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Fractal Dimension as a Prognostic Factor for Laryngeal Carcinoma

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Abstract. *Background: Based on the hypothesis that Fractal Dimension (FD) reflects heterogeneity of tumor tissue, we performed an image analysis study to calculate FD of tissue specimens from patients with laryngeal carcinomas in order to investigate its prognostic value. Materials and Methods: Laryngectomy specimens from 52 patients, who had previously undergone total laryngectomy for squamous cell carcinomas, were examined and their history files reviewed. Ten patients were lost from follow-up. Fractal Analysis software was used to estimate FD of histology sections by the box-counting method. FD of carcinomatous areas was correlated with survival. Results: Patients with a FD lower than the median value of the sample, estimated in sections of carcinomas, had statistically significant higher survival rates. Conclusion: Within the sample of patients studied, FD could be used as a prognostic factor.*

Fractal Geometry, as first introduced by Mandelbrot in 1982 (1), has been used over the past 20 years in medicine for the description of the pattern of various organs and normal tissues such as the bronchi (2), the retina (3), the renal tree (4) *etc.* It has also been used for the analysis of abnormal tissue in either microscopic (5-11) (dysplastic or neoplastic) or radiologic (12-17) images.

One of the main aspects of fractal geometry is the introduction of self-similarity (1). It reflects the geometrical pattern of fractal objects and can be best understood through shapes such as the Koch curve (Figure 1) in which small pieces of the curve reflect the whole and have similar geometrical properties to it.

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The Koch curve is a "regular" fractal object possessing a Fractal Dimension (FD) which characterizes all such objects. The dimension that expresses regular fractals is the so-called *similarity dimension* D_S and is one of the numerous fractal dimensions that has been used since the first introduction of fractal geometry by Mandelbrot in 1982 (1).

The nature of dimension is strongly related to scale. For example, if a straight line of length L, a square plane with area of $A=L^2$ and a cube of volume $V=L^3$, are all equal in unit and divided to a self similar sub-length, sub-area and sub-volume of side ε, and we accept that N such parts compose those shapes then: for straight line: $L=N^*\ \varepsilon=1$ and $\varepsilon=1/N$, for a square plane: $A=N\ \varepsilon^2=1$ and $\varepsilon=1/N^{1/2}$ and for a cube: $V=N\ \varepsilon^3=1$ or $\varepsilon=1/N^{1/3}$.

By studying the above equations, it seems that in each case ε gives an estimate of the self similar dimension of the shapes so in general: $N\varepsilon^{DS}=1$, or $D_S = \frac{\log(N)}{\log(1/\varepsilon)}$, where D_S is the similarity dimension.

The Koch curve is formed by applying a number of iterations to a straight line in the following way (18): in a fixed line of length L, the middle third is removed and replaced by two segments with the length of the removed piece. In that first step (k=1) the line consists of N=4 pieces of length ε=1/3*L. In the next step (k=2), the same process of removal of the middle 1/3 and replacing it with two equal pieces forms a curve of N=16 segments of length ε=1/9*L. After a large number of similar iterations, the Koch curve is formed (Figure 1). When applying the values to the above equation of Similarity Dimension, and accepting that L equals unit, we obtain a result which is the same for all steps of the process:

$$D_S = \frac{\log(N)}{\log(1/\varepsilon)} = \frac{\log(4)}{\log(3)} = \frac{\log(16)}{\log(9)} = 1.2618....$$

So, the Similarity Dimension, which is the FD for the Koch curve, remains constant independently of the scale of iteration or "magnification" and is an estimate of the curves' properties. The actual length of the curve can not be measured, because it is dependent on the scale one chooses to calculate.

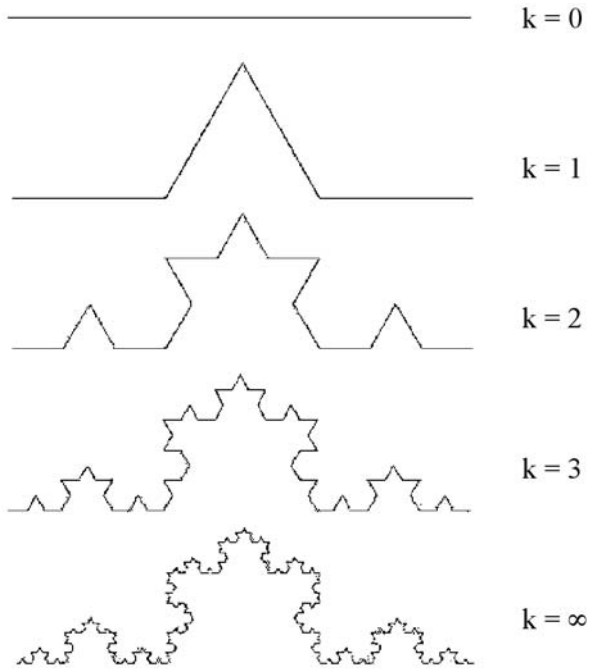

Figure 1. *The process of generating the Koch Curve.*

Apart from regular fractals – regular in the sense that they are composed of scaled-down and rotated identical copies of themselves – there is another group of fractals known as random fractals which are not self-similar but rather statistically self-similar. Each part of a random fractal has the same statistical properties of the whole (18). A typical random fractal is a coastline of an island (19) (or a cell) because each randomly selected segment of the coastline possesses the same statistical properties over all scales of magnification (12). Just as a coastline reveals more details over numerous, actually infinite scale-down magnifications, the perimeter of a cell reveals more details over numerous magnifications.

An exact calculation of the perimeter of an island (19) (or a cell) is impossible since it is dependent on the scale of magnification used. That is, if one uses a certain scale to estimate the perimeter, the same calculation for a lower scale would give a different result, since more details are revealed. Instead, a random fractal such as a coastline is characterized by its FD which is as independent of the scale of magnification as is the Similarity FD for the Koch curve. FD, when dealing with random fractals such as coastlines or biological images such as cells, is best calculated by the box-counting (19) method. The dimension derived by this method is the box-counting dimension and is calculated by first covering the object (island, cell or tissue for example) by squares or "boxes" with a side length of δ. If N is the number of boxes sized δ that completely cover the object, then the box-counting dimension is calculated by the equation:

Table I. *Characteristics of the sample.*

Variable	No.	Percentages (%)
Sex		
Women	5	9.6
Men	47	90.4
Cell Differentiation		
Well	17	32.7
Moderately	19	36.5
Poor	16	30.8
Outcome		
Survived	39	75.0
Dead or censored	13	25.0
Clinical Stage (Tumor size)		
T1	1	1.9
T2	11	21.2
T3	33	63.5
T4	7	13.5

$$D_B = \lim_{\delta \to 0} \frac{d(\log (N))}{d(\log (1/\delta))}$$

which is in the form of a graph of log (N) against log (1/δ). The gradient of the best fit line through the points gives an estimate of the box-counting fractal dimension of the object (18).

Box-counting methods have been used to calculate fractal dimensions in biological objects and images such as mammograms (6, 17), the retina (3) and the pattern of bronchi (2). Neoplasms in organs including the larynx (20) have also been studied by fractal analysis for demonstrating differences between normal, dysplastic and neoplastic tissue or cells (21), estimating angiogenesis (22) and evaluating the response of anticancer therapy (23).

To our knowledge, there are no reports to date of the FD as a prognostic factor. Since the pattern of tumor cells is irregular compared to the cells of the normal epithelium, one could assume that calculating the nonlinear spreading and heterogeneity of neoplastic tissue within the healthy cells would provide an estimate of the tumor's aggressiveness. Presuming that FD describes such an irregularity, it could be used for an independent objective measurement of cancer spreading.

Given such a hypothesis, we estimated FD in laryngeal specimens from patients operated for squamous cell carcinomas of the larynx, in order to evaluate its significance as a prognostic factor.

Patients and Methods

Histology specimens from 52 patients, who underwent total laryngectomy for laryngeal squamous cell carcinoma between 1985 and 1991, were re-evaluated (Table I). Sections from paraffin blocks were cut at 4 μ, stained with haematoxylin-eosin and reviewed by a pathologist. Under the microscope, digital photographs were captured through a CCD camera and stored on a desktop personal

Table II. *Characteristics of measured Fractal Dimensions.*

95% confidence interval for the mean								Data distribution	
mean	Lower bound	Upper bound	median	Standard deviation	Min.	Max.	*N*	*Kolmogorov-Smirnov Z*	*p*
1.6832	1.5727	1.7938	1.6864	0.0695	1.5952	1.7650	40	0.553	0.920

Table III. *Correlation of Fractal Dimensions with survival.*

FD	Mean time (yrs)	Standard Error	95% Confidence interval		Log rank test	df	*p*
			Lower bound	Upper bound			
<1.6864	11.57	1.01	9.58	13.56	5.98	1	0.0144
>1.6864	4.09	0.90	2.33	5.85			

computer. For each case, photographs from neoplastic tissue were obtained under 400x magnification.

A fractal-calculator software (provided by the Foundation of Research and Technology, Crete, Greece) was used, and by the box-counting method the FD was calculated for each image. The software converts the image to black and white and then repeatedly covers it by squares (boxes) of varied size (δ) of 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 pixels. Through the black and white conversion, intensely-stained particles of the tissue that completely fill the plane of the image are exposed as black pixels. The number of "boxes" (*N*), for each of the above box size, that completely cover the image that contain only black pixels are then calculated and values are applied to the aforementioned equation for the calculation of the Box-counting Dimension. The gradient of the line gives the FD, which expresses irregularity and heterogeneity of stained characteristics of cells dominating the tissue studied.

In order to achieve credible results, images captured had to consist entirely of similar tissue throughout the plane without any "empty" areas or artifacts such as stain particles that would be interpreted as white or black pixels, respectively, through the conversion of the image to black and white. Therefore, such images were omitted from the analysis. The actual number of cases is presented in Table II as *N*.

In addition, hospital reports of the patients were reviewed, and survival was analyzed and related to FD calculations. Statistical analysis was achieved by the use of SPSS for Windows, 8.0 SPSS Inc., 1997 software.

Results

The total number of patients was 52, with 47 males (90.4%) and 5 (9.6%) females. Mean age ±SD was 62.6±10.1 years old (range 40-91). Descriptive statistics of the sample are presented in Table I. Mean follow-up time was 3.74 years (44.91 months) after the time of first diagnosis. Ten out of 52 patients were lost from follow-up (Patients No. 21, 22, 27, 28, 29, 34, 37, 45, 46 and 50). Descriptive statistics of measured FD are presented in Table II. A Kolomogorov-Smirnov test was assessed to examine FD data distribution. FD variables were normally distributed. A Kaplan-Meier analysis was assessed to examine possible differences in the survival time of patients (Table III). Survival plots were created using median value of FD as a cut-off (1.6864). Statistically significant differences were noted between patients with FD higher and lower than the median value (Figure II).

Discussion

In 2001 West *et al.* described a General Model for Tissue Growth "based on the premise that the tendency of natural selection to optimise energy transport has led to the evolution of fractal- like distribution networks" (24).

Guiot *et al.* (25) have investigated the extension of this model to the growth of solid malignant tumors. Their results "support the notion that tumor growth follows such a universal law" and its "relevance for tumor metastasis , recurrence, cell turnover rates, angiogenesis and invation" parameters are directly related to prognosis.

Prognosis of malignancies is predominately based on retrospective epidemiological studies. Patients are classified in certain prognostic groups and are expected to follow the prognosis of the group.

Diagnosis and prognosis based on morphometric criteria of specific tumors of patients could lead to more precise information on the pattern of tumor spreading, which would emphasize more individualized prognosis factors and, thus, appropriate modality selection.

Morphometric methods have been used in laryngeal cancer (26, 27). These studies have used nuclear parameters (perimeter, axis, area and shape) based on Euclidean Geometry, assuming that the nucleus has a standard shape such as a circle.

FD differs from these parameters by describing the abnormal, irregular pattern of tumor cells and tissue.

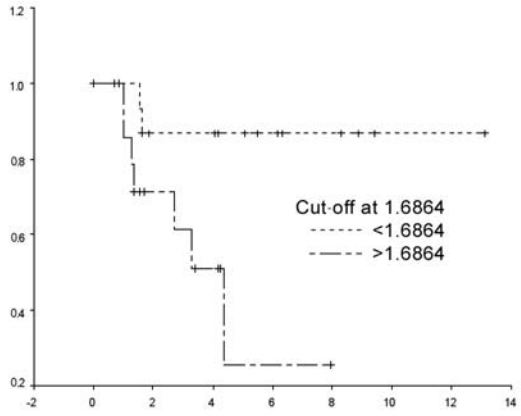

Figure 2. *Survival (years).*

Furthermore, the image analysis system by which it is calculated is an objective, automated method with minimal human interference.

In our study, patients with FD greater than the median value of the sample had a worse prognosis than patients with a value of FD below the median value (within 95% confidence interval). This confirms the hypothesis that the complicity of tumor cells, which is expressed in the present study by their FD, can be used as a prognostic factor.

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