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PAPER

ANTHROPOLOGY

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Assessment of Bone Age Based on Morphometric Study of the Upper End of the Humerus*

ABSTRACT: Assessment of changes in the spongy bone structure of the upper end of the humerus is one of the common methods of age estimation. This method was devised many years ago (1894) and has never been verified in an objective numerical testing. We projected an objective morphometric method assessment of the upper end of the humerus. The study was carried out on humeri from cadavers of 88 men and 84 women. The surface area of atrophy of the spongy structure (medullary cavity) was calculated on the longitudinal section of the humerus. A new morphometric method was applied, which allowed numerical presentation of results. The results of the study show a lack of statistical correlation between atrophy of the spongy structure within the upper end of the humerus and the chronological age. Therefore, it can be concluded that the assessment of humerus structure should be omitted in the forensic medical age estimation.

KEYWORDS: forensic science, bone age, humerus, marrow cavity, surgical neck, morphometric study

Polish, Hungarian, and American literature present anthropological methods of estimating chronological age on the basis of the morphology of the upper ends of the humeri (1–8). Wachholz (1894) described a method based on changes occurring within the epiphyseal cartilage and in the spongy structure of the humerus (1,2,4–7). At a young age, the epiphyseal cartilage undergoes ossification, creating the epiphyseal line, and at a mature age the atrophy of the spongy structure of the bone sets in, leading to enlargement of the medullary cavity, the tip of which, with the passage of time, reaches the surgical neck, then the anatomical neck, and finally takes up nearly the whole head of the humerus. Wachholz describes the characteristic appearance, dependent on age, of the longitudinal cross-section of the humerus (1,2,4–7):

14–15 years in girls, 17–18 years in boys—beginning closure of the middle cartilage;
20 years in women, 21 years in men—closure of the middle cartilage;
28 years in women, 30 years in men—approach of the medullary cavity to the level of the surgical neck; and
35 years—approach and arrival of the medullary cavity to the level of the anatomical neck.

Acsàdi and Nemeskéri (1970) also observed changes in the spongy structure of the upper humerus (3,6). They distinguished six phases of the morphology of the upper humerus, which they ascribed to corresponding age groups:

Phase I (19–28 years)—the medullary cavity extends significantly below the surgical neck, and bone trabeculae have a radiating structure, or, considerably more rarely, form a sharply arched lattice.
Phase II (28–37 years)—the medullary cavity extends to the surgical neck or higher, up to one-fourth of the distance of the epiphyseal line. Trabeculae are brittle, often with a sharply latticed shape.
Phase III (31–40 years)—the medullary cavity can reach the epiphyseal line, and trabeculae have a sharply arched structure. A columnar structure forms along the hard substance of the bone shaft, and single trabeculae are thinner.
Phase IV (37–55 years)—the marrow cavity extends to the epiphyseal line, or through it, and trabeculae within the greater tubercle atrophy, forming fissures. The columnar structure is interrupted.
Phase V (38–56 years)—medullary cavity exceeds the epiphyseal line, and within the greater tubercle appear holes of 2–5 mm size. Apparent are the interrupted remains of the columnar structure.
Phase VI (39–56 years)—medullary cavity connects with the holes in the greater tubercle. Trabeculae are significantly rarer and interrupted in places. Cortical substance within the compact bone becomes thin, brittle, and translucent.

In contrast to the methods described previously, Walker and Lovejoy (9) investigated the humerus using radiological imaging only. Radiograms of the humerus were analyzed using visual seriation qualitative assessment method and objective bone structure relative radiolucency method. As indicated by the results, objective relative radiolucency method failed to demonstrate a correlation between changes in the bone structure and chronological age. On the other hand, subjective visual seriation method demonstrated

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good level of correlation between the observed changes and chronological age (Spearman rank-order $r = 0.5$; $p = 0.001$).

On age estimation in the cadavers of unknown identity, based on humerus morphology, considerable discrepancies between the subsequently determined actual chronological age and bone age have been observed in forensic medical practice in Poland. Therefore, the aim of the study was to determine the usefulness of bone age estimation on the basis of the morphology of the upper end of the humerus to determine chronological age. The methods used to date were devised many years ago and have never been verified by objective testing.

Materials and Methods

Bone material for the study was obtained from remains of 88 men (19–82 years) and 84 women (14–93 years) chosen randomly from among those undergoing forensic medical autopsy in the years 2004–2008 at the Department of Forensic Medicine of the Medical University of Lodz. Before the study was begun, permission was obtained from the Bioethics Committee (Institutional Review Board) of the Medical University of Lodz (no. RNN/198/02/KE).

Remains were selected for the study from subjects of known chronological age, unchanged by trauma or disease that would bias assessment of humeral characteristics. The study was carried out on material collected from cadavers undergoing forensic medical autopsies. The sample was selected on random basis. The autopsied deceased subjects came from different social backgrounds and were of different ages. Although the sample selected for the study could not be representative of the general Polish population, the random selection of subjects was consistent with the structure of the group undergoing forensic medical autopsies. That is why there was no selection of cadavers with respect to the presence of osteopenia or osteoporosis (that factor was not addressed in the study), because the group composition would not be representative of the population of autopsied subjects, including those of unknown identity. The upper humeral epiphyses and proximal one-third of the shafts were extracted from the cadavers and subjected to maceration by boiling in water in a high-pressure vessel without the use of chemical additives. The next step was to remove mechanically any remains of soft tissue and, as far as possible, cartilage. The bone material from each person was placed in a separate, marked container. After having dried, the bone material was washed in hydrogen peroxide solution to remove any potential residual soft tissue or fat. Then, longitudinal cross-sections were cut from the upper humeri. In the attempt to apply the known method of assessing the degree of atrophy of the spongy structure inside the humerus, a considerable difficulty in precise determination of the line held to delimit the surgical neck became apparent, because in fact it is an area of smooth transition between the shaft of the humerus and its head. In other words, precise identification of this place as a level is impossible. Because of this, the reproducibility of the application of such a method cannot be high. It means that using age estimation methods based on the size of the medullary cavity as related to the surgical neck (the location of which cannot be determined precisely as it is a region rather than a level), the examiner is unable to repeat the examination of the same bone material with identical result, because the result is dependent on the site regarded as the surgical neck by the examiner. Different examiners will obtain even more significantly different results from the same material. This fact is attributed to qualitative and, consequently also subjective to some extent, characteristic assessment of the surgical neck in the humerus. Therefore, we undertook an attempt to develop a method that would make it possible to identify changes in the spongy structure of the bone more precisely and objectively.

The atrophic area of spongy structure of the humerus was presumed to be larger in subjects of more advanced age, and in view of the above, it was attempted to find a directly proportional linear correlation between numerically quantified atrophy of spongy structure of the humerus (including the medullary cavity) and the chronological age. On the other hand, it was assumed that the lack of correlation between the size of spongy structure atrophy area and age would indicate uselessness of the methods of age estimation based on qualitative assessment of humerus morphology. Except for the radiological method developed by Walker and Lovejoy (9), the anthropological methods used to date were based on observations of qualitative characteristics of the humerus rather than on quantitative numerical values, which cannot be assessed subjectively by the examiner.

The main objective of works aimed at development of the method was to delimit a comparable surface area of each humerus cross-section, irrespective of individual variations. Therefore, one of the anatomical features of the bone that is always in the same proportion in relation to the remaining bone portion, namely, the anatomical neck, was considered to be the best indicator. It was assumed that the length of the anatomical neck measured on the cross-section for each humerus and then on the same cross-section, marked along the longitudinal axis of the humerus, from the apex of the head, through the surgical neck, to the proximal part of the shaft would allow the area to be investigated. Unfortunately, the anatomical neck was found to be too short to be applied for delimitation of the area of interest within the bone shaft, because its length was not sufficient to include the surgical neck and the proximal part of the shaft of the humerus. Thus, an assumption that extending the anatomical neck length by such value, proportional to each bone, that would enable delimitation of a sufficient area of interest was adopted. However, multiplication of the anatomical neck length by factor 1.5 appeared to be sufficient in each case. Consequently, 1.5 of the anatomical neck length was measured along the longitudinal axis of the humerus starting from the apex of the humerus head. The other end of the measured segment indicated the level within the proximal portion of the shaft. In this way, an area proportional to any humerus, including the head, surgical neck, and proximal portion of the shaft was delimited (Fig. 1).

The next stage involved calculation of the whole area of interest and the area of spongy bone structure atrophy, including the area of the medullary cavity. For this purpose, digital photographs were

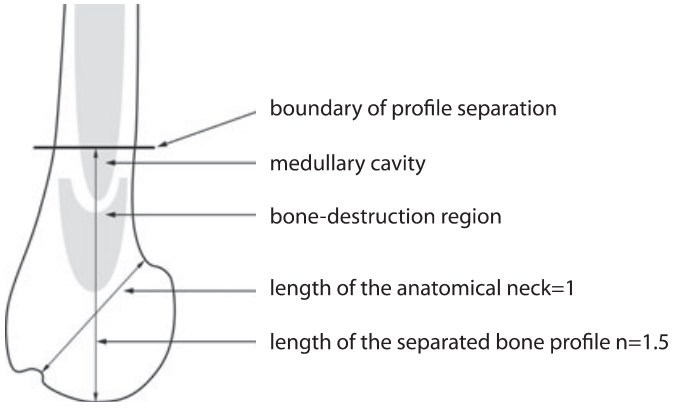

FIG. 1—*Measurement the anatomical neck and determination of the area of regions to be calculated.*

taken perpendicularly to the surface of longitudinal cross-sections of all humeri, with the light falling at 45° angle, and the pictures were subjected to morphometric computer analysis with corresponding measurements. Using a morphometric software package, we delineated for each of the above images of the humerus, a field that was the object of calculations allowing to obtain the surface area, which could be converted into numerical values by the morphometric program. Subsequently, the surface area of the delimited region was calculated, comprising the area of the medullary cavity and the area occupied by atrophic spongy structure referred to herein as "bone-destruction," which were obtained as a result of separate calculations. The observed "bone-destruction" area was the site of reduced trabecular texture in comparison with the normal trabecular structure within the humerus head. On the other side, the "bone-destruction" area showed gradual transition into the medullary cavity with no trabecular texture at all.

Because of various sizes of the humeri, because of individual variability, it was decided to use for the final calculations the values of those structures expressed in percentages, assuming that the entirety of the area of interest was 100%. This allowed to avoid errors resulting from individual variability. All the values obtained were calculated using MultiScan 8.08 software (Computer Scanning Systems Ltd., Warsaw, Poland). Such calculations can be carried out using any morphometric program.

Morphometric Procedure Carried out with MultiScan 8.08

- Change of the resolution of the image to 800 × 600 pixels.
- Uploading the initial image.
- Measurement of the length of the anatomical neck of the bone (Fig. 1).
- Setting the result as the unit-value.
- Scaling the net according to the measurement of the anatomical neck.
- Separation of an image of a length equal to 1.5 times the length of the anatomical neck.
- Making a copy of the image for further measurement.
- Binarization of the image according to hysteresis with parameters (130; 130) (Fig. 2).
- Filling the gaps within the separated profile of the bone (Fig. 3).
- Measurement of the perimeter of the separated bone profile.
- Measurement of the length and width of the separated bone profile.
- Measurement of the surface area of the separated bone profile.
- Measurement of the diameter Fereta H and V of the separated bone profile.
- Recording the report of results.
- Commencement of work on the original image.
- Application of the morphological filter "detection of internal edges" for separating the medullary cavity and bone-destruction region (Fig. 4).
- Application of the nonlinear filter "mediana" with parameters (5; 2; 1) for removing small objects, the external perimeter of the bone, and image background noise (Fig. 5).

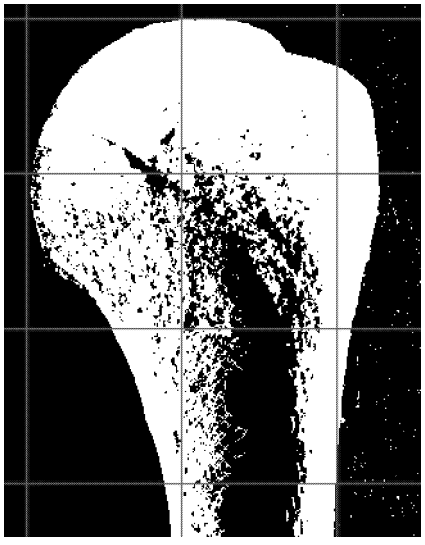

FIG. 2—*Binarization of the image in accordance with hysteresis with parameters (130; 130).*

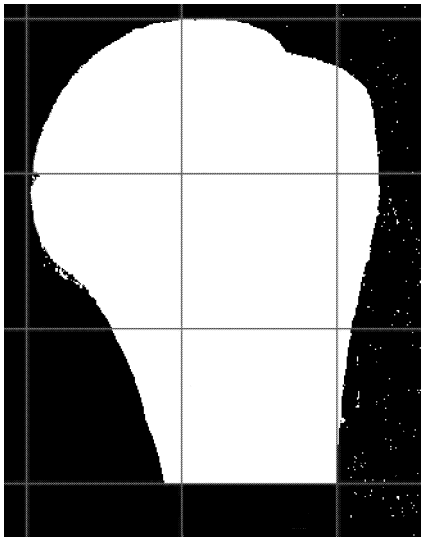

FIG. 3—*Filling gaps within the separated captured bone profile.*

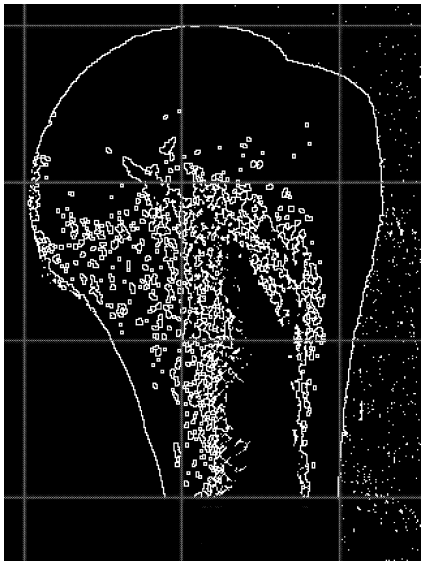

FIG. 4—*Application morphological filter "detection of internal edges" with the purpose of separating the medullary cavity and bone-destruction region.*

FIG. 5—*Application of the nonlinear filter "mediana" with parameters (5; 2; 1) for removal of small objects, external bone outline and background noise.*

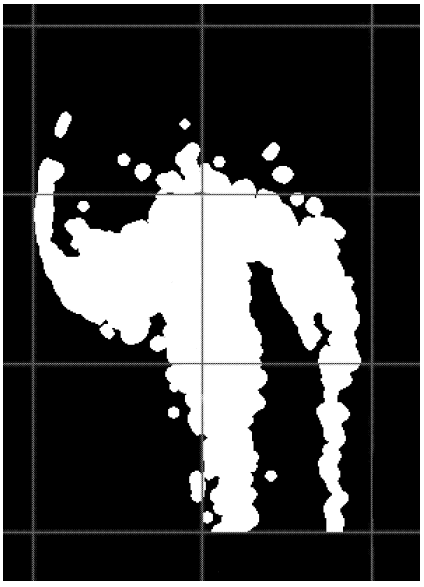

FIG. 6—*Filling gaps within the profile of the bone-destruction region.*

- Application of the nonlinear filters "closure" with parameters (3; 2; 1), and "thickening" with parameters (5; 2; 1) for closing small gaps in the profile.
- Filling gaps within the profile of the bone-destruction region (Fig. 6).
- Measuring the outline of the profile of the medullary cavity and bone-destruction region.
- Measurement of the length and width of the profile of the medullary cavity and bone-destruction region.
- Measuring the surface area of the profile of the medullary cavity and bone-destruction region.
- Measuring the diameter Fereta H and V of the medullary cavity profile and bone-destruction region.
- Recording the report of results.
- Transfer of data to a Microsoft Excel spreadsheet (Microsoft Office Professional PL 2003, Microsoft Corporation).
- End of the procedure.

For the numerical values obtained, a linear regression analysis was carried out for the purpose of finding the coefficients of the correlation and equations of regression. Because of individual variability, the calculations used percentage values for the medullary cavity surface area, for the surface area of the bone-destruction region, and for the sum of the medullary cavity and bone-destruction surface areas, in relation to the entire surface area of the upper humerus.

Results

The statistical analysis of the obtained numerical values allowed to indicate the equations for the medullary cavity surface area, surface area of the bone-destruction region, and the sum of the medullary cavity and destruction region surface areas, together with statistical parameters (Table 1) (Figs 7–12).

The aforementioned linear regression equations should theoretically allow to calculate age on the basis of surface area of the delimited regions. However, because of inappropriate R, F, s, and p parameters, it is impossible to obtain accurate chronological age after substitution of the respective percentage values of surface areas to the equation.

Examples of photographs of the humeri studied are presented below (Figs 13–18).

It can be observed in the figures presented above that the degree of spongy bone atrophy in the humerus differs significantly in subjects of similar chronological age. For instance, it is notable that Figs 16 and 17 present considerable discrepancy between the skeletal and the chronological age.

TABLE 1—*Equations of linear regressions for measurable features of the upper humerus end.*

Age Dependent on	Male	Female
% of marrow cavity surface area	$y = 0.45x + 43.00$ $n = 85$; $R = 0.21$; $F = 3.68$; $s = 13.042$; $p = 0.05864$	$y = 0.23x + 49.50$ $n = 83$; $R = 0.10$; $F = 0.85$; $s = 15.029$; $p = 0.35887$
% of surface area bone-destruction region	$y = 0.099x + 44.83$ $n = 88$; $R = 0.09$; $F = 0.74$; $s = 13.376$; $p = 0.39222$	$y = 0.04x + 50.65$ $n = 84$; $R = 0.04$; $F = 0.11$; $s = 15.594$; $p = 0.74619$
Sum of % of marrow cavity surface area and % bone-destruction region surface area	$y = 0.16x + 41.14$ $n = 85$; $R = 0.16$; $F = 2.13$; $s = 13.160$; $p = 0.14832$	$y = 0.20x + 42.08$ $n = 83$; $R = 0.18$; $F = 2.79$; $s = 14.854$; $p = 0.09849$

n, number of cases analyzed; R, coefficient of Pearson correlation; F, statistic F, serving to verify the hypothesis of the significance of the entire model; s, standard estimation error; p, level of probability, so-called computer level of significance.

Equations presented in graphs (Figs 7–12).

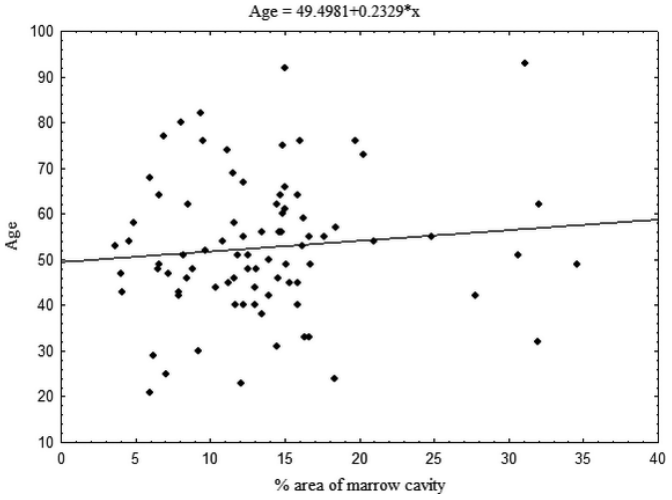

FIG. 7—*Graph of the dependence of % marrow cavity surface area on females age.*

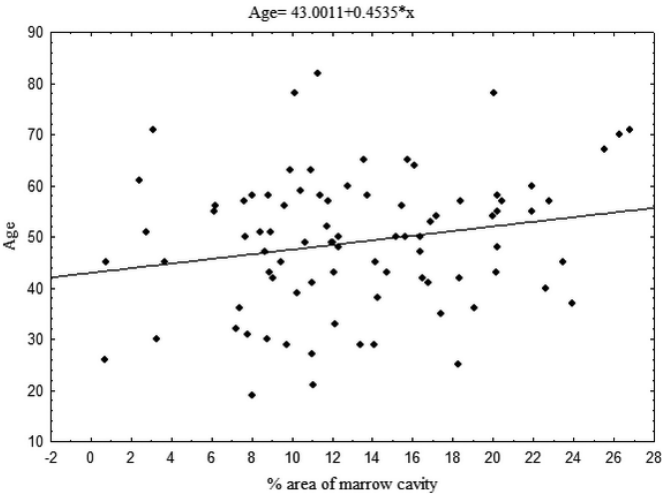

FIG. 10—*Graph of the dependence of % marrow cavity surface area on males age.*

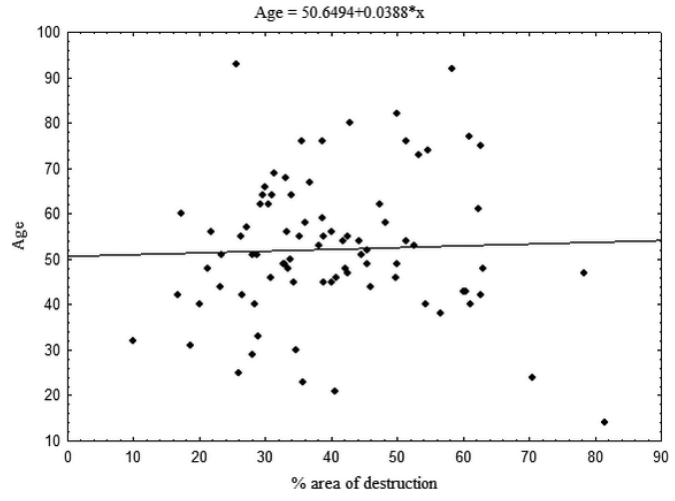

FIG. 8—*Graph of the dependence of % bone-destruction region surface area on females age.*

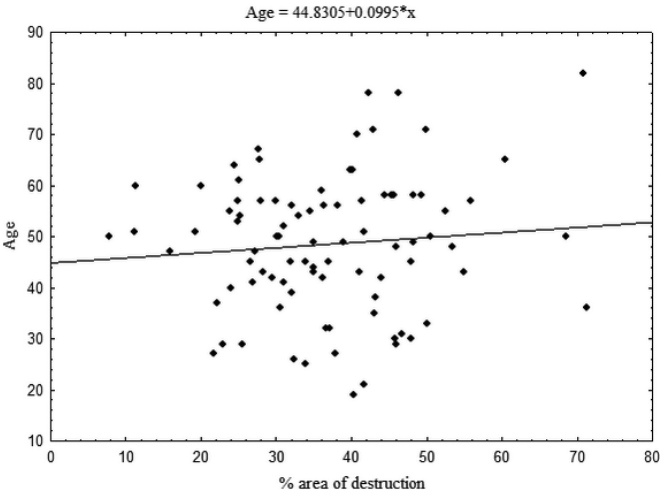

FIG. 11—*Graph of the dependence of % bone-destruction region area on males age.*

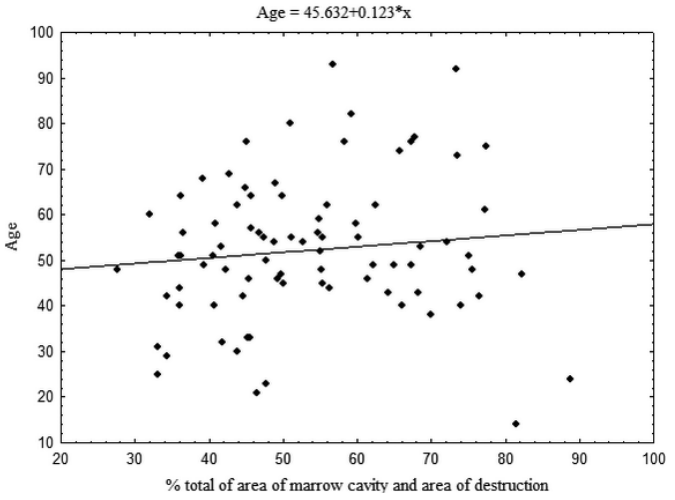

FIG. 9—*Graph of the dependence of % the sum of marrow cavity surface area and bone-destruction region surface area on females age.*

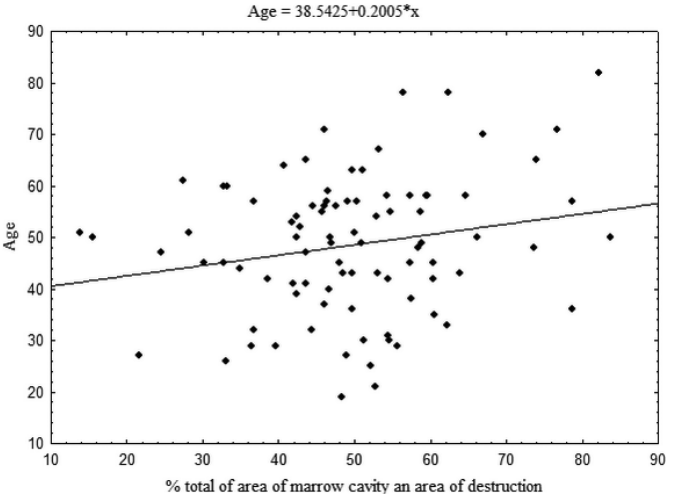

FIG. 12—*Graph of the dependence of % marrow cavity surface area and bone-destruction region surface area on males age.*

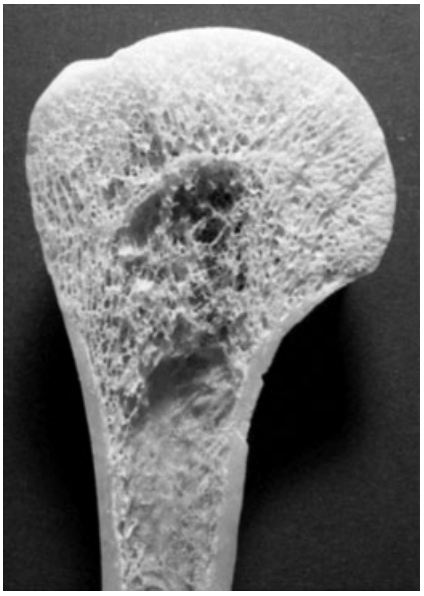

FIG. 13—*Twenty-nine years (13.41% m.cav.; 22.97% b.destr.).*

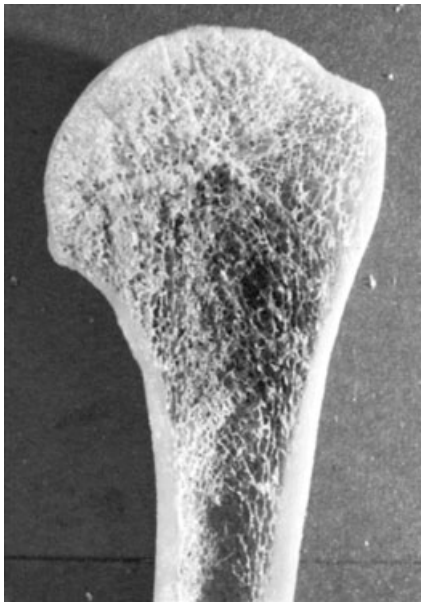

FIG. 14—*Nineteen years (8.04% m.cav.; 40.34% b.destr.).*

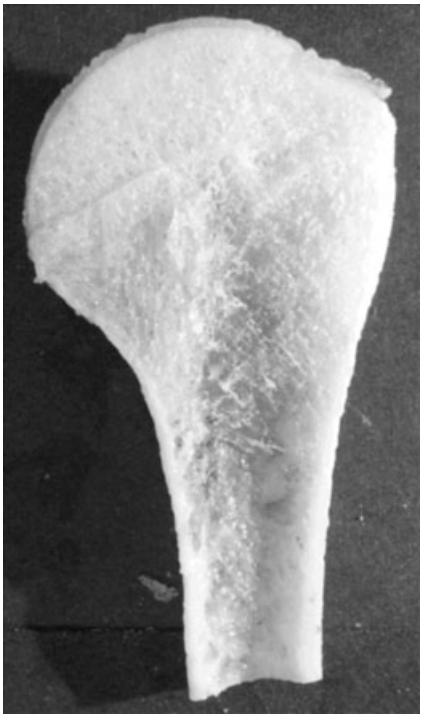

FIG. 15—*Forty-seven years (8.62% m.cav.; 15.86% b.destr.).*

FIG. 16—*Forty-five years (23.47% m.cav.; 36.89% b.destr.).*

Discussion

Identification of deceased persons of unknown identity is an essential task of forensic medicine, and determination of age is one of the most important functions in the process of individual identification. Up to the present, many methods have been devised to estimate age, among which the largest group are the morphological–anthropological methods (9–13). These morphological methods are based on observation of characteristic bone changes in the human skeleton with age and their classification into corresponding age groups. It should be remembered, however, that assessed bone age is a feature of an individual and indicates the degree of advancement of ontogenetic development. Chronological age is, on the other hand, the time that passes from the moment of birth until death. Thus, these are different parameters, and bone age can only to a certain extent be dependent on chronological age. Methods of estimating bone age seek a direct proportional linear dependency between a specific feature studied and age. But the human organism is determined in its ontogenetic development by its genetic variability. In addition, specific populations live in extremely varying environments, and specific subjects carry on individual life styles. This means that even individuals originating from the same populations, and of the same chronological age, can present different

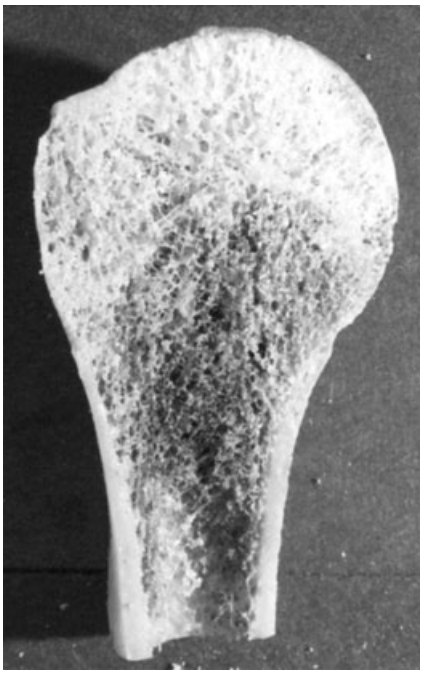

FIG. 17—*Seventy-one years (3.09% m.cav.; 42.89% b.destr.).*

FIG. 18—*Seventy-one years (26.82% m.cav.; 49.88% b.destr.).*

degrees of advancement in ontogenetic development (Figs 13–18). Such a state of affairs permits to suppose that a universal anthropological method most probably does not exist. In other words, it is not possible to devise a method that would allow the use morphological traits that could define a narrow chronological age range, but not in other age groups. The choice of a method of assessing age—from a practical point of view—should be determined by its accuracy as well as its availability. The methods of age estimation—as they are used in practice in the process of identification of unknown subjects—should be as accurate as possible (14). A hypothetical ideal method should make it possible to determine age unequivocally, with an accuracy of 1 year, just like in the case of C^{14} dating (15,16). No such method that would allow in a reliable and exact way to assess the chronological age throughout the entire period of ontogenesis has been devised to date. The continued search for such methods is therefore necessary. The morphological assessment of the longitudinal cross-section of the upper humerus has been long known in the Polish, Hungarian, and American literature (1–8). It is easily available during routine dissection; however, it causes certain diagnostic and interpretative difficulties.

All the morphological–anthropological methods used until now are based on the visual assessment of the relevant skeleton characteristics (10–13). The relative radiolucency method developed by Walker and Lovejoy (9) is an exception. The results of their studies failed to demonstrate the correlation between age and bone radiolucency. However, in the case of morphological methods, such assessment is to a considerable extent dependent on the experience of the examiner. The reproducibility of such methods cannot therefore be complete. Thus, it would seem purposeful to search for more objective methods of visual morphological assessment of the skeleton. The objectivization of studies should arrive at the development of bone age assessment methods, which allow reproducibility of studies, independent of the person or examiner's experience. The results obtained should demonstrate only the dependency between the feature studied and the type of method chosen.

During the study of the longitudinal cross-sections of the upper humeri, we met with the essential difficulty of assessing the size of the medullary cavity and its orientation in relation to the anatomical structures of the surgical and anatomical neck. Therefore, we undertook to objectify the morphological assessment of the humerus. Using a digital morphometric program, the size of the surface area of the entire study area studied was calculated, as well as that of the surface area of the medullary cavity. An additional noticeable element during the calculations was the area of spongy bone atrophy, which, however, did not extend into the region of the medullary cavity. This region, the so-called bone-destruction region, was also separated and its surface area calculated. Transfer of the features of the bone morphology to measurable numerical values allowed application of precise statistical operations. On that basis, direct equations of linear regression corresponding to the percentage value of each parameter were determined separately for both sexes.

For men, in the case of percentage surface area of the medullary cavity, the Pearson correlation coefficient R was only 0.21 with a standard estimation error of 13.042. The area of the bone-destruction region R was only 0.09 with a standard estimation error of 13.376. The percentage value of the sum of those parameters, however, was characterized by Pearson correlation coefficient of $R = 0.16$ and standard estimation error of 13.160. All the results obtained did not reach statistical significance.

Similarly, for the sample of the female population, the percentage surface area of the medullary cavity reached only $R = 0.10$, and the standard estimation error was 15.029. The percentage area of the bone-destruction region R was 0.04, and the standard estimation error was 15.594. Correspondingly, the value of the sum of these parameters reached $R = 0.18$, and the standard estimation error was 14.854. Also in the case of females, all results were statistically nonsignificant.

The morphometric study of longitudinal cross-sections of the upper end of the humerus allows us to say that changes occurring in the humerus are not dependent on chronological age. Thus, in

attempts to determine chronological age, the assessment of humerus structures should be omitted.

Conclusions

Our morphometric study of the longitudinal cross-sections of the upper humerus has shown that the assessed degree of atrophy of the humerus spongy structure does not demonstrate a correlation with chronological age and therefore is independent of age. Because of this, the examination of this feature ought to be excluded from the forensic medical determination of chronological age.

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