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Letter to the Editor

Application of the Minkowski–Bouligand fractal dimension for the differential diagnosis of thyroid follicular neoplasias

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To the Editor,

Nuclear characteristics are important for the differential diagnosis between benign or malignant neoplasias. Subjective interpretation by an observer, however, may cause diagnostic insecurity [5]. Quantitative morphologic analyses can be helpful in this situation [19]. There are several ways to perform karyometry, which may be done by basic morphometry [8,10,14, 16,17,21–24,28], or by sophisticated texture analysis based on digitalized images [2–7,11–13,20,25–27,29–31]. Due to the fractal nature of chromatin organization in interphase nuclei [15], the scale-invariant self similiarity is an important texture feature, which can be estimated by the fractal dimension (FD) [1,3,9,13,31]. For the analysis of nuclear chromatin in routinely stained slides it has been suggested that besides the FD, the goodness-of- fit (GOF) of the regression line in the log-log plots, which are essential for the FD estimation, could be important [3]. In this investigation we tried to find out whether this new parameter GOF could also be useful for differential diagnosis in surgical pathology. We compared the FD and GOF of nuclear chromatin in routinely HE stained paraffin sections of follicular adenomas and minimally invasive follicular carcinomas of the thyroid.

Our study consisted of 18 follicular adenomas and 24 microinvasive follicular carcinomas from our files. Tumors with a diameter up to 6 cm had been completely embedded in paraffin. For larger tumors the number of paraffin blocks taken had been $d + 4$ (d representing the largest diameter in cm). Diagnosis was based on criteria of the World Health Organization Histological Classification [18]. From each tumor 100 nuclei were randomly taken from routinely stained 5 μm HE stained paraffin sections using the Kontron Zeiss KS300 system (0.1 μm/pixel spatial resolution; 1.25 numerical aperture; 100$\times$ oil immersion objective) by one examiner blinded to the diagnosis. The images were converted to grayscale format with levels of luminance ranging between 0 (absence of light) and 255 (very bright). Then a pseudo-three-dimensional "landscape-like" representation was created using the gray level (luminance) of each pixel (picture element) as the height of a z-axis. The fractal dimension (FD) was determined according to Minkowski–Bouligand extended to three dimensions, as described earlier [3]. The linear regression was estimated in a log-log plot composed of 30 points. The goodness-of-fit (GOF) was determined by the R^2 value of the regression between the real and the estimated values. For each nucleus the distribution of the residuals was compared with the normal distribution by the Kolmogorov–Smirnov test. Then for each patient the percentage (P) of cells with normally distributed residuals was calculated [3]. Group comparison was done by the Mann–Whitney test (WinStat software).

Patients of both groups were of similar age with a mean for adenomas of 40.3 years (range 17–72 years) and a mean for minimally invasive carcinomas of 49.5 years (range 19 to 88 years; $p > 0.05$). The same was true for the tumor size (mean for adenomas: 3.6 cm, range 1.0–7.0 cm; mean for minimally invasive carcinomas: 3.4 cm, range 1.3 to 8.5 cm; $p > 0.05$). There was no statistically significant difference ($p > 0.05$) of the FD values between both groups: adenoma mean value 2.424 (range 2.347 to 2.505); minimally invasive carcinomas: 2.432 (range 2.339 to 2.499). The R^2 values, however, were significantly different ($p < 0.05$), ranging between 0.922 and 0.953 (mean 0.939) in adenomas and between 0.929 and 0.952 (mean 0.943) in minimally invasive carcinomas. The percentage of cells with a Gaussian distribution of the residuals ranged from 58 to 97% (mean 79%) for adenomas and between 48 and 94% (mean 75%) for minimally invasive carcinomas.

The fractal dimension is derived from the slope of the curve in a log–log-diagram, assuming that this

curve can be well approximated by a straight line with an excellent GOF, equivalent to a high R^2 value and a normal distribution of the residuals. Since in our study many cells did not fulfill these criteria, we conclude that the calculated FDs should only be interpreted with caution. It is interesting to note that the GOF showed a statistically significant difference between both groups, whereas this was not the case for the FD. This situation is similar to an earlier study [3], where it had been shown that the GOF, but not the Minkowski–Bouligand FD, was an independent prognostic variable for patients suffering from B precursor acute lymphoid leukemia. Therefore we think that the GOF should be considered a new variable for texture analysis, especially for nuclear chromatin, where it was suggested to be a measure of "coarseness" [3]. According to international standards the differential diagnosis between adenoma and minimally invasive follicular carcinoma of the thyroid is only based on the criteria of capsular or vessel invasion but not on nuclear characteristics. Our study shows that there are subtle differences in the coarseness of the chromatin structure between the two entities, normally not visible to the human eye.

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