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MANUSCRIPT VERSION

Age-related distribution of longitudinal pre-strain in abdominal aorta with emphasis on forensic application

Abstract – It is a well-known fact that the length of an artery in situ and the length of an excised artery differs. Retraction of blood vessels is usually observed. This pre-tension plays crucial role in arterial biomechanics. It augments an artery wall load-bearing capacity. This paper presents the longitudinal pre-strain of the human aorta as an index of human age. The length of abdominal aortas was measured during autopsies before and after segment resection. The longitudinal pre-strain was calculated in 130 donors; 100 male and 30 female bodies. The pre-strain was defined as the ratio between in situ length and the length after the excision. The mean pre-strain was found to be 1.18±0.10 for male and 1.14±0.10 for female sample (mean ± standard deviation). The age in the male group was 41.6±15.9 years; and 47.7±17.7 years in the female group. Statistical analysis revealed the correlation coefficient between age and pre-strain r=-0.821 and r=-0.839 in male and female group, respectively. The analysis also confirmed close correlation between aortic circumference and age; and between circumference and pre-strain. Linear and power law regression equations were employed and prediction intervals were computed. The power law estimates the age more accurately than linear one model. Nevertheless, especially for small values of the pre-strain (aged individuals) the linear model can be advantageous.

Keywords: age estimation; ageing; aorta; biomechanics; circumference; pre-strain

INTRODUCTION

The estimation of the age of cadavers of unknown identity is one of the first steps in forensic identification. There are several recommended methods. They can be divided into radiological examination of dental or skeletal development, morphological examination of teeth and skeleton and biochemical analysis based on aspartic acid racemisation rate [1]. The accuracy of the above mentioned methods varies in children and adults and is the most important criterion in forensic practise. Radiological methods are accurate in children and can be used not only for cadavers but also for archeological cases and the living [2]. In adults, aspartic acid racemisation rate in dentine is the gold standard for age estimation, if teeth are available [3,4]. In case teeth are not available, accurate and reproducible results can be obtained by analysis of purified long-living proteins from other tissues [5,6,7].

Age estimation morphological methods are currently used especially for burnt bodies because the racemisation process of aspartic acid is highly temperature dependent [8]. There are also macro-morphological and histo-morphometrical methods, e.g. evaluation of pubic symphyseal and rib ageing patterns and evaluation of bone histology [9,10]. Most of these methods require experienced scientists and/or adequate laboratory equipment.

To the best of authors' knowledge nobody yet considered changes in biomechanical properties of tissues in forensic age estimation. The main goal of the present study is to illustrate that the longitudinal pre-strain of the abdominal aorta is suitable and easily obtainable quantity for this purpose. This phenomenon now will be briefly reviewed.

The non-pressurized artery is not in a stress-free state. This can be confirmed within excision of a tubular segment of an artery from a body; retraction of the sample is usually observed [11-14]. Bergel [11] reported mean shrinkage ranged between 32% - 42% (percentage of original length) for canine samples depending on the position in the arterial tree. Han and Fung [12] confirmed this result when reported monotonically increasing longitudinal pre-strain for canine and porcine aortas from 1.2 to 1.5 with increasing distance from the heart. Learoyd and Taylor [13] performed measurement with 59 samples of arteries obtained from 12 human donors. Their results proved position dependence of the pre-strain of human arteries and also suggested a negative correlation between the age and the retraction. Some studies performed with human subjects gave significantly smaller values of the pre-strain; Schulze-Bauer et al. [14] reported longitudinal pre-strain in aged iliac arteries 1.07±0.09 (mean ± standard deviation).

Different authors call the same phenomenon different names – retraction, pre-stretch, pre-strain. It depends on the used reference configuration of an artery. In what follows we avoid the term "retraction" and only "pre-strain" will be used. This is defined as the ratio between the length in situ of the sample and the length of the sample measured after it was removed from a body.

The longitudinal pre-strain generates the longitudinal pre-stress remaining in an artery after a deflation of the blood pressure. This way generated pre-tension prevents an artery from a tortuosity [15,16]. Moreover, longitudinal pre-strain gives the possibility to carry the pressure pulse wave along an artery without significant change in axial force in the wall [14,17,18,19]. This is advantageous from a mechanical point of view.

The longitudinal pre-strain originates in the biological structure of an artery wall where different cells (smooth muscles, fibroblasts) and matrix proteins (collagen, elastin, and proteoglycans) interact together. They are subjected to growth, remodeling and ageing processes [15,16,20-26]. This results in age-dependent pre-tension of an artery. Our study maps age-dependency of the longitudinal pre-strain in the abdominal aorta and shows that it can be used in forensic age estimation. It also elucidates correlations between other characteristics of cardiovascular system such as aortic circumference, thickness of left ventricle, heart weight, and the degree of atherosclerosis.

MATERIALS AND METHODS

Measurements of the pre-strain in segments of the abdominal aorta were performed in the Department of Forensic Medicine of the Third Faculty of Medicine, Charles University and University Hospital Na Královských Vinohradech in Prague. Postmortem use of human tissue was approved by the Ethic committee of the University Hospital Na Královských Vinohradech in Prague.

Data sample was collected from 130 donors; 100 Caucasian male and 30 Caucasian female individuals. No putrefied bodies were involved. Age [years], longitudinal pre-strain [dimensionless], postmortem interval (PMI) [hour], circumference [cm], stature [cm], heart weight [gram], the thickness of left ventricle [cm], and degree of atherosclerosis [dimensionless] were documented. The thickness of the left ventricle was measured just above the anterior papillary muscle. The degree of atherosclerosis was quantified in the scale from 0 up to 4 according to the morphologic features: 0 – normal artery + fatty streaks; 1 – fibrofatty plaques; 2 – advanced plaques; 3 – calcified plaques; 4 – ruptured plaques [27].

Longitudinal pre-strain. The abdominal aorta was thoroughly preparated during autopsy and two markers were made with permanent ink just below the origin of renal arteries and just above the bifurcation into the iliac arteries. The measurement of the size has been performed two times in situ and then immediately after the excision, using a ruler. Longitudinal pre-strain, λ, was defined by (1).

$$\lambda = \frac{l}{L} \qquad (1)$$

Here l denotes in situ length and L is the length after removal from the body. This definition of the pre-strain is in accordance with [10,12]. The relative retraction mentioned in [9] is obtained as $(\lambda-1)/\lambda$.

Subsequently a ring was cut off from the aortic segment. This ring was then cut to the strip and the circumference of aortic segment was determined as the length of this strip. The measurements of the length were performed two times.

Correlation. Statistical dependence of all documented quantities was evaluated via correlation analysis. It was based on the simple correlation coefficient r defined in (2).

$$r = \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\left(n-1\right) s_x s_y} \qquad (2)$$

Here n is the number of observations, s_x and s_y denote sample standard deviations of quantities x and y. Mean values are written with bands.

Regression analysis. Linear regression equation (3) was suggested in order to employ λ as the age estimator.

$$y = A_j \lambda + B_j \qquad (3)$$

Here the estimated age [years] is denoted y. Regression parameters A_j, B_j, were determined using least square algorithm. Male and female data are distinguished with index j (j=M, and F).

Linear regression model was chosen to keep simplicity. After preliminary computations it was, however, decided to employ also power law model for age–pre-strain equation. Particular form of the model is expressed in (4). C_j and D_j (j=M, F) are the model parameters.

$$y = C_j \lambda^{D_j} \qquad (5)$$

The power law model was transformed by logarithmic transformation into the linear problem and then optimized with least squares algorithm.

Comparison of the models. Two ways were approached in the models' comparison.

First comparison was obtained making use of the correlation coefficient. In case of the linear model it is the same as in (2). The correlation coefficient for the power law model was obtained from (2) after the logarithmic transformation. A correlation coefficient, however, can only indicate the character of statistical relationship nay predictive capability of the model.

Predictive capability evaluation was based on so-called prediction intervals (confidence intervals for model prediction). It means the range in which future observation of the dependent variable will fall with probability equal to α (confidence level). Complete regression model, with implemented prediction intervals, can be written in the form (6).

$$y = y_R \pm t_{\frac{\alpha}{2}}(m)\, S_e \sqrt{1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}} \qquad (6)$$

Here y is predicted variable and x is independent variable. y_R denotes regression equation. $t_{\alpha/2}(m)$ is the quantile of Student's t-distribution with m degrees of freedom (here $m = n - 2$, where n is the number of observations). S_{xx} and S_e are defined with equations (7) and (8).

$$S_{xx} = \sum_{i=1}^{n} (x_i - \bar{x})^2 \qquad (7)$$

$$S_e = \sqrt{\frac{1}{n-2} \sum_{i=1}^{n} (y_i - y_{Ri})^2} \qquad (8)$$

In (8) y_{Ri} denotes model prediction for i-observation. The logarithmic transformation was again used to linearize power law model. Confidence intervals for model parameters were also computed. $\alpha = 0.95$ was considered through entire study.

RESULTS

Data summary for male and female group is presented in Table 1. Female autopsy data were available with complete documentation. Male data, however, were incomplete in five cases. Therefore samples in male population range from 95 to 100.

Correlation. Results of correlation analysis are presented in Table 2. Eight investigated variables generate 28 untrivial correlation coefficients. Some of them, however, do not seem to be worthwhile; e.g. correlation between height and post mortem interval. Thus only ten highest correlation coefficients in each group are reported.

Both populations (male and female) give the highest correlation between age and circumference of abdominal aorta; r_M=0.898 and r_F=0.893 (*M* and *F* denote the population). This is followed by age and pre-strain correlation; r_M=-0.821 and r_F=-0.839. While the circumference increases with the age, longitudinal pre-strain decreases. This is common to both populations.

In what follows male and female data give different order of correlations. Male data indicate tight correlation between age and atherosclerosis (r_M=0.768), circumference and arthrosclerosis (r_M=0.765), and circumference and longitudinal pre-strain (r_M=-0.750). Also the correlation between pre-strain and atherosclerosis should be mentioned (r_M=-0.693), however, statistical dependence weakens rapidly.

Contrary to male population, female data reveal tighter dependence between longitudinal pre-strain and circumference (r_F=-0.802). Also age and degree of arthrosclerosis, and circumference and arthrosclerosis give noticeable correlations (r_F=0.763 and r_F=0.668, respectively). It is worth noting that the correlation between longitudinal pre-strain and degree of atherosclerosis falls in female population to r_F=-0.522, which is in contrast to r_F=-0.693 in male population.

Regression analysis. Obtained regression models are presented in Fig. 1–4. Decreasing trend of longitudinal pre-strain within ageing is clear. Power law models, which were computed after the logarithmic transformation, gave additional correlation coefficients; r_M=-0.860 and r_F=-0.913. They are both higher then obtained in simple linear computations. It strongly suggests that nonlinear model is more suitable than linear one.

The prediction intervals computed with confidence level 0.95 are also presented (Fig. 1– 4). Their widths vary with change in independent variable. It is evident that the power law model gives narrower range of predicted value. Thus, such a way the age can be predicted more reliably. For example mean longitudinal pre-strain gives prediction intervals in the linear model 23.1÷60.1 years; and 27.9÷64.9 years for male and female sample, respectively. Nevertheless, the power law model gives 25.3÷57.0 years; and 30.5÷62.3 years in male and female sample, respectively.

Model parameters are presented in Table 3. They are also supplemented with their confidence intervals computed with α=0.95.

DISCUSSION

The main aim of our study was to investigate the possibility of the estimation of the age based on the longitudinal pre-strain of the abdominal aorta. To this end measurements of the longitudinal pre-strain with 100 male and 30 female subjects were carried out within autopsies. Results have shown that tight correlation between the age and pre-strain exists (r_M=-0.821 and r_F=-0.839). The longitudinal pre-strain decreases with increasing age. The results of the regression analysis and the width of prediction intervals suggest that the power law model is more suitable to fit observed relationship than linear one.

Correlation. Our data confirmed previous observations of Learoyd and Taylor [13] that negative correlation between the pre-strain and the age exists. Langewouters et al. [28] published the list of the retractions found in 20 human abdominal and 45 thoracic aortas. To compare the results with [28] we computed correlation between age and longitudinal pre-strain based on their list. r=-0.790 in abdominal and r=-0.786 in thoracic aorta were obtained. It confirms our observation although our sample gives higher correlation coefficient.

Even higher correlation coefficients were obtained for circumference–age dependence; r_M=0.898 and r_F=0.893. This fact is in accordance with Stefan and Josifko [29] who found the aortic circumference to be suitable indicator of the human age. Because both the age-related increase in the circumference and the decrease of longitudinal pre-strain can be considered as the results of ageing process it is not surprising that the study revealed their tight inter-correlation; r_M=-0.750 and r_F=-0.802. Also the correlation between the age and the degree of atherosclerosis should be presumed; r_M=0.768 and r_F=0.763. Rather surprising fact is low (with respect to previous values) correlation obtained for dependences between the age, heart weight, and thickness of the left ventricle. It suggests the heart weight and thickness of the left ventricle are more dependent on pathologic changes or physical training then ageing.

Regression models. The correlation coefficient computed after the logarithmic transformation of the data (r_M=-0.860 and r_F=-0.913) indicates that the power law model is more suitable. It is also supported by the width of prediction intervals (see Fig. 1–4). The power law model, naturally, gives the widths of prediction intervals variable with measured pre-strain. Nevertheless, they are significantly shorter than in the linear model for pre-strains higher approximately than 1.1. It implies, however, that for elderly subjects the linear model can be advantageous because of gradual increase of the length of prediction interval caused by nonlinearity.

To the authors' knowledge nobody yet considered longitudinal pre-strain as the age estimator. Thus the possibility of comparison with literature is limited. The most precise methods for age estimation in adults based on amino acid racemization can determine the age at death of an individual to ±1.5-4 years with correlation coefficients 0,97-0,99 [1]. Evaluation of skeletal morphology is less accurate and standard errors are ±2-12 years with correlation coefficients 0,60-0,90 [1].

Stefan and Josifko [29] proposed the regression model that predicts the age by means of aortic circumference. Our model can be simply extended with this idea. To keep brevity and also to track only one key idea it is omitted here. Nevertheless, it should be noted that the inter-correlation between age and longitudinal pre-strain was found. This implies that such a model would probably operate with mutually dependent variables.

Biomechanical context. The change of the longitudinal pre-strain is linked to remodeling and adaptation processes (e.g. to hypertension) or other phenomena associated with ageing (e.g. glycation). If the hypothesis of approximately constant longitudinal load in an artery through adulthood is accepted then decreased value of longitudinal pre-strain seems to be explainable with overall stiffening of arteries in increased age. This stiffening was reported widely [30,31]. Alternatively, the wall thickening and increasing of arterial diameter restore the wall stress within a remodeling. Under unchanged material properties it may also lead to a decrease in arterial pre-strain.

However, the biomechanics of the blood vessel wall is determined by its constituents. Histological and biomechanical investigations carried out by Dobrin et al. [32] and Greenwald et al. [33] proved that the amount of longitudinal pre-strain and residual strain correlate closely with the presence of elastin within the artery wall. The study performed with an animal model (haploinsufficiency in elastin) also revealed decreased value of the longitudinal pre-strain in abdominal aorta [34]. It implies the decreasing of longitudinal pre-strain could be attributed to age-related degradation and fragmentation of elastin. The decision between the mentioned hypotheses is still in question and requires detailed studies which will reflect the phenomenon of time-varying longitudinal pre-strain in living subjects.

Conclusion. We conclude that longitudinal pre-strain–age relationship is suitable to be used as a quick and easy preliminary step in estimating the age in forensic practice. The power law estimates the age more accurately than linear one. Nevertheless, especially for small values of the pre-strain (aged individuals) the linear model can be advantageous.

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FIGURE LEGENDS

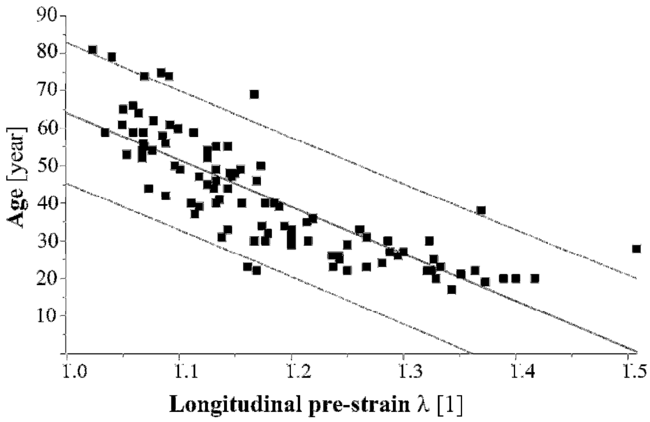

Fig. 1 The age estimated with the linear regression model for male population. Following symbols are used: solid-box as observation points; solid line as the regression model; and dashed curves as limits of prediction intervals (α=0.95). The presence of outliers (especially for small pre-strains) suggests that nonlinear relationship would be more suitable. The correlation coefficient is r=-0.821.

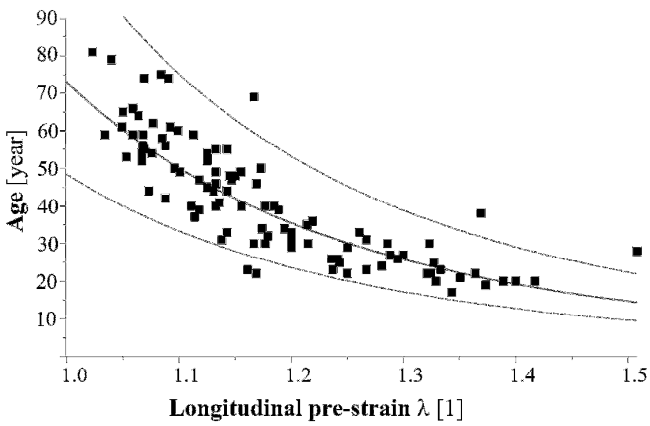

Fig. 2 The age estimated with power law model for male population. Following symbols are used: solid-box as observation points; solid curve as the regression model; and dashed curves as limits of prediction intervals (α=0.95). The nonlinear equation fits data more successfully than linear model (Fig. 1). The correlation coefficient is r=-0.860.

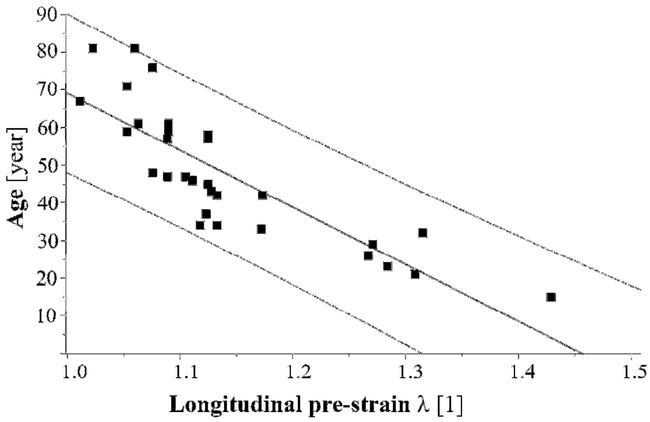

Fig. 3 The age estimated with linear regression model for female population. Following symbols are used: solid-box as observation points; solid line as the regression model; and dashed curves as limits of prediction intervals (α=0.95). The correlation coefficient is r=-0.839.

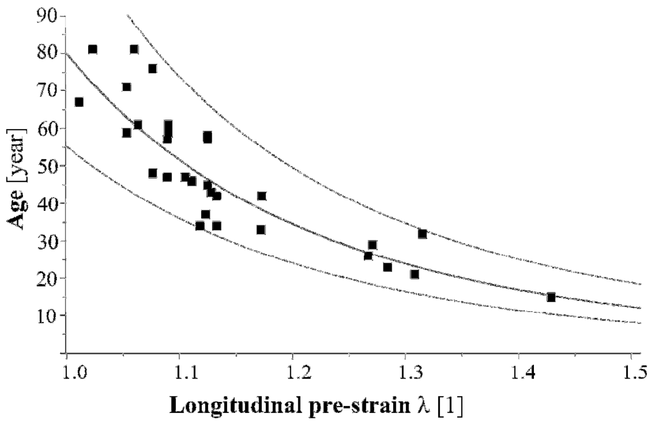

Fig. 4 The age predicted with power law model for female population. Following symbols are used: solid-box as observation points; solid curve as regression model; and dashed curves as limits of prediction intervals (α=0.95). The power law model seems also to be more suitable in female population; the correlation coefficient r=-0.913.

TABLES AND CAPTIONS

Table 1 Summary of documented data; mean ± standard deviation. Following abbreviations were used: AGE – age [years]; PRESTR – longitudinal pre-strain [-]; CIRC – abdominal aortic circumference [cm]; HGHT – height [cm]; HWGHT – heart weight [cm]; TLVENT – thickness of left ventricle [cm]; DEGATHR – degree of atherosclerosis [-]; PMI – post mortem interval [hour]; n – number of observations; M – male; and F – female. The degree of atherosclerosis is characterized rather with the mode (the most frequent observation) than with the arithmetic mean; arithmetic mean gives 1.54 and 1.37 in male and female population, respectively.

	n	M	n	F
AGE	100	41.6±15.9		47.7±17.7
PRESTR	100	1.18±0.10		1.14±0.10
CIRC	99	4.0±0.7		3.7±0.6
HGHT	100	179±7	30	168±5
HWGHT	95	417±119		359±97
TLVENT	96	1.4±0.3		1.3±0.3
DEGATHR	97	0[#]		0[#]
PMI	100	42±25		40±20

Table 2 Ten highest correlations ordered decreasingly. Data reveal significant correlation between age, pre-strain and abdominal aortic circumference. The abbreviations are described in the caption of Table 1.

Correlation coefficient			
Male		Female	
AGE CIRC	0.898	AGE CIRC	0.893
AGE PRESTR	-0.821	AGE PRESTR	-0.839
AGE DEGATHR	0.768	PRESTR CIRC	-0.802
CIRC DEGATHR	0.765	AGE DEGATHR	0.763
PRESTR CIRC	-0.750	CIRC DEGATHR	0.668
PRESTR DEGATHR	-0.693	HWGHT TLVENT	0.591
CIRC HWGHT	0.501	AGE HWGHT	0.564
AGE HWGHT	0.471	CIRC HWGHT	0.558
HWGHT DEGATHR	0.444	HWGHT DEGATHR	0.544
HWGHT TLVENT	0.335	PRESTR DEGATHR	-0.522

Table 3 Estimated model parameters.

A_M	B_M	A_F	B_F
-125.0	189.0	-151.5	220.6
-143÷-107	168÷210	-190÷-114	177÷264
C_M	D_M	C_F	D_F
73.05	-3.968	79.94	-4.614
67.0÷79.6	-4.44÷-3.50	70.9÷90.2	-5.41÷-3.82