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Technical Note

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A Quantitative Method for Estimation of Volume Changes in Arachnoid Foveae with Age

ABSTRACT: Age-related changes of arachnoid foveae have been described, but objective, quantitative analyses are lacking. A new quantitative method is presented for estimation of change in total volume of arachnoid foveae with age. The pilot sample consisted of nine skulls from the Palmer Anatomy Laboratory. Arachnoid foveae were filled with sand, which was extracted using a vacuum pump. Mass was determined with an analytical balance and converted to volume. A reliability analysis was performed using intraclass correlation coefficients. The method was found to be highly reliable (intraobserver ICC = 0.9935, interobserver ICC = 0.9878). The relationship between total volume and age was then examined in a sample of 63 males of accurately known age from the Hamann–Todd collection. Linear regression analysis revealed no statistically significant relationship between total volume and age, or foveae frequency and age ($\alpha = 0.05$). Development of arachnoid foveae may be influenced by health factors, which could limit its usefulness in aging.

KEYWORDS: forensic science, forensic anthropology, arachnoid foveae, arachnoid granulations, endocranial features, age

Arachnoid foveae, also known as granular foveae or Pacchionian depressions (Fig. 1), are pits or depressions found on the endocranial surface. They are concentrated along the anterior two thirds of the sagittal edge of the parietal bones, and midline along the coronal suture on the posterior aspect of the frontal bone (1,2). They may be occasionally found along the transverse sinus (3). They are typically located parasagittally within 3 cm from the midline of the skull (1,2). Arachnoid foveae are closely associated with arachnoid granulations, which are projections of the arachnoid mater into the dural venous sinuses. The arachnoid granulations push outward against the dura mater resulting in the resorption of bone on the inner table of the skull, forming the arachnoid foveae.

Age-related changes of arachnoid foveae have been noted by several authors. Gray's anatomy states that they become "larger and more numerous and obvious as skulls age" (4). Both T.W. Todd and W.M. Cobb observed that these pits or depressions become more marked with age, both in depth and frequency (5). Little work has been done on the measurement of arachnoid foveae. Previous studies include the work of Basmajian (6) and Grossman and Potts (1). Basmajian attempted to correlate changes in foveae with age, but his method was subjective in nature. To characterize depth, Basmajian used a visual inspection of the foveae and assigned a subjectively determined numerical value ranging from one ("just perceptible") to four ("very deep") to describe depth. He concluded that these features do become larger with age, but that they were too variable to be used as a forensic tool for age estimation.

The radiographic study of Grossman and Potts (1) also noted an increase in arachnoid foveae size with age. In this study, the anteroposterior diameters and the depths of foveae were measured on lateral projections, using radiographs from a living clinical sample. Only clearly recognizable arachnoid impressions were measured. Despite the quantitative measurement of depth, the use of only "clearly recognizable arachnoid impressions" suggests that some foveae may have been present, which were excluded from analysis. In addition, the limitations of radiographic imaging may affect the visualization of all foveae present within the skull. It has been stated that a loss of bone density of 30–50% and a lesion size of 1 to 5 centimeters are usually necessary before a feature becomes visible on a radiograph (7,8). It is possible that small foveae would not be observed on a radiograph.

The age-related changes of these osteological features make their examination as a forensic tool worthwhile. In cases where the calvaria is the only skeletal element recovered, these foveae could prove to be a valuable tool for forensic determination of age. Previous studies have been few in number, and often subjective in nature. No study to date has examined the relationship between the quantitatively determined total volume of the arachnoid foveae occupying the skull and age.

The purposes of this study are as follows:

1. To develop and test a new, quantitative method for the estimation of the volume of arachnoid foveae.
2. To use this method to examine the pattern of change in the volume and frequency of arachnoid foveae with age.
3. To determine if these changes can be used for the purpose of estimation of age at death in a forensic context.

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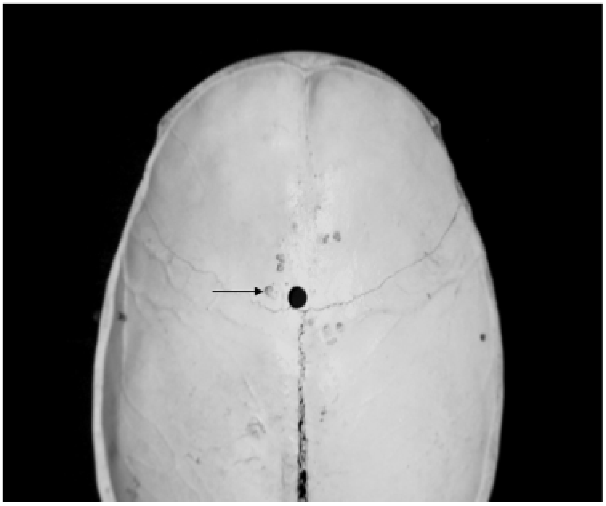

FIG. 1—*Arachnoid foveae. View of the internal surface of calvaria of the skull exhibits arachnoid foveae (black arrow) located parasagittally along the sulcus for the superior sagittal sinus.*

Methods

Two samples were used in this study. The first sample was selected for the reliability analysis and consisted of nine dry skulls from the Gross Anatomy Laboratory at Palmer College of Chiropractic. The age, sex, and race of these skulls were not known. Because the purpose of this analysis was to determine whether the measurements on the arachnoid foveae of each skull could be repeated by a single observer and between two observers with high reliability, knowledge of age at death of these specimens was not necessary. The second sample used in this study was selected to examine the relationship between arachnoid foveae and age. It was derived from the Hamann–Todd Osteological Collection housed at the Cleveland Museum of Natural History (Cleveland, OH). Hamann–Todd is an extensive skeletal collection that includes files documenting the age, sex, ethnicity, cause of death, and over 70 measurements for each individual. This collection consists primarily of unclaimed bodies from the Cuyahoga County morgue in Cleveland and from local hospitals, which were collected during the early 20th century (9).

Knowledge of the accurate ages of the specimens in the Hamann–Todd Osteological Collection is essential because of the fact that this is an aging study. Although the Hamann–Todd Collection is accompanied by extensive documentation, the stated age at death has often been questioned by examining researchers. Discrepancies between stated and observed age of up to 15 to 20 years have been noted (10). In response to these discrepancies, Lovejoy and colleagues performed a multifactorial aging analysis on the 3157 available adult skeletons with a stated age at death. Based on this study, they concluded that only 512 of the 3000+skeletons had stated ages that corresponded accurately (within $\pm$ 5 years) with their observed age (10). The sample used in the present study was drawn from a list of 131 specimens used in the multifactorial aging study of Lovejoy (Test II), which was derived from this subpopulation of most accurately aged specimens. The initial sample of 67 skulls ranged in age from 21 to 66 years. In an effort to limit heterogeneity and avoid creating subsample sizes that were too small for statistical analysis, this study was limited to African American (black) and European American (white) males. Examination of sex-related changes may be addressed in future studies.

In the present study, the observer (S. S. M.) was kept unaware of the age and race of the study specimens with the help of an impartial assistant. Any specimens demonstrating pathological processes that may mimic or alter the appearance of arachnoid foveae were excluded from the sample. This was not a problem however, as the foveae formed by arachnoid granulations are easily differentiated from other skull markings because of their parasagittal location (11,12). It is uncommon for arachnoid granulations to become unusually large producing calvarial defects that simulate other lesions (13,14). It is rare that the foveae will affect the outer table of the skull. Care was taken to distinguish foveae from features such as foramina of emissary and diploic veins. Skulls in which the sagittal cut destroyed more than one fovea of any size were excluded from the sample. Any skulls that could not be analyzed for any reason (such as fragmentation) were also excluded from the sample. Application of these exclusion criteria yielded a final sample of 63 skulls. Of these remaining specimens, 50 (79.4%) were from African American individuals, while 13 (20.6%) were from European American individuals.

For each skull in the sample, the number of arachnoid foveae was counted. Foveae that appeared to be formed by the convergence of two or more foveae were counted as one and were measured as one unit. The location (bone and side) of the fovea on the skull was recorded. The following measurements were taken and recorded on the data sheet: maximum length, which is defined for this study as the longest diameter in the fovea, maximum width measured perpendicular to the length, and the total mass of sand (Silicone Dioxide, Fisher Scientific, Acros Organics, Fair Lawn, NJ) that can occupy each fovea (for the purpose of gravimetric analysis). All linear measurements were taken using 4-inch sliding calipers (Mitutoyo Digimatic Calipers, Model number 500-195, MSI-Viking Gage, Duncan, SC).

A gravimetric analysis was used to determine the mass of clean, dry sand of known specific gravity that could be contained within each fovea. A specific gravity was listed on the package of sand, but to ensure the validity of the volume conversion, the specific gravity of the sand was tested by the Augustana College Chemistry Laboratory using a bulk density measurement method. The specific gravity of the sand was determined to be 1.636 ± 0.007.

Each fovea was completely filled with sand until the sand was level with the surrounding internal surface of the skull. Care was

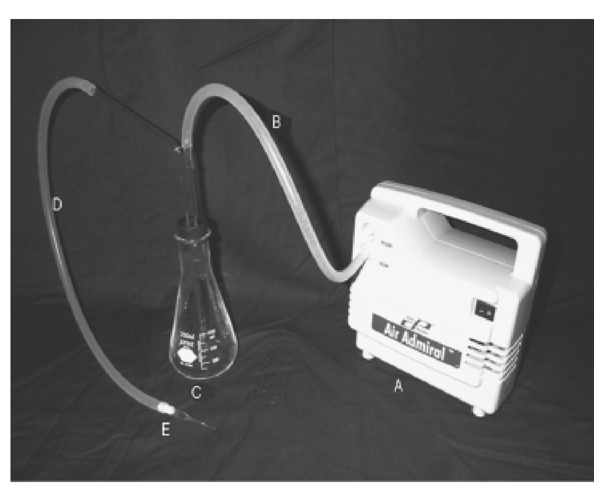

FIG. 2—*Equipment setup. The vacuum pump (A) is connected via a piece of rubber tubing (B) to the flask (C). A second piece of rubber tubing (D) runs from the flask and holds a pipette containing a cotton plug (E) to capture sand in its distal end.*

taken to avoid mounding the sand or compressing it into the fovea. Any excess sand surrounding the fovea was removed using a small cotton swab. When only the sand within the fovea of interest remained, it was suctioned from the fovea using a vacuum/pressure pump (Air Admiral Vacuum/Pressure Pump, Model number 79202-00, Cole-Parmer Instrument Company, Vernon Hills, IL), which was connected by rubber tubing to a reservoir flask. A second piece of rubber tubing connected the flask to a pipette with a cotton plug in the end of the tubing (Fig. 2). The sand was captured in the glass pipette plugged with cotton at its distal end (the mass of which had been previously determined with the balance) and the balance zeroed to that mass. This was done using an analytical balance that measures with a precision of 0.1 mg. The pipette with cotton plug and suctioned sand was then placed on the analytical balance in order to determine the mass. Following each determination of mass, the balance was zeroed to the new mass of the pipette with plug and sand using the tare feature, which is a deduction from the gross mass of a substance and its container made in allowance for the mass of the container. This process was repeated for all arachnoid foveae.

Specific gravity is defined as the density of a substance relative to the density of a reference substance. If the substance is a solid or liquid, the reference substance is water. The density of water is nearly 1 g/cm^3. Therefore, the specific gravity of a substance is equal to the density of that substance, so long as that substance is either solid or liquid. The specific gravity of the sand used in this study was determined to be 1.636; therefore its density is 1.636 g/cm^3. A known density of the sand and a known mass of the sand occupying each arachnoid fovea as measured by the analytical balance allows for a simple conversion from mass to volume using the formula $V = m/d$.

Statistics

All data analysis for this study was performed with the SPSS for Windows, Version 9.0 statistical package (SPSS for Windows, Version 9.0, SPSS Inc., Chicago, IL). To examine the relationship between total volume of arachnoid foveae and age, the data were first plotted on scatterplots and boxplots. Scatterplots and boxplots were utilized to provide a descriptive, visual representation of the data and determine if there appeared to be a linear relationship between the two variables. Pearson's correlation coefficient and a linear regression model were then used. In order to equalize variance and normalize the data, the primary outcome variable of total volume was transformed using the log 10 function. The relationship between age and the frequency of foveae per skull was also analyzed using these methods. For this study, any p-value less than 0.05 was considered statistically significant.

Results

Reliability Analysis

The intraobserver portion of this analysis consisted of the observer (S. S. M.) executing the volume estimation technique on the nine skulls from the Palmer Anatomy Laboratory sample. The maximum length, maximum width, and mass of sand occupying each fovea was measured and recorded for each of the nine skulls (observer 1—trial 1). The masses were converted into volumes and then totaled, yielding a measurement defined as total volume for each skull. The measurements were then repeated after a period of several days (observer 1—trial 2). Using SPSS 9.0, the two sets of data were compared using intraclass correlation coefficients (15,16) and 95% confidence intervals. The intraclass correlation model used was the two-way mixed effect model (consistency agreement) or ICC (1,3). The single measure ICC for the intraobserver analysis was 0.9935, with a confidence interval of 0.9717–0.9985.

In order to analyze interobserver reliability, a second judge was utilized. Following a training period of 1 h, in which the second observer was instructed on how to perform the technique, the mass of sand occupying each fovea was measured and recorded for the same nine skulls (observer 2—trial 1). The masses were converted into volumes and totaled for each skull. The total volumes for the nine skulls were then compared with the first set of measurements by observer one (observer 1—trial 1), once again using the two-way mixed effect model. The ICC for the interobserver analysis was 0.9878, with a confidence interval of 0.9471–0.9972 (Table 1).

TABLE 1—*Reliability analysis.*

Study	Intraclass Correlation Coefficient	95% Confidence Interval
Intrarater reliability	0.9935	0.9717–0.9985
Interrater reliability	0.9878	0.9471–0.9972

TABLE 2—*Hamann–Todd sample by age group.*

Age Group (years)	n	Frequency of Foveae*	Total Volume (cm^3)*	Length (mm)*	Width (mm)*
20–24	6	10.3 (4.3)	0.354 (.48)	8.45 (4.5)	6.07 (3.6)
25–29	7	9.3 (3.1)	0.233 (.11)	7.37 (4.0)	4.98 (2.5)
30–34	9	9.8 (2.7)	0.245 (.19)	7.10 (4.0)	4.88 (2.8)
35–39	11	7.6 (3.7)	0.259 (.18)	7.98 (4.4)	5.61 (3.1)
40–44	9	10.2 (5.2)	0.344 (.26)	7.53 (3.9)	5.40 (2.8)
45–49	9	10.9 (3.0)	0.408 (.30)	7.23 (4.7)	4.91 (3.2)
50–54	8	11.3 (5.4)	0.294 (.11)	6.85 (3.6)	4.69 (2.7)
55–59	2	10.0 (2.8)	0.158 (.14)	6.47 (3.5)	4.46 (2.9)
60–64	1	6.0 (0.0)	0.484 (.00)	12.44 (9.0)	7.47 (4.6)
65+	1	13.0 (0.0)	0.445 (.00)	7.12 (4.3)	5.29 (3.0)
Total	63				

*Indicates mean (standard deviation).

Analysis of Foveae and Age

The 63 Hamann–Todd specimens observed contained a total of 618 foveae. The mean total volume for the entire sample was 0.188 cm^3, ranging from 0.025 to 0.816 cm^3. The mean frequency of foveae for each skull was 9.81, ranging from three to 23 foveae. A summary of the Hamann–Todd sample by age group can be found in Table 2.

Of the 618 foveae observed, 254 (41.1%) were located entirely on the frontal bone, while 333 (53.9%) were located solely on the parietal bones. There were also 16 foveae (2.6%) located along the coronal suture between the frontal and parietal bones. The remaining foveae were located on the occipital (1.9%) and temporal

TABLE 3—*Foveae location and frequency.*

Bone	Right	Left	Midline	Total	%
Frontal	127	126	1	254	41.1
Parietal	162	169	2	333	53.9
Occipital	10	2	0	12	1.9
Temporal	2	1	0	3	0.5
Coronal suture	7	9	0	16	2.6
Total	308	307	3	618	100

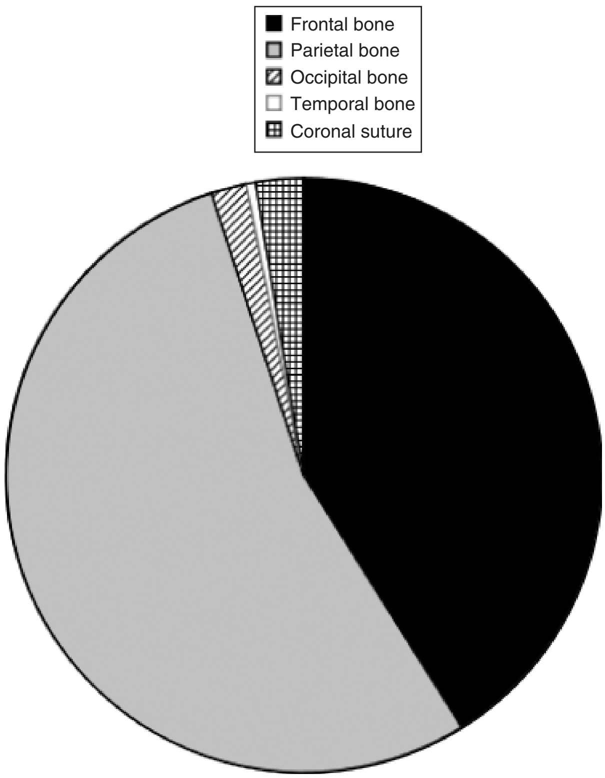

FIG. 3—*The distribution of arachnoid foveae by location in the skull.*

bones (0.5%). Foveae were nearly equally distributed by side: 308 (49.8%) were located on the right half of the skull while 307 (49.7%) were located on the left. Three foveae (0.5%) were found to be located in the midline. A complete description of foveae location is shown in Table 3, and illustrated graphically in Fig. 3.

Total Volume vs. Age—When the sample was analyzed using the log 10 of total volume, the correlation between total volume and age did not approach statistical significance with an r-value of 0.188 ($p = 0.14$). The regression line shown on the scatterplot in Fig. 4 indicates a slightly positive (although nonsignificant) relationship between total volume and age. The boxplot in Fig. 4 appears to demonstrate a slight increase in total volume with age, but the total volume reaches a peak in the 40–44-year age group and then declines, only to rise again in the 60+ age group. The data also appear to be widely distributed within age classes.

Foveae Frequency vs. Age—The relationship between the total number of arachnoid foveae occupying each skull and age was also analyzed. When the total number of foveae per skull was compared with age, the results were similar to those for total volume compared to age. A very low correlation between the total number of foveae per skull and age was found with an r-value of 0.093 ($p = 0.47$). The scatterplot in Fig. 5 confirms this with data points that are widely dispersed and a regression line with just a slightly positive slope. The boxplot in Fig. 5 does not indicate an increase in foveae frequency with age. The median values for foveae frequency are consistently near 10 for the first eight age groups, with a lower value occurring only in the 35–39-year age category.

Discussion

The high correlations for both interrater and intrarater reliability suggest that the developed method is highly reliable. A potential negative influence on the accuracy (validity) of the method would be use of an incorrect specific gravity in the conversion from mass to volume. This would result in a systematically biased volume estimate. The laboratory determination of the specific gravity of the sand used in this study addresses this issue. The confidence interval of ± 0.007, based on six subsamples, supports the accuracy of the volume conversion.

The lack of correlation between arachnoid foveae volume and age, found in this study, remains to be addressed. In order for a biological feature to be useful as a predictor of age at the time of death, that feature must undergo consistent changes with increasing age. Aging methods in adults are typically based on degenerative processes that occur normally with age. To understand the age-related changes of arachnoid foveae, it is essential to understand the function of the anatomical features that produce these impressions: the arachnoid granulations. Although it is widely

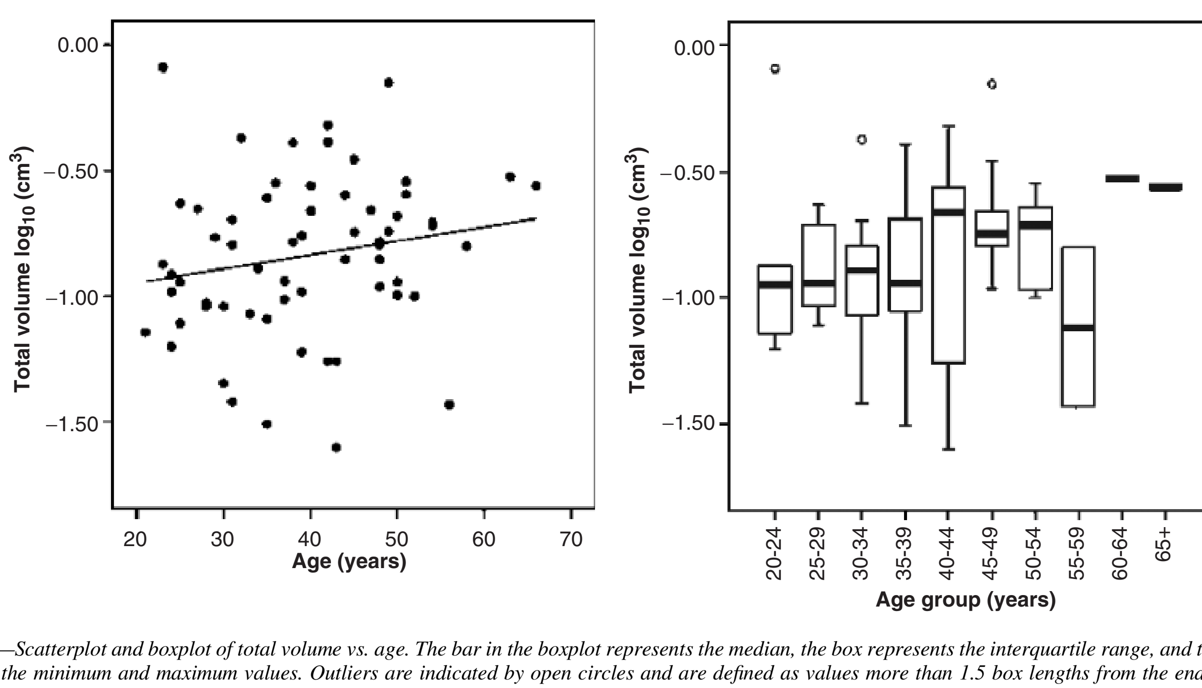

FIG. 4—*Scatterplot and boxplot of total volume vs. age. The bar in the boxplot represents the median, the box represents the interquartile range, and the end lines represent the minimum and maximum values. Outliers are indicated by open circles and are defined as values more than 1.5 box lengths from the end of the box.*

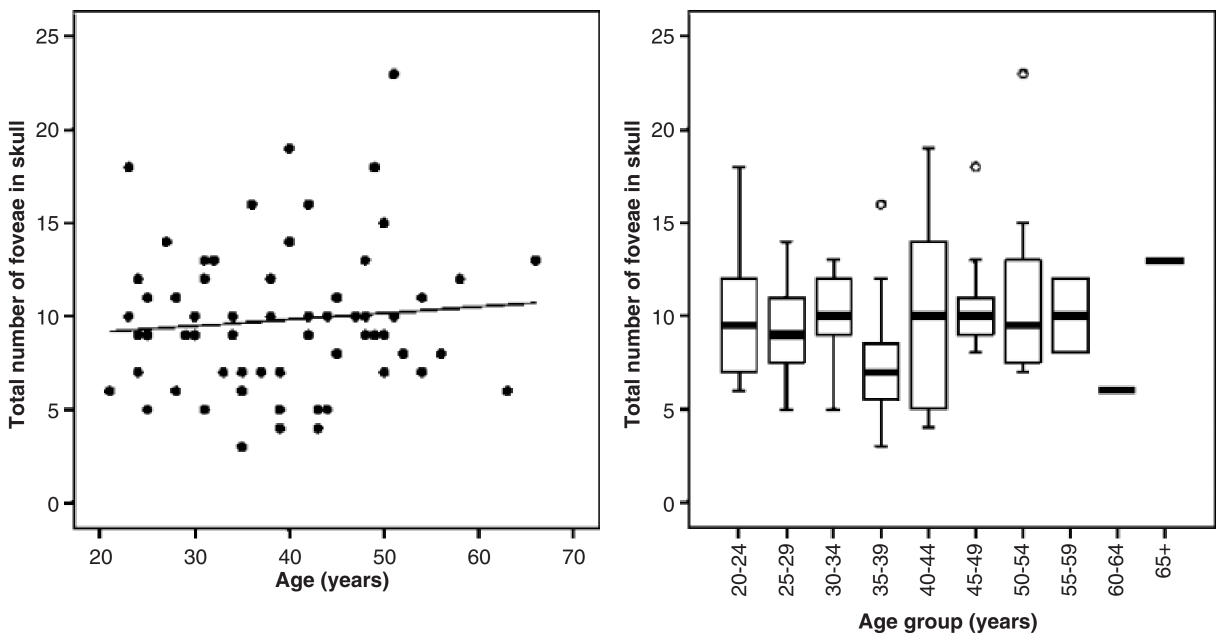

FIG. 5—*Scatterplot and boxplot of foveae frequency vs. age. The bar in the boxplot represents the median, the box represents the interquartile range, and the end lines represent the minimum and maximum values. Outliers are indicated by open circles and are defined as values more than 1.5 box lengths from the end of the box.*

accepted that arachnoid granulations increase in frequency with age, the exact function of these structures is somewhat controversial. They are commonly reported to be the primary site of CSF absorption into the venous system, but some studies have suggested that this may not be the case. Some argue that arachnoid granulations may play only a minor role in CSF reabsorption and that other pathways, such as cervical lymphatic channels, are more significant (17–19). It has also been stated that arachnoid granulations may only provide an alternate route for CSF drainage under conditions of increased CSF pressure. Proponents of this school of thought point out that the CSF producing choroid plexus develops prior to the appearance of arachnoid villi, suggesting the presence of an absorption route other than the villi (17). Also lending support to this theory are studies that have examined the relationship between the frequency of arachnoid granulations in an individual and the development of hydrocephalus. Hydrocephalus is a pathological condition resulting from an increase in CSF volume within the skull. Cases have been observed in which individuals have few or no arachnoid granulations, but fail to develop hydrocephalus. If arachnoid granulations are the primary site of CSF reabsorption, it is argued that hydrocephalus would be a near certainty in individuals who lack them (19). As an alternative theory, it has been suggested that arachnoid granulations are the primary site for the absorption of CSF proteins, which are waste products of normal neuronal metabolism (19). Under normal physiological conditions, metabolic waste products from the central nervous system progressively accumulate in the CSF as it circulates, reaching maximum concentrations at the superior aspect of the brain and the lower aspect of the spinal cord. Pathological conditions may result in the production of excess waste products. The prevalence of arachnoid granulations in these two locations (along the superior sagittal sinus and lower spinal cord) would be necessary to filter the proteins from the CSF (19).

In addition to serving as a filter for metabolic waste products, arachnoid granulations have also been suggested to play a role in the immune response. Cerebrospinal fluid contains immunoglobulins under pathological conditions such as infection. It has been shown that the lining cells of the pia mater, arachnoid mater, and arachnoid granulations not only exhibit nonreceptor-mediated phagocytic functions, but also contain specialization for receptor-mediated immune clearance (20). These findings suggest that the flow of CSF to the arachnoid granulations may be of importance in the clearance of immunoglobulins (specifically IgG and IgG-coated antigens), thereby playing a protective role in CNS infections and immune-mediated conditions (20). A more recent study involving monkey arachnoid villi adds support to this theory. Upon inspection of villi under a scanning electron microscope, aggregations of immune cells such as melanocytes, plasma cells, macrophages, polymorphonuclear leukocytes, lymphocytes, and fibroblasts were commonly observed (17). If it is true that arachnoid granulations play an integral role in the immune response, it may be that the morphology of these features may be affected by the general health of the individual rather than by age-related degenerative processes alone. This may help to explain the results of this study. For example, the individual with the highest total arachnoid foveae volume found in this study was a 23-year-old, who is reported to have died from tuberculosis. Perhaps the large total volume is a reflection of this individual's poor state of health and struggle with an infectious disease that claimed his life at such a young age.

Le Gros Clark recognized as early as the 1920s that the size of arachnoid granulations appeared to be affected by health conditions. He believed that the arachnoid granulations were a reflection of cerebrospinal fluid pressure; therefore, any condition which elevated CSF pressure should increase the size and number of granulations. He pointed to a case of Dementia Paralytica, a condition resulting in increased CSF pressure, in which the individual displayed an increased number and size of arachnoid granulations. He noticed similar results in individuals with brain tumors that produce an increase in CSF pressure, and patients suffering from chronic nephritis and arteriosclerosis (21). Arachnoid granulations have been found to respond to changes in CSF pressure. In an experimental study using monkeys, Takahashi et al. (22) found that arachnoid granulations tend to decrease in size with an induced state of CSF hypotension, while normalizing the pressure tends to return them to their normal size. Arachnoid granulations have also been described as safety balloons, preventing compression of brain tissue during abrupt increases in intracranial pressure (17). Vitamin deficiency has also been found to affect CSF pressure, specifically vitamin A. Under conditions of vitamin A deficiency, increases in CSF pressure are among the first changes to occur (23). Studies have noted that arachnoid granulations were found to be larger in rats suffering from vitamin A deficiency when compared with rats with adequate levels of the vitamin (23).

Although several older studies report that arachnoid granulations do change with age, a recent magnetic resonance imaging (MRI) study found no correlation between either size or frequency of arachnoid granulations and age (24). This supports the results of the present study, particularly since an examination of the actual granulations would be expected to yield more accurate results than the impressions caused by them, as some granulations may not result in the resorption of bone.

The results of the MRI study and the present study do not prove that arachnoid granulations and foveae do not increase in number and size with age, but do suggest that they do not change predictably with age from one individual to the next, certainly not predictably enough to be used as a forensic technique. The high intra- and interrater correlation coefficients suggest that the lack of correlation between total volume and age was not a result of unreliable methods. Indeed, we believe that the method presented here represents the most reliable and accurate method used to date for the analysis of arachnoid foveae. Reasons for this lack of correlation between arachnoid foveae volume and age are most likely related to the fact that arachnoid granulations are influenced by multiple factors, including overall health of the individual, any condition that affects CSF pressure, and vitamin deficiency. The use of specimens from the Hamann–Todd collection, representing individuals of low socioeconomic status, and dating to the preantibiotic era, may have accentuated health-related variability when compared to modern samples. The aim of the present study was to develop and test a method, and then apply it to a limited skeletal sample of known age. Future studies should focus on application of the method to a larger sample of known age and health history, which includes females.

While more work is needed, the results of the present study do not support the hypothesis that a useful aging technique can be developed based on changes in arachnoid foveae with age. Certainly, any aging technique that requires that the deceased be healthy at the time of death would not be very robust. However, the association of arachnoid granulations with immune response, and their enlargement in a variety of health conditions, suggests that the usefulness of arachnoid foveae volume as an indicator of health status should be explored.

Conclusion

A new quantitative method is presented for estimating the volume of arachnoid foveae. The method demonstrates high intraobserver and interobserver reliability. No significant relationship was found between arachnoid foveae volume and age in a sample of 63 crania of accurately known age. Development of arachnoid foveae may be affected by a variety of health-related factors. Use of specimens from the Hamann–Todd collection, representing individuals of low socioeconomic status and dating to the preantibiotic era, may have accentuated health-related variability when compared with modern samples.

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