The Image Analysis of Colour Changes of Different Human Tissues in the Relation to the Age Part 2: Practical applicability.

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Abstract

The method of image analysis of intervertebral disc, Achilles tendon and rib cartilage was applied for assessment of colour changes of these tissues in the relation to the human age. It was proved that colour of tested tissues changes with age which is most obvious on rib cartilage and intervertebral disc, while Achilles tendon does not display important changes. The parameters MeanBlue, MeanSaturation and MeanBrightness are the best for age estimation based on colour analysis.

Key words: Age estimation, colour changes of tissues, non-enzymatic browning, image analysis, AGEęs, Lucia G

Souhrn

Analýza barevných změn různých lidských tkání ve vztahu k věku

Pro vyhodnocení barevných změn meziobratlového disku, Achillovy šlachy a žeberní chrupavky byla použita metoda obrazové analýzy. Bylo prokázáno, že barva těchto tkání se s věkem mění, což je nezřetelněji patrné na žeberní chrupavce, zatímco meziobratlový disk a Achillova šlacha nevykazují významné změny. Nejlépe jsou barevné změny charakterizovány parametry MeanBlue, MeanSaturation and MeanBrightness

Klíčová slova: odhad věku, barevné změny tkání, AGEęS, Lucia G

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Introduction

The estimation of age can be made by different methods. However, the problem may rise when only a part of the body is found or tissues which are usually used for age estimation are absent or changed by pathologic conditions. The method of image analysis of the colour of tissues was used (1). This part of study deals with the dependence of selected parameters on age. The causes of colour changes and appreciation of practical applicability are discussed.

Results:

Colour of intervertebral disc vs. age.

MeanBlue, MeanGreen, MeanRed (Fig. 1): a decrease in intensity of all three channels in correlation with age has been found; the correlation coefficients for three channels were as follows: $r_{MeanBlue} = -0.82$, $r_{MeanGreen} = -0.78$, $r_{MeanRed} = -0.54$. The dependence on age in the decrease of intensity of all three RGB channels is evident (the negative correlation is significant on 0.99 confidence level). The most distinct is the decrease of the values of intensity for the blue constituent (as opposite to yelow colour increase).

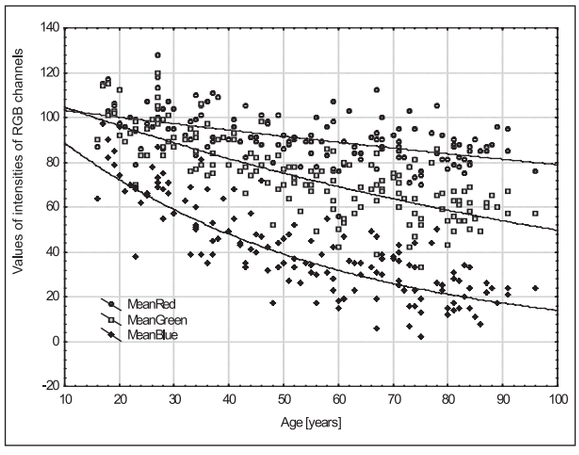

Fig. 1. Changes of values of RGB channels on the age - intervertebral disc

Age: MeanRed: r = -0.54; p = 0.0000;
y = 106.014 - 0.275x
Age: MeanGreen: r = -0.78; p = 00.0000;
y = 108.107 - 0.619x
Age: MeanBlue: r = -0.82; p = 00.0000;
y = 84.451 - 0.792x
MeanRed = 106.244exp$^{-0.003x}$
MeanGreen = 113.701exp$^{-0.0083x}$
MeanBlue = 108.864exp$^{-0.0205x}$

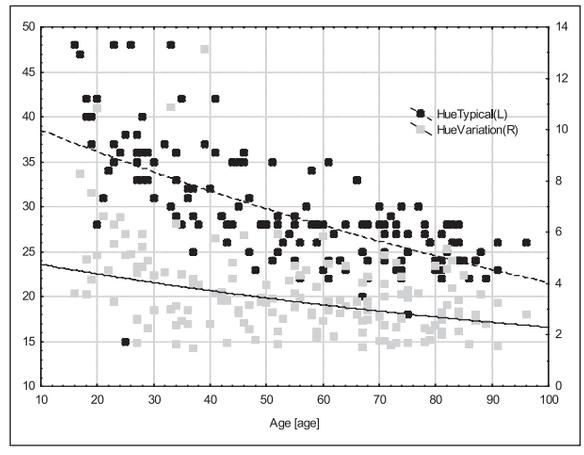

Fig. 3: Relation of HueVariation and HueTypical on age - intervertebral disc. Values for HueTypical are on the left Y axis, those for HueVariation are on the right Y axis.
Age: HueVariation: r = -0.41; p = 0.0000001;
y = 5.494 - 0.034x
Age: HueTypical: r = -0.69; p = 00.0000;
y = 40.593 - 0.203x
HueTypical = 41.083exp$^{-0.0064x}$
HueVariation = 5.152exp$^{-0.0081x}$

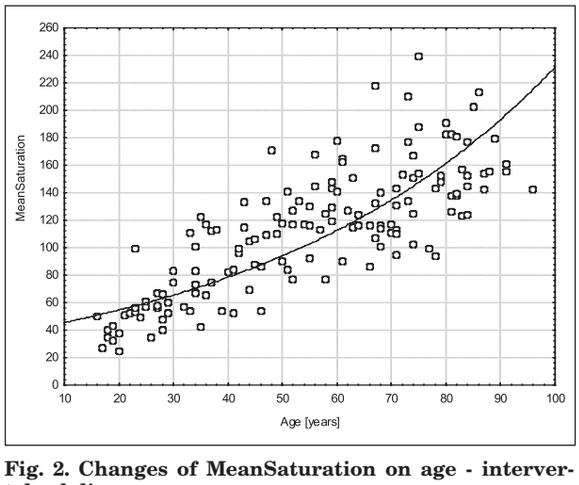

Fig. 2. Changes of MeanSaturation on age - intervertebral disc.
Age: MeanSaturation: r = 0.80; p = 00.0000;
y = 19.458 + 1.705x
MeanSaturation = 38.124exp^{0,018x}

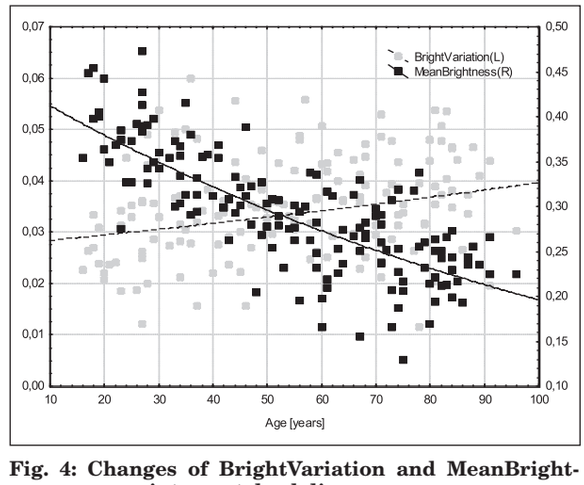

Fig. 4: Changes of BrightVariation and MeanBrightness on age - intervertebral disc.
Values for MeanBrightness are on the left Y axis, those for BrightVariation are on the right Y axis.
Age: BrightVariation: r = 0.27; p = 0.0010;
y = 0.0284 + 0.00012x
Age: MeanBrightness: r = -0.7754; p = 00.0000;
y = 0.426 - 0.0024x
BrightVariation = 0.027exp$^{0.0037x}$
MeanBrightness = 0.447exp$^{-0.0082x}$

MeanSaturation (Fig. 2): an increase of values of this parameter which can be seen in people aged 20 to 90 years old has been found (r=0.8). The spread of values is lowest in young persons up to 40 years. Above this age, values of MeanSaturation reflect only an accumulation of substances in tissue.

HueVariation (Fig. 3): as indicator of colour homogeneity, states that a tissue modifies its colour in a quite steady manner, i.e. cumulated substances are deposited into a tissue equally in all age groups except the youngest ones (sharp decrease of values approximately till 30 years is evident).

HueTypical (Fig. 3): it is evident from the graph that the colour changes with age and it corresponds to the observation of the change from pure chalky white in young people to yellowish in middle age and up to yellow-brown in the elderly persons. However, the spread of values lowers the correlation coefficient (r=-0.698).

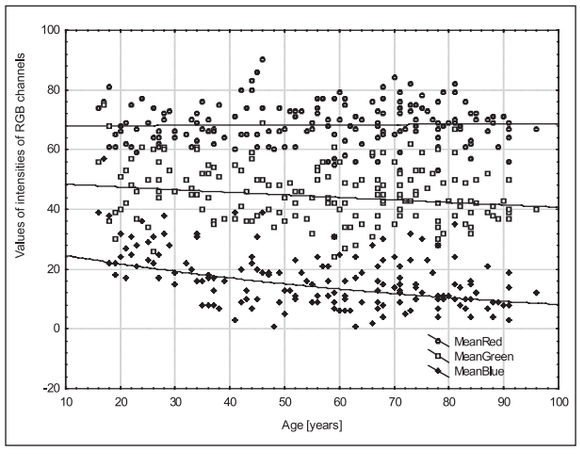

Fig. 5: Changes of values of RGB channels on age - Achilles tendon.
Age: MeanRed: r = 0.028; p = 0.73; y = 68.142 + 0.0089x
Age: MeanGreen: r = -0.22; p = 0.0058; y = 50.566 - 0.094x
Age: MeanBlue: r = -0.47; p = 0.0000; y = 28.151 - 0.203x
MeanRed = 67.864exp$^{0.0001x}$
MeanGreen = 49.388exp$^{-0.0019x}$
MeanBlue = 27.803exp$^{-0.0122x}$

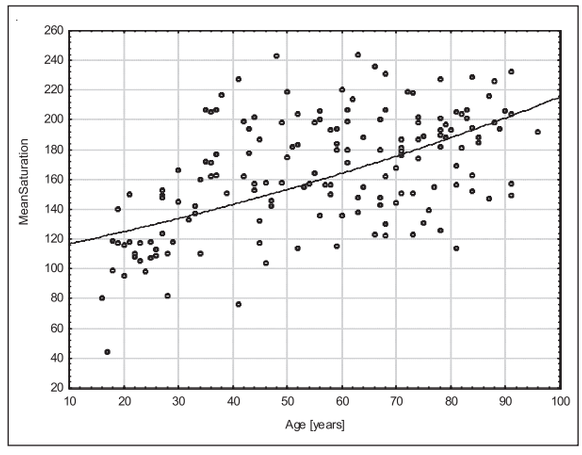

Fig 6.: Changes of MeanSaturation on age - Achilles tendon.
Age: MeanSaturation: r = 0.54; p = 0.0000;
y = 110.359 + 0.975x
MeanSaturation = 109.114exp0.0068x

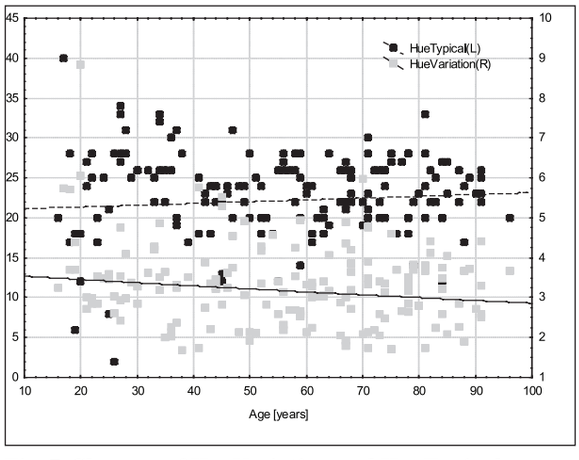

Fig. 7: Changes of HueVariation and HueTypical on age - Achilles tendon
Age: HueTypical: r = -0.031; p = 0.7;
y = 23.414 - 0.0075x
Age: HueVariation: r = -0.2; p = 0.0138;
y = 3.834 - 0.0092x
HueTypical = 20.943exp$^{0.001x}$
HueVariation = 3.624exp$^{-0.0024x}$

BrightVariation (Fig. 4): this parameter indicates the tissue colour homogeneity in terms of brightness. Only a low value of age correlation coefficient (r=0.264) was found. Mean Brightness (Fig. 4): indicates a clear decrease of tissue brightness, i.e. increasing darkness of tissue related to age (r=-0.78). It corresponds with tissue colour change as mentioned above.

Colour of tendon vs. age.

MeanBlue, MeanGreen, MeanRed (Fig. 5): the decrease of the channel intensity in terms of red and green does not closely depend on age: $r_{MeanRed}$ = 0.028 (no significant correlation), $r_{MeanGreen}$ = 0.22. A more significant decrease of MeanBlue is evident in persons younger than 30 years, followed by a huge spread showing only low dependence on this parameter on age (r=-0.467). MeanSaturation (Fig. 6): this parameter shows only a slight increase of values according to age (r=0.534). The spread is so high that this parameter is not relevant with respect to an individual age. HueVariation (Fig. 7): as a dimension of colour homogeneity, it shows almost no dependence on age and a low correlation coefficient (r=-0.196) testifies about a relatively homogenous distribution of substances in the tissue.

HueTypical (Fig. 7): in the tissue of the Achilles tendon, no substantial changes of colour depending on age were found, contrary to IVD and rib cartilage. No correlation of this parameter to age was found. It is not possible to make conclusions about the age of a person according to these values except young persons (about 20 years) where the tendon is white.

BrightVariation (Fig. 7): a wide spread of values can be seen from the diagram. The correlation coefficient (r=0.147) indicates that this parameter cannot be used for an age estimation. Most probably, not only accumulation of substances but also other factors affect the colour of this tissue.

MeanBrightness (Fig. 8): this parameter expresses “darkness of tissue”. The variation is very high while the decrease of values being imponderable, no significant age dependency was found (r=-0.221).

Colour of a rib cartilage vs. age:

MeanBlue, MeanGreen, MeanRed (Fig. 9): the age-related decrease of intensity in all three

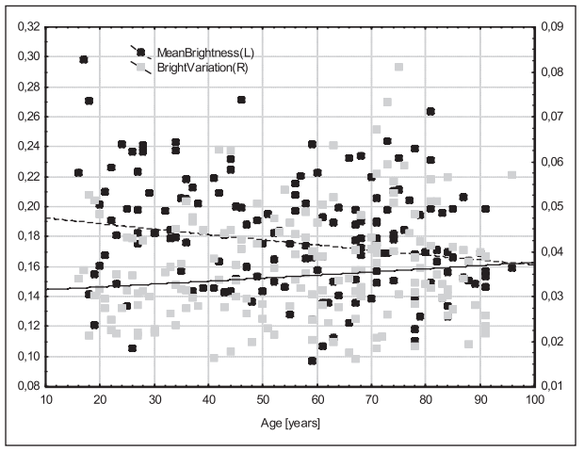

Fig. 8: Changes of BrightVariation and MeanBrightness on age - Achilles tendon.
Values for MeanBrightness are on left Y axis, those for BrightVariation are on the right Y axis.
Age: MeanBrightness: r = -0.22; p = 0.0046;
y = 0.201 - 0.000378x
Age: BrightVariation: r = 0.15; p = 0.0615;
y = 0.0318 + 0.0000821x
MeanBrightness = $0.196\exp^{-0.002x}$
BrightVariation = $0.031\exp^{0.002x}$

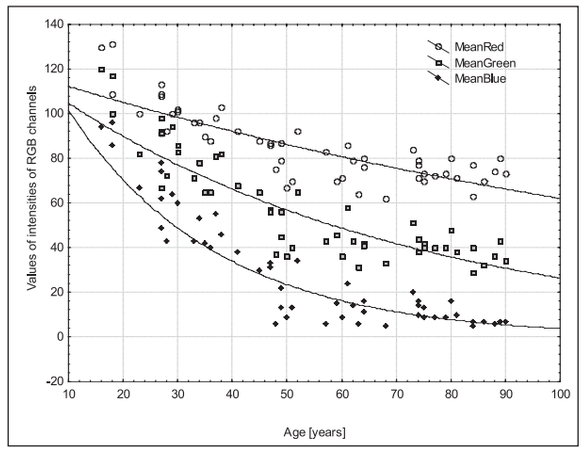

Fig. 9: Changes of intensities of values of RGB channels on the age - rib cartilage.
Age: MeanRed: r = -0.82; p = 0.0000;
y = 117.098 - 0.589x
Age: MeanGreen: r = -0.87; p = 0.0000;
y = 107.537 - 0.9264x
Age: MeanBlue: r = -0.87; p = 0.0000;
y = 85.624 - 1.0329x

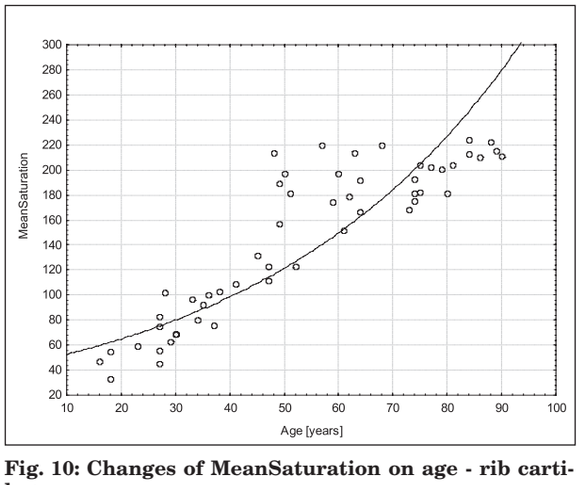

Fig. 10: Changes of MeanSaturation on age - rib cartilage.
Age: MeanSaturation: r = 0.90; p = 00.0000;
y = 12.863 + 2.492x

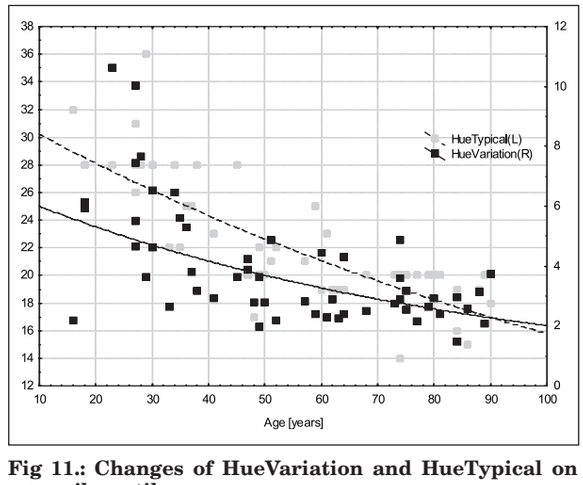

Fig 11.: Changes of HueVariation and HueTypical on age - rib cartilage.
Values for HueTypical are on the left Y axis, those for HueVariation are on the right Y axis.
Age: HueTypical: r = -0.8; p = 0.0000;
y = 31.398- 0.166x
Age: HueVariation: r=-0.61; p=0.00002; y= 6.79-0.054x
HueTypical = $32.464\exp^{-0.0072x}$
HueVariation = $6.7696\exp^{-0.0121x}$

channels was found, correlation coefficients being $r_{MeanBlue}$ = -0.87, $r_{MeanGreen}$ = -0.86, $r_{MeanRed}$ = -0.81. The diagram shows a sharp intensity decrease especially in MeanBlue and MeanGreen approximately up to the age of 40. After this age limit, the values of intensities remain constant.

MeanSaturation (Fig.10): an increase corresponds to the amount of accumulated compounds in tissue (r=0.901). This is evident until the age of about 50 years. In older individuals, values of this parameter do not change with age and a significant spread also appears. It can be concluded that tissue is "saturated" by accumulated compounds.

HueVariation (Fig. 11): can be an indicator of colour homogeneity of the tissue slice. The spread of values is higher in persons under 40 years compared to older than 40 years. This shows that saturation of tissue with accumulated compounds is uneven, which can be seen by the naked eye – the colour of transversally sliced tissue in younger person is not homogenous while it is in older persons.

HueTypical (Fig. 11): a diagram shows that changes of colour hue can be found in three age groups approximately: between 20-40 years, 40-60 years, and older than 60 years. The tissue

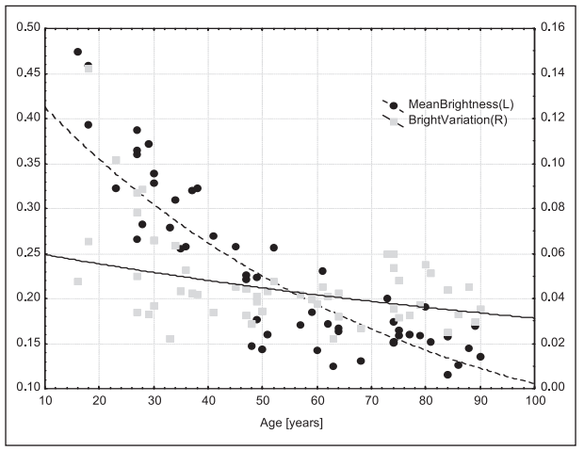

Fig 12.: Changes of BrightVariation and MeanBrightness on age - rib cartilage.
Values fo MeanBrightness are on the left Y axis, values for BrightVariation are on the right Y axis.
Age: MeanBrightness: r = -0.87; p = 0.0000;
y = 0.424- 0.0036x
Age: BrightVariation: r = -0.45; p = 0.0007;
y = 0.0703 - 0.000436x
MeanBrightness = 0.48exp$^{-0.0152x}$
BrightVariation = 0.064exp$^{-0.0072x}$

colour changes in young people correspond to this finding - cartilage in young people is chalky white and subsequently becomes yellow (in middle age) and yellow-brown in the elderly. The correlation coefficient (r=-0.798) reflects a quite tight dependence on age of colour change.

BrightVariation (Fig.12): decrease of Bright Variation values brings evidence that brightness homogeneity changes significantly only in young people, while in the elderly people it changes little.

MeanBrightness (Fig.12): the sharpest decrease comes in persons younger than 40 years, then is stabilises. The curve of changes is very similar to that of values of MeanSaturation and these parameters correspond – accumulation of substances marks tissue saturation, which is accompanied by darkening. The link to an age is quite evident (r=-0.865).

Discussion

The examined phenomenon, i.e. change of colour in relation to age is most probably the result of non-enzymic browning of tissues which is a biochemical reaction where molecules of pentose or hexose react with amino acids residues in proteins, mainly with lysin and arginin. The tissues chosen for image analysis consist of bundles of collagen fibrils and proteoglycans. Both of these compounds contain the large amounts of lysin and arginin that are necessary for glycation of proteins. It was proved that the AGEęs like -(N $^{@}$ (carboxymethyl)lysine, N $^{@}$ (carboxyethyl)lysine or pentosidin as well as chemically unidentified compounds accumulate during the life in cartilage which the result in protein-bound browning or fluorescence and cross-linking [2]. Another product leading to the tissue browning is a lipofuscin but it is not present in the examined tissues, as we can know, and it originates from another type of biochemical reaction than AGE@s.

The accumulation of products of non-enzymic browning absorbs mainly the blue portion from white light that causes the change of the hue of tissue. That is the reason why the MeanBlue parameter evaluated by image analysis has good negative correlation with age. The parameter, which describes changes of hue, is HueTypical. The hue modifies with age from white in the youngest persons – 15–20 years of age, then the tissue becomes yellow up to the middle age and then a yellow brown hue starts to prevail. Both RGB values and HueTypical change indicates the accumulation of different substances in tissues, or modification of their physicochemical properties.

Another parameter that corresponds well to age is MeanSaturation, and it can be considered as an indicator of the amount of substances that accumulate in tissues. This effect is visible to the naked eye; however, it is not apparent equally in all examined tissues. The changes of colour can be observed best in rib cartilage then in the intervertebral discs and the weakest in the Achilles tendon. The change of tissue colour depends on its composition and with high probability depends on the amount of proteoglycans. The composition of investigated tissues [3-7] corresponds to this fact. The distribution of accumulated compounds in the tissue is not uniform during life. This fact is supported by the values of HueVariation parameter, which is an indicator of colour homogeneity of the tissue section. It is apparent in diagrams of IVD and rib cartilage where the decrease of values in persons up to approximately 40 years old can be seen, and then values remain almost constant. The values of HueVariation in Achilles tendons show remarkable spread and no dependence on age.

Another characteristic which was evaluated was brightness – the parameter BrightVariation. This parameter describes changes from the point of view of “dark – light”. A value of “1” indicates a pure white for all colours, while “0” means pure black. This parameter reflects the distribution of light and dark points and indicates heterogeneity of material from the point of view of the structure and deposition of coloured substances. The spread of this parameter is high in all tissues and therefore it cannot be used for age estimation. This spread can be brought about not only by tissue properties but also by other factors like uneven surface, typical for intervertebral disc which is formed by concentric bundles of collagen, or fascicular arrangement of Achilles tendon that can lead to shadows on the surface (i.e. darker places). Most probably, such effects play a part in the changes of this parameter rather then the ageing

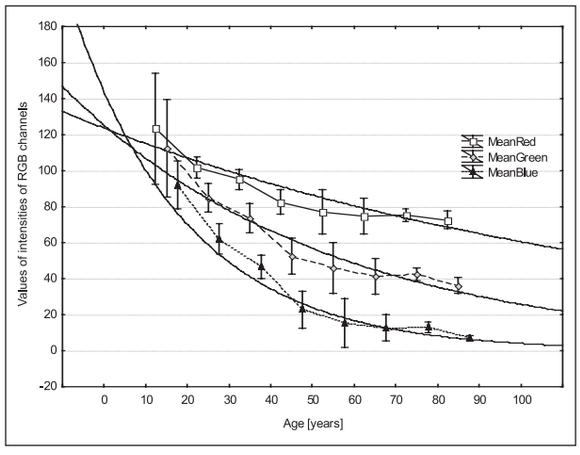

Fig 13.: Changes of intensities of values of RGB channels on the age - rib cartilage (average ± 0.95 Int.of confidence).
MeanRed Average = 123.5855exp$^{-0.0072x}$
MeanGreen Average = 125.2932exp$^{-0.0159x}$
MeanBlue Average = 143.8886exp$^{-0.0359x}$

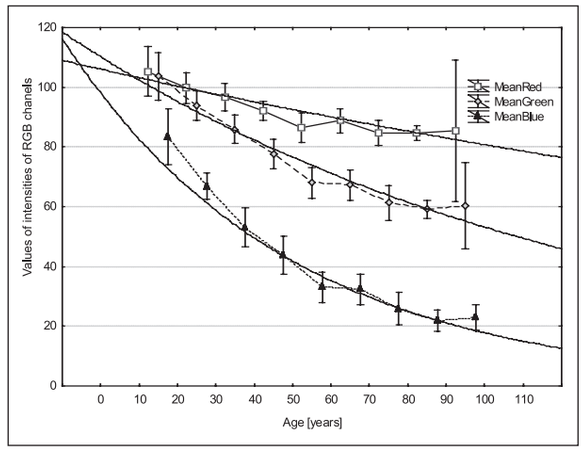

Fig 14.: Changes of intensities of values of RGB channels on the age - intervertebral disc (average ± 0.95 Int. of confidence).
MeanRed Average = 106.0327exp$^{-0.0027x}$
MeanGreen Average = 110.1635exp$^{-0.0073x}$
MeanBlue Average = 98.0548exp$^{-0.017x}$

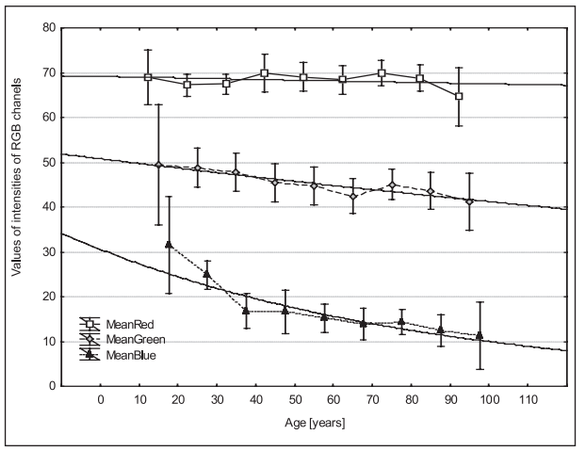

Fig. 15: Changes of intesities of values of RGB channels on the age – Achilles tendon (average ± 0.95 Int. of confidence).
MeanRed Average = 69.391exp$^{-0.002x}$
MeanGreen Average = 50.754exp$^{-0.021x}$
MeanBlue Average = 30.037exp$^{-0.112x}$

of the tissue itself, as can be deduced from the spread of values even in young persons. On the contrary, rib cartilages that have a smooth surface show a very sharp decrease of values in very young people while no other significant decrease is evident in persons older than 30 years.

Whilst BrightVariation is a measure of the homogeneity of tissue brightness, the MeanBrightness parameter reflects how the tissue becomes dark throughout the life and it is most pronounced in intervertebral disc and rib cartilage. A decrease of values suggests that approximately up to 40 years of age the tissue becomes dark quickly, which is probably connected to an accumulation of colour substances in tissue, but deposition slows down after this age.

The graphs of average RGB intensity values in particular age decades illustrates the proper usefulness of this method for age estimation. The interval of confidence of average values overlaps at the least for values of intensity for blue channel. The MeanBlue parameter could be used for age calculation. This is evident from the Figs 13–15 that show the dependence of colour changes (MeanBlue) on age in the analysed tissues: the best correlation of colour changes to age was found in rib cartilages (Fig. 13) followed by intervertebral discs (Fig. 14) and the lowest relationship was found in Achilles tendon (Fig. 15). Compared to intervertebral disc or intercostals cartilages, excisions of Achilles tendon are somewhat transparent which makes evaluation of colours changes considerably difficult. Another factor that influences colour changes is probably linked to the amount of proteoglycans – the highest amounts of which can be detected in rib cartilage. The opinion that the amount of non-enzymatic browning products leading to visible colour changes depends on the proteoglycans content is supported by an observation of tissue formed almost exclusively by collagen (e.g. dura matter, pericardium) where no visible colour changes can be found.

This method was worked out for the purposes of rapid age estimation in unidentified dead adult persons where other method can not be applied. These are such cases where the tooth set is not present or it cannot be used due to pathologic changes (typical examples are homeless people or persons depending on illicit drugs), or only a part of body was found. The importance of the method lies in rapid age estimation and in sorting out the persons according to age groups. The study results lead to a conclusion that a reliable age estimation based on colour changes can be performed up to the age of 45. Linear regression could be used during this time interval in spite of the fact that this relationship is not line-

ar in all data sets and exponential curves give the best description. After 45 years of age, the spread of values increases, which makes the usability of this method low in this time period.

Conclusion

The study deals with changes of colour of tissues that are caused by accumulation of non-enzymic browning product in the relation to the age. As far as we know; this phenomenon has not been described from the point of view of examined tissues and method nor used in forensic medicine yet. The rapid age estimation can be performed especially from rib cartilage where colour changes are most evident; however, this conclusion still needs confirmation based on a larger group. The parameters MeanBlue, MeanSaturation and MeanBrightness are the best for age estimation based on colour analysis.

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Súdne lekárstvo je tradičný medicínsky obor, ktorý však najmä v poslednej dobe trpí v našich krajinách nedostatkom aktuálnej literatúry. Našťastie je možné túto prekážku prekonať veľmi jednoduchým spôsobom – s pomocou zahraničnej odbornej literatúry. Nemecko je krajinou, kde tradične vychádza veľmi kvalitná literatúra z nášho odboru.

Autorský kolektív pod vedením profesora Madeu (Bonn) vytvoril originálnu a prakticky orientovanú príručku, ktorá vyšla už v druhom vydaní. Tá na svojich vyše 700 stranách obsahuje všetko potrebné pre každodenný výkon súdnolekárskej činnosti, pregraduálne a postgraduálne vzdelávanie.

Editor s pomocou vynikajúco zvládnutej grafiky štruktúry dosiahol pridanú hodnotu diela v podobe prehľadnosti a jednoduchej orientácie. Každá téma má kľúčové poznatky farebne zvýraznené, definície sú zreteľne oddelené od textu a tabuľky vhodne dopĺňajú text. Samozrejmosťou sú aj štruktúrované „checklisty“ pre prax.

Text je rozdelený do dvanástich kapitol. Prvá pojednáva o úlohách a štruktúre odboru a potom už nasledujú kapitoly venované tanalógii, súdnolekárskej traumatológii, náhlym a neočakávaným úmrtiam. Pomerne rozsiahle sú kapitoly venované klinickému súdnemu lekárstvu a forenznej psychopatológii. Toxikológia, dopravná medicína, forenzná sérologia a molekulárna genetika sú takisto štandardnými a očakávanými kapitolami. V nemeckých krajinách je tradíciou zaraďovať kapitoly venované medicínskemu právu, poistnému súdnemu lekárstvu a právnym normám v nemecky hovoriacich krajinách. Pozitívne hodnotíme aj zaradenie slovníka pojmov.

Túto knihu môžeme každému vrelo odporúčať.

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