial clavicular epiphyseal cartilage ossification status which encompassed the maximal number of 308 clavicles.

The study data were comprised of CT examinations performed by Siemens SOMATOM Sensation Open. The included CT scans involved contrast-enhanced 3 mm-reconstructed images from 5 mm-width slices (120 kV, Care Dose 4D, slice collimation 1.2 mm, pitch factor 1.20, increment 5.0 mm, recon increment 3.0 mm, kernel B70f). The analyses process was conducted on SIEMENS Syngo Multimodality Leonardo Workplace using bone window.

CT scan images allowed the assessment of the ossification status of medial clavicular epiphyseal cartilage following five-phase scoring system developed by Schmeling et al. [12, 27]. According to the provided instructions, if ossification centre was not ossified, the first stage was assigned (Fig. 1a). The presence of ossification centre with unossified epiphyseal cartilage was considered as the second stage (Fig. 1b). The third stage stood for partly ossified epiphyseal cartilage (Fig. 1c). If epiphyseal cartilage was fully ossified and epiphyseal scar was still visible, the fourth stage was assigned (Fig. 1d). However, fully ossified epiphyseal cartilage with no longer visible epiphyseal scar represented the fifth stage (Fig. 1e).

The clavicular analyses also involved direct recording of the following measurements: radio density of the sternal epiphyseal-meta physeal region (Ep.Dn) (Fig. 2), thickness of the anterior (Ct.Th$_{ant}$) and posterior cortex (Ct.Th$_{post}$) (Fig. 3a), clavicular shaft diameter (Dp.Wi) (Fig. 3b), and diameter of the medullar canal (Ca.Wi) (Fig. 3c) [28–30]. Subsequently, acquired data permitted calculation of the

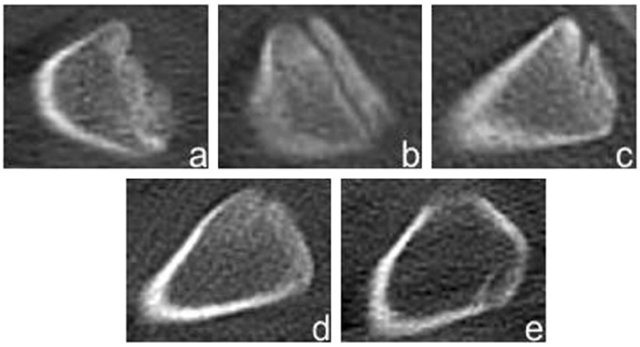

Fig. 1 CT scan images of the ossification status of the medial clavicular epiphyseal cartilage (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, original axial CT plane): **a** the first stage, **b** the second stage, **c** the third stage, **d** the fourth stage, **e** the fifth stage

following derived indices: anterior to posterior cortical thickness ratio ($Ct.Th_{ant}/Ct.Th_{post}$) and medullar to shaft diameter ratio (Ca.Wi/Dp.Wi). The measurements of clavicular diaphysis were obtained in the middle third of the shaft, always recording of all data at the same site of one clavicle. The radiodensity of a surface of the sternal epiphyseal-metaphyseal region was taken on the most representative slice by manually shape-adjusted freehand ROI tool and expressed in Hounsfield units (HU). In addition, all data were recorded in the original CT axial plane. For all observed features, the expression of sexual and side dimorphism was analysed as well.

This study also examined whether morphological appearances of the calcifications in the first costal cartilage correlate to age. The conducted analyses excluded two cartilages (one per both sides) due to image artefacts and therefore were performed on total of 306 cartilages.

Fig. 2 The radio density of the sternal clavicular epiphyseal-meta physeal region was taken on the most representative slice by manually shape-adjusted freehand ROI tool and expressed in Hounsfield units (HU) (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, original axial CT plane)

The assessment of the bony changes of anterior and posterior margin of costal face which extend through cartilage medially to sternum was carried out in concordance with published methodology of Moskovitch et al. [8]. Hence, the earliest stage, named “stage 0”, was obtained in cases with no signs of the beaks or calcifications projecting medially in the costal cartilage (Fig. 4).

The first and second stages were assigned in the cases of ossified and calcified linear projections (OCP). Thus, the first stage was recorded if projection’s length (OCP.Le) did not exceed 50 % of distance between costal face and sternum (CS.Le) (Fig. 5); otherwise, it was marked as the second stage (Fig. 6).

However, all non-linear appearances of calcifications projecting medially towards sternum were ascribed to the third stage (Fig. 7).

In addition, following Moskovitch’s methodology, all data were recorded in multi planar reconstruction (MPR) viewer window in the oblique plane which was derived from an original axial plane adjusted to the plane of the first cartilage in order to perform the most objective measuring [8]. The projection’s length was measured by manually shape-adjusted 2D freehand distance tool.

The analyses also made distinction in the sex, lateralization, number of present linear projections i.e. beaks, and radiodensity of a surface of costal cartilage (FC.Dn) which was recorded on the most representative slice by manually shape-adjusted freehand ROI tool and expressed in Hounsfield units (HU) (Fig. 8).

Statistical analyses were performed using SPSS for Windows, version 15. In all analyses, the significance level was set at 0.05.

During the analyses, the normality of data distribution was assessed by Kolmogorov–Smirnov test. Subsequently, the parametric or non-parametric tests were applied in order to achieve research goals. Thus, the relation between obtained parametric data and individual’s age was tested by linear regression analysis while Spearman correlation was applied in the case of non-Gaussian distribution. The assessment of relation between obtained ordinal data and age involved analysis of variance (ANOVA).

The discrepancy between body sides was identified by paired Student’s *t* test for recorded parametric data and Wilcoxon test for non-parametric data. The estimation of sexual dimorphism involved unpaired Student’s *t* test or Mann–Whitney *U* test, depending on the data distribution. The evaluation of intersex relations among ordinal data was conducted by ordinal regression analyses.

Overall, if the data expressed intersex variation, the results were presented independently for male- and female-sample part. Otherwise, the sex differentiation was disregarded.

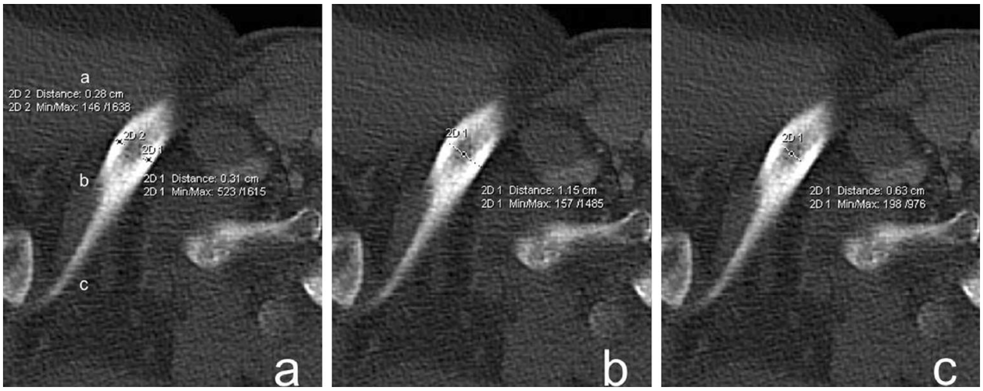

Fig. 3 The analyses of clavicular diaphysis involved direct recording of the following measurements: **a** thickness of the anterior (Ct.Th$_{ant}$) and posterior cortex (Ct.Th$_{post}$); **b** clavicular shaft diameter (Dp.Wi); **c** diameter of the medullar canal (Ca.Wi). (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, original axial CT plane)

Likewise, if the conducted analyses determined side variability, the results were given for right and left sides separately.

The recordings were performed by two independent observers. The first observer was the author who is a resident in radiology with previous experience in the field of forensic anthropology. The second observation was conducted by the radiology specialist who is a teaching assistant at Institute of Anatomy and with extensive experience in the field of forensic anthropology. Both the observers were ignorant of individuals' age and sex. The entire thoracic CT examinations were available to observers without predefined slices for staging procedure. Each clavicle and the first costal cartilage were subjected to analyses of discerning radiographic appearances. Intraobserver reliability was tested by the first observer scoring the whole sample with an 8-month time lapse. The Cohen's Kappa test was used for assessment of intra and interobserver reliability of observed macroscopic features of medial clavicles and macroscopically estimated epiphyseal union stages as well as radiologically analysed stages of the medial clavicle and the first costal cartilage. The instructions were provided by Landis and Koch [31]. The interclass correlation coefficient was applied in order to evaluate reproducibility of quantitative measurements made by observers.

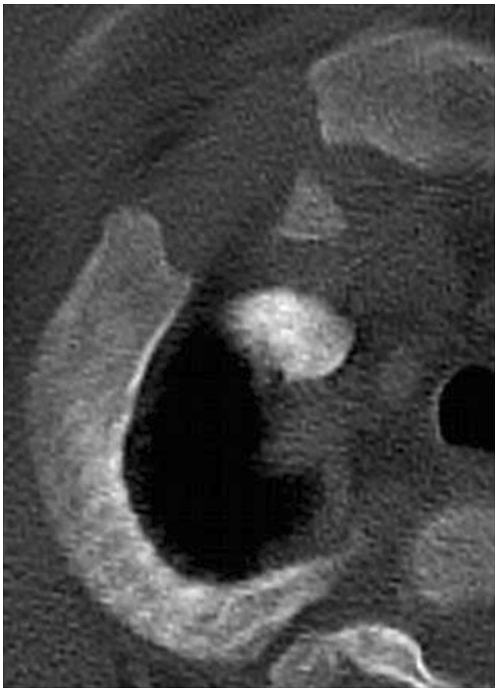

Fig. 4 The CT image of the earliest cartilage stage (stage 0) (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, multi planar reconstruction—MPR, oblique plane adjusted to the plane of the first cartilage)

Results

Clavicles

Ossification status of the medial clavicular epiphyseal cartilage did not show significant side differences ($p>0.05$). Although females showed slight tendency towards higher epiphyseal union stages, the observed sexual dimorphism was not statistically significant (OR=0.86, $p=0.498$). Therefore, in further analyses, sex and side were not considered (Table 1). The first epiphyseal union stage was recorded predominantly in 15-year-old individuals (63.6 %) and could be seen until the age of 17. The second stage almost entirely overlapped with the first, being present in the range between 15 and 18 years. However, the second stage was mainly recorded (70.0 %) among 17- and 18-year-old individuals. Considering the third stage, while its latest detection was at the age of 25, it could be sometimes observed from the age of 17 with even one recording at the age of 15 (2.1 %). The fourth stage of epiphyseal fusion was observed in the age range from 19 to 30 years, with three quarters (75.5 %) of recordings in the range between 20 and 25 years of age. Finally, the last (fifth) stage thoroughly overlapped with the fourth showing the earliest detection at the age of 19 and extended mainly (98.8 %) from 22 to 35 years of age. The statistical analysis confirmed significant correlation between the stage of epiphyseal fusion and age ($p<0.001$), revealing the low level of stage discrimination only between the first two stages ($p>0.05$) while mutual distinctions between all other stages were statistically significant ($p<0.05$).

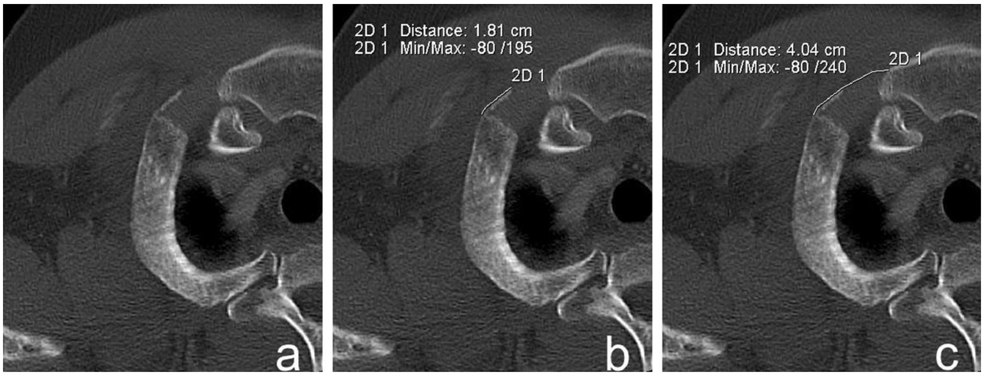

Fig. 5 The CT images of the first cartilage stage: **a** image without measures, **b** OCP.Le, **c** CS.Le. (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, multi planar reconstruction—MPR, oblique plane adjusted to the plane of the first cartilage)

The sex variability in radio density of the sternal epiphyseal-metaphyseal region was not detected ($p>0.05$). However, the radio density differed among sides both in the whole sample ($p=0.036$) and separately analysed female part of the sample ($p=0.015$). The average values of 276.78 HU for the right side and 269.16 HU for the left side were observed in the whole sample (Table 2), with mean values of involved surface area of 3.1 cm^2.

Further analyses revealed that observed radiodensity of the sternal epiphyseal-metaphyseal region in the whole sample depended on the individual's age ($R^2=0.446$, $p<0.001$ for the right side; $R^2=0.389$, $p<0.001$ for the left side). Furthermore, separate analyses without side differentiation in males showed statistical significance ($R^2=0.399$; $p<0.001$). The obtained relations allowed implementation of the following equations:

– for the whole sample, right clavicle:

$$\text{Ep.Dn} = 490.059 - 8.655 \cdot \text{Age}$$

– for the whole sample, left clavicle:

$$\text{Ep.Dn} = 479.954 - 8.565 \cdot \text{Age}$$

– additional equation for the male part of the sample:

$$\text{Ep.Dn} = 493.032 - 8.879 \cdot \text{Age}$$

Obtained thickness of the anterior (Table 3) and posterior (Table 4) cortex allowed side discrimination in the whole sample, as well as in the separately analysed male and female parts of the sample ($p<0.05$). Furthermore, sex difference was only noted in recorded thickness of anterior cortex of the right side clavicles ($p=0.042$).

The observed anterior and posterior cortex thicknesses in the male and female parts of the sample did not express age dependence in either side ($p>0.05$). The calculated anterior to posterior cortical thickness ratio although differed among sexes ($p=0.003$) did not express statistically significant side differences ($p>0.05$). There was slight age dependence of the calculated anterior to posterior cortical thickness ratio in males ($R^2=0.022$, $p=0.041$).

The conducted tests could not assign sexual equality ($p<0.001$) to analysed diameter of the medullar canal (Table 5), diameter of the clavicular shaft (Table 6), and calculated medullar to shaft diameter ratio.

Considering both male and female subsamples, as well as the whole sample, side differentiation was observed in the diameter of the medullar canal ($p<0.001$) and calculated medullar to shaft diameter ratio ($p<0.05$), while recorded diameter of clavicular shaft did not express statistically significant inter-side variability ($p>0.05$). In males, there was a significant age dependence of the recorded diameter of clavicular shaft ($R^2=0.064$, $p<0.001$) as well as bilaterally observed diameters of the medullar canal ($R^2=0.056$, $p=0.020$; $R^2=0.078$, $p=0.006$; for the right and left sides, respectively).

Nevertheless, the calculated medullar to shaft diameter ratio did not depend on age either in the whole sample or in separately analysed male and female parts of the sample in both sides ($p>0.05$). Finally, the analyses of the diameter of

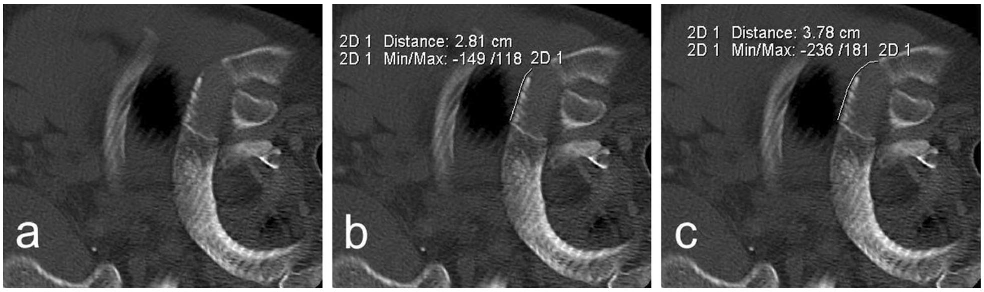

Fig. 6 The CT images of the second cartilage stage: **a** image without measures, **b** OCP.Le, **c** CS.Le. (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, multi planar reconstruction—MPR, oblique plane adjusted to the plane of the first cartilage)

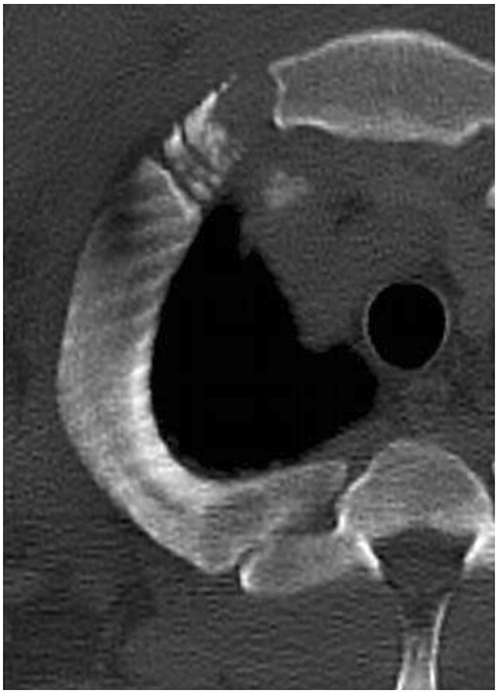

Fig. 7 The CT image of the third cartilage stage (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, multi planar reconstruction—MPR, oblique plane adjusted to the plane of the first cartilage)

Table 1 Distribution of the medial clavicular epiphyseal cartilage ossification status by individual's age in the whole sample

Stage	Frequency		Individual's age				
	N	%	Min	Max	Mean	Mod	SD
1	22	7.14	15	17	15.5	15	0.7
2	20	6.49	15	18	16.9	17	1.1
3	48	15.58	15	25	19.7	20	2.0
4	49	15.91	19	30	23.8	25	2.8
5	169	54.87	19	35	28.3	24	4.0

N number of cases, *SD* standard deviation

the medullar canal (Table 5) and diameter of the clavicular shaft (Table 6), conducted in the female-sample-part did not confirm any dependence on age ($p>0.05$).

Concerning epiphyseal union stages, the applied Cohen's Kappa test determined almost perfect agreement regarding intraobserver reliability (right side: Kappa=0.817, $p<0.001$; left side: Kappa=0.832, $p<0.001$), while substantial agreement was observed between two observers (right side: Kappa=0.708, $p<0.001$; left side: Kappa=0.678, $p<0.001$). On the other hand, in order to assess reproducibility of quantitative measurements made by observers the interclass correlation coefficient was applied. Therefore, the calculated values demonstrated excellent agreement concerning radiodensity of the sternal epiphyseal-metaphyseal region between two observers (right side: ICC=0.918, $p<0.001$; left side: ICC=0.914, $p<0.001$), as well as during intraobserver evaluation (right side: ICC=0.921, $p<0.001$; left side: ICC=0.935, $p<0.001$). The excellent agreement was also estimated during intra- (right side: ICC=0.938, $p<0.001$; left side: ICC=0.920, $p<0.001$) and interobserver analyses (right side: ICC=0.902, $p<0.001$; left side: ICC=0.916, $p<0.001$) of involved surface area in radiodensity measurements. The analyses of the clavicular midshaft included testing of interobserver reliability, which was excellent regarding enlargement of medullar canal (right side: ICC=0.928, $p<0.001$; left side: ICC=0.926, $p<0.001$), and diameter of clavicular shaft (right side: ICC=0.950, $p<0.001$; left side: ICC=0.957, $p<0.001$). However, the interobserver agreement was optimal in the case of thickness of the anterior (right side: ICC=0.708, $p<0.001$; left side: ICC=0.751, $p<0.001$) and posterior cortex (right side: ICC=0.767, $p<0.001$; left side: ICC=0.725, $p<0.001$).

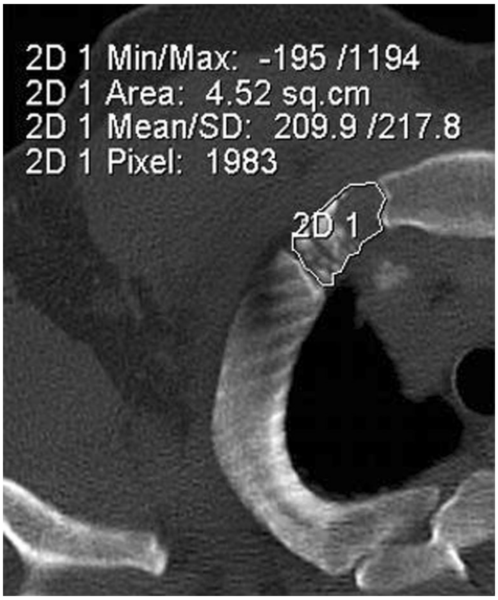

Fig. 8 The radiodensity of costal cartilage (FC.Dn) was recorded on the most representative slice by manually shape-adjusted freehand ROI tool and expressed in Hounsfield units (HU) (SIEMENS Syngo MultiModality Leonardo Workplace, bone window, multi planar reconstruction – MPR, oblique plane adjusted to the plane of the first cartilage)

The first costal cartilage

Considering the analysed features of the first costal cartilage, the results revealed side ($p>0.05$) and sex ($p>0.05$) uniformity among selected 154 individuals.

The measuring of costal cartilage radiodensity (Table 7) revealed average value of 130.39 HU, with mean value of involved surface area of 4.20 cm^2 (SE 0.06).

There was significant age dependence of cartilage radiodensity ($R^2=0.307$, $p<0.001$) which was even more reliable after the process of data normalisation ($R^2=0.378$,

Table 2 The average values of recorded sternal metaphyses radio density (presented in HU)

		Min	Max	Mean	Mod	SE
Female	Right	108.50	430.50	271.46	216.30	9.87
	Left	72.10	411.80	258.18	381.20	9.86
	Right and left	72.10	430.50	264.76	216.30*	6.97
Male	Right	120.90	458.70	279.86	185.00*	7.50
	Left	103.70	492.90	275.61	234.20*	8.10
	Right and left	103.70	492.90	277.73	185.00*	5.51
Whole sample	Right	108.50	458.70	276.78	185.00*	5.96
	Left	72.10	492.90	269.16	232.10*	6.29
	Right and left	72.10	492.90	272.96	185.00*	4.33

HU Hounsfield unit, *SE* standard error

*Multiple modes exist, the smallest value is shown

$p<0.001$). The estimated relations were presented in the following equations:

- relation between the first cartilage radio density and individual's age:

$$FC.Dn = 6.101 \cdot Age - 19.911$$

- relation between the first cartilage radio density and individual's age, after data normalisation:

$$1nFC.Dn = 3.603 + 0.047 \cdot Age$$

Most frequently, analysis of the first costal cartilage revealed one calcified linear projection (47.1 %), while two beaks were recorded in 9.7 %. Considering both single OCP and two OCPs, the first detection of projections was made in a 19-year-old-individual and could be followed until the 35 years of age. The non-linear appearances of calcifications were noted in only 4.5 % of all examined cartilages, always after the age of 23. The absence of linear projections was noted in 38.6 % and could be seen in almost all ages from 15 to 34 years of age, with almost half (48.7 %) of all observations in the age range between 15 and 18 years. The analyses revealed significant influence of calculated stages of the bony changes of the anterior and posterior margins of the costal face upon individual's age ($p<0.001$) (Table 8). However, results revealed satisfying distinction only between the earliest—"zero" stage and all subsequent stages ($p<0.001$), while the mutual difference between the first, second, and third stages could not be identified ($p>0.05$). Nevertheless, the projection's length to distance between the costal face and sternum (Table 7) depended significantly on individual's age ($R^2=0.065$, $p=0.001$).

The analyses of observer reliability revealed almost perfect agreement concerning calculated stages of the morphological appearances of the first costal cartilage between two observers (right side: Kappa=0.888, $p<0.001$; left side: Kappa=0.887, $p<0.001$), as well as during intraobserver evaluation (right side: Kappa=0.821, $p<0.001$; left side: Kappa=0.830, $p<0.001$). The almost perfect agreement was also determined

Table 3 The average values of recorded thickness of the anterior cortex (presented in mm)

		Min	Max	Mean	SE
Female	Right	0.13	0.45	0.23	0.008
	Left	0.15	0.37	0.25	0.006
	Right and left	0.13	0.45	0.24	0.005
Male	Right	0.10	0.36	0.21	0.005
	Left	0.14	0.43	0.24	0.006
	Right and left	0.10	0.43	0.23	0.004
Whole sample	Right	0.10	0.45	0.22	0.005
	Left	0.14	0.43	0.24	0.004
	Right and left	0.10	0.45	0.23	0.003

SE standard error

Table 4 The average values of recorded thickness of the posterior cortex (presented in mm)

		Min	Max	Mean	SE
Female	Right	0.17	0.35	0.25	0.005
	Left	0.18	0.35	0.27	0.005
	Right and left	0.17	0.35	0.26	0.004
Male	Right	0.14	0.45	0.25	0.005
	Left	0.13	0.47	0.28	0.005
	Right and left	0.13	0.47	0.26	0.004
Whole sample	Right	0.14	0.45	0.25	0.004
	Left	0.13	0.47	0.27	0.004
	Right and left	0.13	0.47	0.26	0.003

SE standard error

Table 5 The average values of recorded diameter of medullar canal (presented in cm)

		Min	Max	Mean	SE
Female	Right	0.37	1.15	0.69	0.021
	Left	0.39	1.07	0.64	0.020
	Right and left	0.37	1.15	0.66	0.015
Male	Right	0.43	1.52	0.94	0.022
	Left	0.55	1.32	0.88	0.018
	Right and left	0.43	1.52	0.91	0.014
Whole sample	Right	0.37	1.52	0.85	0.019
	Left	0.39	1.32	0.79	0.017
	Right and left	0.37	1.52	0.82	0.013

SE standard error

during inter (right side: Kappa=0.906, $p<0.001$; left side: Kappa=0.815, $p<0.001$) and intraobserver analyses at the right side (right side: Kappa=0.887, $p<0.001$) for the number and type of the bony changes of anterior and posterior margins of the costal face, while substantial agreement was estimated regarding intra observer analyses at the left side (left side: Kappa=0.803, $p<0.001$). Concerning acquired quantitative measurements, the applied Interclass correlation coefficient detected excellent agreement regarding obtained radiodensity of the costal cartilage during both intra (right side: ICC=0.961, $p<0.001$; left side: ICC=0.978, $p<0.001$) and interobserver analyses (right side: ICC=0.982, $p<0.001$; left side: ICC=0.984, $p<0.001$). Furthermore, the excellent agreement was determined regarding analyses of involved surface area in radiodensity measurements during intraobserver testing (right side: ICC=0.918, $p<0.001$; left side: ICC=0.934, $p<0.001$), while optimal agreement was detected between two observers (right side: ICC=0.884, $p<0.001$; left side: ICC=0.876, $p<0.001$). The excellent agreement was also estimated during intraobserver analyses of beak length measures (right side: ICC=0.961, $p<0.001$; left side: ICC=0.961, $p<0.001$) while agreement was optimal in case of distance between costal face and sternum (right side: ICC=0.872, $p<0.001$; left side: ICC=0.813, $p<0.001$). The interobserver analyses of measured beak length demonstrated excellent agreement at the left side (ICC=0.962, $p<0.001$) and optimal agreement regarding measured distance between costal face and sternum also at the left side (ICC=0.890, $p<0.001$). Unexpectedly, the interobserver agreement was not satisfactory concerning measurements acquired at the right side such as beak length (ICC=0.469, $p=0.002$) and distance between costal face and sternum (ICC=0.182, $p=0.181$). However, subsequently conducted analyses of the relation of projection's length to distance between costal face and sternum revealed excellent agreement between observers at both sides (right side: ICC=0.946, $p<0.001$; left side: ICC=0.952, $p<0.001$) as well as during intraobserver evaluation (right side: ICC=0.958, $p<0.001$; left side: ICC=0.934, $p<0.001$).

Table 6 The average values of recorded diameter of clavicular shaft (presented in cm)

		Min	Max	Mean	SE
Female	Right	0.77	1.53	1.16	0.020
	Left	0.93	1.62	1.17	0.020
	Right and left	0.77	1.62	1.17	0.008
Male	Right	1.00	1.91	1.40	0.020
	Left	0.98	1.85	1.41	0.018
	Right and left	0.98	1.91	1.41	0.013
Whole sample	Right	0.77	1.91	1.31	0.017
	Left	0.93	1.85	1.32	0.016
	Right and left	0.77	1.91	1.32	0.012

SE standard error

Table 7 The average values of recorded radiodensity of the first costal cartilage and calculated relation of projection's length to distance between costal face and sternum in the whole sample

	Min	Max	Mean	SE
Radiodensity of the first costal cartilage (presented in HU)	40.70	391.50	130.39	3.57
Relation of projection's length to distance between costal face and sternum	0.12	1.00	0.66	0.01

HU Hounsfield unit, *SE* standard error

Mutual analyses of clavicles and the first costal cartilage

Subsequently conducted analyses of mutual relation of observed clavicular and the first costal cartilage features to individual's age encompassed only those features that independently already expressed statistically significant correlation to age without intersex variability. The side differentiation was disregarded in further analyses.

Although separately analysed stages of the ossification status of medial clavicular epiphyseal cartilage and stages of the bony changes of the first costal cartilage were statistically

Table 8 Distribution of stages of the bony changes of anterior and posterior margin of costal face in the whole sample

Stage	Frequency		Individual's age			
	N	%	Min	Max	Mean	SD
0	119	38.64	15	34	20.08	4.37
1	34	11.04	19	34	25.59	4.39
2	141	45.78	19	35	27.75	4.37
3	14	4.55	23	35	29.07	3.71

N number of cases, *SD* standard deviation significantly influenced by age, when their mutual relation was tested to individual's age the results revealed that the observed interaction did not correlate with age ($p=0.564$).

Finally, in order to resolve age classification problem, we tested age in relation to acquired values of radiodensity of sternal epiphyseal-metaphyseal region and calculated relation of OCP's length to distance between costal face and sternum ($R^2=0.372, p<0.05$). Similar results were attained after testing age in relation to three acquired variables: radiodensity of sternal epiphyseal-metaphyseal region, radio density of the first costal cartilage, and calculated relation of OCP's length to distance between costal face and sternum ($R^2=0.398$, $p<0.05$). However, statistical results excluded calculated relation of OCP's length to distance between costal face and sternum from this equation ($p=0.579$). Nevertheless, the analyses suggested individual usage of radiodensity of sternal epiphyseal-metaphyseal region as more precise predictor of age ($R^2=0.414, p<0.001$). Furthermore, the mutual relation of radiodensity of sternal epiphyseal-metaphyseal region and radiodensity of the first costal cartilage allowed creation of more reliable equation ($R^2=0.557; p<0.001$), which was even more pronounce after the process of data normalisation ($R^2=0.588; p<0.05$). Thus, two equations were created:

$$\text{Age} = 0.035\cdot\text{FC.Dn} - 0.039\cdot\text{Ep.Dn} + 30.831$$

$$\text{Age} = 5.695\cdot\text{1nFC.Dn} - 0.037\cdot\text{Ep.Dn} + 7.534$$

Discussion

Considering limitations of each methodological approach, usage of scoring systems for age estimation was suggested only for sample types for which they were developed i.e. gross samples demand scoring methodology developed for direct skeletal inspection whereas radiographs require radiology-based scoring systems [1, 14, 21, 27, 32]. While radiological approach suffers from intrinsic optical and tissue superimpositions especially in PA projections, it is still more successful in early detection of ossification than macroscopic observation methods. Introduction of modern radiological techniques such as computed tomography allowed more precise age classification [6]. Nevertheless, the four-phase scoring system which was based on Webb and Suchey's methodology was primary applied in CT analyses although it was basically developed for direct skeletal inspection [6, 33]. The existing CT methodology sustained the only modifications in 2005, after the publication of Schulz et al. [12, 13, 27, 34, 35]. Conversely to Kreitner, Schulz et al. introduced new five-phase scoring system which was in accordance with classification methodology for conventional radiology developed by Schmeling et al. [12, 27].

Although Schulz was the first who recommended slice thickness, his and Kreitner's publications were criticised because of wide slice thickness ranges of encompassed samples (Table 9) [12, 13, 21, 35, 36]. Mühler et al. even went a step further, evaluating age estimation process on different slice thicknesses among the same individuals [36]. The authors observed higher ossification stages at increased slice thicknesses. This was explained by arousal of partial volume effect which was able to partly or fully mask fine radiological features that are mandatory for stage distinction. Therefore, increasing of slice thickness led to identification of different ossification stages in the same cases. Overall, in order to achieve maximum accuracy, the authors recommended the slice thickness of not larger than 1 mm for CT examinations [12, 36, 37].

Unfortunately, in some countries including Serbia, CTs are available only at clinics and not at forensic or research institutes. Therefore, the testing of developed age estimation methods and its adjustments to a specific population require using the data obtained during standard CT examinations of the living subjects. The standard clinical protocols are always in concordance with radiation protection "as low as is reasonably achievable" (ALARA) principle [38]. Therefore, as slice thickness is defined as a dose-related parameter, the available CT protocol in the current study involved slice thickness of only 3 mm [39–41].

Although the present study tested five-stage scoring system on CT studies with thicker slices than recommended, the results were fairly in concordance with the literature (Table 9). Thus, the first epiphyseal union stage was recorded until the age of 17 which was similar to Kreitner and Kellinghaus who both reported 16 years of age [6, 35]. The Wittschieber's study provided somewhat earlier boundary at about 15 years of age [37]. Nevertheless, Bassed et al. published rather higher age limits in males and females, 21 and 19 years of age, respectively [13]. Unfortunately, other authors did not provide information on their results concerning the first stage [12, 34]. The signs of the second stage could be seen until the age of 18 in the current study. Similarly, Kellinghaus and Wittschieber both reported the upper limits at 20 years of age in males, and 19 and 18 years in females, respectively. However, the upper age limit of the second stage in other studies was in the range between 21 and 25 years, with exception of Schulz who did not provide necessary data [6, 12, 13, 34]. According to the results of the present study, the signs of partly ossified epiphyseal cartilage were observed in the range between 15 and 25 years, which was almost absolutely in concordance to the all cited authors except Wittschieber who reported the upper limit of 37 years of age in males [6, 12, 13, 34, 35, 37]. In addition, Schulz published only the earliest age of partly ossified epiphyseal cartilage without providing the data about the latest age [12]. The earliest signs of completely ossified epiphyseal cartilage were recorded at 17 years of

Table 9 Comparative overview of the epiphyseal union stage timing through different studies with applied CT methodology

Study group	Year	Population	Staging system	Slice thickness		Sample size	Males	Females	Age range (in years)		Latest age of not ossified epiphyseal cartilage male/female (in years)	Earliest age of partly ossified epiphyseal cartilage male/female (in years)	Latest age of partly ossified epiphyseal cartilage male/female (in years)	Earliest age of completely ossified epiphyseal cartilage with visible epiphyseal scar male/female (in years)
				mm	*N*	*N*	*N*	*N*	Min	Max				
Wittschieber D. et al. [28]	2014	German	5-Phase	0.6	493	493	336	157	10	40	20/18	16	37/27	22/21
The Current Study	2013	Serbian	5-Phase	3	154	154	97	57	15	35	18	15	25	19
Bassed R. B. et al. [13]	2011	Australian	5-Phase	1 2	391 283	674	455	219	15	25	25/21	17	25	17/19
Kellinghaus M. et al. [29]	2010	German	5-Phase	0.6 1.0 1.25 1.5	3 301 122 77	502	288	214	10	35	20/19	18/17	26/26	22/21
Schulze D. et al. [29]	2006	German	4-Phase	1 3 5 6 7 10	1 15 20 1 62 1	100	50	50	16	25.9	24	16	25	19
Schulz R. et al. [12]	2005	European	5-Phase	1 2 3 5 7	3 1 4 2 546	556	417	139	15	30	NA	17/16	NA	21
Kreitner K.F. et al. [6]	1998	European	4-Phase	1 2 3 4 5 8	36 54 88 202	380	229	151	0	30	22	16	26	22

N number of cases, *NA* not available

age in Australian male population, while all other studies including the current study reported higher boundary [13]. Namely, the present study and Schulze's survey reported 19 years of age as lower boundary [34]. The same age was observed in the female Australian population, while the published results of other studies set the age boundary at 21–22 [6, 12, 13, 35, 37]. Finally, although the fifth stage was mainly recorded (98.8 %) in the age range from 22 to 35 years, the first appearance was noted at the age of 19 in the present study. Conversely, Kellinghaus and Wittschieber reported the fifth stage since the ages of 26 and 27 years, respectively, while Schulz published rather earlier age of 22 years [35, 37]. The youngest individuals with recorded fully ossified epiphyseal cartilage with no longer visible epiphyseal scar were observed in the Australian study at the ages of 17 and 20 in males and females, respectively [13].

The observed discrepancy in ossification timing could possibly be caused by thicker slices used in the current study and therefore aroused partial volume effect. Considering the fourth and especially the fifth stage, we could expect that our results would correspond to studies that used thicker scan slices (Table 9). However, the only study which reported even lower boundaries for those stages was Australian study that used fairly thin slices. Therefore, the discrepancy could be explained not only by different slice thicknesses used in the scanning but also probably by different sample populations.

Conversely to previously published macroscopic analyses, we could not compare the result of the current study to some socioeconomically similar populations [11, 15, 16, 25, 26, 42]. Bassed encompassed Australian population, while Kreitner and Schulz involved European population similarly to Kellinghaus, Schulze, and Wittschieber who derived their samples from typical German population [6, 12, 13, 34, 35]. Hence, the involved samples belonged to countries with very high human development index (HDI) [26]. However, according to the official Eurostat data, Germany is the country with the highest rate of foreigners who are mostly citizens of non-EU countries [43]. Furthermore, a half of foreigners in European Union derive from the countries with medium human development index (HDI), about a third from high HDI countries, while only 3.1 % originates from the candidate countries which correspond to our population [43]. Overall, although Eurostat classified Serbian population separately to candidate countries, the data might still be comparable as UNDP ranked Serbia in countries with high HDI [26].

Finally, the possible source of the observed discrepancy could be explained by human error. Namely, although observers were experienced in CT imaging, as well as in anatomy and forensic anthropology, they were the first to apply this method of age estimation on Serbian population and therefore ignorant of certain staging limitations. According to recently published study of Wittschieber, the diversity of anatomic shape variants of the medial clavicular epiphyses play an important role in the staging process as it appears to be the major source of error [44]. Thus, the possible inadmissible stage evaluation of anatomic shape variants, represent another important limitation of the current study as it might strongly interfere with the presented results. We also emphasise the importance of special training programs which could facilitate the introduction of this age estimation methodology in certain countries.

The role of clavicular CT morphology

The analyses of clavicular shaft revealed variable data which resulted in inability to create appropriate age-related patterns. Calculated anterior to posterior cortical thickness ratio and the diameters of the medullar canal and clavicular shaft showed age-related variability only in men. However, the further appliance of the tested radiological features could not be advisable in the process of age estimation as the expressed statistical reliability was not satisfying.

The usefulness of medial clavicular density

For a long period of time, the only developed histological methods for age estimation from clavicles have been based on osteon density of the clavicular midshaft [45, 46]. However, our recent study of clavicles in a Serbian population applied histomorphometric approach like in other skeletal sites in order to assess age-related deterioration in bone micro-architecture of the medial clavicular end [16, 47, 48]. The results suggested that quantitative architectural parameters of trabecular bone may represent an age-distinctive feature in clavicles, i.e. trabecular bone volume fraction and trabecular width linearly declined with age in the medial end of clavicle in men [16].

However, the histomorphometric analyses are available only in cadaver studies, while justification for their use in living subjects is very limited. Nevertheless, it is known that the quantity of trabecular and cortical bone influences bone density which could be measured by CT [49–52]. This method is practical and easily achievable, and therefore, it could be used as an additional tool in age estimation process. The results of the present study provided several equations in clavicles, based on estimated age dependence of bilaterally observed radiodensity of the sternal epiphyseal-metaphyseal region.

The significance of the first costal cartilage involvement

The analyses of radiodensity were also conducted on the first costal cartilage. As expected, the signs of mineralization and ossification altered its radiodensity which expressed statistically significant dependence on individual's age. The first costal cartilage already received considerable attention as an age indicator in the literature. However, the only previous CT study was published in 2010 by Moskovitch et al. on a population of 160 individuals between 15 and 30 years of age [8].

Their published results of the two-dimensional analyses revealed new age-related features which cannot be seen by direct skeletal inspection. Actually, the authors observed osseous and calcified projections (OCP) within costal cartilage and presented a new staging system based on the presence, length, and shape of OCPs [8]. According to the results, several conclusions were developed: OCP's were absent until the age of 25 in males; females with observed two OCPs could only be older than 20 years of age; a non-linear appearance of extensive cartilaginous calcifications was observed after the ages of 20 and 25 in males and females, respectively [8]. However, although results were present separately for males and females, the conducted statistics did not reveal sexual dimorphism. Unfortunately, analyses of body side variation were not provided as study sample involved only right side ribs [8].

This is the first time to test the cartilage-staging system developed by Moskovitch [8]. Although we also found that OCP's features correlate with age, the results were somewhat different. Thus, in the present study, single OCP and two OCPs were observed at the same age, and this detection was made in 19-year-old-individuals, while the non-linear appearances of calcifications were noted after the age of 23. In contrast to Moskovitch who reported the absence of OCPs until the ages of 24 and 26 in males and females, respectively, the absence of linear projections in the present study was noted in almost all encompassed ages, from 15 to 34 years of age [8]. Still almost half of all those observations we made in the age range between 15 and 18 years. Overall, the present study detected statistically significant relation between individual's age and calculated stages of the OCPs. However, the results did not reveal satisfying distinction between all stages, except for the earliest stage. Therefore, we additionally tested the correlation of individual's age to calculated relation of projection's length to distance between costal face and sternum.

The results of the current study suggested that the mutual enrollment of the applied clavicular and the first costal cartilage scoring systems would not improve age estimation process. However, the interaction between radiodensity of the sternal epiphyseal-metaphyseal region and the first costal cartilage represented an interesting new age predictor, which was more reliable in comparison to all other tested measurement-based predictors.

The question of intersex variability

The influence of sexual maturation on the epiphyseal union progress remained questionable. The macroscopic analyses conducted on a Serbian population identified females' tendency towards the higher epiphyseal union stages; however, statistical significance was not confirmed in the present study [16]. Similar findings were previously reported by Singh and Chavali [14]. Furthermore, the earlier maturity of female population was also observed by CT [13]. The differences among the sexes in some phases of epiphyseal union were reported by Schmeling et al. who developed five-stage scoring system based on applied conventional radiology method [27]. Afterwards, statistically significant intersex variability was detected only in the second stage of epiphyseal cartilage ossification process according to the surveys of Schulz et al. and Kellinghaus et al. which were based on CT methodology with applied Schmeling's five-stage scoring system [12, 35]. Wittschieber also reported intersex variability in certain stages and sub-stages of epiphyseal union progress [37]. However, the results of the present study as well as that of Kreitner et al. and Schulze et al. could not detect intersex variation [6, 34]. Nevertheless, the current study identified several sex-dependent features: thickness of anterior cortex of the right side clavicles, calculated anterior to posterior cortical thickness ratio, diameter of the medullar canal, diameter of the clavicular shaft, and calculated medullar to shaft diameter ratio. Conversely, the conducted analyses confirmed sexual equality among the observed features of the first cartilage as it was previously reported by Moskovitch [8].

The presence of side differences

Heretofore, statistically significant signs of side variability were not found [3, 6, 12, 14, 34, 37]. It is compatible with the findings of the present study which also could not detect side disparity in ossification stages of the medial clavicular epiphyseal cartilage. Nevertheless, the newly introduced features of CT analyses which correlated with age expressed left-right asymmetry: radiodensity of the sternal epiphyseal-metaphyseal region of the clavicle and acquired diameter of the medullar canal. Although, authors excluded cases with visible image artefacts, the possible explanation for the observed discrepancy in the recorded radiodensity medial clavicles could be assigned to the conducted CT study protocol as it used intravenously injected contrast during acquisition. However, if that was the case, we had to expect the same effect on the conducted radiodensity analyses of the first costal cartilage. Conversely, our analyses did not reveal any signs of side differences in the first costal cartilage and therefore aforementioned cause was ruled out. The other possible causes should be evaluated through the other comparable reference samples in the future analyses.

The reproducibility

Overall, the estimated intra and interobserver reliability of observed radiological stages were marked as substantial to almost perfect agreement. Similarly, the interobserver analyses of acquired quantitative measurements were in the range of optimal to excellent with only exception concerning some measurements acquired at the right sided first cartilage;

however, subsequently calculated index values revealed excellent agreement between observers at both sides. Therefore, the current study demonstrated satisfying reproducibility of tested age estimation methodology.

The role of radiation-free imaging methods

Following ALARA principles, the development of reliable radiation-free imaging methods for age estimation in living represents an imperative nowadays. Such methods would possibly allow involvement of several anatomic sites without increasing absorbed radiation dose [53, 54]. The contribution of magnetic resonance in the field of age estimation has recently been marked as valuable [53–55]. Although considerably subjective method, ultrasonography represents another interesting radiation-free method which could successfully be applied in age estimation process. Namely, ultrasonography is low cost, fast, portable, and easily performed method which could be applied post-mortem as well as in living [56]. However, observed limitations of both radiation-free methods require further investigation and testing on different sample populations [53–57].

Conclusion

Age estimation applied post-mortem as well as in living individuals is essential for the identification process. Accuracy and reliability of every method developed for age assessment are in relation to geographic region and socioeconomic status of the reference population. Unfortunately, very few methods have been tested and applied in the Balkan region so far.

Our findings of the conducted CT analyses of the sternal epiphyseal-metaphyseal region of the clavicle led to the implementation of several age-related equations. Besides radiodensity and the stages of epiphyseal cartilage ossification of medial clavicles, the study also detected other age-related features among males, such as calculated anterior to posterior cortical thickness ratio, or diameters of the medullar canal and clavicular shaft.

Although the analyses of the first costal cartilage revealed statistically significant age dependence of the calculated stages of the OCPs, the distinction between the stages was not satisfying.

The interaction between applied scoring systems for the ossification status of the medial clavicular epiphyseal cartilage and OCP's stages was not significantly influenced by age, and their mutual enrollment might not be beneficial. However, the results of the current study suggested that acquired radio density of the sternal epiphyseal-metaphyseal region and radiodensity of the first costal cartilage stood out as interesting new age predictors with mutual relationship which could represent an additional useful tool in future analyses.

In addition, evaluation of sex differences revealed intersex variability in several age-related features: calculated anterior to posterior cortical thickness ratio, diameter of the medullar canal, and diameter of the clavicular shaft.

Overall, this is the first study that has been carried out in a Balkan population with the aim to examine whether radiological analysis of medial clavicles as well as radiological examination of the first costal cartilage could be applied with success in age assessment of individuals in anthropological and forensic practice. Finally, the results suggested multifactorial approach as more reliable.

The provided results could especially benefit the age estimation process applied in cases of advanced decomposition state. Thus, identifying appropriate age range, it would narrow and lead further investigation. However, the future researchers should consider that radiological analyses encompassed only living individuals, and therefore, the obtained results should be used with caution in cases of advanced decomposition as the influence of the natural process of decay is yet to be tested. Moreover, as the analyses involved 3 mm thick slices, the present study does not meet the criteria of a reference study in forensic age estimation. Hence, the provided conclusions should be applied as an additional guidance for age estimation in each specific case.

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Conflicts of interest The authors declare that they have no conflict of interest.

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